FACULTY OF PHARMACEUTICAL SCIENCES

THE INFLUENCE OF INDOXYL SULFATE AND P-CRESYL SULFATE ON THE RENAL CLEARANCE AND ON THE PLASMA PROTEIN BINDING OF DRUGS USED IN CKD

Rozalie Vandaele

A Master dissertation for the study programme Master in Pharmaceutical Care

Academic year: 2022 – 2023

GHENT UNIVERSITY

FACULTY OF PHARMACEUTICAL SCIENCES

THE INFLUENCE OF INDOXYL SULFATE AND P-CRESYL SULFATE ON THE RENAL CLEARANCE AND ON THE PLASMA PROTEIN BINDING OF DRUGS USED IN CKD

Rozalie Vandaele

A Master dissertation for the study programme Master in Pharmaceutical Care

Academic year: 2022 – 2023

GHENT UNIVERSITY

SUMMARY

During chronic kidney disease (CKD), the protein-bound uremic toxins (PBUTs), indoxyl sulfate (IS) and p-cresyl sulfate (pCS) accumulate. This is due to the decreased kidney function that occurs. On the other hand, the accumulation may also be the result of the changed composition of the microbiome. Accumulation of these substances has a negative effect on the patient's quality of life. Patients suffering from this condition are treated with drugs to manage the risk factors associated with the disease. These PBUTs are to a large extent bound to albumin, which provides transport. For elimination from the body, they depend almost exclusively on the organic anion transporters (OAT) 1 and 3, located at the kidney.

The aim of this master's thesis was to investigate the effect of the accumulation of the PBUTs on the renal clearance of drugs administered to patients with CKD. The main focus was on drugs, which, like the studied PBUTs, binds to albumin and rely on the OAT transporters for elimination, to check competition. This was done by conducting an extensive literature search.

Results found showed that the accumulation of the PBUTs is not due to changes in the microbiome. At the level of albumin, several changes have been found, which occur with the condition. For example, more post-translational modifications were observed, as well as decreased binding capacity and hypoalbuminemia. Minimal differences were found between binding affinities of the studied PBUTs for albumin, for the different stages of CKD. For furosemide, which binds to a large extent with albumin, a shift in the volume of distribution was observed from 0.11 L/kg to 0.18 L/kg, at normal and high indoxyl sulfate concentrations, respectively. Implying competition between the two. Furthermore, a competitive experiment showed that different drugs (such as: furosemide, valsartan, simvastatin) have an inhibitory effect on the OAT 1 transporter. The addition of IS and pCS enhanced the inhibitory effect.

Given the competition with uremic toxins, altered pharmacokinetics are expected for drugs used in CKD, if these drugs bind to a large extent with albumin and the drugs depend on the OAT transporters for their elimination. But this is difficult to predict. However, in order to be able to make concrete statements about these interactions and the influence on clearance, further research in humans will have to be conducted. The results showed potential interactions, but this has not been verified.

SAMENVATTING

Tijdens chronisch nierziekte stappelen de proteïne-gebonden uremische toxines (PGUTs), indoxyl sulfaat (IS) en p-cresyl sulfaat (pCS) zich op. Dit is het gevolg van de gedaalde nierfunctie die optreedt. Anderzijds kan de accumulatie mogelijks ook het gevolg zijn van de gewijzigde samenstelling van het microbioom. Accumulatie van deze stoffen heeft een negatief effect op de levenskwaliteit van de patiënt. Patiënten die leiden aan deze aandoening worden behandelt met geneesmiddelen, dit voor het management van de risicofactoren die de ziekte met zich mee brengt. Deze BGUTs zijn in grote mate gebonden aan albumine, die zorgt voor het transport. Voor eliminatie uit het lichaam hangen ze zo goed als volledig af van de organische anion transporters (OAT) 1 en 3, gelokaliseerd ter hoogte van de nier.

Het doel van deze masterproef was om na te gaan wat het effect van de accumulatie is op de renale klaring van geneesmiddelen toegediend aan patiënten met chronisch nierfalen. Voornamelijk werd er gefocust op geneesmiddelen, die net als de onderzochte uremische toxines, in belangrijke mate binden met albumine en afhankelijk zijn van de OAT transporters voor eliminatie, om competitie tussen beide na te gaan. Dit werd gedaan aan de hand van een uitgebreid literatuuronderzoek.

Gevonden resultaten toonden aan dat de accumulatie van de BGUTs niet te wijten is aan veranderingen in het microbioom. Op het niveau van albumine zijn er verschillende wijzigingen gevonden, die optreden bij de aandoening. Zo stelde men meer post-translationele modificaties vast, alsook een gedaalde bindingscapaciteit en hypoalbuminemie. Er werden minimale verschillen gevonden tussen de bindingsaffiniteiten van de bestudeerde PBUT's voor albumine, voor de verschillende stadia van CKD. Voor furosemide, die in grote mate bindt met albumine, zag men een verschuiving van het verdelingsvolume van 0.11 L/kg naar 0.18 L/kg, bij respectievelijk normale en hoge indoxyl sulfaat concentraties, wat competitie tussen beide impliceert. Verder kon er via een competitief experiment aangetoond worden dat verschillende geneesmiddelen (zoals: furosemide, valsartan, simvastatine) een inhiberend effect hebben op de OAT 1 transporter. Toevoeging van IS en pCS zorgden voor versterking van het inhiberend effect.

Gezien de competitie met de uremische toxines wordt er een gewijzigde farmacokinetiek verwacht voor gebruikte geneesmiddelen bij chronisch nierziekte,

indien deze geneesmiddelen in grote mate binden met albumine en de geneesmiddelen afhankelijk zijn van de OAT transporters voor hun eliminatie. Doch valt dit moeilijk te voorspellen. Om echter concrete uitspraken te kunnen doen over deze interacties en de invloed op de klaring, zal verder onderzoek bij mensen moeten worden uitgevoerd. De resultaten toonden potentiële interacties, maar dit is niet geverifieerd.

ACