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Wpływ inhibitora hydroksylazy tryptofanu – LP533401 na parakrynny układ kinureninowy tkanki kostnej w doświadczalnym modelu przewlekłej choroby nerek

Rozprawa doktorska w oparciu o cykl publikacji naukowych

w dziedzinie nauk medycznych i nauk o zdrowiu

w dyscyplinie nauki farmaceutyczne

Białystok 2021

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Spis treści

Rozdział 1. Wykaz publikacji będących podstawą rozprawy doktorskiej.

Łączna wartość Impact Factor dla cyklu publikacji: 4.556
Łączna ilość punktów MNiSW dla cyklu publikacji: 240 pkt

Lista publikacji stanowiących rozprawę doktorską:

1. Mor A, Kałaska B, Pawlak D. Kynurenine Pathway in Chronic Kidney Disease: What's Old, What's New, and What's Next? International Journal of Tryptophan Research 2020; 13:1-18. Doi: 10.1177/1178646920954882.
   MNiSW = 100 pkt
2. Mor A, Pawlak K, Kałaska B, Domaniewski T, Sieklucka B, Ziemińska M, Cylwik B, Pawlak D. Modulation of the Paracrine Kynurenic System in Bone as a New Regulator of Osteoblastogenesis and Bone Mineral Status in an Animal Model of Chronic Kidney Disease Treated with LP533401. International Journal of Molecular Sciences 2020; 21:E5979. Doi: 10.3390/ijms21175979.
   IF = 4,556; MNiSW = 140 pkt

Wykaz stosowanych skrótów:

25(OH)D, 25-hydroxyvitamin D, 25-hydroksywitamina D

5-HT, 5-hydroxytryptamine, 5-hydroksytryptamina, serotonina

AhR, aryl hydrocarbon receptor, receptor węglowodorów aromatycznych

ATF4, activating transcription factor 4, czynnik transkrypcyjny 4

BMD, bone mineral density, gęstość mineralna kości

cDNA, complementary DNA, komplementarny DNA

CKD, chronic kidney disease, przewlekła choroba nerek

CKD-MBD, chronic kidney disease mineral and bone disorders, zaburzenia mineralno-kostne w przebiegu przewlekłej choroby nerek

CREB, cyclic AMP-responsive element-binding protein, białko wiążące element odpowiedzi cyklicznego AMP

FOXO1, forkhead box protein O1, czynnik transkrypcyjny O z rodziny forkhead

GFR, glomerular filtration rate, współczynnik filtracji kłębuszkowej

HPLC, high-performance liquid chromatography, wysokosprawna chromatografia cieczowa

IDO, indoleamine 2,3-dioxygenase, 2,3-dioksygenaza indolowa

IL-1, interleukin 1, interleukina 1

IL-6, interleukin 6, interleukina 6

KYN, kynurenine, kinurenina

OUN, ośrodkowy układ nerwowy

PChN, przewlekła choroba nerek

qRT-PCR, quantitative real-time reverse transcriptase polymerase chain reaction, ilościowa łańcuchowa reakcja polimerazy w czasie rzeczywistym

RNA, ribonucleic acid, kwas rybonukleinowy

SD, standard deviation, odchylenie standardowe

TDO, tryptophan 2,3-dioksygenase, 2,3-dioksygenaza tryptofanowa

TPH, tryptophan hydroxylase, hydroksylaza tryptofanu

TRP, tryptophan, tryptofan

Rozdział 2. Wprowadzenie

Przewlekła choroba nerek (PChN) jest wieloobjawowym zespołem chorobowym. Przyczyną jego jest zmniejszenie ilości czynnych nefronów, które ulegają degradacji na skutek różnych procesów chorobowych toczących się w miąższu nerek. Podstawowe kryteria rozpoznania PChN według National Kidney Foundation-Kidney Disease Outcomes Quality Initiative obejmują po pierwsze - zmiany strukturalne lub czynnościowe narządu w postaci nieprawidłowości morfologicznych lub wskaźników uszkodzenia nerek w badaniach biochemicznych oraz zmian w składzie krwi, moczu lub badaniach obrazowych, które utrzymują się przez co najmniej 3 miesiące, z prawidłową lub obniżoną filtracją kłębuszkową (GFR), po drugie - utrzymujące się przez ponad 3 miesiące, niższe niż 60 ml/min/1,73 m^2 powierzchni ciała wartości GFR z lub bez uszkodzenia nerek [1-7].

Globalnie schorzenie to dotyka około 9% osób dorosłych, zaś występowanie PChN w stadium przynajmniej umiarkowanym w populacji światowej w przedziale powyżej 65 roku życia szacuje się na poziomie oscylującym w granicach 30% [2,4-6]. PChN charakteryzuje się postępującym upośledzeniem czynności wydalniczej, wewnątrzwydzielniczej i metabolicznej nerek. Zmianom tym towarzyszy uszkodzenie innych narządów i układów, przy czym mechanizmy leżące u ich podstaw nie zostały w pełni poznane. Najczęstsze zaburzenia homeostazy organizmu związane z rozwojem PChN obejmują niedokrwistość, kwasicę metaboliczną, miażdżycę tętnic i zakrzepicę prowadzące do częstego występowania incydentów sercowo-naczyniowych, a także zaburzenia endokrynologiczne, neurologiczne oraz mineralne i kostne [6-10]. U pacjentów z PChN obserwuje się również nieprawidłowości w obrębie gospodarki aminokwasowej. Szczególnie istotnym elementem w aspekcie toksycznym jest kumulacja w organizmie produktów degradacji tryptofanu (TRP). Wzrost ich stężenia w przebiegu PChN jest istotnym czynnikiem leżącym u podstaw rozwoju wielu objawów mocznicowych ze strony układu krążenia, endokrynologicznego, ośrodkowego układu nerwowego (OUN), a także kostnego [3,10-21].

Zaburzenia gospodarki hormonalnej, mineralnej oraz metabolizmu kostnego należą do powszechnie występujących, a zarazem najbardziej złożonych pod względem etiologii, powikłań układowych towarzyszących PChN. Zmiany patologiczne w tkance kostnej, ze względu na wysoką częstość występowania, zostały odrębnie sklasyfikowane, jako zaburzenia mineralno-kostne w przebiegu PChN, określane mianem CKD-MBD (chronic kidney disease mineral and bone disorders) [6,7,22,23]. Dane wskazują, że zespół CKD-MBD może rozwijać się już we wczesnych stadiach PChN, kiedy choroba nerek nie manifestuje jeszcze swoich objawów [22]. Badania nad nieprawidłowościami w metabolizmie kostnym w przebiegu PChN wykazały, że chorobie tej towarzyszą postępujące zmiany w gospodarce fosforanowo-wapniowej i hormonalnej. Zaburzenia endokrynologiczne obejmują, między innymi, zmiany stężeń parathormonu, 25-hydroksywitaminy D (25(OH)D), kalcytriolu i innych metabolitów witaminy D, czynnika wzrostu fibroblastów-23 oraz hormonu wzrostu [24-26]. Podczas progresji PChN nerki tracą zdolność do wydalania fosforanów, co prowadzi do hiperfosfatemii. To z kolei powoduje wzrost poziomu parathormonu i czynnika wzrostu fibroblastów-23 oraz obniżenie podaży kalcytriolu, w skutek czego zmniejsza się wchłanianie wapnia w jelicie [25]. Zmiany te powiązane są z zahamowaniem procesów osteoanabolicznych oraz nasileniem resorpcji kości, co prowadzi do utraty masy kostnej, obniżenia wytrzymałości kości, a tym samym zwiększonego ryzyka złamań, w tym także tych samoistnych [24,26]. Z tego powodu w sposób znaczący obniżają one jakość życia chorych na PChN oraz mogą przyczyniać się do ich kalectwa i zwiększonej śmiertelności.

Ostatnie doniesienia wskazują, że zaburzenia metabolizmu aminokwasów obserwowane w przebiegu PChN odgrywają istotną rolę w rozwoju CKD-MBD. Dowody na kumulację metabolitów

TRP w przebiegu przewlekłej niewydolności nerek oraz na ich wpływ na tkankę kostną [16-20,27-31] stały się podstawą do dalszych badań, które pozwoliły szerzej poznać zależności między występowaniem PChN, zaburzeniami przemian TRP, a metabolizmem tkanki kostnej [24-26,32].

TRP jest egzogennym aminokwasem biogennym, który ulega przemianom na drodze kilku szlaków metabolicznych. W skutek tego, powstaje szeroka gama biologicznie aktywnych związków wywierających zróżnicowany wpływ na liczne procesy zachodzące w organizmie [33-35]. TRP jest prekursorem serotoniny (5-HT), a jego konwersja do tej aminy jest inicjowana przez hydroksylazę tryptofanu (TPH). W organizmie zaobserwowano dwie izoformy tego enzymu (TPH-1 i TPH-2). TPH-1 inicjuje obwodową syntezę 5-HT, głównie w dwunastnicy, podczas gdy ekspresja drugiej izoformy - TPH2 - obserwowana jest wyłącznie w OUN. 5-HT pełni rolę przede wszystkim ważnego neuroprzekaźnika w OUN, modulującego szeroki zakres procesów fizjologicznych, takich jak: percepcja, nastrój, apetyt, termoregulacja, funkcje poznawcze, wrażliwość na ból, zachowania seksualne, sen i rytm okołodobowy, ponadto obwodowo wykazuje ona aktywność hormonu tkankowego [32,36-40]. W ostatnich latach dowiedziono, iż współuczestniczy między innymi w regulacji metabolizmu kostnego. Zagadnienia dotyczące 5-HT i jej wpływu na metabolizm tkanki kostnej wciąż budzą kontrowersje [32,40,41]. Udowodniono istnienie dwóch niezależnych pul 5-HT: centralnej, syntezowanej w mózgu i uwalnianej w obrębie OUN oraz obwodowej wytwarzanej w przez enterocyty i wydzielanej do układu krążenia. Są one całkowicie niezależne od siebie, gdyż amina ta nie przenika przez barierę krew-mózg [37]. 5-HT wywiera przeciwstawny wpływ na strukturę kostną, a jej efekt metaboliczny jest ściśle związany z miejscem występowania. 5-HT w OUN, na drodze modulacji aktywności układu współczulnego, wykazuje pośrednie działanie osteoanaboliczne i promuje proliferację osteoblastów [41-43]. Z kolei obwodowa 5-HT silnie hamuje proliferację osteoblastów nie wpływając na procesy resorpcji kości [32,44,45].

Dotychczasowe badania z wykorzystaniem zwierzęcego modelu PChN, prowadzone w naszym Zakładzie, dowiodły, iż obwodowe stężenie 5-HT oraz jej metabolitu - kwasu 5-hydroksyindolooctowego istotnie rośnie w przebiegu niewydolności nerek. Efekt ten skutkuje osłabieniem metabolizmu struktury kostnej, co przekłada się na obniżenie parametrów wytrzymałościowych kości [32]. Istnieją realne przesłanki, że osłabienie biosyntezy obwodowej 5-HT może stanowić potencjalną opcję terapeutyczną w leczeniu stanów chorobowych, którym towarzyszy spadek masy kostnej [24,37,46]. Jednym ze związków zdolnych do blokowania aktywności izoformy TPH-1 jest małocząsteczkowy inhibitor tego enzymu - LP533401. Nie przenika on przez barierę krew-mózg, dzięki czemu nie wpływa na ośrodkową produkcję 5-HT [45]. Badania wykazały, że spadek stężenia 5-HT w osoczu spowodowany podaniem LP533401 w przypadku owariektomizowanych zwierząt przekładał się u nich na wzrost gęstości mineralnej kości (BMD) oraz poprawę ich parametrów biomechanicznych [47,48]. Udowodniono również, że inhibicja obwodowej syntezy 5-HT przez LP533401 w zwierzęcym modelu CKD-MBD skutkowała zahamowaniem, a nawet cofaniem się niekorzystnych zmian w gospodarce mineralnej oraz strukturze tkanki kostnej, do stanu porównywalnego z obserwowanym u zdrowych zwierząt, jednakże zagadnienie to do dnia dzisiejszego nie zostało w pełni wyjaśnione [24,25,49].

Obwodowy TRP metabolizowany jest szlakiem serotoninowym zaledwie w 1%, zdecydowana większość tego aminokwasu (ok. 94%) jest enzymatycznie degradowana szlakiem kinureninowym, w wyniku czego powstają liczne substancje obdarzone aktywnością biologiczną. Końcowym produktem przemian kinurenin jest utleniona forma dinukleotydu nikotynamidoadeninowego. Koenzym ten uczestniczy w wielu procesach na poziomie komórkowym, w tym oddychania komórkowego [33-35,46,50-55].

Kinurenina (KYN) i jej pochodne odgrywają znaczącą rolę w modulowaniu szeregu procesów fizjologicznych, a także przyczyniają się w dużej mierze do pojawienia się zmian patologicznych - pełnią istotną rolę w generowaniu stresu oksydacyjnego, są składową procesów zapalnych, modulują glukoneogenezę, a także zaburzenia homeostazy wapniowej, co w konsekwencji upośledza funkcję mitochondriów. Wewnątrzkomórkowy wzrost stężenia KYN może generować apoptozę. Aktywacja przemian kinureniny odgrywa istotną rolę w patogenezie licznych schorzeń, między innymi neurodegeneracji, retinopatii cukrzycowej, zmian nowotworowych, czy chorób układu krążenia. [13-15,27,28,54-61]. KYN i jej metabolity są agonistami receptora węglowodorów aromatycznych (AhR). Należy on do rodziny czynników transkrypcyjnych, występuje w cytoplazmie i odgrywa kluczową rolę w regulowaniu aktywności licznych szlaków sygnałowych, odpowiedzialnych za utrzymanie homeostazy komórkowej. Nadmierna aktywacja AhR, skutkuje zaburzeniami homeostazy komórkowej, co z kolei przekłada się na aktywację procesów starzenia komórek oraz tempa ich apoptozy [14,15,55-66].

W przebiegu PChN dochodzi do kumulacji licznych produktów przemiany materii, wśród nich obserwuje się także wzrost osoczowego i tkankowego stężenia metabolitów szlaku kinureninowego. Powyższy efekt jest wypadkową dwóch mechanizmów, z jednej strony aktywacji procesów zapalnych towarzyszących PChN i aktywacji przemian kinureniny, z drugiej - upośledzonej nerkowej eliminacji produktów degradacji TRP [2,3,16-19,54,56,64-75]. Przewlekły proces zapalny prowadzi do wzrostu stężenia interleukin (IL-1 i IL-6), czynnika martwicy nowotworów α oraz interferonów (w odpowiedzi na infekcję wirusową), które aktywują 2,3-dioksygenazę indolową (IDO) [64-69]. Jest to enzym, który podobnie jak 2,3-dioksygenaza tryptofanowa (TDO), zapoczątkowuje procesy degradacji TRP szlakiem kinureninowym [2,3,56,70-73]. Należy jednak pamiętać, że zmiany aktywności wielu enzymów tego szlaku często nie idą w parze ze stężeniem jego składowych obserwowanym podczas progresji PChN [72]. Aktywność aminotransferazy kinureninowej, kinureninazy i 3,4-dioksygenazy 3-hydroksyantranilowej pozostaje niezmieniona lub nawet zmniejszona, niezależnie od stopnia zaawansowania PChN i nasilenia stanu zapalnego. Pomimo tego, stężenia większości metabolitów szlaku kinureninowego we krwi i tkankach (mięśnie, jelita, płuca, wątroba, śledziona i mózg) dodatnio korelują ze stopniem niewydolności nerek [16,17,72,73]. W tym miejscu warto zwrócić uwagę na fakt, iż metabolity tego szlaku, podobnie jak większość toksyn mocznicowych, są wydalane z moczem, na drodze filtracji kłębuszkowej [54,75]. Dlatego spadek funkcji wydalniczej nerek, towarzyszący zmianom degeneracyjnym w ich miąższu związanym z rozwojem PChN, prowadzi do zahamowania wydalania tych związków.

Dotychczasowe doniesienia wskazują, iż towarzyszące rozwojowi PChN zaburzenia aktywności szlaku kinureninowego, prowadzące do kumulacji jego toksycznych metabolitów, wydają się jednym z istotniejszych czynników leżących u podstaw rozwoju wielu powikłań układowych towarzyszących mocznicy oraz szerzej opisanych poniżej zaburzeń metabolizmu kostnego [2,3,9,13-15,26-28,56-61,66,69,70].

Badania z ostatnich lat wskazują, że KYN posiada zdolność modulowania procesów metabolicznych w tkance kostnej. Kałaska i wsp. udowodnili, że KYN, podobnie jak 5-HT, syntezowana w OUN wpływa stymulująco na procesy osteoanaboliczne, podczas gdy KYN produkowana i wydzielana w tkankach poza OUN przyczynia się do rozwoju patologicznych zmian w strukturze kości. Zaobserwowano dodatnią korelację między stężeniem KYN w korze czołowej i TRP w podwzgórzu i prążkowiu oraz parametrami biomechanicznymi i geometrycznymi kości oraz ujemną zależność pomiędzy analizowanymi parametrami kostnymi i stężeniem KYN w surowicy [26,76]. Mechanizm leżący u podstaw protekcyjnego działania KYN syntetyzowanej i uwalnianej ośrodkowo wobec metabolizmu kostnego, pomimo, że nie został dokładnie poznany, wydaje się być zależnym od

takich obszarów mózgu, jak: podwzgórze, kora czołowa i prążkowie [76]. Z kolei wysoce prawdopodobnym jest, że KYN syntetyzowana w tkankach obwodowych może wpływać na tkankę kostną modulując aktywność receptora AhR [26]. W badaniach na modelu zwierzęcym dowiedziono bowiem, że nasilona aktywacja AhR może prowadzić do niekorzystnych zmian w metabolizmie kostnym. W osteoblastach powoduje ona zahamowanie ich proliferacji i różnicowania [77], natomiast w przypadku osteoklastów stymulacja AhR powoduje wzrost ich aktywności i nasilenie procesu resorpcji kości [78,79]. Powyższe obserwacje sugerują, że aktywacja AhR może mieć kluczowe znaczenie dla utrzymania odpowiedniej masy kostnej, a zmiany w parametrach kostnych mogą być w dużym stopniu zależne od stopnia ekspresji tego receptora w tkance kostnej. Potwierdzają to między innymi doniesienia, iż podawanie egzogennych agonistów AhR wpływa negatywnie na parametry wytrzymałościowe kości, jak również fakt, że genetyczny niedobór tego receptora przekłada się na wzrost masy kości i zahamowanie procesu ich resorpcji przez osteoklasty [30,79]. W jednym z wcześniejszych badań realizowanych w naszym Zakładzie oceniono poziom ekspresji genu AhR w tkance kostnej w zwierzęcym modelu PChN. U zwierząt nefrektomizowanych był on istotnie podwyższony, w porównaniu ze zwierzętami zdrowymi [26]. Niewątpliwie, kumulacja innych metabolitów KYN (kwasu kinurenowego, 3-hydroksykinureniny) może także być powodem aktywacji receptora AhR. [80]. Występowanie dodatnich korelacji między poziomem ekspresji genu AhR w tkance kostnej, a stężeniem KYN w surowicy oraz ujemnych między ekspresją AhR, a parametrami wytrzymałościowymi i densytometrycznymi tkanki kostnej wskazuje, że wyżej opisany mechanizm wydaje się być przynajmniej częściowo odpowiedzialny za obwodowe efekty działania KYN na tkankę kostną [26].

Bez wątpienia, konieczne są dalsze badania i analizy udziału szlaku kinureninowego i jego ewentualnych powiązań ze szlakiem serotoninowym oraz metabolizmem kostnym w przebiegu PChN. Tego typu wiedza może stać się kluczem do opracowania nowych rozwiązań terapeutycznych, które przełożą się na wyższą skuteczność leczenia zaburzeń metabolizmu kostnego związanych z PChN.

Rozdział 3. Cel pracy z uzasadnieniem podjętej tematyki badawczej

Zaburzenia mineralno-kostne rozwijające się u pacjentów z PChN, określane mianem CKD-MBD, w sposób znaczący obniżają jakość ich życia. Mogą one prowadzić do występowania samoistnych złamań kości, przyczyniając się do kalectwa oraz zwiększonej śmiertelności wśród tej populacji pacjentów [6,7,22-26]. Doniesienia z ostatnich lat dowiodły, że obwodowa 5-HT, jeden z metabolitów TRP, może odgrywać ważną rolę w rozwoju CKD-MBD. Okazało się bowiem, iż 5-HT uwalniana z enterocytów do krążenia hamuje aktywność osteoblastów i procesy kościotworzenia. Z kolei 5-HT pochodząca z OUN, działając jako neuroprzekaźnik, w sposób pośredni wywiera całkowicie przeciwny efekt na tkankę kostną [32,36-46]. Co więcej, inhibicja obwodowej syntezy 5-HT przez LP533401 hamuje, a nawet cofna niekorzystne zmiany w gospodarce mineralnej oraz w strukturze tkanki kostnej [24,25,45,47-49]. Jednak, jedynie niewielka część całkowitej puli TRP w organizmie, gdyż zaledwie około 1%, metabolizowana jest do 5-HT, natomiast ogromna jego większość ulega przemianom na drodze szlaku kinureninowego [33-35]. Liczne dane wskazują na istotną rolę głównej składowej tego szlaku - KYN oraz jej metabolitów w rozwoju objawów PChN i CKD-MBD [2,3,9,13-15,26,28,56-61,66,69,70]. Badania prowadzone w ostatnim czasie dowiodły, że podobnie jak 5-HT, KYN wytwarzana w OUN pozytywnie wpływa na parametry wytrzymałościowe kości, podczas gdy KYN uwalniana obwodowo wywiera na nie całkowicie odwrotny efekt [26,76]. Brak jest jednak jakichkolwiek informacji dotyczących występowania metabolitów szlaku kinureninowego w obrębie samej tkanki kostnej oraz ich ewentualnego wpływu na jej metabolizm. Oprócz tego zagadką pozostaje kwestia, czy hamowanie syntezy 5-HT może w jakikolwiek sposób wpłynąć na aktywność szlaku kinureninowego oraz jakie mogą być tego konsekwencje dla gospodarki mineralnej i parametrów tkanki kostnej. To pytanie wydaje się uzasadnione w związku z faktem, iż zarówno 5-HT, jak i KYN są produktami metabolizmu tego samego związku.

Biorąc powyższe pod uwagę celem niniejszej rozprawy było:

1. Ustalenie czy tkanka kostna posiada parakrynny układ kinureninowy oraz wskazanie jego ewentualnego wpływu na metabolizm kostny.

2. Ocena czy farmakologiczna modulacja aktywności obwodowej hydroksylazy tryptofanowej (TPH-1) przez LP533401 może wpływać na aktywność szlaku kinureninowego w tkance kostnej szczurów poddanych nefrektomii oraz określenie podłoża i potencjalnych konsekwencji tego procesu związanych z osteogenezą, regulacją gospodarki mineralnej i metabolizmem kostnym.

Rozdział 4. Realizacja celów naukowych, materiały i metody badawcze, podsumowanie wyników badań i dyskusja

Materiały i metody badawcze

Część doświadczalna niniejszej rozprawy jest kontynuacją prowadzonych przez nasz zespół badań związanych z osteoporozą towarzyszącą PChN (numer pozwolenia Lokalnej Komisji Bioetycznej ds. Badań na Zwierzętach: uchwała nr 29/2013 z dnia 24 kwietnia 2013 roku) [24,25,49]. W ramach grantu pt.: „Hamowanie aktywności hydroksylazy tryptofanu typu I - nowa koncepcja leczenia zaburzeń metabolizmu kostnego w przewlekłej niewydolności nerek", finansowanego przez Narodowe Centrum Nauki, wykorzystano materiał biologiczny uzyskany od zwierząt z doświadczalną niewydolnością nerek.

Szczury zostały sklasyfikowane w pięciu grupach. Wybrane losowo zwierzęta, które trafiły do grupy kontrolnej poddano tzw. „operacji pozorowanej", która polegała na przecięciu powłok skórnych oraz wypreparowaniu z otoczek łącznotkankowych obu nerek, bez naruszenia ich struktury (n= 10). U pozostałych wywołano indukowaną eksperymentalnie niewydolność nerek, poprzez zabieg 5/6 subtotalnej nefrektomii. Następnie, nefrektomizowane zwierzęta podzielono losowo na 4 równej wielkości grupy (n= 16): nieleczone, otrzymujące rozpuszczalnik oraz leczone LP533401 w dawkach 30 mg/kg i 100 mg/kg przez 8 tygodni. Wielkość dawek LP533401 oparto na wcześniejszych badaniach [45,48,81]. Rozpuszczalnik dla LP533401 stanowiła mieszanina glikolu polietylenowego z 5% dekstrozą w stosunku 40/60. Do badań wchodzących w skład niniejszej rozprawy wykorzystano materiał biologiczny (surowica, mocz, kość udowa, jelito cienkie) wcześniej pobrany i zabezpieczony (temp. -80°C) w trakcie badań prowadzonych w ramach powyższego grantu.

W ramach niniejszej rozprawy doktorskiej oznaczono stężenia TRP i KYN w surowicy, moczu (oceniono ich dobową eliminację) oraz w homogenacie uzyskanym z tkanki kostnej (część korowa, część beleczkowa), a także w homogenacie jelita cienkiego. Stężenie TRP i KYN oceniono techniką wysokosprawnej chromatografii cieczowej (HPLC). Pomiar przeprowadzono według metody opracowanej i opisanej przez Holmes'a [82].

Ekspresję genu TDO w tkance kostnej przeprowadzono z wykorzystaniem metody ilościowej reakcji łańcuchowej polimerazy w czasie rzeczywistym (qRT-PCR). RNA został wyizolowany z komórek metodą kolumienkową przy użyciu komercjalnego zestawu do izolacji AxyPrep Multisource RNA miniprep kit (Axygen, USA) według instrukcji producenta. Pomiar ilości i jakości RNA został przeprowadzony na bioanalizatorze Agilent 2100 Bioanalyzer (Agilent Technologies, USA). Synteza cDNA została przeprowadzona za pomocą komercyjnego zestawu RevertAid First Stand cDNA Synthesis Kit (Fermentas, Canada) przy użyciu termocyklera DNAEngine (Bio-Rad). Reakcja amplifikacji PCR była przeprowadzona przy użyciu komercyjnego zestawu odczynników do qRT-PCR Maxima SYBR Green qPCR Master Mix (Fermentas, Canada). Poziom ekspresji TDO został oceniony względem ekspresji referencyjnego genu dehydrogenazy aldehydu 3-fosfoglicerynowego.

Do oceny rozkładu danych zastosowano test normalności Shapiro-Wilka. Dane o rozkładzie normalnym wyrażono w postaci średniej ± SD, natomiast dane niegaussowskie przedstawiono jako medianę i pełny zakres wartości. Przeprowadzono wiele porównań grupowych, stosując jednokierunkową analizę wariancji (ANOVA), a istotne różnice między grupami przeanalizowano za pomocą testu posthoc Duncana przy wartości $p<0,05$. Korelacje między badanymi zmiennymi obliczono za pomocą analizy korelacji rang Spearmana. Dwustronna wartość $p<0,05$ została uznana za statystycznie znaczącą. Wszystkie obliczenia przeprowadzono za pomocą oprogramowania komputerowego Statistica ver. 13 (StatSoft, Tulsa, OK, USA). Graficzną prezentację wyników

przeprowadzono przy użyciu oprogramowania R w wersji 3.6.1 lub oprogramowania GraphPad Prism 6.0 (GraphPad Software, San Diego, CA, USA).

Podsumowanie wyników i dyskusja

Wyniki badań stanowiących podstawę mojej rozprawy doktorskiej opublikowano na łamach czasopisma **International Journal of Molecular Sciences** - artykuł oryginalny zatytułowany „*The modulation of the paracrine kynurenic system in bone as a new regulator of osteoblastogenesis and bone mineral status in animal model of chronic kidney disease treated with LP533401*". Jednoznacznie wykazano w nich obecność, parakrynnego układu kinureninowego występującego w tkance kostnej szczurów, który pozostawał elementem niezależnym od obwodowej puli metabolitów tego układu, które pochodziły z innych tkanek. Dowiedziono także, że modulacja aktywności szlaku kinureninowego zaobserwowana podczas hamowania aktywności hydroksylazy tryptofanowej (TPH-1) przez LP533401 wiązała się z istotnym pogorszeniem parametrów mineralnych kości. Oprócz tego, zaobserwowano występowanie zależności pomiędzy zmianami w poziomie ekspresji genu 2,3-dioksygenazy tryptofanowej (TDO), odpowiedzialnej za aktywność szlaku kinureninowego w tkance kostnej zwierząt po podaniu LP533401, a zaburzeniami równowagi między obwodowymi stężeniami 5-HT i 25(OH)D. Ponadto, stwierdzono obecność ścisłych powiązań między poziomem ekspresji genów uczestniczących w regulacji osteoblastogenezy i zależną od TDO aktywacją szlaku KYN w tkance kostnej szczurów mocznicowych otrzymujących LP533401.

Jak już zaznaczono powyżej, badania prowadzone w ramach niniejszej rozprawy stanowią kontynuację cyklu badań dotyczących oceny wpływu hamowania obwodowej syntezy 5-HT przez LP533401 na parametry kostne, zaburzenia hormonów kalciotropowych i ekspresję genów zależnych od 5-HT zaangażowanych w osteoblastogenezę w zwierzęcym modelu niewydolności nerek [24,25,49]. W poprzednim cyklu doświadczeń, dotyczącym wpływu ośrodkowego i obwodowego układu kinureninowego u młodych szczurów z łagodnym do umiarkowanego PChN na tkankę kostną, Kałaska i wsp. wykazali, że aktywacja układu kinureninowego w obrębie OUN stymuluję osteogenezę u tych zwierząt. Z kolei, pobudzenie komponenty obwodowej tego układu prowadzi do rozwinięcia się niekorzystnych zmian w obrębie geometrii, wytrzymałości i mikroarchitektury kości [26,76]. W niniejszej rozprawie przeprowadzono ocenę stężenia TRP, KYN i współczynnika KYN/TRP, który powszechnie wykorzystywany jest jako wskaźnik aktywności szlaku kinureninowego. Aktywacja obwodowego układu kinureninowego u szczurów z mocznicą, zaobserwowana w trakcie obecnych badań, była zgodna z wcześniejszymi eksperymentami prowadzonymi na zwierzęcym modelu PChN, jak również z danymi klinicznymi u pacjentów z PChN [16,26,70,75,83,84]. Po raz pierwszy wykazano także obecność TRP i KYN w tkance kostnej we wszystkich grupach eksperymentalnych. Spośród trzech enzymów, zdolnych metabolizować TRP do KYN (IDO-1, IDO-2 i TDO), w obrębie tkanki kostnej wykazano jedynie ekspresję genu TDO. Co więcej, korelowała ona ze stężeniem KYN i współczynnikiem KYN/TRP w strukturze kostnej badanych zwierząt. Nie zaobserwowano zależności między stężeniem KYN w surowicy i tkance kostnej. Powyższe wyniki jednoznacznie wskazują, iż tkanka kostna posiada własny, parakrynny, zależny od TDO, układ kinureninowy pozostający niezależnym od obwodowego, który zdolny jest do lokalnej produkcji KYN [85]. Do tej pory fizjologiczna, konstytutywna ekspresja TDO była obserwowana jedynie w wątrobie i mózgu ssaków [86]. Wydaje się, że odkrycie wskazujące TDO, jako jedyny enzym biorący udział w degradacji TRP do KYN w tkance kostnej, może znacznie poszerzyć istniejącą wiedzę na temat obwodowego metabolizmu TRP.

Warto również zwrócić uwagę na fakt, iż pomimo zaobserwowanego w niniejszych badaniach znacznego spadku ekspresji genu TDO u szczurów z PChN bez hamowania aktywności TPH-1 oraz w grupie otrzymującej sam rozpuszczalnik, w porównaniu do zdrowych zwierząt, wartości stężeń KYN i współczynnika KYN/TRP w tkance kostnej były porównywalne między tymi grupami. Sugeruje to nasilenie degradacji TRP do KYN u zwierząt z mocznicą. Dodatkowo, najsłabsza aktywacja szlaku kinureninowego w tkance kostnej, którą zaobserwowano u szczurów mocznicowych po podaniu LP533401 w dawce 30 mg/kg korespondowała z najsilniejszym spadkiem poziomu ekspresji TDO u tych zwierząt, w porównaniu z innymi grupami. Potwierdza to zależność aktywności szlaku kinureninowego w tkance kostnej od TDO. Patofizjologiczną konsekwencją obniżonej aktywności tego szlaku w tkance kostnej szczurów, które otrzymywały LP533401 była poprawa jej parametrów mineralnych, szczególnie widoczna w obszarze przynasady dalszej kości udowej, który bogaty jest w bardziej aktywną metabolicznie kość beleczkową. Biorąc pod uwagę wyniki powyższych badań oraz wcześniejsze obserwacje wskazujące, iż poziom KYN krążącej we krwi ujemnie koreluje z parametrami biomechanicznymi, geometrycznymi i stopniem mineralizacji kości nefrektomizowanych szczurów [26], można wysunąć wniosek, że aktywacja zarówno obwodowego układu kinureninowego, jak i jego lokalnej formy obecnej w tkance kostnej wydaje się być powiązana z zaburzeniami metabolizmu kostnego u szczurów z PChN. Pozostaje to w zgodzie z wcześniejszymi obserwacjami poczynionymi przez El Refaey i wsp. dowodzącymi, że zwiększenie stężenia obwodowej KYN powoduje przyspieszone starzenie się szkieletu i spadek masy kostnej u myszy [30]. Również Apalset i wsp. potwierdzili istnienie ujemnej korelacji między stężeniem kwasu kinureninowego, jednego z metabolitów szlaku kinureninowego i wartościami BMD u ludzi [28]. Najbardziej prawdopodobne wytłumaczenie niekorzystnego wpływu KYN na kondycję kości, zaobserwowanego także w trakcie powyższych badań, może obejmować wspominaną już wcześniej aktywację AhR w komórkach tkanki kostnej, w tym przypadku przez parakrynnie wydzielaną KYN, prowadzącą do uruchomienia szlaków sygnałowych powodujących nasilenie resorpcji tkanki kostnej i zahamowanie osteogenezy [26,87]. Nie można również wykluczyć występowania innych patologicznych interakcji spowodowanych podwyższonym poziomem KYN, prowadzących do modulowania aktywności innych niż AhR szlaków sygnałowych, skutkujących zaburzeniami metabolizmu kostnego [87,88].

Kolejnym etapem badań była identyfikacja potencjalnego mechanizmu molekularnego prowadzącego do nasilenia ekspresji genu TDO w tkance kostnej szczurów mocznicowych otrzymujących LP533401 i jego możliwych konsekwencji. Dotychczasowe doniesienia wskazują, że podawanie LP533401 w wysokiej dawce szczurom z PChN, oprócz zmniejszenia obwodowego stężenia 5-HT, wywołuje zaburzenia hormonów kalciotropowych, co skutkuje przewagą 25(OH)D nad obwodową 5-HT [24,49]. W niniejszym badaniu dodatkowo wykazano, że zarówno stężenie 25(OH)D w surowicy, jak i współczynnik 25(OH)D/5-HT dodatnio korelowały z poziomem ekspresji genu TDO w tkance kostnej, podczas gdy między obwodowym stężeniem 5-HT, a ekspresją tego genu taka korelacja nie występowała. Zatem, zaburzenia równowagi między stężeniami 25(OH)D i 5-HT w surowicy mogą być jednym z czynników wpływających na aktywację szlaku kinureninowego w tkance kostnej w trakcie podawania LP533401. Ustalenia z poprzednich badań prowadzonych w ramach tego cyklu wskazują również, że brak równowagi między 5-HT, a 25(OH)D towarzyszący podawaniu LP533401 prowadzi do zaburzenia równowagi między składowymi kompleksu CREB-ATF4-FOXO-1 [49], uznawanego za kluczowy, zależny od 5-HT, szlak sygnałowy zaangażowany w procesy kościotworzenia [89]. Skutkuje to normalizacją ekspresji genów biorących udział w osteoblastogenezie, która stała się mniej intensywna w porównaniu do szczurów z PChN nieotrzymujących LP533401. Normalizacja procesu osteoblastogenezy była powiązana z równoczesną poprawą parametrów mineralnych tkanki kostnej tych zwierząt [49]. Analiza interakcji występujących pomiędzy ekspresją genów biorących udział w osteoblastogenezie i stężeniami KYN obserwowanymi

w tkance kostnej ujawniła związek między ekspresją aktywującego czynnika transkrypcyjnego 4 (ATF-4) - markera proliferacji i wczesnych etapów różnicowania osteoblastów, a aktywnością układu kinureninowego w tkance kostnej. Udało się również ustalić istnienie silnych dodatnich korelacji między ekspresją TDO, współczynnikiem KYN/TRP, a poziomem ekspresji genów sklerostyny i osteokalcyny, markerów końcowych etapów dojrzewania osteoblastów i ich różnicowania w osteocyty. Wyniki te sugerują, że aktywacja układu kinureninowego jest w większym stopniu powiązana z procesem dojrzewania osteoblastów i ich przemiany w osteocyty, niż z proliferacją i wczesnymi etapami różnicowania. Silna, ujemna korelacja między ekspresją TDO i cykliny D1 - kluczowego czynnika odpowiadającego za regulację cyklu komórkowego w przebiegu proliferacji i różnicowania osteoblastów [90], wydaje się potwierdzać tę hipotezę. Podobne ujemne korelacje zostały wcześniej zaobserwowane między nasileniem ekspresji cykliny D1 oraz osteokalcyny i sklerostyny [49].

W dostępnej literaturze istnieje niewielka liczba doniesień sugerujących, że degradacja TRP do KYN może być potencjalnie niezbędna w procesie różnicowania komórek kostnych. W tej kwestii nie brakuje jednak sprzecznych informacji. El Refaey i wsp. wykazali, że egzogenne podawanie KYN w warunkach *in vitro* silnie hamuje proliferację mezenchymalnych komórek macierzystych szpiku kostnego oraz proces ich różnicowania w osteoblasty [88]. Z kolei Vidal i wsp. uzyskali przeciwstawne rezultaty. Dowiedli oni bowiem, iż blokowanie szlaku kinureninowego, poprzez hamowanie aktywności IDO-1, prowadzi do upośledzenia procesu różnicowania osteoblastycznego w warunkach *in vitro*, a także, że u transgenicznych myszy z niedoborem IDO-1 obserwowana jest wyraźna osteopenia [91].

Warto zauważyć, że również w niniejszym badaniu stężenie ocenianych składowych szlaku kinureninowego, szczególnie w obszarach kości o przewadze tkanki beleczkowej, było dodatnio skorelowane z poziomem ekspresji ATF4, czynnika odpowiedzialnego za nasilenie proliferacji i wczesnych etapów różnicowania osteoblastów. W związku z tym nie można wykluczyć, że KYN może także działać stymulująco na wczesny etap różnicowania osteoblastów, szczególnie nasilony w przebiegu PChN. Z kolei ujemna korelacja między poziomami ekspresji genów cykliny D1 i TDO może ilustrować próbę normalizacji tego procesu, mającą na celu uzyskanie zwiększonej puli dojrzałych osteocytów, zdolnych do pełnienia swojej fizjologicznej funkcji, w szczególności do udziału w procesie mineralizacji tkanki kostnej [49,92]. Taki wniosek jest zgodny z wynikami poprzedniego badania, w którym zaobserwowano, że spowolnienie tempa nasilonej osteoblastogenezy u szczurów mocznicowych po podaniu LP533401 skutkowało poprawą parametrów mineralnych ich kości [49].

Niewątpliwie, opisane powyżej dane stanowią kolejny krok w poznawaniu wpływu metabolitów TRP na rozwój osteodystrofii nerkowej oraz dają podstawę do dalszych prac nad rolą szlaku kinureninowego w rozwoju PChN. Co więcej, stwarzają one fundamenty pod badania w zakresie modulowania aktywności tego szlaku w przebiegu niewydolności nerek. Jeżeli w przyszłości uda się dowieść, że modulacja aktywności enzymów tego szlaku jest skuteczną metodą przeciwdziałania niekorzystnym zmianom parametrów kostnych, wywołanych akumulacją jego metabolitów w przebiegu PChN, wtedy odkrycie to może przyczynić się do opracowania nowych rozwiązań pozwalających skuteczniej przeciwdziałać zaburzeniom w metabolizmie kostnym związanym z progresją choroby nerek.

Rozdział 5. Wnioski

1. W tkance kostnej szczurów obecny jest zależny od 2,3-dioksygenzy tryptofanowej (TDO) parakrynny układ kinureninowy.

2. Zahamowanie obwodowej syntezy serotoniny przy użyciu inhibitora hydroksylazy tryptofanowej (LP533401) może pośrednio aktywować szlak kinureninowy, prowadząc do pogorszenia parametrów mineralnych kości.

3. Zmiany ekspresji TDO w tkance kostnej zwierząt z przewlekłą niewydolnością nerek po podaniu LP533401 mogą być powiązane z zaburzeniami w równowadze stężeń pomiędzy obwodową serotoniną a 25-hydroksywitaminą D.

4. Istnieją ścisłe powiązania pomiędzy nasileniem ekspresji genów uczestniczących w osteoblastogenezie, szczególnie markerów dojrzewania osteoblastów, a zależną od TDO aktywacją szlaku kinureninowego w tkance kostnej szczurów mocznicowych po podaniu LP533401.

Rozdział 6. Piśmiennictwo

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Rozdział 7. Streszczenie w języku polskim

Nieprawidłowości w metabolizmie kostnym, określane mianem CKD-MBD (chronic kidney disease mineral and bone disorders), stanowią powszechne powikłanie towarzyszące rozwojowi przewlekłej choroby nerek (PChN). Znacząco obniżają one jakość życia osób z tym schorzeniem i mogą prowadzić do występowania samoistnych złamań kości, przyczyniając się do kalectwa oraz zwiększonej śmiertelność wśród pacjentów z PChN. W ostatnich latach pojawiły się doniesienia, które wskazują, że serotonina i kinurenina, będące produktami metabolizmu tryptofanu, mogą pełnić ważną rolę w rozwoju objawów CKD-MBD. Okazało się bowiem, że zarówno serotonina, jak i kinurenina uwalniane obwodowo do krążenia hamują aktywność osteoblastów i procesy formowania kości. Dodatkowo kinurenina poprzez aktywację receptora węglowododorów aromatycznych prowadzi do wzrostu aktywności osteoklastów i nasilenia procesów resorpcji kości. Dowiedziono również, że progresji PChN towarzyszy akumulacja obu tych związków, której konsekwencją jest pogorszenie parametrów biomechanicznych i geometrycznych kości. LP533401 jest inhibitorem hydroksylazy-1 tryptofanu (TPH-1), enzymu odpowiedzialnego za obwodową syntezę serotoniny. Wcześniejsze badania potwierdziły, że hamowanie aktywności TPH-1 przez LP533401 obniża stężenie serotoniny w osoczu, co przekłada się na poprawę mineralizacji kości u szczurów z eksperymentalnie wywołaną PChN. Wpływ zahamowania obwodowej syntezy serotoniny na aktywność szlaku kinureninowego pozostawał jednak niewyjaśniony.

Celem badań wchodzących w zakres niniejszej rozprawy było ustalenie czy tkanka kostna posiada własny układ kinureninowy, wykazanie jego potencjalnego wpływu na metabolizm kostny i regulację gospodarki mineralnej w eksperymentalnym, szczurzym modelu PChN oraz sprawdzenie, czy hamowanie syntezy obwodowej serotoniny przez zastosowanie preparatu LP533401 może modulować aktywność powyższego układu.

Nefrektomizowane szczury losowo podzielono na: nieleczone, otrzymujące rozpuszczalnik oraz leczone LP533401 w dawce 30 i 100 mg/kg dziennie przez 8 tygodni. Stężenia tryptofanu i kinureniny określono za pomocą wysokosprawnej chromatografii cieczowej. Poziom ekspresji 2,3-dioksygenazy tryptofanu (TDO) oceniono za pomocą metody ilościowej reakcji łańcuchowej polimerazy w czasie rzeczywistym.

Po raz pierwszy wykazano obecność zależnego od TDO, parakrynnego układu kinureninowego występującego w tkance kostnej szczurów z PChN, funkcjonującego niezależnie od jego obwodowej puli. Zahamowanie obwodowej syntezy serotoniny przez LP533401 może w sposób pośredni modulować aktywność tego układu. Obserwowane zmiany w jego aktywności wiązały się z upośledzeniem statusu mineralnego kości. Zmiany ekspresji TDO, odpowiedzialnego za aktywność szlaku kinureninowego w tkance kostnej zwierząt otrzymujących LP533401, związane były z zaburzeniami w równowadze stężeń między obwodową serotoniną a 25-hydroksywitaminą D. Istnieją również bliskie powiązania między ekspresją genów uczestniczących w osteoblastogenezie, szczególnie markerów dojrzewania osteoblastów oraz zależną od TDO aktywacją szlaku kinureninowego w tkance kostnej szczurów z PChN otrzymujących LP533401.

Chociaż nie jest jeszcze możliwe dopasowanie wszystkich przedstawionych w niniejszej rozprawie danych do prostej mechanistycznej hipotezy postępu osteoporozy w PChN, uzyskane wyniki stanowią kolejny krok w poznaniu roli metabolitów tryptofanu w rozwoju osteodystrofii nerkowej. Stworzyły one też podstawy pod dalsze badania nad rolą szlaku kinureninowego w rozwoju PChN oraz możliwością modulowania jego aktywności w przebiegu niewydolności nerek.

Streszczenie w języku angielskim

Abnormalities in bone metabolism, classified as chronic kidney disease mineral and bone disorders (CKD-MBD), are common systemic disorders accompanying the development of CKD. They lead to morbidity, mortality, increased the risk of fractures, and significantly decreased the quality of CKD patients' life. In recent years, reports indicated that serotonin and kynurenine, belonging to products of tryptophan metabolism, may play an important role in the development of CKD-MBD. The researchers proved that both serotonin and kynurenine released peripherally to circulation could inhibit the activity of osteoblasts and bone formation processes. Additionally, the kynurenine leads to an increase in the activity of osteoclasts and intensification of bone resorption processes by activation of the aryl hydrocarbon receptor. It has been also proved that the progression of CKD is accompanied by an accumulation of both of these compounds, which results in the deterioration of bone biomechanical and geometric parameters. LP533401 is an inhibitor of tryptophan hydroxylase-1 (TPH-1), an enzyme responsible for the synthesis of peripheral serotonin. Previously we found that inhibition of TPH-1 by LP533401 decreases a plasma serotonin concentration and may improve bone mineralization in rats with experimentally induced CKD. The effect of inhibition of peripheral serotonin synthesis on the kynurenine pathway activity remained unexplained.

This doctoral dissertation aimed to establish, whether the bone tissue possesses its own kynurenic system and evaluate its potential effect on bone metabolism, as well as to assess if the inhibition of serotonin synthesis by LP533401 may affect kynurenine pathway activity in bone tissue of nephrectomized rats and to determine the background and potential consequence of this process in relation to osteogenesis and regulation of bone mineral status.

Nephrectomized rats were randomized into: untreated, treated with a vehicle, and treated with LP533401 at a dose of 30 and 100 mg/kg daily for 8 weeks. Tryptophan and kynurenine concentrations were determined using high-performance liquid chromatography. The expression level of tryptophan 2,3-dioxygenase (TDO) was assessed using the quantitative real-time polymerase chain reaction method.

The results of my research demonstrated for the first time the presence of a TDO-dependent paracrine kynurenic system, occurring in the bone tissue of CKD rats, functioning regardless of the peripheral one. Inhibition of peripheral serotonin synthesis by LP533401 may indirectly modulate the activity of this pathway. The observed changes in its activity were associated with impaired bone mineral status. The changes in TDO expression level affecting the kynurenine pathway activity in the bone tissue of the LP533401-treated animals were related to the imbalance between peripheral serotonin and 25-hydroxyvitamin D levels. There were also close associations between the expression of genes that participated in osteoblastogenesis, particularly with osteoblast maturation markers, and TDO-dependent activation of the kynurenine pathway in the bone tissue of CKD rats treated with LP533401.

Although it is not yet possible to match all the data presented in this dissertation to the simple mechanistic hypothesis of the progression of osteoporosis in CKD, the obtained results represent the next step in studying the role of tryptophan metabolites in the development of renal osteodystrophy. They also provide the basis for further research on the role of the kynurenine pathway in the development of CKD and the modulation of its activity in the course of renal failure.

Kynurenine Pathway in Chronic Kidney Disease: What's Old, What's New, and What's Next?

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International Journal of Tryptophan Research
Volume 13: 1–18

DOI: 10.1177/1178646920954882

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ABSTRACT: Impaired kidney function and increased inflammatory process occurring in the course of Chronic Kidney Disease (CKD) contribute to the development of complex amino-acid alterations. The essential amino-acid tryptophan (TRP) undergoes extensive metabolism along several pathways, resulting in the production of many biologically active compounds. The results of many studies have shown that its metabolism via the kynurenine pathway is potently increased in the course of CKD. Metabolites of this pathway exhibit differential, sometimes opposite, roles in several biological processes. Their accumulation in the course of CKD may induce oxidative cell damage which stimulates inflammatory processes. They can also modulate the activity of numerous cellular signaling pathways through activation of the aryl hydrocarbon receptor, leading to the disruption of homeostasis of various organs. As a result, they can contribute to the development of the systemic disorders accompanying the course of chronic renal failure. This review gathers and systematizes reports concerning the knowledge connecting the kynurenine pathway metabolites to systemic disorders accompanying the development of CKD.

KEYWORDS: Tryptophan, kynurenine, chronic kidney disease, thrombosis, neurological disorders, mineral and bone disorders

RECEIVED: April 27, 2020. **ACCEPTED:** August 6, 2020.

TYPE: Review

FUNDING: The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study was supported by Medical University of Bialystok project number SUB/2/DN/20/001/2211.

DECLARATION OF CONFLICTING INTERESTS: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Introduction

The essential amino-acid tryptophan (TRP) undergoes extensive metabolism along several pathways, resulting in the production of many biologically active compounds, which exert differential effects on many physiological and pathological processes.[1-3] The vast majority of TRP is metabolized by the kynurenine pathway, leading to the synthesis of the oxidized form of nicotinamide adenine dinucleotide (NAD^+), a coenzyme participating in many cellular processes.[2-4]

In the first step of the kynurenine pathway, TRP is oxidized to N-formylkynurenine using oxidase tryptophan 2,3-dioxygenase (TDO) and two 2,3-indole dioxygenase isoforms (IDO-1 and IDO-2).[5,6] Under physiological conditions, TDO activity is highest in the liver.[7-11] However, the presence of this enzyme has also been confirmed in other organs such as the brain or endometrium. TDO is an enzyme induced by the high substrate availability, as well as glucocorticosteroids, and its activity is allosterically modulated by NADPH in a negative feedback mechanism controlling the kynurenine pathway activity.[7-12] Kynurenic acid (KYNA) activates this enzyme possibly by increasing liver synthesis of another metabolite of this pathway, 3-hydroxyanthranilic acid (3-HAA), which enhances liver TDO.[10,11] IDO-1 occurs in almost all body tissues. Its high activity was detected in small intestine, spleen, lungs, epididymis, kidneys, blood vessel endothelium, and the brain.[10] Under physiological conditions, the IDO-1 expression is low, while interferon-γ (IFN-γ), amyloid peptides, lipopolysaccharide, and other pro-inflammatory factors are potent inductors of its expression and activity.[13,14] IDO-2 has been recently discovered and its physiological significance is still the subject of research. IDO and TDO catalyze the same reaction in parallel in physiological conditions.[10] In the next step, N-formylokinurenine is converted to kynurenine (KYN) by the enzyme formamidase.[6] The presence of KYN has been confirmed in blood, brain, and many peripheral tissues of the body. KYN is metabolized by the 3 pathways, resulting in the formation of KYNA, anthranilic acid (AA), and 3-hydroxykynurenine (3-HKYN).[15-18] The conversion of KYN to 3-HKYN occurs with the participation of kynurenine 3-monooxygenase. The local concentration of 3-HKYN depends on its synthesis and degradation rate as well as transport between different compartments of the body.[19] 3-HKYN is converted to xanthurenic acid (XA) by the kynurenine aminotransferase and to 3-HAA after modification with the kynureninase. The presence of enzymes that catalyze the transformation of both KYN to 3-HKYN and 3-HKYN to XA and 3-HAA was confirmed in almost all tissues of the body.[20,21] 3-HAA is degraded to unstable aminocarboxymuconatesemialdehyde (ACMS), which is enzymatically metabolized by ACMS decarboxylase to aminomuconicsemialdehyde. This compound undergoes non-enzymatic cyclization to picolinic acid, or is transformed non-enzymatically to quinolinic acid (QA).[22] QA is a direct substrate from which NAD^+ is formed.[23-25]

Most of the metabolites of the kynurenine pathway show diverse biological activity and the impact of its biologically active components on body homeostasis cannot be omitted in the course of diseases where significant changes in its activity are observed.[10,26] They play a significant role in the modulation of several physiological, as well as pathological processes, including redox homeostasis, gluconeogenesis, diabetic retinopathy, inflammation, carcinogenesis, and apoptosis.[19,27-36] Numerous KYN derivatives demonstrated toxic effects on the body's cells at higher concentrations. To a large extent, this effect is related to their ability to induce and potentiate

Table 1. The summary of negative effects of kynurenine pathway metabolites in selected organs and systems associated with disorders accompanying CKD.

ORGAN OR SYSTEM	IMPACT OF KYNURENINE PATHWAY
Kidneys	▪ Elevated KYN level promotes mesangial cells proliferation, while the excess of AA and 3-HKYN significantly suppresses mesangial cells proliferation.[45] ▪ Increased ROS production, induced by 3-HKYN and QA, leads to intensified cell damage and the accelerated rate of apoptosis in renal tissue.[46-49] ▪ QA contributes glomerular fibrosis processes.[45]
Hematopoietic and cardiovascular system	▪ Induction of oxidative stress and AhR activation by KYN and its metabolites increase inflammation and contributes to the development of anemia of inflammation and thrombosis.[50-53] ▪ KYN/TRP ratio is related to carotid intima-media thickness, a presymptomatic predictor of atherosclerosis.[50-52] ▪ In patients with recent ischemic stroke occurs the association between serum KYNA levels and patient mortality.[54,55] ▪ 3-HAA is positively correlated with the concentration of CCL-2 and CCL-4 chemokines. It may be one of the mechanisms involved in the development of atherosclerosis.[56] ▪ KYN increases up-regulates cell tissue factor expression via AhR activation in human arterial smooth muscle cells and regulates thrombosis in an AhR-dependent way.[50,54,57,58] ▪ 3-HKYN levels were independently associated with the presence of cardiovascular disease in uremic patients.[57,58] ▪ Concentration of KYN, 3-HKYN, AA, and QA were positively associated with the level of the crucial factors associated with the development of atherosclerosis, such as like von Willebrand factor, cell tissue factor, thrombomodulin, soluble intercellular adhesion molecule-1, soluble circulating vascular cell adhesion molecule-1, and prothrombin fragments F(1+2). In the end-stage CKD these coagulation factors also showed significant and independent positive associations with increased KYN, 3-HKYN, AA, and QA levels, and AA/KYN and QA/KYN ratios.[40,50,52,53,59-62] ▪ AA also is negatively correlated with the urokinase/urokinase receptor fibrinolytic system in uremic patients.[57,58] ▪ KYN, 3-HKYN, 3-HAA, KYNA, and XA were significantly increased in patients with hypertension, compared to people without this disease.[63] ▪ Elevated KYN concentration is significantly associated with an adverse clinical course in patients with both renovascular hypertension and pulmonary arterial hypertension.[64,65]
Nervous system	▪ QA is the agonist of the N-Methyl-d-aspartic acid receptor and is considered as a brain endogenous excitotoxin. Its neurotoxicity is also connected with pro-oxidant properties, leading to increased lipid peroxidation, and cytoskeletal destabilization, by increasing the phosphorylation of cellular structural proteins in neurons.[66-77] ▪ QA contributes to the increased release of glutamate by neurons, and inhibited its uptake by astrocytes, and block astroglial glutamine synthetase, leading to neurotoxicity associated with excessive glutamate.[78-80] ▪ 3-HKYN contributes to the increase in neuronal cell death rate via apoptosis. The neurotoxicity of 3-HKYN is associated with its pro-oxidative properties.[19,46,81-83] ▪ The excess of 3-HAA is converted to QA, indirectly leading to the intensification of its neurotoxic effects.[84,85]
Pancreas	▪ Accumulation of KYN and its metabolites induced by chronic stress and chronic low-grade inflammation, promotes the development of the type 2 diabetes.[25,86-90] ▪ Formation of the complexes by XA with insulin which reduces insulin activity.[91-97] ▪ 3-HKYN due to its properties can oxidatively damages pancreatic cells.[86,87,90-94] ▪ KYN, KYNA, and other metabolites can also induce pancreatic cells apoptosis probably via AhR activation.[86,91-97]
Skeletal system	▪ KYN and 3-HKYN in bone tissue lead to a decrease in marrow stromal cells cell proliferation and differentiation, though the mechanism directly associated with their pro-oxidative properties, leading to changes in miRNA expression level.[98-102] ▪ Excess of AA and 3-HKYN is associated with reduced viability of BMSC cells *in vitro*.[98] ▪ Peripherally-secreted KYN causes pathological changes in bone structure, probably via the AhR receptor activating the CYP1A1-dependent pathway.[103]

Abbreviations: 3-HAA, 3-hydroxyanthranilic acid; 3-HKYN, 3-hydroxykynurenine; AA, Anthranillic acid; AhR, Aryl hydrocarbon receptor; CKD, Chronic kidney disease; CCL2, Chemokine (C-C motif) ligand 2; CCL4, Chemokine (C-C motif) ligand 4; KYN, Kynurenine; KYNA, Kynurenic acid; QA, Quinolinic acid; ROS, Reactive oxygen species; TRP, Tryptophan; XA, Xanthurenic acid.

oxidative stress, dysregulation of calcium homeostasis and mitochondrial dysfunction in cells, causing severe disorders in cellular metabolism, including disturbances in the functioning of the respiratory chain, leading to cell damage, an increased rate of apoptosis and triggering the inflammatory proces ses.[26-41] This is manifested as a disorder of the homeostasis of various organs and systems.[10,26-41] The authors demonstrated that the toxic effects of KYN and its metabolites are also associated with their ability to activate their physiological receptor, the aryl hydrocarbon receptor (AhR). The AhR belongs to the transcription factors family and has recently been highlighted as playing a key role in regulating the numerous cellular signaling pathways and due to its impact on the maintenance of cellular homeostasis. Therefore, its excessive activation, by the elevated concentration of KYN and its metabolites, may accelerate cell aging processes and their death rate.[26-41]

The effects of the toxic properties of accumulated metabolites of the kynurenine pathway, both through the propagation of oxidative stress by KYN, 3-HKYN, 3-HAA, and QA and overstimulation of the AhR mainly by KYN and KYNA, as well as other mechanisms, such as the formation of complexes with insulin by XA or QA excitotoxic properties, may manifest themselves clinically in the form of systemic disorders, such as anemia, hypercoagulability, atherosclerosis, insulin resistance, kidney tissue damage, neurological disorders, changes in blood pressure, and osteodystrophy (Table 1).[26-36,41-43] Over the years,

Table 2. Changes of kynurenine pathway components in CKD and pathological states accompanying its development with the division into animal models and patients.

COMPOUND	CHANGES IN ANIMAL MODEL	CHANGES IN PATIENTS
Tryptophan	▪ Decrease in plasma, cerebrospinal fluid, kidney, liver, lung, intestine, spleen, brain, and muscle tissue.[44,106,107]	▪ Unchanged or decreased in plasma of CKD patients.[24,50,54,107] ▪ Decreased in plasma of patients with anemia of inflammation and hypertension.[52,63] ▪ Increased or unchanged in plasma of patients with diabetes.[63,86]
Kynurenine	▪ Increase in plasma, cerebrospinal fluid, kidney, liver, lung, intestine, spleen, brain, and muscle tissue.[44,106,107]	▪ Increased in plasma of patients with CKD, anemia of inflammation, diabetes, hypertension, atherosclerosis and increased risk of thrombosis.[50,52,54,56,57,59-61,63,86,111]
3-Hydroksy-kynurenine	▪ Increase in plasma, kidney, liver, lung, intestine, spleen muscle, and brain tissue.[29,44,106]	▪ Increased in plasma of patients with CKD, diabetes, hypertension, atherosclerosis and increased risk of thrombosis.[24,44,50,57,60,61,63]
Kynurenic acid	▪ Increase in plasma, kidney, liver, lung, intestine, spleen, and muscle tissue.[44,106]	▪ Increased in plasma of patients with CKD, diabetes, hypertension, atherosclerosis and increased risk of thrombosis.[50,54,57,59-61,63,86,87]
3-Hydorksy-anthranillic acid	▪ N/A	▪ Increased in plasma of patients with CKD, diabetes, hypertension, atherosclerosis and increased risk of thrombosis.[50,57,60,61,63,87,109,111]
Anthranillic acid	▪ Increase in plasma, kidney, liver, lung, intestine, spleen, and muscle tissue.[44,106]	▪ Increased in plasma of patients with CKD, diabetes, atherosclerosis and increased risk of thrombosis.[50,56,57,59,60,61,63,109]
Xanthurenic acid	▪ Increase in plasma, kidney, liver, lung, intestine, spleen, and muscle tissue.[44,106]	▪ Increased in plasma of patients with CKD, obesity, diabetes, and hypertension.[24,63,86]
Quinolinic acid	▪ Increase in plasma, cerebrospinal fluid, kidney, liver, lung, intestine, brain, and spleen tissue.[44,106,107]	▪ Increased in plasma of patients with CKD, atherosclerosis and increased risk of thrombosis.[24,44,50,54,57,60,61,107,111]

Abbreviations: CKD, Chronic kidney disease; N/A, Data not available.

the excessive supply of these compounds has been observed in patients suffering from a renal failure.[13,14,24,43-44] Therefore, in this review, we present the current state of knowledge linking the kynurenine pathway metabolites to chronic kidney disease (CKD) and main disorders associated with its progression. We focus here on the alterations of the kynurenine pathway activity in the course of CKD and their effect on the severity of inflammatory processes, and functional disorders of organs associated with the development of the systemic disorders accompanying in the course of renal failure.

The alterations of the kynurenine pathway activity in the course of CKD

CKD is a general term relating to heterogeneous disorders in the structure or function of the kidneys affecting about 7% of adults aged 30 and older. The occurrence of at least moderate CKD in the population aged over 65 is estimated to be about 30%.[42,43] Kidney damage leads to a decrease in the excretory, endocrine, and metabolic functions of this organ. Although many indicators of renal damage are nonspecific for the CKD, their early assessment may enable the diagnosis of the disease process before a clinically apparent decrease in renal glomerular filtration.[24,42,43]

In the course of the CKD, the induction of IDO is primarily a consequence of the chronic inflammatory process, especially an increased IFN-γ concentration. An increase in activity of IDO leads to a decrease in tissue and plasma TRP concentration with a simultaneous increase in kynurenine pathway metabolites synthesis.[11,12,39-41] The metabolites of the kynurenine pathway are excreted in the urine.[10,104] They get into the urine during the glomerular filtration process. 3-HKYN, similarly to most of the uremic toxins, is also subject to the tubular secretion process, because its clearance was higher than creatinine, in particular, at low plasma levels, while KYN, due to its low clearance, undergoes resorption in kidney tubules to a significant degree.[24,43,44,104,105] Therefore, the decrease in the glomerular filtration rate (GFR) in the course of the CKD also contributes to their accumulation in the plasma and tissues. The level of KYN, 3-HKYN, XA, KYNA, AA, and QA in the patients' blood and animal tissues (muscles, intestine, lungs, liver, spleen, and brain) positively correlated with the degree of renal insufficiency (Table 2).[24,29,43-44,105-107] In turn, changes in the activity of most of the enzymes of the kynurenine pathway do not correlate with the level of metabolites of this pathway during the progression of CKD.[47] The results of our research confirmed that the activity of kynurenine aminotransferase, kynureninase, 3-hydroxyanthranilate-3,4-dioxygenase, and ACMS decarboxylase is unchanged or even decreased. Only in the case of IDO, TDO, kynurenine 3-monooxygenase, the other authors confirmed a significant increase in their activity in

Table 3. The changes of activity of main kynurenine pathway enzymes in CKD with the division into animal models and patients.

ENZYME	CHANGES IN ACTIVITY IN ANIMAL MODELS OF CKD	CHANGES IN ACTIVITY IN PATIENTS WITH CKD
Tryptophan 2,3-dioxygenase	Increase in liver tissue.[106,107]	N/A
Indoleamine 2,3-dioxygenase	Unchanged in kidney, lung, intestine, spleen, and muscle tissue.[106]	Increase in serum.[54,108]
Kynurenine aminotransferase	Increased in kidney and decreased in liver tissue.[106]	N/A
Kynureninase	Decreased in kidney, and spleen tissue.[106] Increased or decreased in liver tissue.[54,106]	N/A
Kynurenine 3-hydroxylase	Increase in liver and kidney tissue.[106,107]	N/A
3-hydroxyanthranilate-3,4-dioxygenase	Decrease in kidney liver and lung tissue.[106,107]	N/A
Quinolinic acid phosphoribosyltransferase	Decreased in liver tissue.[54]	N/A
Aminocarboxymuconate-semialdehyde decarboxylase	Decreased in liver tissue.[54,107]	N/A

Abbreviations: CKD, Chronic kidney disease; N/A, Data not available.

the course of CKD (Table 3).[54,106-108] It is worth noting that in some cases we discovered some ambiguities. For example, in our studies, unlike the others, we have shown that, in an animal model of CKD, IDO activity in CKD remains unchanged, while kynurenine aminotransferase was increased, but only in the liver tissue.[54,106,108] However, the observed increase level of the above-mentioned TRP metabolites bases on two main causes. Firstly, an enhanced immunological activity leads to an increase in pro-inflammatory factors' levels during the first period of uremia, which causes an increase in the activity of the kynurenine pathway upstream enzymes, and secondly, a decrease in renal excretion of these metabolites due to kidney dysfunction developing during the CKD, leads to their accumulation in peripheral circulation and body tissues of uremic patients.[10,24,29,43,44,56,59,104-110]

Current research indicates that the disturbances in the TRP metabolism and accumulation of its toxic metabolites in the body seem to be one of the most important factors underlying the development of the uremic symptoms, such as neurological disorders, as well as impaired lipid metabolism, and vascular endothelial dysfunction, leading to atherosclerosis, hypercoagulability, bone metabolism disorders, and calcification of the vessels connected with higher prevalence the cardiovascular incidents.[10,26,54,56,59,108-110,112,113]

The role of metabolites of the kynurenine pathway in the development of mitochondrial dysfunction in CKD

In the CKD, increased mitochondrial dysfunction is frequently reported occurrence.[46,47,114-116] Except that, it was proved that the oxidative stress-mediated mitochondrial damage is also an important part of the pathogenesis of diabetic nephropathy.[117] Disruption in mitochondrial function certainly belongs to one of the most important factors responsible for homeostasis disorders at the cellular level responsible for the organs dysfunctions developing in the course of uremia.[46-49,114-121] Disturbances of renal mitochondrial homeostasis could lead to the microvasculature damage, inflammation, fibrosis, and kidney failure. Except that, mitochondrial damage is one of the mechanisms contributing to the development of most of the CKD-associated systemic disorders, because this process is also observed in the cells of all body tissues.[46,47,119-121] Numerous factors are responsible for mitochondrial dysfunction, but at least two are potently related to the kynurenine pathway, overactivated in the CKD course.[46,47] Toxic KYN metabolites may directly interfere with mitochondrial function, for example, by AhR activation. In turn, the excess of 3-HKYN intensifies the reactive oxygen species (ROS) generation, leading to mitochondria function impairment.[19,46] It is associated with a decrease in the activity of peroxisome proliferator-activated receptor-gamma coactivator.[46] It belongs to the family of transcription factors regulating mitochondrial biogenesis and function. Its decrease correlates with the elevated oxidative stress level and may lead to damage to the mitochondrial respiratory chain, alterations in membrane permeability, as well as impairment in Ca^{2+} homeostasis, and mitochondrial antioxidant defense systems.[46,119] Moreover, 3-HKYN disturbs the respiratory chain parameters, decreases the respiratory control index, and lowers the adenosine diphosphate/oxygen and glutamate/malate ratio in mitochondria.[46,47] Research has also shown that an increase in the level of the 3-HAA negatively affects mitochondrial mechanisms, hinders oxygen uptake by mitochondrial respiration with NAD^+-dependent substrates, and uncouples the respiratory chain, and oxidative phosphorylation.[48,49] This is probably due to the increase in ROS concentration associated with its intensified auto-oxidation process accompanying its accumulation in the CKD.

This indicates that the prooxidative properties of KYN and its downstream metabolites in combination with their direct AhR-related toxic effects, result in cellular metabolic disorders and seem to be connected with the induction of increased cell death, via the apoptosis, which leads to the increasing damage

and dysfunction of kidney and other organs, and probably is one of the mechanisms, which contributes, at the cellular level, the development of CKD symptoms and accompanying systemic disorders.[19,49,56,81,109,114-121]

The accumulation of the kynurenine pathway metabolites and functional disorders of renal cells in CKD

Many factors are involved in kidney cell damage in CKD. In turn, most of the metabolites of the kynurenine pathway revealed cellular toxic effects.[11,12] Therefore, their increase observed during renal failure formed the basis for the research on the animal model, which showed that KYN metabolites can regulate mesangial cell proliferation rate and impact gene expression levels in these cells (Table 2).[13] Besides, the increase in level of KYN and its metabolites is associated with the progression of renal failure and glomerular fibrosis. The excess of AA and 3-HKYN suppresses mesangial cells proliferation.[45] In contrast, QA and KYN promoted their proliferation, although this process slows down at their higher concentrations. Besides, QA contributed to the upregulated expression of collagen type-Iα1, type-IVα1, and platelet-derived growth factor-B genes (Table 1).[45] However, the expression level of the hepatocyte growth factor was downregulated, while the insulin-like growth factor-1 was increased with the kynurenine pathway activity. Changes in the level of these factors were accompanied by the increase in the expression of three kynurenine pathway enzyme (kynurenine 3-monooxygenase, kynurenine aminotransferase, phosphoribosyltransferase) in mesangial renal cells.[45] These results suggest that abnormalities in the kynurenine pathway are connected with the dysfunction of mesangial cells and might be associated with the progression of renal failure and glomerular fibrosis. In vitro research also suggests that an accumulation of the above-mentioned KYN metabolites in kidney cells during the CKD progression, through their ability to produce ROS, contributes to increased oxidative stress level severity in renal cells, leading to exacerbated cell damage and the accelerated rate of apoptosis in renal tissue, associated with enhanced inflammatory process within the tissue (Table 1).[45-49]

All this suggests that the accumulation of the metabolites of the kynurenine pathway may contribute to the development of pathological changes in kidney cells, leading to their damage and loss of function, resulting in organ failure. Thus, inhibition of the kynurenine pathway activity in uremic patients could potentially slow or even stop destructive processes in kidney tissue in the course of CKD.

The associations between the overactivity of the kynurenine pathway and inflammatory processes in CKD

Pathological conditions, such as progression of renal failure, the chronic inflammation, and oxidative stress lead to significant changes in the activity of many metabolic pathways, including the kynurenine pathway.[13,14] The accumulation of its metabolites caused by its activation by pro-inflammatory factors contributes to processes leading to the development of many disorders. A loss of kidney function is associated with an increase in the concentration of the inflammation marker, such as neopterin and the activity of the kynurenine pathway.[122,123] Furthermore, research conducted on patients with CKD proved, that IDO activity positively correlated with the severity of CKD (Table 3).[54,56,108] It is also associated with the level of key inflammation biomarkers, such as C reactive protein (CRP) and tumor necrosis factor-α, soluble tumor necrosis factor receptor-1 (sTNFR-1), independently from other parameters, such as age, body weight, and serum creatinine level.[25,54-56,108,109,124] This is another evidence confirming that the activity kynurenine pathway increases with the CKD severity, as a consequence of chronic inflammation. Except IDO, another kynurenine pathway enzyme—kynureninase also plays a pro-inflammatory role in human disease. It has been shown, that its downstream metabolites can induce overexpression of pro-inflammatory factors.[59,109] Kynurenine 3-monooxygenase is known as another crucial regulator of inflammation. Its overactivity leads to the excess of 3-HKYN, contributing to the increase in the apoptosis rate and the enhanced secretion of pro-inflammatory cytokines.[59,109,110] Except that, the concentrations of many kynurenic system components, such as KYN, KYNA, 3-HKYN, AA, and QA, and KYN/TRP ratio, increasing with the severity of CKD, remain independent from serum creatinine level, age, and body weight (Table 2).[24,43,44,59,109,124,125] They are also positively correlated with the levels of before-mentioned inflammatory markers, and other factors, such as cellular adhesion molecules, like soluble intercellular adhesion molecule-1 (sICAM-1) and circulating vascular cell adhesion molecule-1 (sVCAM-1), especially in dialyzed patients.[23,44,56,104-106,109,110] Correlations between them are particularly strong, especially in end-stage renal disease.[23,44,49,54,56,104-106,109,110] Apart from the fact that, the activation of the kynurenine pathway, in the CKD is mainly a consequence of chronic inflammation, some research demonstrated that increase of serum level of kynurenine pathway components, especially KYN, and QA, as well as a QA/KYN ratio, is also associated with oxidative stress markers, such as superoxide dismutase, total peroxide, and malondialdehyde, particularly in patients with end-stage renal disease.[23,26,59,109,110,124,125] Several studies also support the hypothesis that AhR activation by TRP-derived uremic toxins is at least partially involved in worsening inflammation associated with the CKD course. KYN and its metabolites induce T-lymphocytes differentiation, and KYNA activates IL-6 in MCF-7 cells in an AhR-dependent manner.[125]

Interestingly, not all components of the kynurenine pathway present the pro-inflammatory effects. Studies proved, that KYNA, AA, and PA possess anti-inflammatory

properties.[10,126-130] In turn, research on 3-HAA is a source of controversial conclusions. It has been shown that 3-HAA exhibits an anti-inflammatory properties.[49,81,84,126-128] Although Reyes-Ocampo et al. demonstrated that 3-HAA shows a potent pro-inflammatory effect. It is connected with mechanisms that include impairment of cellular energy metabolism, which are not related to early ROS production.[56] However, it may be at least partly related to the fact, that excess of 3-HAA leads to increased QA synthesis, and results in enhanced ROS generation and oxidative cell damage, due to its accumulation.[46,48,49,131] This also may lead to increased secretion of pro-inflammatory factors by damaged cells.

The above reports indicate a significant share of metabolites of the kynurenine pathway in inflammatory processes, including these occurring in the course of CKD. Importantly, among the components of this pathway, some compounds also exhibit anti-inflammatory properties.[84,126-130] Although their degradation products are mostly characterized by pro-inflammatory properties, thus their undue supply leads to an increase in the concentration of their pro-inflammatory metabolites. Therefore, the excessive activation of the kynurenine pathway and increased supply of its metabolites, in general, leads to an exacerbation of the inflammatory process in the body. So, counteracting the accumulation of KYN and its metabolites could potentially be an effective method of controlling inflammation level in uremic patients.

The importance of accumulation of the kynurenine pathway components in the development of anemia atherosclerosis, and thrombosis during CKD progression

As described before, recent studies have proved that the increased activation of the kynurenine pathway, associated with increased oxidative stress, and inflammation levels is related to the risk of the development of anemia, atherosclerosis, and thrombosis in patients with chronic renal dysfunction.[10,25,26,54-56,108,124] The elevated level of 3-HKYN is most frequent in patients with cardiovascular diseases. In the patients with recent ischemic stroke, the authors found that the KYN/TRP ratio inversely correlates with kidney function, and positively with carotid intima-media thickness, a presymptomatic predictor of atherosclerosis. In turn, higher IDO activity is associated with larger carotid plaque, while high KYN and 3-HKYN levels and KYN/TRP ratio are related to oxidative stress markers (Table 1).[54,55]

The disturbances in the peripheral kynurenine pathway activity result in decrease of the TRP plasma level and an increase in the concentration of its toxic metabolites (Table 2).[50-52] The research indicates at least indirect correlations between changes in the level of KYN and its metabolites and the risk of the development of anemia of inflammation in the course of CKD (Table 1).[51,52] Pro-inflammatory cytokines like IFN-γ and tumor necrosis factor-α suppress the growth and differentiation of erythroid progenitor cells, inhibit erythropoietin secretion, and increase hepcidin synthesis.[50-52] Besides, serum concentrations of 3-HKYN, AA, KYNA, XA, and QA are negatively correlated with main hematological parameters in CKD patients.[51,52] Due to the biological properties of KYN metabolites, their long-term accumulation may at least partly worse the parameters of the erythrocytic system in patients. The authors indicated, that in this case, the impact of KYN on hematopoiesis, except induction of oxidative stress mechanisms, may be also connected with AhR-dependent pathway activation.[51-53]

Due to the accumulation of uremic toxins, which show detrimental effects on blood and the vessel wall, the patients with CKD also have an increased risk of thrombosis.[50] This is confirmed by the presence of the positive associations between elevated plasma levels of KYN, 3-HKYN, KYNA, QA, and KYN/TRP ratio, endothelial dysfunction, and the risk of myocardial infarction (Table 2).[60,61] Kynurenine pathway metabolites could be involved in the development of thrombosis by deregulation of plasma coagulation factors, induction of endothelial cell dysfunction, and stimulating cell tissue factor (TF) overexpression, through AhR-pathway (Table 1).[40,50,59,62,132-134] Thus, up-regulation of IDO in coronary atherosclerotic plaques in combination with increasing level of inflammatory factors might contribute to thrombus formation through TF upregulation in activated macrophages (Figure 1).[50,57,59,111]

The research concerning the relationship between the cytokines and kynurenine pathway activity at the background of the CKD-associated complications indicates, that CC-chemokines and KYN metabolites are associated with the accelerated development of atherosclerosis in CKD patients. In these patients there was discovered a significant increase in plasma concentrations of CCL2, CCL4 chemokines, inflammation markers, such as superoxide dismutase, and CRP, as well as several KYN metabolites, like AA, 3-HAA, and KYN/TRP ratio (Table 2).[56] Increase in these compounds, and decrease in the GFR and 3-HAA levels have been recognized as the independent factors significantly associated with increased CCL2 and CCL4 concentrations[56,109] The above-mentioned relationship may represent one of the mechanisms involved in the accelerated atherosclerosis in CKD patients (Table 1).[56] Other studies concerning the effects of changes in the kynurenine pathway activity on endothelial dysfunction and coagulation markers in the CKD patients have shown that an increase in the TRP metabolites level, such as KYN, KYNA, and crucial factors associated with the development of atherosclerosis, like von Willebrand factor (vWF), thrombomodulin (TM), sICAM-1, sVCAM-1 were elevated and positively associated with the stage of CKD and severity of inflammatory processes (Table 2).[59,109] Except that, KYN, 3-HKYN, AA, and QA positively correlated with vWF, and other endothelial dysfunction markers, such as TM, TF, TF-pathway inhibitor system, sICAM-1 and sVCAM-1 level, as well as with prothrombin fragments

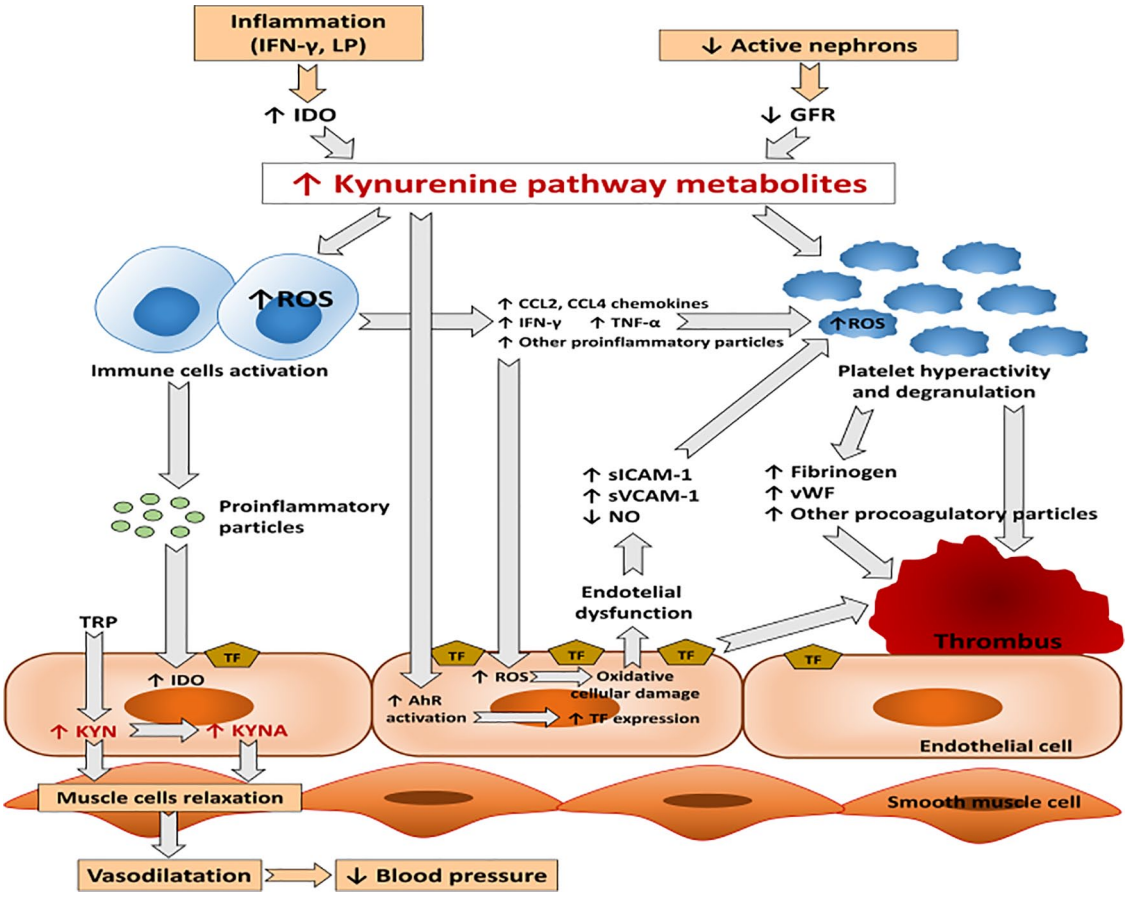

Figure 1. Mechanisms of peripheral kynurenines promoting vasodilatation and thrombosis. Kynurenine pathway metabolites can be involved in the development of thrombosis by deregulation of plasma coagulation factors, induction of endothelial cell dysfunction, and stimulating cell TF overexpression, through AhR-pathway. The up-regulation of IDO in coronary atherosclerotic plaques in combination with the increasing level of inflammatory factors leads to the increased oxidative stress level and the AhR pathway stimulation. It might contribute to thrombus formation through TF upregulation in activated macrophages. Parallel the increase in KYN and KYNA concentration in endothelial cells induce arterial relaxation by activation of the adenylate cyclase and soluble guanylate cyclase pathways. It indicates that the activation of the kynurenine pathway may also represent a local compensatory mechanism counteracting hypertension the increase in blood pressure.
Abbreviations: AhR, Aryl hydrocarbon receptor; CCL2, Chemokine (C-C motif) ligand 2; CCL4, Chemokine (C-C motif) ligand 4; GFR, Glomerular filtration rate; IDO, 2,3-indole dioxygenase; INF-γ, Interferon gamma; KYN, Kynurenine; KYNA, Kynurenic acid; LP, Lipopolysaccharide; NO, nitric oxide; ROS, Reactive oxygen species; sICAM-1, Soluble intercellular adhesion molecule-1; TF, Cell tissue factor; TNF-α, Tumor necrosis factor alpha; TRP, Tryptophan; sVCAM-1, Soluble circulating vascular cell adhesion molecule-1; vWF, von Willebrand factor.

F(1+2) concentration, even in the early stages of the CKD.[40,50,52,53,57-62] Except that, AA was negatively correlated with the urokinase-type plasminogen activator and its receptor levels in uremic patients, also entailing an increase in the prothrombotic potential.[57,58] In the end-stage CKD, the above-mentioned coagulation factors demonstrated additional positive associations with AA/KYN and QA/KYN ratios, not observed in the early stages of CKD.[135-137] This confirms the links between enhanced coagulation in uremic patients and the activation of the kynurenine pathway, especially in the advanced stages of the CKD. KYN and its metabolites are also associated with hyperfibrinolysis, which also is causally related to the development of atherosclerosis and cardiovascular disorders (Table 1).[135-138]

IDO was in turn recognized as a novel marker of immune activation in the early stages of atherosclerosis.[59] Moreover, it is a potential novel contributor to vessel relaxation and metabolism in systemic infections, also activated in acute severe heart attacks.[139-141] Changes in its activity suggest that the kynurenine pathway plays a key role in the increased prevalence of cardiovascular disease due to the presence of a positive relationship between IDO-dependent kynurenine pathway activation, and cardiovascular disease prevalence in end-stage renal disease patients.[57,139-141]

The above-mentioned associations indicate the relationships between kynurenine pathway activity, endothelial dysfunction, hypercoagulability, and the progression of atherosclerosis in CKD.[58] The significant increase in these parameters observed in uremic patients, even at the early stage of the disease, suggests that hypercoagulability and atherosclerosis belong to the early complications of the CKD. It also indicates that 3-HKYN levels were independently associated with the presence of cardiovascular disease in uremic patients (Table 1).[57,58]

Not all metabolites of the kynurenine pathway have a proatherogenic effect. The inverse correlations have been observed between KYNA levels and cardiovascular disease prevalence in dialyzed end-stage renal disease patients. The authors demonstrated that KYN, KYNA, and QA levels are significantly higher in peritoneal dialysis (PD) patients than in healthy ones, whereas TRP was lower in these patients. PD patients with cardiovascular disease have lower KYNA levels compared to patients without heart disease. Therefore, low KYNA levels were independently associated with the presence of cardiovascular disease in PD patients.[142,143] Other important relationships were observed in another study concerning patients undergoing continuous ambulatory PD. In this case, KYNA concentration, and the KYNA/KYN ratio were significantly lower in patients without cardiovascular disease, and they were positively associated with homocysteine levels in all continuous ambulatory PD patients, and with a hyperhomocysteinemia severity in patients with cardiovascular disease.[143-145] These findings are in agreement with previous reports concerning a KYNA protective effect on the vascular wall epithelium against the homocysteine-induced inhibition of endothelial cell proliferation and migration. KYNA mechanism of action appears to be associated with the inhibition of homocysteine-induced cytotoxicity.[143,144] They also indicate, that KYNA may be liberated from epithelial cells in response to a high level of homocysteine or cellular damages caused by the excess of this compound.[143-145] Another anti-atherosclerotic mechanism related to kynurenine pathway activity seems to be related to 3-HAA activity. Its increase in plasma concentration was associated with a decrease in cholesterol and triglyceride levels, inhibition of the uptake of oxidized low-density lipoproteins by macrophages, and the resultant inhibition of atherogenesis in the murine model.[146-148]

The above reports indicate that the above-mentioned TRP metabolites are independently and potently associated with the development of anemia, atherosclerosis, and thrombosis in renal disease patients. They confirm the occurrence of links between disorders in hematopoietic and vascular systems in uremic patients and the activity of the kynurenine pathway. As most of them have a detrimental effect on these systems, they indicate the validity of therapeutic steps aimed at protecting patients against the effects of the accumulation of its metabolites in the course of CKD and research towards more effective methods of reducing the concentration of KYN and its metabolites in patients exposed to their harmful impact.[138-143,149-166]

The importance of the kynurenine pathway metabolites in blood pressure alterations in CKD

Kidney disease is the most common cause of secondary hypertension. Functional or structural renal damage is a much more common cause of hypertension than previously thought. CKD leads to impaired renal sodium and water excretion and excessive secretion of decongestants in the kidneys, with insufficient production of vasodilators.[43,64,65] CKD is the cause of the increased activity of the sympathetic nervous system and hormonal disorders leading to an increase in blood pressure.[43,65] However, the issue of the impact of metabolites of the kynurenine pathway on blood pressure remains a controversial matter. Studies concerning the impact of components of this pathway on blood pressure are still scarce. Only several studies show that the pathobiology and development of renovascular hypertension and pulmonary arterial hypertension are associated with the increase in the kynurenine pathway activity.[64,65] In turn, the Hordaland Health Study proved that hypertensive patients have significantly increased KYN, 3-HKYN, 3-HAA, KYNA, and XA plasma concentrations compared to healthy people (Tables 1 and 2).[63] The alterations of these pathway activity remain in close relationship with blood pressure values, as well as with the activity of pro-inflammatory mechanisms. Also, changes in angiotensin II concentration level and vascular remodeling processes suggest the presence of relationships between them and KYN metabolites levels.[65] Elevated KYN concentration is associated with an adverse clinical course in patients with both renovascular hypertension and pulmonary arterial hypertension (Table 1).[64,65] As the progression of CKD is often accompanied by the development of renovascular hypertension, we could assume that the accumulation of the kynurenine pathway components in the course of renal failure may stimulate the development of this form of hypertension.[64] However, we cannot forget that the impact of KYN and its metabolites on blood pressure remains not fully understood because some studies suggest that certain metabolites of the kynurenic system play a previously unknown contradictory role in the field of vascular tension.[64,65] It has been proved that KYN may also induce arterial relaxation by the activation of the adenylate cyclase and soluble guanylate cyclase pathways. An intravenous administration of KYN transiently and in a dose-dependent way decreases the mean blood pressure in hypertensive rats *in vivo*.[65] In turn, the increase in IDO activity in endothelial cells in response to worsening inflammation during the CKD is recognized as a factor stimulating vasodilation and a contributor to hypotension.[167,168] It suggests that IDO up-regulation may represent a local compensatory mechanism counteracting the increase in blood pressure. The other data from studies both on animals and patients indicate that KYNA and XA may also have vasorelaxation and antihypertension properties (Figure 1). [143,144,169]

These data indicate that the components of the kynurenine pathway can play diverse, sometimes opposite roles in the development of hypertension. Their accumulation in the course of chronic renal failure seems to be at least partly associated with the development of CKD-related hypertension. The amount of research concerning this topic is scarce, while some studies demonstrated that certain kynurenine pathway metabolites may be also components of antihypertensive compensatory mechanisms. Therefore, this issue

requires further research, to better understand the impact of alterations in the kynurenine pathway activity on the development of hypertension.

The impact of the accumulation of the kynurenine pathway metabolites on the development of neurological disorders accompanying the progression of CKD

TRP metabolism via the kynurenine pathway leads to the formation of several neuroactive substances, including KYN, 3-HKYN, KYNA, 3-HAA, AA, and QA. They are involved in the pathogenesis of numerous neurodegenerative diseases.[19,29,30,170] Also, the development of chronic renal insufficiency, accompanied by the accumulation of these compounds, is associated with the worsening of neurological disturbances.[29,30,43] Alterations in the TRP, KYN, 3-HKYN, and QA concentrations were observed within CNS homogenates in uremic rats.[27,29,30,171,172] These substances seem to be responsible for neuronal dysfunction in uremia, especially 3-HKYN, QA, and indirectly AA.[29,30,171] QA is the N-Methyl-d-aspartic acid (NMDA) agonist, while 3-HKYN neurotoxicity is related to its high redox-activity. KYNA, a glutamate antagonist possibly participates in neurodegenerative disorder, as a neuroprotective agent.[171,173] Also AA revealed beneficial effects in neurological disorders related to an enhanced inflammation. However, at the high levels, it becomes a source of increased QA synthesis.[173] Accumulation of the neurotoxic TRP metabolites in the CNS results from the enhanced entry of serum KYN to CNS, resulting from its increase accompanying renal failure, as well as from the elevated activity of IDO, kynurenine 3-monooxygenase, and anthranilate oxidase, and decreased of ACMS decarboxylase in the brain, associated with the increase of systemic inflammation. As a result, the supply of before-mentioned neurotoxic KYN metabolites in the CNS increases, although their brain uptake is very limited.[27,29,30,107,171,173-177]

Within the CNS, QA is produced and released by penetrating macrophages and activated microglial cells. Both types of cells play an important role during neuroinflammatory process development.[27,66,67] Increased release of QA in the course of CKD is related to the above-mentioned changes in the KYN concentration and the activity of the kynurenic system enzymes, observed in the course of CKD.[27,29,30,107,171,174-177] QA belongs to the agonists of the NMDA receptor and is considered as a brain endogenous excitotoxin. The activity and toxicity of QA depends on its levels. In high levels, it launch a chain of detrimental effects, which may induce functional disorders or even apoptotic death of neurons.[66-68] The toxic effect of QA is also connected with its pro-oxidant properties responsible for increased lipid peroxidation, and cytoskeletal destabilization, by increasing the phosphorylation of cellular structural proteins in neurons.[69-77] The gliotoxic effects of QA amplify the inflammatory response. It contributes to the increased release of glutamate by neurons, inhibition of its uptake by astrocytes, and blocks astroglial glutamine synthetase, leading to neurotoxic effect associated with excessive glutamate supply in the brain.[78-80] It affects neurons located mainly in the hippocampus, striatum, and neocortex, due to the selectivity of QA for the specific types of NMDA receptors occurring in those regions.[30,63-80,148-183] Excessive synthesis of QA, along with the exacerbation of the inflammatory process, leads to overexcitation of the NMDA receptor, causing an increased influx of Ca^{2+} into the neuron, which triggers the activation of destructive enzymatic pathways, including protein kinases, phospholipases, nitric oxide synthase, and proteases in neurons (Table 1).[27,131,184] They intensify the decomposition of crucial proteins in the cell and increase nitric oxide levels, leading to an apoptotic response of oligodendrocytes, neurons, and astrocytes connected with intensified NMDA-dependent ROS formation.[185] Finally, the accumulation of QA can trigger neuronal lesions linked to excitotoxicity, including convulsions.[186]

3-HKYN is another metabolite of the kynurenine pathway well-known for its neurotoxic properties, due to its pro-oxidative potential.[19,19,81,82] Its excessive concentration in the CNS leads to increased ROS generation.[19,19,81,82] Similarly to QA, it contributes to the increase in neuronal cell death rate via apoptosis.[19,46,82] The activity of endogenous xanthine oxidase is involved in 3-HKYN-induced H_2O_2 generation, and exacerbates cell damage.[46,81-83] Furthermore, 3-HKYN can reduce copper to generate superoxide and H_2O_2 in a copper-dependent manner.[46] The level of KYN and 3-HKYN were elevated both in the plasma and different brain regions of the uremic animals. KYN concentrations were higher in the cerebellum, midbrain, and cortex compared to the healthy ones, while 3-HKYN in the striatum and medulla than in other structures.[187] This data suggests that the CKD results in deep disturbances on the kynurenine pathway in the CNS, which seem to be at least partly responsible for neurological abnormalities observed in uremia.[30] The direct metabolite of 3-HKYN, 3-HAA also revealed the ability to generate ROS such as hydrogen peroxide and superoxide. However, it is also a highly efficient scavenger of free radicals.[46,48,49] Under physiological conditions, 3-HAA is regularly auto-oxidized to unstable ACMS, which is converted to QA, which, in turn, is transformed to NAD^+, and the concentrations of these compounds remain at the level that does not disturb homeostasis in the CNS.[84,85] In turn, in the course of CKD, their accumulation both directly, and by increased QA synthesis leads exacerbation of neuronal apoptosis rate associated with an increased oxidative stress level and NMDA overexcitation (Table 1).[46,82,85,131,184]

Among the neuroactive KYN metabolites, KYNA is well-recognized as a neuroprotective agent. It abolishes the neurotoxic effects of QA due to its ability to block glutamate receptors, especially the NMDA receptor where it inhibits the actions of the glycine coagonist.[188-190] It also counteracts neurotoxicity through its potent antioxidant activity affecting glutamate signaling through scavenging free radicals. It means that it is also able to counteract the prooxidative effects of 3-HKYN and 3-HAA in physiological states.[74,127,190] Except that, in vitro studies proved that it can block the effects of

exogenously applied acetylcholine or nicotinic receptor-selective agonists, as well as excitatory postsynaptic potentials (EPSPs) mediated by the activation of cholinergic, and nicotinic receptors in hippocampal interneurons.[74,190] KYNA reduced the amplitude of these potentials and was more potent in blocking the nicotinic than the glutamate-mediated EPSPs.[190] During physiological conditions, there is a balance between KYNA and neurotoxic KYN metabolites concentrations. In chronic inflammatory states, such as CKD, the KYNA level is decreased, while 3-HKYN, 3-HAA, and QA are increased. In turn, AA may have beneficial effects in neurological disorders, associated with an inflammatory state. However, its molecular mechanism received relatively little attention so far. Although its excess also leads to an increase in QA synthesis, thus it may indirectly contribute to the exacerbation of neurological symptoms.[173] The chronic imbalance between neurotoxic and neuroprotective kynurenine metabolites may potentiate brain damage.[188]

The presented reports clearly show that the accumulation of KYN and its metabolites, associated with the exacerbation of inflammatory processes and impaired renal excretory capacity, have a significant impact on the neurons damage and development of neurological disorders in the course of CKD. Although some of the intermediate metabolites of the kynurenine pathway has a neuroprotective effect, the excessive activation of this pathway leads to an increase in the imbalance between its neurotoxic and neuroprotective metabolites, resulting in an accumulation of neurotoxic ones. This explains why the inhibitors of the kynurenic system enzymes are considered as potential therapeutics in neurodegenerative disorders.[159-166]

Accumulation of the components of the kynurenine pathway in CKD and the development of diabetes mellitus

CKD can be a consequence or complication of all other civilization diseases, from obesity through diabetes to cardiovascular diseases.[39] It has been also proved that diabetic patients have also increased TRP degradation via the kynurenine pathway, resulting from increased activity of IDO, kynurenine 3-monooxygenase, kynureninase and kynurenine aminotransferase, leading to elevated serum concentration of diabetogenic KYN, 3-HKYN, 3-HAA, KYNA, XA, and AA in comparison with healthy people (Tables 1 and 2).[25,63,86-90] It seems to be related to the increase in the activity of the enzymes of the kynurenine pathway accompanying long-lasting inflammation.[25,86] This relationship prompted researchers to study if the accumulation of uremic toxins, including KYN and its metabolites induced by chronic stress and chronic low-grade inflammation can be one of the mechanisms promoting the development of insulin resistance and type 2 diabetes.[86-89] They proved that KYN and its metabolites may directly contribute to the higher incidence of insulin resistance due to their pro-inflammatory and pro-oxidative properties.[10,19,26] In turn, an increase in 3-HKYN and its metabolite XA concentrations, observed in the course of CKD, is recognized as a factor disturbing insulin secretion and activity, and therefore glucose homeostasis in uremic patients.[86,87,90-93] Therefore, it can be concluded that the changes in the kynurenine pathway activity observed in the course of renal failure may also contribute to the development of diabetes.[86,90-93] Experimental studies suggested diabetogenic effects of numerous kynurenine pathway metabolites, such as XA-induced hyperglycemia, due to the formation of the XA-insulin complex, which reduces insulin activity, and impairment of biosynthesis of insulin by KYN, and KYNA, probably related with the AhR activation, and by 3-HKYN and QA, mainly associated with the induction of oxidative damage of pancreatic cells (Tables 1 and 2).[86,90-97] Therefore, it can be concluded that the changes in the kynurenine pathway activity observed in the course of renal failure may also contribute to the development of diabetes.[86,90-94]

The potent changes in TRP metabolism with the accompanying rise in several bioactive KYN metabolites concentrations also correlates with the severity of kidney function disorders in type 2 diabetic patients with diabetic nephropathy symptoms.[86] It is worth recalling, that the concentration of KYN in the course of CKD was positively associated with circulating pro-inflammatory factors level, such as CRP, tumor necrosis factor-α, independently of changes in glomerular filtration in the kidney.[25,56,55,86,93,124] These data indicate the existence of links between the systemic effect of accumulation of KYN and its metabolites and the inflammatory process in the progression of chronic renal insufficiency accompanying diabetic nephropathy development in type 2 diabetes.[25]

Thus, the chronic stress, as well as chronic inflammation, even low-grade, directly activate enzymes of the upstream steps of the kynurenine pathway and divert downstream steps of this pathway from the biosynthesis of NAD towards the formation and accumulation of diabetogenic downstream metabolites. This combined with the accumulation of uremic toxins, including KYN and its metabolites, due to impaired excretory function of the kidneys seems to be related to an increased risk of type 2 diabetes development.[25,86,91-97]

Associations between the increased activity of the kynurenine pathway and bone metabolism disorders in the course of CKD

Abnormalities in endocrine, mineral, and bone metabolism represent the most complex complications during the CKD progression. Pathological changes in the bone tissue, as the one of the most commonly occurring during the CKD progression, have been classified as the CKD-mineral and bone disorder (CKD-MBD).[42,191,192] Evidence for the accumulation of the metabolites of the kynurenine pathway in the course of chronic renal insufficiency and their impact on bone tissue, prompted some researchers, including our team, to deal with the topic of connections between the CKD, changes in kynurenine system activation and bone metabolism.[31,35,98,187,192-194]

In vitro studies have proven, that the accumulation of KYN and 3-HKYN in bone marrow stromal cells (BMSCs) have been associated with a decrease in cell proliferation and differentiation, though the mechanism which is directly mediated by their pro-oxidative properties. Microarray analysis identified 50 upregulated, and 36 downregulated micro RNAs (miRNAs) in KYN-treated BMSCs. Differentially expressed miRNA includes, among other miR-1281, miR-330-3p, let-7f-5p, and miR-493-5p, important for proper BMSCs proliferation and differentiation.[98-102] Upregulated miRNA fragments are related to glutathione metabolism critical for removing oxidative species (Table 1).[98] It can be assumed that these changes in miRNA expression pattern are the response to KYN-mediated increases in oxidative stress level, disrupting cellular processes important in normal metabolism.[98-102] The high levels of AA and 3-HKYN were also associated with reduced viability of the BMSCs in vitro (Table 1).[98] The increased production of the above-mentioned metabolites may exert cumulative negative effects on BMSCs metabolism and increased their susceptibility to oxidative damage.[99-102] It is known that elevated plasma concentration of KYN, of AA, and 3-HKYN observed in the animal model of CKD, may be associated with an increased risk of femoral and hip fractures and a decrease in bone densitometry parameters.[35,103,193,194] The concentration level of these compounds positively correlates with IDO activity, which in turn is dependent on the severity of inflammation in the body. This allows to conclude that there is a link between inflammation-induced kynurenine pathway overactivity and risk of bone fractures.[35,195]

Research conducted previously by our team established the ability of KYN to modulate metabolic processes in bone tissue. Kalaska et al proved that KYN present in the CNS positively influences on bone formation processes, while peripherally-secreted KYN causes pathological changes in bone structure. Our team observed a positive correlation between KYN in the frontal cortex and TRP in the hypothalamus and striatum and the main parameters of bone biomechanics and geometry, as well as negative between KYN in serum and bone biomechanical and geometric parameters.[103,187] The impact of the KYN in the CNS may be associated with the role of the hypothalamus in the bone regulation and suggests that the frontal cortex and striatum may also take part in the regulation of bone changes in the CKD. However, the exact mechanism associated with the protective effect of KYN in the brain on bone metabolism remains unexplained.[187] The effect of peripheral KYN on bone tissue may be explained by the interaction of KYN with the AhR. Its activation may lead to unfavorable changes in bone metabolism through induction of CYP1A1 transcription and activation of the CYP1A1-dependent pathway (Table 1).[103] Stimulation of the AhR in osteoblasts leads to the inhibition of their proliferation and differentiation in a collagen-induced arthritis mouse model by activation of the signaling pathway dependent on extracellular signal-regulated kinases.[196] In turn, its activation in osteoclasts leads to an increase in their activity and the bone resorption processes.[197] It indicates, that AhR may be crucial in maintaining adequate bone mass and the altered bone properties are highly dependent on the functional AhR. Recent research also indicates the negative effects of exogenous AhR agonists on bone strength in the animal model.[198] We cannot exclude that another pathological mechanism related to the influence of KYN on bone metabolism is responsible for the observed changes. The other authors proved that the elevated level of peripheral KYN in the CKD also led to changes in the activity of other signaling factors, such as the receptor activator of nuclear factor-κB ligand/osteoprotegerin axis, extracellular signal-regulated kinases, and histone deacetylase-3 or runt-related transcription factor 2 expression levels.[103,194,199] We also do not exclude that the observed process was induced by the accumulation of other AhR ligands. Many of them, such as indoxyl sulfate, circulate in the serum of the CKD patients.[200] However, the AhR gene expression in the bone tissue was positively associated with serum KYN concentration, and negatively with the main parameters of bone biomechanics, geometry, and density.[103] It indicates that the above-described mechanism appears to be at least partially complementary to the effect of bone KYN, due to similar correlations between bones and serum KYN levels, and bone densitometry parameters (Figure 2).

It is worth noting that not all metabolites of the kynurenine pathway exert a peripherally unfavorable effect on the bone formation processes. The picolinic acid, one of the end products of the kynurenine pathway shows a strong and dose-dependent osteogenic effect on the hBMSCs in vitro.[181,201] Moreover, TRP degradation by the kynurenine pathway is also increased during osteoblastogenesis.[201] This suggests that its activation plays an important role during the development of the hBMSCs into the osteoblast lineage in vitro and that this process can be even accelerated by exogenous addition of IFN-γ. Besides, the lack of IDO activity in the animal model led to the development of osteopenia. These data, support a newly discovered role for the kynurenine pathway and especially picolinic acid as positive regulators of osteoblastogenesis and bone-formation processes.[181,201]

Results obtained by our team, and other authors indicate that CKD-induced accumulation of peripheral KYN, 3-HKYN, and AA negatively affects the osteoblasts function and bone-forming processes and contributes to the development pathological changes in bone structure. This is associated with the inhibition of catabolic and the acceleration of anabolic processes within bone tissue. Interestingly, not all of the kynurenine system components exert an adverse effect on bone tissue. What is more, the same metabolites, like KYN, may have the opposite influences on bone metabolism, depending on the site of synthesis. So far, the impact of inhibition of the peripheral activity of the kynurenine pathway on bone health has not been recognized. This could be an

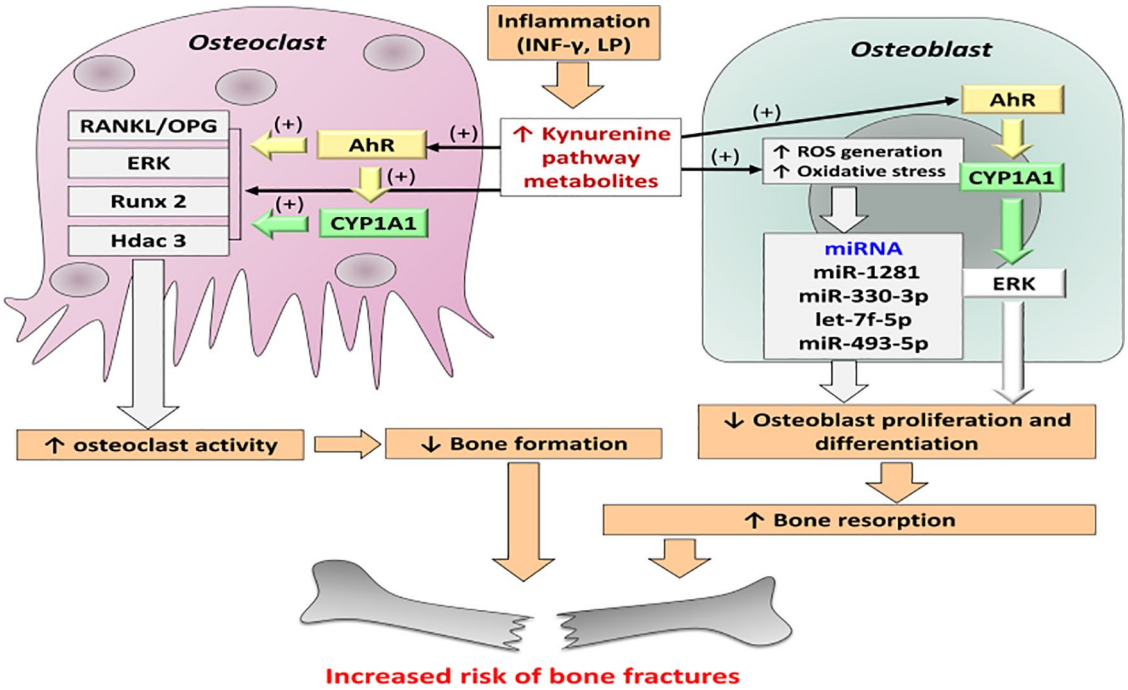

Figure 2. Mechanisms of peripheral kynurenines promoting bone fractures. The effect of circulating kynurenine pathway metabolites on bone tissue may be explained by their interaction with a cytosolic receptor AhR. Its activation may lead to unfavorable changes in bone metabolism through induction of CYP1A1 transcription and activation of the CYP1A1-dependent pathway. In turn, stimulation of the AhR in osteoblasts, through the CYP1A1 pathway stimulation, leads to the inhibition of proliferation and differentiation of osteoblasts in a collagen-induced arthritis mouse model by activating the signalling pathway dependent on extracellular signal-regulated kinases. At the same time, the activation of CYP1A1 in osteoclasts leads to an increase in their activity and exacerbation of the bone resorption process. Elevated level of peripheral kynurenine pathway metabolites in the course of CKD may also induce changes in the activity of other signalling factors, such as the receptor activator of nuclear factor-κB ligand/osteoprotegerin axis, extracellular signal-regulated kinases, and histone deacetylase-3 or runt-related transcription factor 2 expression levels, which also may stimulate osteoclasts and inhibit osteoblast activity.
Abbreviations: 3-HKYN, 3-hydroxykynurenine; AhR, Aryl hydrocarbon receptor; CYP1A1, AhR-dependent cytochrome P450, family 1, subfamily A, polypeptide 1; ERK, Extracellular signal-regulated kinases; Hdac3, Histone deacetylase-3; INF- γ, Interferon-gamma; KYN, Kynurenine; LP, Lipopolysaccharide; miRNA, micro RNA; RANKL/OPG, Receptor activator of NF-kB ligand/osteoprotegerin axis; ROS, Reactive oxygen species; Runx2, Runt-related transcription factor 2.

effective method of counteracting the development of CKD-associated osteodystrophy.

The role of the dialysis therapy in minimizing the harmful effects of accumulation of tryptophan degradation products in the end stages of CKD

IDO and TDO activity and kynurenine pathway's metabolites plasma levels were potently increased in dialyzed patients in the end-stage of chronic renal failure. These increases are positively associated with the level of oxidative stress in the patients' bodies.[19,24,57,98,120,138,142-145] Renal replacement therapy was proposed as the one of the solutions effective in the reduction of the effects of the accumulation of toxic metabolites in patients with advanced CKD stages.[24,138,142,143] Hemodialysis and PD significantly reduce the concentration of all the kynurenine pathway metabolites in the uremic patients plasma.[24,137,138,142,143] More than one research demonstrated strong relationships between kynurenine pathway upregulation and the increase in the oxidative stress level, inflammation and the progression of hemostatic and biochemical disturbances, as well as cardiovascular diseases, in patients with end-stage renal disease.[57,135-143] Therefore, the use of dialysis leads to a reduction in the inflammation, oxidative stress level, and the severity of the hemostatic and biochemical disturbances occurring in the advanced stages of CKD.[24,142,143] It may weaken the uremic symptoms and decrease the prevalence of CKD-associated systemic disorders, resulting from the accumulation of KYN and its metabolites. However, it should not be forgotten, that the efficiency of this solution seems to be limited. Although, the KYN, KYNA, 3-HKYN, AA, XA, and QA plasma concentrations in patients after dialysis were distinctly decreased, they still significantly exceeded a range typical for the healthy people.[24,107,137,138,142,202] It suggests that dialysis does not fully protect patients against the accumulation of these compounds, and the detrimental effects of their chronic influence. What is more, Yilmaz et al showed that the patients undergoing PD therapy have increased the IDO level in comparison with, hemodialysed. Patients on hemodialysis were also less prone to oxidative stress, compared to the PD group. This indicates the greater importance of oxidative stress and IDO activity in patients on PD than in those on hemodialysis.[203]

Another important point is that KYN is not a metabolic end product and is not normally cleared by the kidney. Therefore, in this case, the authors observed a markedly different pattern of accumulation in the CKD than other uremic toxins, normally cleared by renal secretion. The plasma KYN

level is elevated in the course of CKD, but it does not rise proportionally to the decrease in the GFR value.[25,56,105,177,204] This is probably because KYN is rather an intermediary metabolite, mostly reabsorbed by the renal tubules. Consequently, a very small amount of KYN is normally observed in the urine.[105] Most KYN is produced in the liver, and its production depends largely on the dietary intake of TRP. In the advanced stages of CKD, a serum albumin level is strongly decreased. It may lead to the potent increase in the free TRP supply in plasma, which can get into the liver with the bloodstream and become metabolized by TDO, and thus increase KYN and its downstream metabolite synthesis.[205-209] In turn, increased extrahepatic KYN synthesis observed in states with increased inflammation contributes to the increase in plasma KYN level in renal insufficiency.[10] Therefore, although KYN is removed from circulation by dialysis, but it is not clear if the amount of KYN removed from plasma has a notable effect on its average plasma levels in dialyzed patients.[25,142,204] Besides, the authors hypothesized that in serum KYN and other its metabolites such as 3-HKYN or 3-HAA were expected to increase in conditions of renal insufficiency and might transverse the blood-brain barrier and be converted to QA, but they could not prove it.[210] In turn, a considerable rise in the QA plasma concentration is observed in uremic patients and its removal in significant quantities by hemodialysis, discovered in the one our previous studies might be explained by decreased activity of ACMS decarboxylase during the progression of uremia.[25,107,202]

These results indicate that renal replacement therapy, despite its partial effectiveness, is not a fully efficient method of counteracting the accumulation of KYN and its metabolites in the end-stages of CKD. The increased availability of the free TRP supply and the activity of kynurenine pathway enzymes contributes to such a significant increase in the production of KYN and its metabolites, that even dialysis does not fully prevent patients against their accumulation, and thus detrimental effects of their chronic influence. This justifies the search for other ways to protect patients from harmful effects of the accumulation of the kynurenine pathway metabolites. Inhibition of the peripheral activity of the kynurenine pathway could be an effective method of preventing systemic disorders related to the accumulation of its components in patients with advanced CKD.

Summary and future directions

The results of numerous recent research indicate the significant impact of the accumulation of the components of the kynurenine pathway on the general body homeostasis, as well as on the condition of many organs, mainly by their ability to induce oxidative stress, enhance apoptosis rate and exacerbate inflammation. This review indicates that the changes in the activity of the kynurenine pathway accompanying renal insufficiency have a significant effect on most of the important systemic disorders accompanying the CKD progression, such as anemia, hypercoagulability atherosclerosis, neurological disorders, changes in blood pressure, and osteodystrophy.

The discovery of the impact of the TRP active metabolites on numerous metabolic processes in the course of CKD demonstrates a need for the continuation and expansion of studies concerning the role of compounds, such as KYN and its derivatives in the CKD and main systemic disorders associated with its progression. The arguments for this include their occurrence in the brain, blood plasma, and most of the peripheral tissues, extensive metabolic activity, and participation in many physiological and pathological processes in different types of tissue proven by numerous studies. A broader study of this area would open the new possibilities and may give the grounds to the development of new, more effective solutions in the prevention, diagnosis, and treatment of the CKD and systemic disorders related with its progression, which could improve the therapy of this disease and effectively reduce the harmful effects of disorders accompanying its courses, such as atherosclerosis, anemia, thrombosis, neurological disorders, and CKD-MDB.

The main problems associated with disturbances in the kynurenine pathway, observed in patients with the CKD, are connected with the increase in the activity of upstream enzymes responsible for the synthesis of biologically active TRP metabolites, which exert harmful effects on the body and simultaneous inhibition of downstream enzymes responsible for the degradation of these compounds into their low-toxic derivatives. Except that, their accumulation in the body to a certain degree is also independent of enzymatic activity due to impaired renal function. That is why the efforts of experimental pharmacologists should be directed towards new drugs that would allow for efficient and favorable modulation of the kynurenine pathway activity. It can be hypothesized that if KYN and its derivatives are positively correlated with the CKD severity and the occurrence of associated systemic disorders, consequently the efficient modulation of this pathway activity may be an effective method to reduce the severity of some disorders associated with the its progression. In the case of other diseases, certain compounds able to modify the kynurenic system activity have been recently considered as potential drugs.[149-166] Kynurenine aminotransferase inhibitors are considered as the agents for the treatment of neurodegenerative diseases, cognitive disorders, and schizophrenia but their usefulness in the case of CKD-associated neurological disorders seems to be debatable because in the most tissues no increase in this enzyme activity in the CKD was observed.[44,149-153] IDO inhibitors are found as therapeutics useful in cancer therapy.[154-158] As IDO is upregulated both in malignancy and in the course of CKD, the research concerning the use of its inhibitors in the reduction of systemic uremic symptoms appears to be potentially justified.[54,56,154-158] Another enzyme whose activity increases during the CKD progression is kynurenine 3-monooxygenase. Its inhibitors are considered as potential therapeutics in pancreatitis and neurodegenerative diseases.[159-166] This suggests that

they could be potentially useful in the treatment of CKD-associated neurological disorders. The systemic administration of IDO and kynurenine 3-monooxygenase inhibitors seems to be a rational approach to the treatment of the kynurenine pathway abnormalities because it should result in a decrease in the systemic level of cytotoxic kynurenine metabolites, such as KYN, 3-HKYN, and QA. Concerning the metabolites accumulation problem, it should be noted that the dialysis is not a completely effective method of reduction of the kynurenine pathway derivatives concentration in the patients with limited renal excretory capacity. Therefore it seems to be a good direction of studies in the field of experimental pharmacology. If such treatment would prove to be effective it should also at least partly contribute to the reduction of the severity of the CKD-associated disorders. In turn, all actions taken towards minimizing the harmful effects of toxic uremic metabolites, including the components of the kynurenine pathway should result in improved quality of life and reduced mortality in the patients with CKD in the future.

Author contributions

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International Journal of
Molecular Sciences

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Article

Modulation of the Paracrine Kynurenic System in Bone as a New Regulator of Osteoblastogenesis and Bone Mineral Status in an Animal Model of Chronic Kidney Disease Treated with LP533401

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Received: 30 June 2020; Accepted: 18 August 2020; Published: 19 August 2020

Abstract: An increase in the peripheral synthesis of serotonin and kynurenine, observed during the chronic kidney disease (CKD) course, is negatively associated with bone health. Serotonin and kynurenine are connected by the common precursor, tryptophan. LP533401 is an inhibitor of peripheral serotonin synthesis. This study aimed to establish if the inhibition of serotonin synthesis by LP533401 may affect the kynurenine pathway activity in bone tissue and its potential consequence with regard to osteogenesis and bone mineral status. Nephrectomized rats were treated with LP533401 at a dose of 30 and 100 mg/kg daily for eight weeks. Tryptophan and kynurenine concentrations were determined, and tryptophan 2,3-dioxygenase (TDO) expression was assessed. We discovered the presence of a TDO-dependent, paracrine kynurenic system in the bone of rats with CKD. Its modulation during LP533401 treatment was associated with impaired bone mineral status. Changes in TDO expression affecting the kynurenine pathway activity were related to the imbalance between peripheral serotonin and 25-hydroxyvitamin D. There were also close associations between the expression of genes participating in osteoblastogenesis and activation of the kynurenine pathway in the bones of LP53301-treated rats. Our results represent the next step in studying the role of tryptophan metabolites in renal osteodystrophy.

Keywords: LP533401; gut-derived serotonin; chronic kidney disease; mineral and bone disorders; tryptophan metabolism; tryptophan 2,3-dioxygenase; kynurenine pathway; kynurenine; uremic toxins

1. Introduction

Abnormalities in bone metabolism represent the most complex complications accompanying chronic kidney disease (CKD) development [1,2]. The systemic CKD mineral and bone disorders (CKD-MBD) are associated with the disturbances in calcium and phosphorus metabolism, secondary hyperparathyroidism, deficiency of vitamin D, vascular calcification, and bone tissue disorders [2].

In recent decades, serotonin (5-HT) received intensive attention due to its potential role in bone metabolism [3]. However, the issue of 5-HT and bone biology is still controversial, and it is closely dependent on the site of its synthesis; gut-derived 5-HT has unfavorable effects on bone health, while

brain-derived 5-HT has an osteoanabolic effect [4–7]. Our previous study showed that the elevated levels of peripherally synthesized 5-HT may influence the strength and metabolism of a long bone in nephrectomized rats [8]. We also identified a new molecular pathway, via which elevated circulating 5-HT can affect the expression of 5-HT-dependent genes in bone, shifting in forkhead box protein O1 (FOXO1) target genes from a cAMP-responsive element-binding protein (CREB)- to an activating transcription factor 4 (ATF4)-dependent response, resulting in enhanced osteoblast differentiation in this model [9].

Tryptophan (TRP) is the only precursor of 5-HT, and its conversion into 5-HT is initiated by tryptophan hydroxylase (TPH). Two isoforms of this enzyme (TPH-1 and TPH-2) occur. TPH-1 initiates the synthesis of peripheral 5-HT, mainly in the duodenum, while the second isoform, TPH-2, is exclusively expressed in the central nervous system. LP533401 is a small-molecule inhibitor of TPH-1. LP533401 does not penetrate the blood–brain barrier and does not lead to disturbances of the central 5-HT [10]. Yadav et al. also showed that pharmacological inhibition of TPH-1 by LP533401 was able to prevent bone loss in ovariectomized animals [10]. Recently, we demonstrated that LP533401 decreases plasma 5-HT concentration and may improve bone mineralization in nephrectomized rats [11].

Kynurenine (KYN), the major metabolite of TRP, is synthesized in the body by the tryptophan 2,3-dioxygenase (TDO) and indoleamine 2,3-dioxygenase (IDO) [12–14]. Metabolites of the kynurenine pathway play crucial roles in several physiological and pathophysiological processes [15–24]. Recent reports indicate that they are also connected with osteoblast proliferation and differentiation, and they can be related to the pathophysiology of osteoporosis [25,26]. Recently, we demonstrated that KYN produced in the central nervous system positively affects bone strength, while peripherally secreted KYN has the opposite effect [27,28].

The aim of this study was to establish whether the inhibition of 5-HT synthesis by LP533401 may modulate kynurenine pathway activity in bone tissue of nephrectomized rats and to determine the potential consequence of this process in relation to osteogenesis and regulation of bone mineral status.

2. Results

2.1. Effect of LP533401 Treatment on Tryptophan Utilization via the Kynurenine Pathway in Serum, Urine, and Intestinal Homogenate

We observed that 5/6 nephrectomy caused a significant decrease in TRP concentrations and an increase in KYN levels and KYN/TRP ratio, being a marker of KYN pathway activation, in comparison with sham-operated animals. The administration of the LP533401 at the dose of 30 mg/kg (LP 30) caused a significant increase in serum KYN concentration, especially in relation to the vehicle group. We also found a significant decrease in the KYN/TRP ratio in the group treated with LP533401 at the dose of 100 mg/kg (LP 100) in comparison to LP 30 (Figure 1A–C).

As shown in Figure 1D–F, there were no differences in the diurnal KYN component excretion in urine, apart from increased TRP excretion in the CKD and vehicle groups compared to controls and LP533401-treated animals. Similarly, there were no differences in TRP, KYN levels, and the KYN/TRP ratio among all studied groups in the intestinal homogenate (Figure 1G–I).

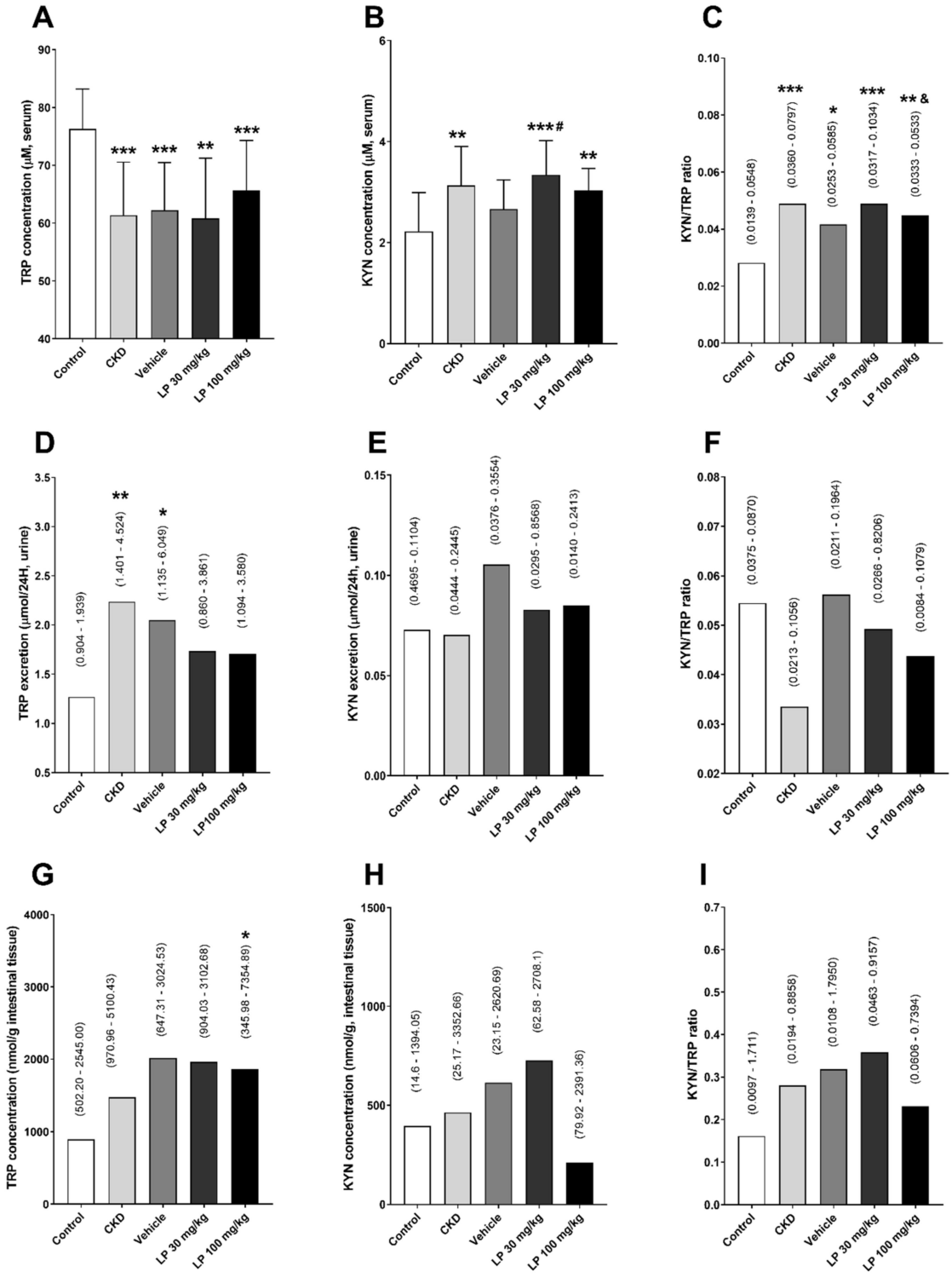

Figure 1. Changes in tryptophan (TRP) and kynurenine (KYN) concentrations, and KYN/TRP ratio among experimental groups in serum (**A**–**C**), diurnal urinary excretion (**D**–**F**), and intestinal homogenate (**G**–**I**). Data are shown as mean ± SD (**A**,**B**) or median and range (**C**–**I**). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, control vs. others; # $p < 0.05$, vehicle vs. others; & $p < 0.05$, LP 30 mg/kg vs. LP 100 mg/kg.

2.2. *Effect of LP533401 Treatment on KYN Pathway Activation in Bone Tissue*

The concentrations of TRP and KYN, and the KYN/TRP ratio were measured separately in homogenates from the trabecular and cortical bone region. Lower TRP levels were observed in rats

with CKD treated with LP 100 in comparison with the control (CON) and LP 30 groups. Despite this, the animals treated with LP at the dose of 30 mg/kg LP (LP 30) had lower KYN concentrations compared to CON and CKD in the trabecular bone. The higher dose of LP533401 resulted in an intensification of the KYN pathway activation in this bone region compared to other uremic groups, especially in relation to the LP 30 group (Figure 2A–C).

A similar tendency was shown in the cortical bone tissue homogenate. A significant decrease in KYN concentration was noticed in LP-treated groups compared to controls. We also found a significant increase in the KYN/TRP ratio after administration of the vehicle compared to the untreated CKD rats, as well as its decrease in the LP 30 group compared to the vehicle group and healthy controls (Figure 2D–F). Importantly, we did not observe an association between components of the KYN pathway in serum and bone tissue, which indicated an endogenous KYN synthesis in the bone.

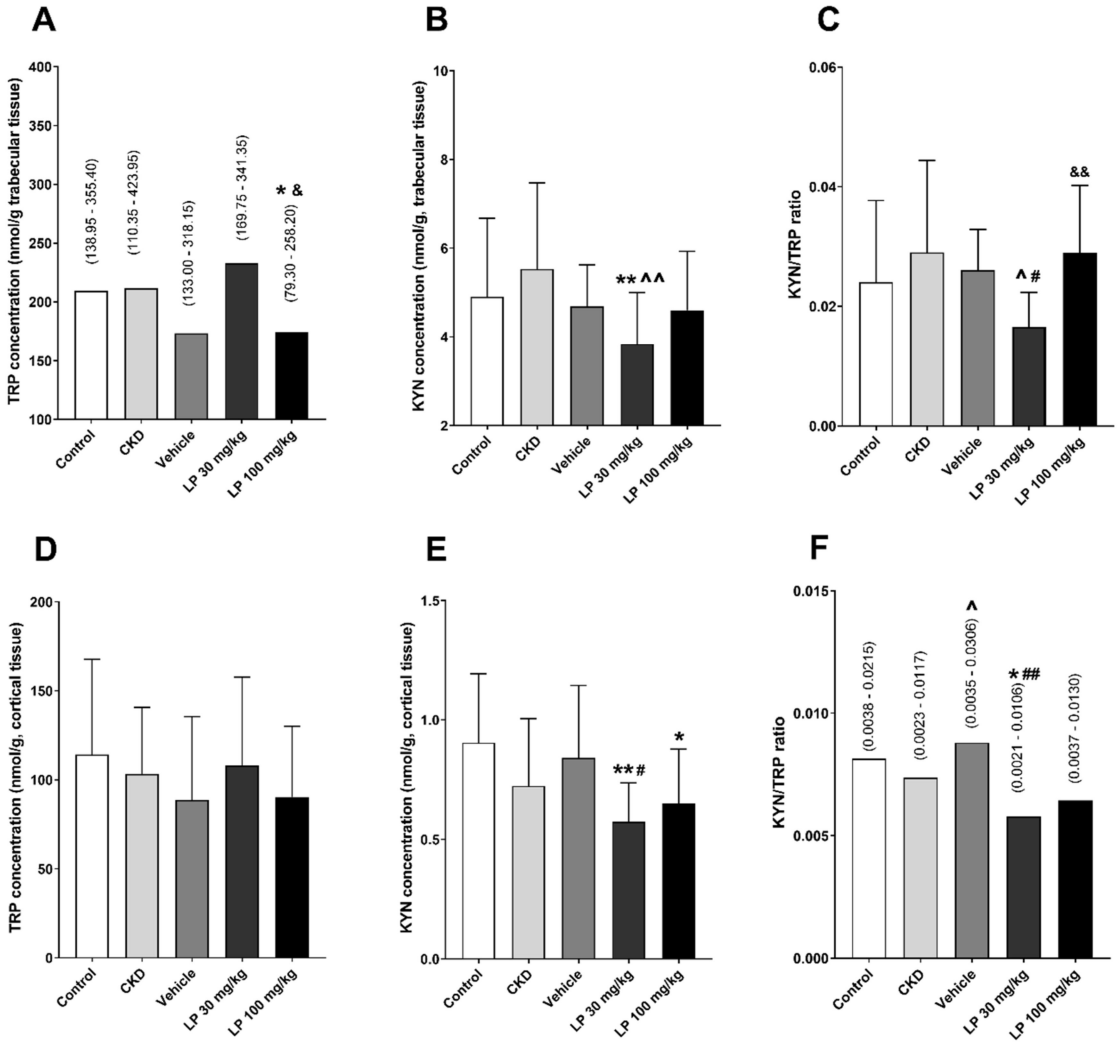

Figure 2. Changes of tryptophan (TRP) and kynurenine (KYN) concentrations, and KYN/TRP ratio among experimental groups in the femoral trabecular (**A**–**C**) and cortical (**D**–**F**) bone regions. Data are shown as median and range (**A**,**F**) or mean ± SD (**B**–**E**). * $p < 0.05$, ** $p < 0.01$, control vs. others; ^ $p < 0.05$, ^^ $p < 0.01$ CKD vs. others; # $p < 0.05$, ## $p < 0.01$, vehicle vs. others; & $p < 0.05$, && $p < 0.01$, LP 30 mg/kg vs. LP 100 mg/kg.

Because KYN pathway activation can be IDO- or TDO-dependent [29,30], we tried to establish which of these enzymes may be responsible for KYN synthesis in the bone tissue, by measuring the expression of relevant genes. There was no IDO-1 and IDO-2 expression in bone tissue (data not shown), whereas TDO expression was present in all studied groups (Figure 3A). We observed

a significant decrease in TDO messenger RNA (mRNA) level in the bone tissue of nephrectomized animals in comparison with the control. The administration of vehicle or LP 30 resulted in further attenuation of TDO expression in comparison with CKD. In the LP 100 group, the TDO mRNA level was still lower than in the control, but significantly higher than in the vehicle- and LP 30-treated groups (Figure 3A).

We also noticed that TDO expression was positively associated with KYN concentration and KYN/TRP ratio in the trabecular bone region (Figure 3B,C), and that KYN concentration in this bone region was positively related to KYN level in the cortical bone region (Figure 3D).

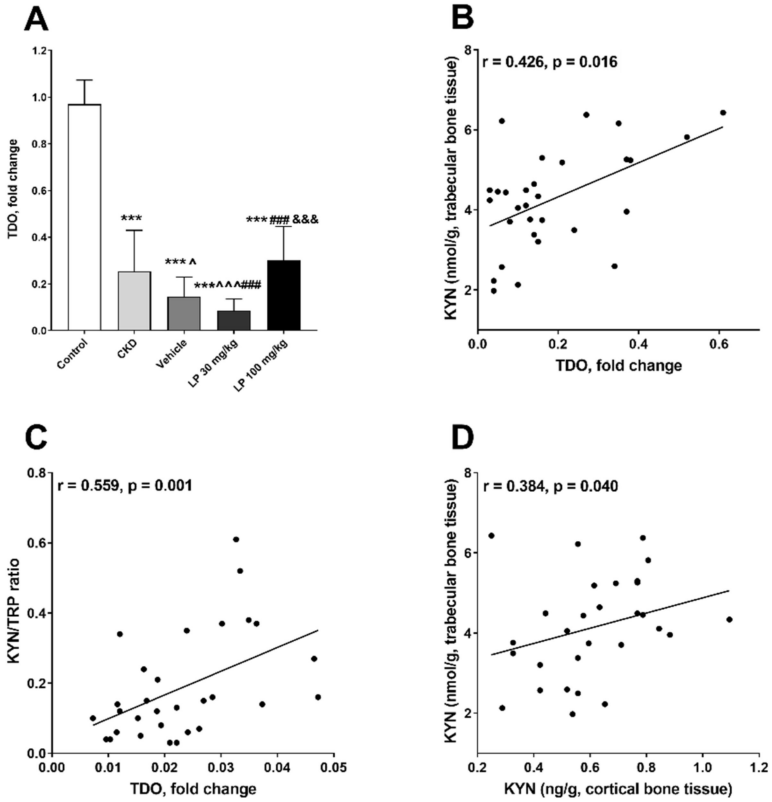

Figure 3. Changes in tryptophan 2,3-dioxygenase (TDO) expression level in the femoral bone (**A**) among experimental groups, and the association between TDO messenger RNA (mRNA) level and kynurenine pathway activation in bone tissue of CKD rats treated with LP533401 (**B**–**D**). Data are shown as mean ± SD. *** $p < 0.001$, control vs. others; ^ $p < 0.05$, ^^^ $p < 0.001$, CKD vs. others; ### $p < 0.001$, vehicle vs. others; &&& $p < 0.001$, LP 30 mg/kg vs. LP 100 mg/kg.

2.3. The Associations between Bone Mineral Status and Components of the Bone Kynurenine Pathway

The effect of LP533401 on femoral bone densitometry parameters was presented in detail in our previous study. Briefly, rats with CKD had significantly decreased bone mineral status compared with controls, while treatment with vehicle and LP533401 caused a significant increase in these parameters in comparison with untreated animals [11]. The evaluation of relationships between the components of the bone kynurenine pathway in individual bone regions and the bone mineral status in LP533401 treated rats revealed that TDO gene expression, as well as KYN concentration and KYN/TRP ratio in trabecular homogenates, was strongly and inversely related to bone mineral area (BMA), bone mineral content (BMC), and bone mineral density (BMD) values, particularly in the distal metaphysis (R1) bone region. In contrast, the TRP level in trabecular tissue was positively associated with BMA, especially in the R1 bone region. Similar, although weaker correlations were found between KYN levels in cortical homogenates and the studied parameters of femoral mineral status (Figure 4).

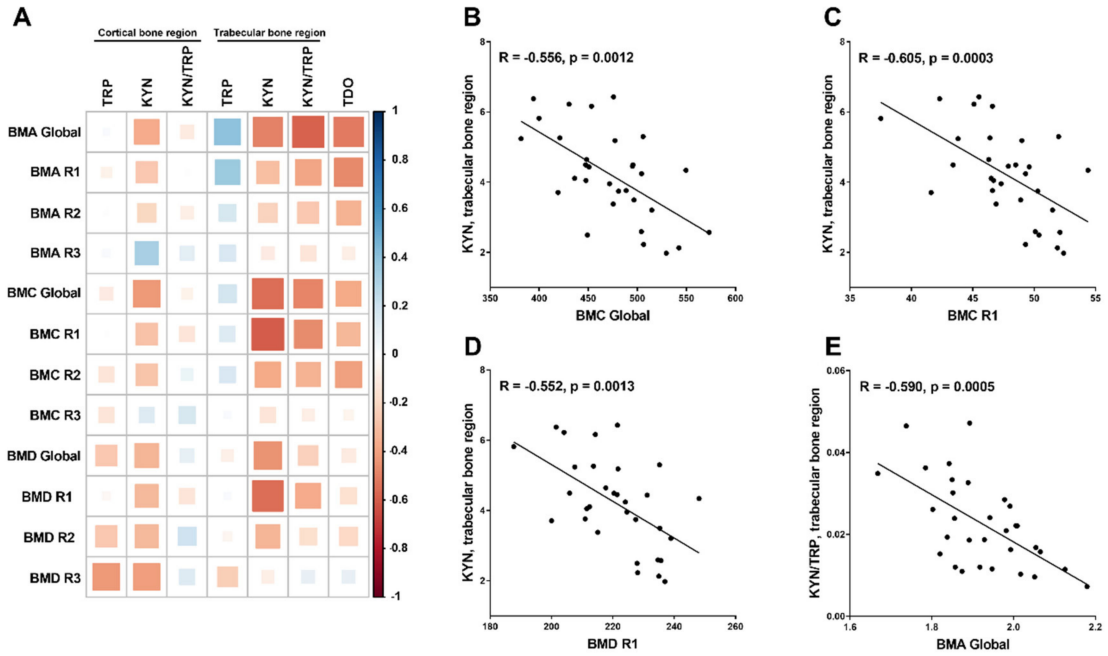

Figure 4. The association between components of the kynurenic system in bone homogenates and the parameters of bone mineral status in rats with CKD treated with LP533401 (**A**) and a detailed graphic presentation of the strongest among them (**B**–**E**). The size and the color intensification demonstrate the strength of the correlation (larger and darker circles represent a strong correlation). Blue colors—positive correlations; red colors—negative correlations. BMA—bone mineral area, BMC—bone mineral content, BMD—bone mineral density. R1—the distal metaphysis subregion, composed mostly of the trabecular tissue bone. R2—midshaft area subregion, constituted mostly of cortical bone tissue. R3—femoral neck subregion, built of a similar proportion of trabecular and cortical bone tissue.

2.4. The Imbalance between Peripheral Serotonin and 25-Hydroxyvitamin D (25(OH)D) Status Affects TDO Expression in the Bone of CKD Rats Treated with LP533401

In the next step of our work, we tried to find the factors that influenced bone TDO gene expression in CKD rats treated with LP533401. Previously, we showed that LP533401 administration to rats with CKD reduced turnover of circulating 5-HT [11], and it simultaneously caused the disturbances in serum 25-hydroxyvitamin D (25(OH)D) status [31]. In general, the dose-dependent rise in its concentration was observed in animals treated with LP533401 [31]. Recently, we demonstrated that not only peripheral 5-HT [32], but also the disturbances in calciotropic hormones, namely, the advantage of 25(OH)D over 5-HT, reflected by the 25(OH)D/5-HT ratio, can affect osteoblastogenesis in LP-treated animals. As shown in Figure 5A, 25(OH)D/5-HT ratio in the LP 100 group was comparable to the control, and it was significantly higher in this group than in the LP 30 and vehicle groups, where we observed a significant decrease in the 25(OH)D/5-HT ratio in comparison with the control. Moreover, both serum 25(OH)D levels, as well as 25(OH)D/5-HT ratio, were positively associated with TDO gene expression (Figure 5B,C), while serum 5-HT level did not correlate with TDO mRNA expression (Figure 5D).

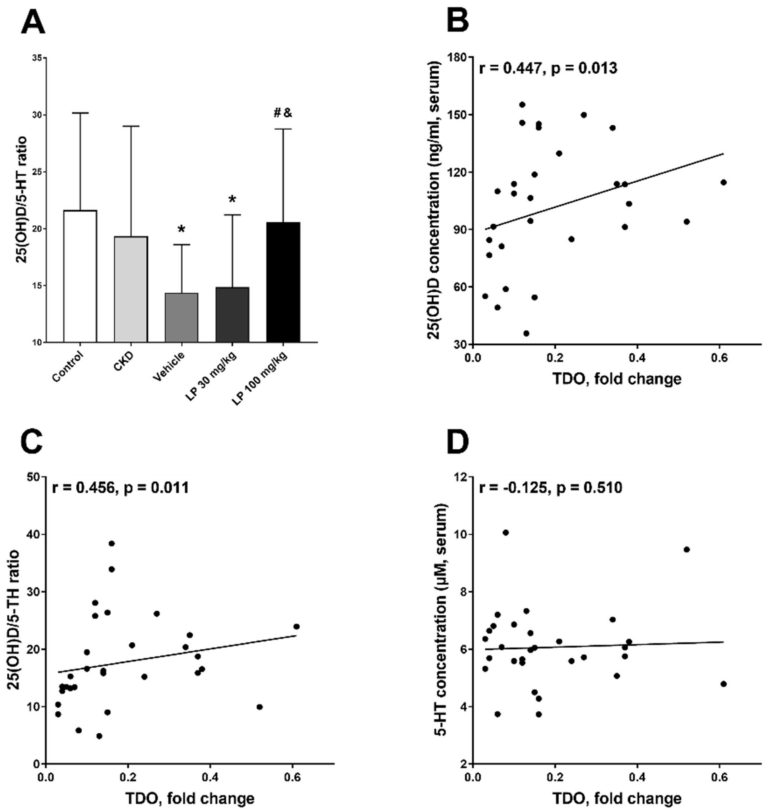

Figure 5. Changes in serum 25-hydroxyvitamin D to serotonin (25(OH)D/5-HT) ratio (**A**) among experimental groups, and the association between bone tryptophan 2,3-dioxygenase (TDO) expression and serum 25(OH)D concentration (**B**), 25(OH)D/5-HT ratio (**C**), and 5-HT level (**D**) in rats with CKD treated with LP533401. Data are shown as mean ± SD. * $p < 0.05$, control vs. others; # $p < 0.05$, vehicle vs. others; & $p < 0.05$, LP 30 mg/kg vs. LP 100 mg/kg.

2.5. The Association between Serotonin-Dependent Molecular Pathway Involved in Osteoblast Formation and Activity of Kynurenic System in the Bone of Rats with CKD Treated with LP533401

Data obtained from an in vitro experiment by El Refaey et al. suggest that KYN can impair osteoblastic differentiation from bone marrow mesenchymal stem cells and, via this mechanism, it can play a role in bone loss [33]. Herein, we analyzed the associations between the expression of 5-HT-dependent genes involved in osteoblast proliferation and differentiation processes [32] and the activity of the bone kynurenic system, represented as TDO expression and KYN/TRP ratio. As schematically presented in Figure 6A, activating transcription factor (ATF4) gene expression was similar in LP 30 and control groups, whereas it was significantly higher in other studied groups compared to healthy animals, and a positive relationship existed between ATF4 and TDO expression (Figure 6B), as well as between ATF4 gene expression and KYN/TRP ratio both in trabecular (Figure 6C) and in cortical bone tissue ($r = 0.421$, $p = 0.023$) of LP-treated rats. Moreover, a positive and strong association was also observed between ATF4 gene expression and trabecular KYN concentration ($r = 0.474$, $p = 0.007$). In contrast, cyclin D1 mRNA level was lowest in the LP 100 group compared to other studied groups (Figure 6D), and a strong inverse relationship existed between this gene's expression and TDO expression (Figure 6E), whereas there was only a slight, non-significant relationship between cyclin D1 expression and the KYN pathway activation marker in rats with CKD treated with LP533401 (Figure 6F).

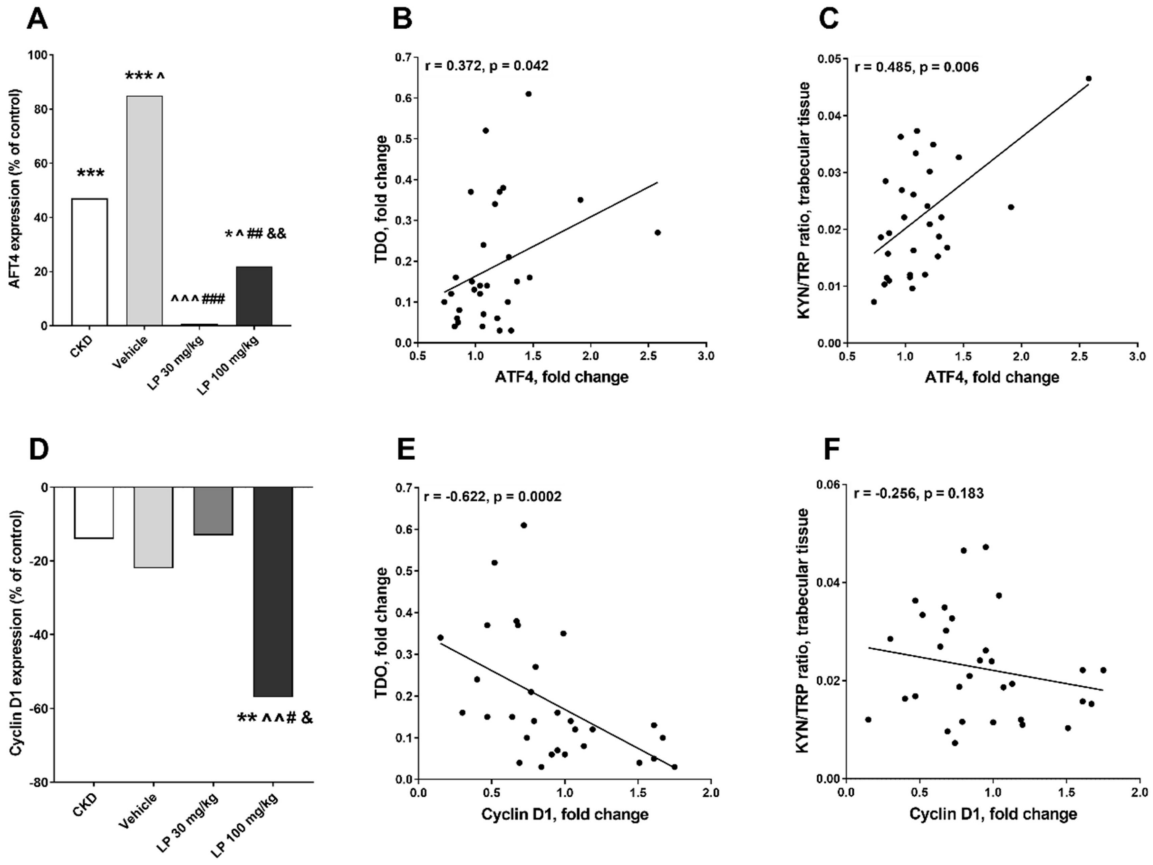

Figure 6. Changes in activating transcription factor (ATF4) and cyclin D1 gene expression among experimental groups (**A**,**D**), and their associations with tryptophan 2,3-dioxygenase (TDO) expression (**B**,**E**), and kynurenine to tryptophan (KYN/TRP) ratio in trabecular bone tissue (**C**,**F**) in rats with CKD treated with LP533401. Data relate to the control taken as 0%. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, control vs. others; ^ $p < 0.05$, ^^ $p < 0.01$, ^^^ $p < 0.001$, CKD vs. others; # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$, vehicle vs. others; & $p < 0.05$, && $p < 0.01$, LP 30 mg/kg vs. LP 100 mg/kg.

Osteocalcin (Bglap) is a marker of the late stage of osteoblast differentiation, whereas sclerostin (Sost) is considered as an indicator of osteoblast transition to osteocyte [34]. As shown in Figure 7A, Bglap gene expression was higher in CKD and especially in the vehicle group compared to the control group. CKD rats treated with LP 30 had the lowest Bglap mRNA levels among all analyzed uremic groups, and a positive relationship existed between this gene's expression and both TDO expression (Figure 7B) and KYN/TRP ratio in trabecular bone (Figure 7C) of uremic rats treated with LP533401. Similarly, Sost gene expression was significantly reduced in the LP 30 group in comparison with other studied groups (Figure 7D), and it was positively correlated with TDO expression (Figure 7E) and KYN/TRP ratio (Figure 7F) in trabecular bone tissue of rats treated with LP533401.

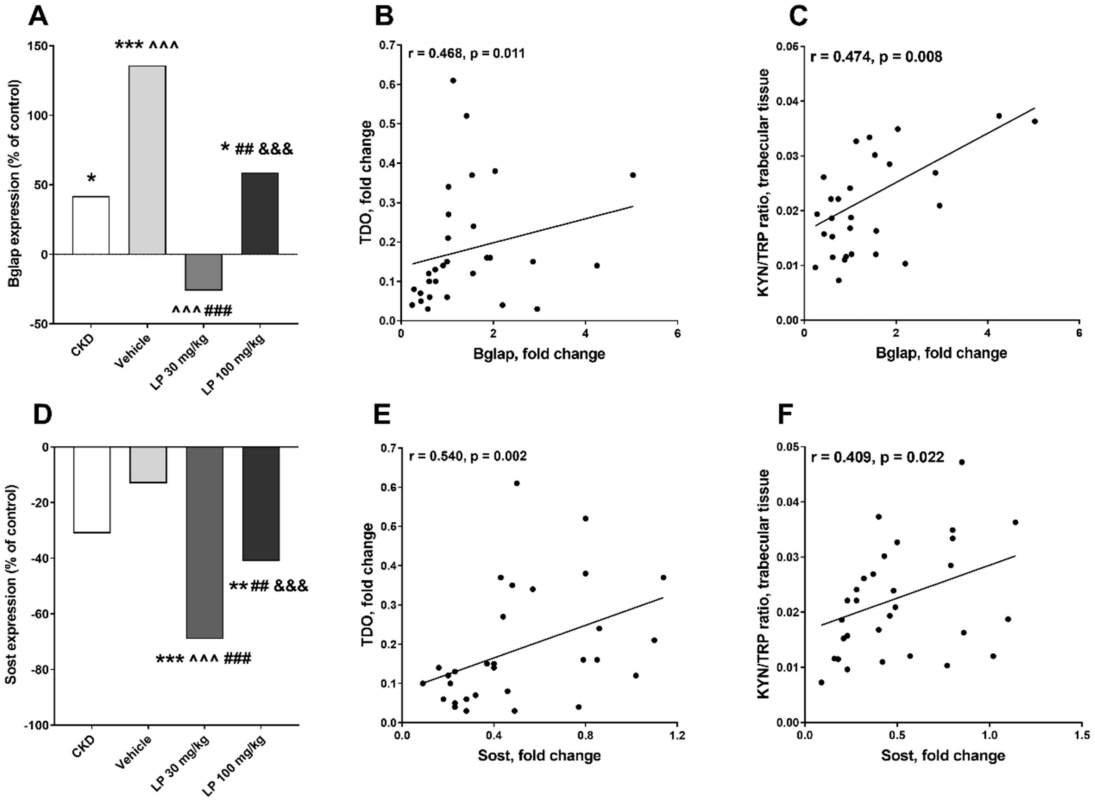

Figure 7. Changes in osteocalcin (Bglap) and (sclerostin) Sost gene expression among experimental groups (**A**,**D**), and their associations with tryptophan 2,3-dioxygenase (TDO) expression (**B**,**E**), and kynurenine to tryptophan (KYN/TRP) ratio in trabecular bone tissue (**C**,**F**) in rats with CKD treated with LP533401. Data relate to the control taken as 0%. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, control vs. others; ^^^ $p < 0.001$, CKD vs. others; ## $p < 0.01$, ### $p < 0.001$, vehicle vs. others; &&& $p < 0.001$, LP 30 mg/kg vs. LP 100 mg/kg.

3. Discussion

Our study shows four main findings. Firstly, we established the presence of a TDO-dependent, paracrine kynurenic system in the bone of rats with CKD. Secondly, the modulation of this system during LP533401 treatment was associated with impaired bone mineral status. Thirdly, the imbalance between peripheral 5-HT and 25(OH)D affects TDO expression level and, consequently, KYN pathway activation in the bone of animals treated with LP533401. Fourthly, there are close associations between genes participating in osteoblastogenesis and TDO-dependent activation of the KYN pathway in the bone of uremic rats treated with LP533401.

The present study is a continuation and an extension of our earlier investigation concerning the impact of the inhibition of peripheral 5-HT synthesis by LP533401 on bone health, disturbance of calciotropic hormones, and the expression of 5-HT-dependent genes involved in osteoblastogenesis in uremic rats [11,31,32]. Previously, we also showed that the activation of the peripheral kynurenine system in young rats with mild to moderate CKD unfavorably affected bone microarchitecture geometry and strength [27]. In the present study, we measured the components of the KYN pathway, namely, TRP and KYN, and we determined KYN/TRP ratio, as a marker of this system's activity in the blood, urine, intestinal, and bone tissue of rats with CKD treated with LP533401. The activation of the peripheral KYN system in uremic rats, observed in the present study, was in agreement with our previous experiments performed in the rat model of CKD [27,35,36], as well as with clinical data from CKD patients [37–39]. In the present study, we demonstrated for the first time the presence of TRP and KYN in bone tissue of all experimental groups. Next, we discovered that, among the three enzymes which can degrade TRP into KYN (IDO-1, IDO-2, and TDO), only TDO gene expression was

present in bone, and it was associated with bone KYN levels and KYN/TRP ratio of studied animals. Moreover, there was no association between KYN levels in serum and bone tissue in these animals. The above results suggest that bone possesses its own, paracrine, TDO-dependent system, which is able to locally produce KYN [40]. Until now, the physiological constitutive expression of TDO was demonstrated only in liver and neurons [41]; thus, our unexpected finding pointing to TDO as an exclusive enzyme participating in TRP degradation to KYN in bone tissue may significantly expand the existing knowledge about peripheral TRP metabolism.

Despite significantly reduced TDO gene expression in rats with CKD and vehicle groups compared to the control, KYN concentrations and KYN/TRP ratio in bone tissue were comparable between these groups, suggesting the intensification of TRP degradation to KYN in uremic animals. The lowest activation of the bone kynurenic system observed in uremic rats treated with LP533401 at the dose of 30 mg/kg was compatible with the strongest reduction of TDO gene expression in these animals, compared to other groups. The pathophysiological consequence of the reduced activity of the KYN pathway in the bone of rats treated with LP533401 was an improvement of their mineral status, especially visible in the distal metaphysis (R1) bone region, which is rich in the more metabolically active trabecular bone. Previously, we showed that peripheral kynurenine levels correlated inversely with the parameters of bone biomechanics, bone geometry, and bone mineral status of young nephrectomized rats [27]. Taking these results together, the activation of both systemic and local KYN pathways in bone tissue seems to be related to an impairment in bone integrity in rats with CKD. This finding is in agreement with the observation made previously by El Refaey et al., where increasing the level of peripheral KYN resulted in accelerated skeletal aging and bone loss in mice [42]. In addition, a study reported an inverse connection between kynurenic acid, one of KYN metabolites, and BMD in humans [23]. The most likely explanation for this unfavorable effect of KYN on bone health may include the activation by KYN of the aryl hydrocarbon receptor-dependent pathway in the bone of CKD animals and humans [27,43]. This time, we also cannot rule out the presence of other pathological KYN interactions, leading to modulation of other signaling factors, such as the factor receptor activator of nuclear factor-κB ligand/osteoprotegerin axis and histone deacetylase-3 or runt-related transcription factor 2 expression [33,43].

In the next step of our study, we tried to identify the potential molecular mechanism leading to the increased TDO gene expression in the bone of CKD rats treated with LP533401 and its potential consequences. Previously, we noticed that the administration of LP533401 to rats with CKD, apart from the reduction of peripheral 5-HT, evoked disturbances in calciotropic hormones, resulting in an advantage of 25(OH)D over 5-HT [32]. Herein, we demonstrated that both serum 25(OH)D levels and 25(OH)D/5-HT ratio were positively associated with TDO gene expression. Thus, the imbalance between circulating 25(OH)D and 5-HT may be one of the factors affecting the activation of the bone KYN pathway during LP533401 treatment. Findings from our previous investigation also demonstrated that the imbalance between 5-HT and calciotropic hormones during LP533401 administration led to disruption of the interactions in the CREB–ATF4–FOXO-1 complex [32], which is is recognized as a crucial, 5-HT-dependent molecular pathway involved in bone formation [44]. The above mechanism resulted in the sequential and exclusive expression of genes involved in osteoblast growth, differentiation, maturation, and the improvement of bone mineral status in LP-treated animals [32]. The analysis of the interaction between genes involved in osteoblastogenesis and components of the bone KYN pathway revealed the association between the marker of the early stage of osteoblast proliferation/differentiation (ATF4) and the bone kynurenic system. On the other hand, stronger positive associations were noticed among TDO expression, KYN/TRP ratio, and the markers of terminally differentiated osteoblasts/osteocytes—Bglap and Sost gene expression (Figure 8). These results suggest that the activation of the kynurenic system is rather related to osteoblast maturation than to their differentiation process. The strong, inverse relationship between cyclin D1 (the key cell-cycle regulatory factor in proliferating/differentiating osteoblasts [45]) and TDO gene expression seems to confirm this hypothesis. Similar inverse associations were previously noticed by us between

cyclin D1 and Bglap/Sost expression in this model [32]. In the available literature, there is scarce and contradictory evidence suggesting that degradation of TRP into KYN is essential for osteogenic differentiation. El Refaey et al. showed that KYN under in vitro conditions significantly inhibited bone marrow mesenchymal stem cell proliferation and differentiation into osteoblasts [33]. Opposing results were obtained by Vidal et al., who demonstrated that blocking the KYN pathway through IDO-1 inhibition led to impaired osteoblastic differentiation in vitro, and that IDO-1$^{-/-}$ deficient mice were osteopenic [25].

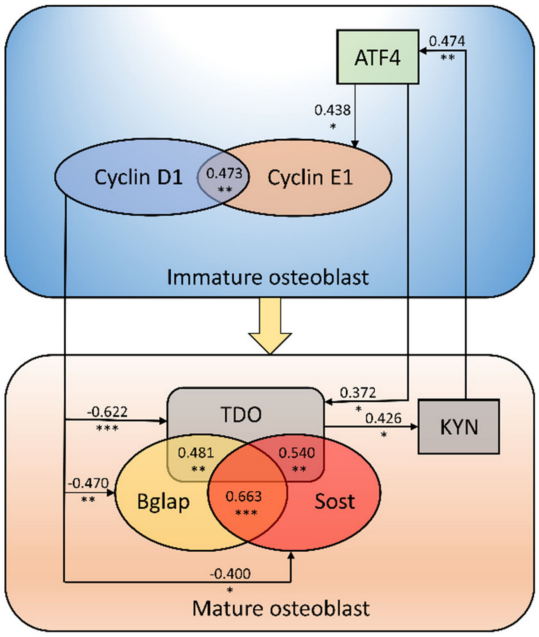

Figure 8. Schematic presentation of the possible role of bone kynurenic pathway activation in the osteoblast maturation process. Each value is Spearman's rank correlation coefficient (*R*) value between the genes. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ indicate statistically significant values; a negative value indicates an inverse correlation between parameters. ATF4—activating transcription factor 4; Bglap—osteocalcin; KYN—kynurenine; Sost—sclerostin; TDO—tryptophan 2,3-dioxygenase.

It is worth noting that, in the present study, the components of the kynurenic system, especially in the trabecular bone region, were also positively related to ATF4 expression. Thus, it cannot be excluded that KYN may support the early stage of osteoblast differentiation, which is particularly intensified in CKD [32,46], while the inverse relationship between cyclin D1 and TDO gene expression may be an attempt to compensate for this phenomenon, in order to obtain mature osteoblasts (Figure 8) capable of performing their physiological functions, e.g., participation in the mineralization process. This is in line with the results of our previous study, in which the mitigation of intensified osteoblastogenesis in rats with CKD treated with LP533401 resulted in improvement of their bone mineral status [32].

In conclusion, our study for the first time demonstrated the presence of an active, paracrine kynurenic system in rat bone, which is independent of the peripheral one. The treatment of uremic rats with LP533401, which is an inhibitor of peripheral 5-HT synthesis, can indirectly activate this pathway, resulting in impaired bone mineral status. There are close associations among the expression of genes that participate in osteoblastogenesis, particularly with osteoblast maturation markers, and TDO-dependent activation of the KYN pathway in the bone of uremic rats treated with LP533401. Although it is not yet possible to fit all these data into a simple mechanistic hypothesis of the progression of osteoporosis in CKD, our results represent the next step in studying the role of tryptophan metabolites in renal osteodystrophy.

4. Materials and Methods

4.1. Animals and Experimental Design

Seventy-four male Wistar rats aged four weeks were purchased from and housed in the Centre of Experimental Medicine of the Medical University of Bialystok (Poland). The study was carried out in accordance with European Union (EU) Directive 2010/63/EU for animal experiments and was approved by the Local Ethical Committee on Animal Testing in Olsztyn (Permit Number: 29/2013, approved: 04/2013). The animals were housed in conventional cages, grouped as appropriate in vivarium conditions, with a 12-h light/dark cycle at controlled temperature (22 ± 2 °C) and humidity (55 ± 5%). They were allowed access to sterilized tap water and standard rat chow, containing 1% calcium, 0.7% phosphorus, and 19% protein. The animals' health status was monitored throughout the experiments by a health surveillance program according to the Federation of European Laboratory Animal Science Associations (FELASA) guidelines. The rats and mice were free of all viral, bacterial, and parasitic pathogens listed in the FELASA recommendations. Ten of them were randomly chosen and sham-operated (control). The other 64 rats had induced CKD by surgical 5/6 subtotal nephrectomy using the surgical procedure described in detail previously [8]. Nephrectomized rats were randomized into four groups (n = 16 in each): untreated (CKD), administered with a vehicle, treated with LP533401 at a dose of 30 mg/kg for eight weeks, and treated with LP533401 at the dose of 100 mg/kg for eight weeks. After this period, the animals were subjected to analysis. The vehicle was polyethylene glycol and 5% dextrose in a ratio of 40:60. The doses of LP533401 were based on previous studies [10,47,48]. The vehicle or its solution with LP533401 was administered daily by gavage, while untreated nephrectomized and sham-operated rats received sterile water in this same regimen. At the end of the experiment, they were anesthetized until unconscious and euthanized by exsanguination via cardiac puncture. Procedures were conducted in the light phase of the cycle in the surgical room of our laboratory. Details about the experimental design, housing conditions, tissue collection, and general characteristics of animals were described in detail previously [11].

4.2. TRP and KYN Quantification in Serum, Urine, and Bone and Intestinal Tissues

Deproteinized serum samples were prepared by adding 50 µL of 2 M perchloric acid into 200 µL of a thawed sample. Then, 20 µL of urine samples were firstly diluted 10 times with distilled water and then acidified by adding 20 µL of 2 M perchloric acid. The acidified serum and urine samples were vortexed, kept at 4 °C for 20 min, and then centrifuged for 30 min at 14,000× g at 4 °C. The obtained supernatant was stored at −80 °C until assayed by high-performance liquid chromatography (HPLC, Agilent Technologies, Palo Alto, CA, USA).

Frozen small intestine samples were weighed and homogenized in 20% trichloroacetic acid in a ratio of 1:5. Obtained homogenates were placed at 4 °C for 30 min, and then the samples were centrifuged at 12,000× g for 20 min at 4 °C. After centrifugation, the supernatant was filtered (0.45-mm Millipore filter) and subjected to HPLC analysis.

Immediately after densitometric measurements, slices of bone tissue were taken from the distal femoral epiphysis (trabecular bone domination region) and femoral diaphysis (cortical bone domination region) subregions. Subsequently, they were weighed, thoroughly rinsed, and homogenized in a cold potassium phosphate buffer (50 mM, pH = 7.4; Polish Chemicals Reagents, Gliwice, Poland) using a high-performance homogenizer (Ultra-Turrax T25; IKA, Staufen, Germany) equipped with a stainless-steel dispersing element (S25N-8G; IKA, Staufen, Germany) to receive 10% homogenates. The homogenate was deproteinized by 20% trichloroacetic acid in a ratio of 1:4 and centrifuged at 14,000× g for 20 min at 4 °C; then, the supernatant was collected, filtered (0.45-mm Millipore filter), and stored at −80 °C. Immediately before performing the analysis, the supernatant was thawed and then injected into the HPLC system for the analysis.

The concentrations of TRP and KYN in serum, urine, intestinal homogenate, and trabecular and cortical bone tissue homogenates were determined using the HPLC method (Agilent 1260 series

LC system, Agilent Technologies, Palo Alto, CA, USA) according to Holmes [49]. The prepared samples (2 µL) were separated on the ODS column (Waters Spherisorb 3 µm ODS2, 2.1 × 150 mm, Waters Corporation, Milford, MA, USA). The column effluent was monitored with a diode array detector (KYN-365 nm, TRP-260 nm, Agilent Technologies, Palo Alto, CA, USA). The mobile phase was composed of 0.1 M acetic acid and 0.1 M ammonium acetate (pH 4.6) containing 1.8% of acetonitrile, and it was pumped at a flow rate of 0.2 mL/min.

4.3. Tryptophan 2,3-Dioxygenase mRNA Expression Level Assessment

Total RNA was isolated from femoral tissue using a Thermo Scientific GeneJET RNA Purification Kit (Thermo Scientific, Vilnius, Lithuania), and a quantitative real-time polymerase chain reaction assay was performed, as described in detail previously [50]. Primers were designed using Primer-BLAST software. During the study, the expression of the TDO gene was assessed. The primer sequences were AGCGTCATGACTACCTTCTG and TGTCCATAAGTGAGGTCAGC(5′–3′ forward and reverse, respectively). All results were normalized to the endogenous reference glyceraldehyde 3-phosphate dehydrogenase. The comparative cycle threshold method was used for relative quantification of gene expression.

4.4. Statistical Analysis

Shapiro–Wilk's test of normality was used for data distribution evaluation. Normally distributed data were expressed in the form of mean ± SD, while non-Gaussian data were shown as median and a full range. Multiple group comparisons were performed using the one-way analysis of variance (ANOVA), and significant differences between the groups were analyzed with the help of Duncan's post hoc test at $p < 0.05$. The correlations between study variables were calculated with Spearman's rank correlation analysis. A two-tailed p-value <0.05 was considered statistically significant. All computations for statistical analysis were performed using Statistica ver. 13.3 computer software (StatSoft, Tulsa, OK, USA). Graphic design presentation of the results was performed using R statistical software version 3.6.1 (The R Foundation for Statistical Computing, Vienna, Austria) or GraphPad Prism 6.0 software (GraphPad Software, San Diego, CA, USA).

References

Author Contributions: Conceptualization, A.M., K.P., B.K., and D.P.; methodology, A.M., K.P., B.K., and D.P.; formal analysis, K.P. and D.P.; investigation, A.M., K.P., B.K, T.D., B.S., M.Z., B.C., and D.P.; data curation, A.M., K.P., and B.K.; writing—original draft preparation, A.M. and B.K.; writing—review and editing, K.P., B.K., and D.P.; visualization, A.M., K.P., and B.K.; supervision, B.K. and D.P.; funding acquisition, B.K., T.D., and B.S. All authors read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Science Centre, Poland (grant number 2015/19/N/NZ4/01347) and grants No. N/ST/ZB/18/002/2228 and No. N/ST/ZB/18/003/2228 financed by the Medical University of Bialystok, Poland.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

25(OH)D	25-Hydroxyvitamin D
5-HT	Serotonin
ATF4	Activating transcription factor 4
Bglap	Osteocalcin
BMA	Bone mineral area
BMC	Bone mineral content
BMD	Bone mineral density
cAMP	Cyclic adenosine monophosphate
CKD	Chronic kidney disease
CREB	cAMP-responsive element-binding protein

Abbreviations

FOXO1	Forkhead box protein O1
HPLC	High-performance liquid chromatography
IDO	Indoleamine 2,3-dioxygenase
KYN	Kynurenine
MBD	Mineral and bone disorders
RNA	Ribonucleic acid
Sost	Sclerostin
TDO	Tryptophan 2,3-dioxygenase
TPH	Tryptophan hydroxylase
TRP	Tryptophan

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Białystok, 4 luty 2021 r.

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1. Mor A, Kałaska B, Pawlak D. Kynurenine Pathway in Chronic Kidney Disease: What's Old, What's New, and What's Next? International Journal of Tryptophan Research 2020; 13:1-18.Doi: 10.1177/1178646920954882.

wchodzącej w skład mojej rozprawy doktorskiej polegał na zebraniu i przeglądzie literatury, analizie i interpretacji danych oraz przygotowaniu manuskryptu artykułu, co określam jako 80% udziału w przygotowaniu wyżej wymienionej publikacji.*

2. Mor A, Pawlak K, Kałaska B, Domaniewski T, Sieklucka B, Ziemińska M, Cylwik B, Pawlak D. Modulation of the Paracrine Kynurenic System in Bone as a New Regulator of Osteoblastogenesis and Bone Mineral Status in an Animal Model of Chronic Kidney Disease Treated with LP533401. International Journal of Molecular Sciences 2020; 21:E5979. Doi: 10.3390/ijms21175979.

wchodzącej w skład mojej rozprawy doktorskiej polegał na opracowaniu koncepcji i planu badań oraz metod badawczych, prowadzeniu części eksperymentalnej badań, analizie statystycznej wyników i ich interpretacji oraz przygotowaniu manuskryptu artykułu, co określam jako 75% udziału w przygotowaniu wyżej wymienionej publikacji.*

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**W przypadku każdej z włączonych do cyklu prac zaleca się złożenie oświadczenia przez autora wskazujące na jego merytoryczny oraz procentowy wkład w powstanie pracy [np. twórca hipotezy badawczej, pomysłodawca badań, wykonanie specyficznych badań (np. przeprowadzenie konkretnych doświadczeń, opracowanie i zebranie danych, wykonanie zestawień statystycznych itp.), wykonanie analizy wyników, przygotowanie manuskryptu artykułu, i inne]. Określenie wkładu autora powinno być na tyle precyzyjne, aby umożliwić dokładną ocenę jego udziału i roli w powstaniu każdej z prac.*

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1. Mor A, Kałaska B, Pawlak D. Kynurenine Pathway in Chronic Kidney Disease: What's Old, What's New, and What's Next? International Journal of Tryptophan Research 2020; 13:1-18. Doi: 10.1177/1178646920954882.

wchodzącej w skład rozprawy doktorskiej Pana mgr Adriana Łukasza Mora polegał na współuczestniczeniu w opracowaniu koncepcji manuskryptu oraz jego edytowaniu.*

2. Miklosz J, Kałaska B, Kamiński K, Rusak M, Szczubiałka K, Nowakowska M, Pawlak D, Mogielnicki A, The inhibitory effect of protamine on platelets is attenuated by heparin without inducing thrombocytopenia in rodents, Marine Drugs (Multidisciplinary Digital Publishing Institute), 2019, 17, 9, 18 pp.

wchodzącej w skład rozprawy doktorskiej Pana mgr Adriana Łukasza Mora polegał na współuczestniczeniu w opracowaniu koncepcji i planu badań oraz metod badawczych i interpretacji wyników badań oraz edytowaniu manuskryptu artykułu.*

Jednocześnie wyrażam zgodę na przedłożenie wyżej wymienionych prac przez Pana mgr Adriana Łukasza Mora jako część rozprawy doktorskiej w formie spójnego tematycznie cyklu prac opublikowanych w czasopismach naukowych.

Dariusz Pawlak

.....

Podpis (czytelny)

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Białystok, 4 luty 2021 r.

Bartłomiej Kałaska

Uniwersytet Medyczny w Białymstoku
Zakład Farmakodynamiki
ul. Mickiewicza 2C
15-089 Białystok

Oświadczenie współautora

Oświadczam, iż mój udział w przygotowaniu publikacji:

1. Mor A, Kałaska B, Pawlak D. Kynurenine Pathway in Chronic Kidney Disease: What's Old, What's New, and What's Next? International Journal of Tryptophan Research 2020; 13:1-18. Doi: 10.1177/1178646920954882.

wchodzącej w skład rozprawy doktorskiej Pana mgr Adriana Łukasza Mora polegał na edytowaniu manuskryptu artykułu.*

2. *Mor A, Pawlak K, Kałaska B, Domaniewski T, Sieklucka B, Ziemińska M, Cylwik B, Pawlak D. Modulation of the Paracrine Kynurenic System in Bone as a New Regulator of Osteoblastogenesis and Bone Mineral Status in an Animal Model of Chronic Kidney Disease Treated with LP533401. International Journal of Molecular Sciences 2020; 21:E5979. Doi: 10.3390/ijms21175979.*

wchodzącej w skład rozprawy doktorskiej Pana mgr Adriana Łukasza Mora polegał pozyskaniu funduszy, współuczestniczeniu w opracowaniu koncepcji i planu badań oraz metod badawczych, prowadzeniu części eksperymentalnej badań i analizie wyników oraz edytowaniu manuskryptu artykułu.*

Jednocześnie wyrażam zgodę na przedłożenie wyżej wymienionych prac przez Pana mgr Adriana Łukasza Mora jako część rozprawy doktorskiej w formie spójnego tematycznie cyklu prac opublikowanych w czasopismach naukowych.

Bartłomiej Kałaska
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Podpis (czytelny)

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Białystok, 4 luty 2021 r.

Krystyna Pawlak

Uniwersytet Medyczny w Białymstoku
Zakład Farmakoterapii Monitorowanej
ul. Mickiewicza 2C
15-089 Białystok

Oświadczenie współautora

Oświadczam, iż mój udział w przygotowaniu publikacji:

2. *Mor A, Pawlak K, Kałaska B, Domaniewski T, Sieklucka B, Ziemińska M, Cylwik B, Pawlak D. Modulation of the Paracrine Kynurenic System in Bone as a New Regulator of Osteoblastogenesis and Bone Mineral Status in an Animal Model of Chronic Kidney Disease Treated with LP533401. International Journal of Molecular Sciences 2020; 21:E5979. Doi: 10.3390/ijms21175979.*

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Krystyna Pawlak
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Podpis (czytelny)

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Białystok, 4 luty 2021 r.

Tomasz Domaniewski

Uniwersytet Medyczny w Białymstoku
Zakład Farmakoterapii Monitorowanej
ul. Mickiewicza 2C
15-089 Białystok

Oświadczenie współautora

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2. *Mor A, Pawlak K, Kałaska B, Domaniewski T, Sieklucka B, Ziemińska M, Cylwik B, Pawlak D. Modulation of the Paracrine Kynurenic System in Bone as a New Regulator of Osteoblastogenesis and Bone Mineral Status in an Animal Model of Chronic Kidney Disease Treated with LP533401. International Journal of Molecular Sciences 2020; 21:E5979. Doi: 10.3390/ijms21175979.*

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Tomasz Domaniewski

Podpis (czytelny)

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Białystok, 4 luty 2021 r.

Beata Sieklucka

Uniwersytet Medyczny w Białymstoku
Zakład Farmakoterapii Monitorowanej
ul. Mickiewicza 2C
15-089 Białystok

Oświadczenie współautora

Oświadczam, iż mój udział w przygotowaniu publikacji:

2. *Mor A, Pawlak K, Kałaska B, Domaniewski T, Sieklucka B, Ziemińska M, Cylwik B, Pawlak D. Modulation of the Paracrine Kynurenic System in Bone as a New Regulator of Osteoblastogenesis and Bone Mineral Status in an Animal Model of Chronic Kidney Disease Treated with LP533401. International Journal of Molecular Sciences 2020; 21:E5979. Doi: 10.3390/ijms21175979.*

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Podpis (czytelny)

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Białystok, 4 luty 2021 r.

Marta Ziemińska

Uniwersytet Medyczny w Białymstoku
Zakład Farmakoterapii Monitorowanej
ul. Mickiewicza 2C
15-089 Białystok

Oświadczenie współautora

Oświadczam, iż mój udział w przygotowaniu publikacji:

2. *Mor A, Pawlak K, Kałaska B, Domaniewski T, Sieklucka B, Ziemińska M, Cylwik B, Pawlak D. Modulation of the Paracrine Kynurenic System in Bone as a New Regulator of Osteoblastogenesis and Bone Mineral Status in an Animal Model of Chronic Kidney Disease Treated with LP533401. International Journal of Molecular Sciences 2020; 21:E5979. Doi: 10.3390/ijms21175979.*

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Ziemińska Marta

.....

Podpis (czytelny)

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Białystok, 4 luty 2021 r.

Bogdan Cylwik

Uniwersytet Medyczny w Białymstoku
Zakład Laboratoryjnej Diagnostyki Pediatrycznej
ul. Waszyngtona 17
15-269 Białystok

Oświadczenie współautora

Oświadczam, iż mój udział w przygotowaniu publikacji:

2. *Mor A, Pawlak K, Kałaska B, Domaniewski T, Sieklucka B, Ziemińska M, Cylwik B, Pawlak D. Modulation of the Paracrine Kynurenic System in Bone as a New Regulator of Osteoblastogenesis and Bone Mineral Status in an Animal Model of Chronic Kidney Disease Treated with LP533401. International Journal of Molecular Sciences 2020; 21:E5979. Doi: 10.3390/ijms21175979.*

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Bogdan Cylwik
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Podpis (czytelny)

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UCHWAŁA NR 29/2013 w sprawie wniosku nr 2013/24
z dnia 24.04.2013 r.
Lokalnej Komisji Etycznej do Spraw Doświadczeń na Zwierzętach w Białymstoku

§ 1

Na podstawie art. 30 ust. 1 pkt 1 ustawy z dnia 21 stycznia 2005r. o doświadczeniach na zwierzętach (Dz. U. Nr 33, poz. 289) i § 14 ust. 3 rozporządzenia Ministra Nauki i Informatyzacji z dnia 29 lipca 2005r. w sprawie Krajowej Komisji Etycznej do Spraw Doświadczeń na Zwierzętach oraz lokalnych komisji etycznych do spraw doświadczeń na zwierzętach (Dz. U. Nr 153, poz. 1275), po rozpatrzeniu wniosku, pt.:

Hamowanie aktywności hydroksylazy tryptofanu typu I- nowa koncepcja leczenia zaburzeń metabolizmu kostnego w przewlekłej niewydolności nerek.

z dnia 12.04.2013 r. złożonego przez prof. dr hab. Krystynę Pawlak z Zakładu Farmakoterapii Monitorowanej, Uniwersytet Medyczny w Białymstoku, lokalna komisja etyczna,

WYRAŻA ZGODĘ / ~~NIE WYRAŻA ZGODY~~

na przeprowadzenie doświadczeń na zwierzętach w zakresie wniosku.

§ 2

W wyniku rozpatrzenia wniosku, o którym mowa w § 1, lokalna komisja etyczna ustaliła, że:

1. Wniosek należy zaliczyć do kategorii:

Badania naukowe na zwierzętach

2. Najwyższy stopień inwazyjności proponowanych procedur nie przekracza wartości: 4
3. Doświadczenia będą przeprowadzone na zwierzętach:

szczury stada *Wistar-Crl:WI(Han)* **-95 sztuk**

4. Doświadczenia będą przeprowadzone przez: prof. dr hab. Dariusz Pawlak z Zakładu Farmakodynamiki UMB, dr Tomasz Domaniewski oraz mgr Ewa Oksztulska z Zakładu Farmakoterapii Monitorowanej UMB

§ 3

Integralną część niniejszej uchwały stanowi uzasadnienie i kopia wniosku, o którym mowa w § 1.

Podpisy członków lokalnej komisji etycznej biorących udział w głosowaniu:

[Pieczęć lokalnej komisji etycznej]

1. ..... Przewodnicząca LKE w Białymstoku
2. .....
3. .....
4. .....
5. .....
6. .....
7. .....
8. .....
9. .....

UNIWERSYTET MEDYCZNY W BIAŁYMSTOKU
LOKALNA KOMISJA ETYCZNA
do Spraw Doświadczeń na Zwierzętach w Białymstoku
ZAKŁAD FIZJOLOGII DOŚWIADCZALNEJ
15-222 Białystok, ul. A. Mickiewicza 2A
tel./fax 0-85 748 56 99

Otrzymują:
1. Wnioskodawca,
2. Prorektor ds. Nauki UMwB
3. a/a

Pouczenie
Strona niezadowolona z niniejszej uchwały może wnieść odwołanie do Krajowej Komisji Etycznej do Spraw Doświadczeń na Zwierzętach w terminie 14 dni od dnia otrzymania uchwały.
Odwołanie składa się za pośrednictwem lokalnej komisji etycznej, która wydała uchwałę zgodnie z § 20 rozporządzenia Ministra Nauki i Informatyzacji z dnia 29 lipca 2005r.
w sprawie Krajowej Komisji Etycznej do Spraw Doświadczeń na Zwierzętach oraz lokalnych komisji etycznych do spraw doświadczeń na zwierzętach (Dz. U. Nr 153 poz. 1275).

Białystok, dnia 4.02.2021r.

Zgoda kierownika jednostki na wykorzystanie materiału badawczego

Wyrażam zgodę na wykorzystanie zgromadzonego wcześniej zwierzęcego materiału badawczego w postaci pobranych i zabezpieczonych, zamrożonych próbek surowicy, moczu, tkanki kostnej oraz homogenatu jelitowego szczurów szczepu Wistar przez magistra Adriana Łukasza Mora, doktoranta w Zakładzie Farmakodynamiki UMB, w celu przeprowadzenia badań własnych do przygotowywanej przez niego rozprawy doktorskiej.

Materiał zwierzęcy został pobrany i zabezpieczony w ramach grantu pt.: „*Hamowanie aktywności hydroksylazy tryptofanu typu I - nowa koncepcja leczenia zaburzeń metabolizmu kostnego w przewlekłej niewydolności nerek*", dotyczącego badań związanych z osteoporozą towarzyszącą przewlekłej choroby nerek (Numer pozwolenia Lokalnej Komisji Bioetycznej ds. Badań na Zwierzętach: uchwała nr 29/2013 z dnia 24 kwietnia 2013 roku).

Kierownik Zakładu Farmakodynamiki UMB

Prof. dr hab. n. med. Dariusz Pawlak

.....

(Podpis kierownika jednostki)

Rozdział 13. Dorobek naukowy:

Łączna wartość Impact Factor: 9,921
Łączna ilość punktów MNiSW: 420 pkt

Lista publikacji stanowiących rozprawę doktorską:

1. Mor A, Kałaska B, Pawlak D. Kynurenine Pathway in Chronic Kidney Disease: What's Old, What's New, and What's Next? International Journal of Tryptophan Research 2020; 13:1-18. Doi: 10.1177/1178646920954882.
   MNiSW = 100 pkt
2. Mor A, Pawlak K, Kałaska B, Domaniewski T, Sieklucka B, Ziemińska M, Cylwik B, Pawlak D. Modulation of the Paracrine Kynurenic System in Bone as a New Regulator of Osteoblastogenesis and Bone Mineral Status in an Animal Model of Chronic Kidney Disease Treated with LP533401. International Journal of Molecular Sciences 2020; 21:E5979. Doi: 10.3390/ijms21175979.
   IF = 4,556; MNiSW = 140 pkt

Wykaz innych publikacji naukowych:

1. Mor AL, Kobus K, Leszczyńska UM, Reszeć J. Markery różnicujące dysplazję w przełyku Barretta i raka gruczołowego przełyku. Postępy Higieny i Medycyny Doświadczalnej 2019; 73:608-625. doi: 10.5604/01.3001.0013.5643.
   IF = 0,878; MNiSW = 40 pkt
2. Kamiński TW, Pawlak K, Karbowska M, Znorko B, Mor AL, Myśliwiec M, Pawlak D. The impact of antihypertensive pharmacotherapy on interplay between protein-bound uremic toxin (indoxyl sulfate) and markers of inflammation in patients with chronic kidney disease. International Urology and Nephrology 2019; 51:491-502. doi: 10.1007/s11255-018-02064-3.
   IF = 1,843; MNiSW = 70 pkt
3. Mor AL, Kamiński TW, Karbowska M, Pawlak D. New insight into Organic Anion Transporters from the perspective of clinically important interactions. Journal of Phisiology and Pharmacology 2018; 69:307-324. doi: 10.26402/jpp.2018.3.01.
   IF = 2,644; MNiSW = 70 pkt

Wykaz doniesień zjazdowych:

1. Pawlak D, Mor A, Kałaska B, Domaniewski T, Sieklucka B, Pawlak A, Ziemińska M, Pawlak K. The activation of kynurenic system in bone tissue as a new regulator of osteoblastogenesis in rats with experimental chronic kidney disease during LP533401 therapy. 57th ERA-EDTA Congress. Fully Virtual. June 6-9th, 2020.
2. Kalaska B, Mor A.L, Sieklucka B, Domaniewski T, Zawadzki R, Łebkowska U, Pawlak K, Pawlak D. The influence of tryptophan hydroxylase inhibitor LP533401 on kynurenine concentration in bone tissue in the experimental model of chronic kidney disease. 56th ERA-EDTA Congress, Nephrology Dialysis Transplantation, an International Basic Science and Clinical Renal Journal. Budapest, Hungary. 13th-16th June 2019.
3. Pawlak D, Domaniewski T, Znorko B, Mor AL, Pawlak K. Effect of tryptophan hydroxylase inhibitor - LP533401 on the vitamin D metabolism in the experimental model of renal

insufficiency. XXIV PNA Scientific Conference "Nephrological problems in an aging population". Warsaw, Poland, 21-23th June, 2018.
4. Kałaska B, Pawlak K, Domaniewski T, Znorko B, Karbowska M, Mor AL, Lipowicz M, Doroszko M, Pryczynicz A, Pawlak D. Kynurenine modulates bone strength in rats with chronic kidney diseases depending on the place of its occurrence. WCO-IOF-ESCEO World Congress on Osteoporosis, Osteoarthritis and Musculoskeletal Diseases, Krakow, Poland, 19-22th April, 2018.
5. Muszyński P, Groblewska M, Mroczko B, Zboch M, Kulczyńska-Przybik A, Barański D, Mor AL, Szmitkowski M, Komhuber J, Lewczuk P. The diagnostic usefulness of determination of matrix metalloproteinase 3 (MMP-3) in patients with mild cognitive impairment (MCI).: XXVIIth International Symposium on Cerebral Blood Flow, Metabolism and Function & XIIth International Conference on Quantification of Brain Function with PET, Vancouver, Canada, June 27-30, 2015.
6. Mor AL, Barański D, Łukaszewicz-Zając M, Mroczko B, Szmitkowski M, Kozłowski M, Nikliński J. Stem cell factor (SCF) in patients with Esophageal cancer (EC). 10th BIMC Bialystok International Medical Congress for Young Scienctists, Bialystok, May 14-16th 2015.
7. Kucharski R, Mor A.L, Zajkowska A, Zboch M, Mroczko B. Matrix metalloproteinase-3 (MMP-3) as biomarker of Alzheimer's disease. 9th Bialystok International Medical Congress for Young Scientists. Bialystok, April 24-26th 2014.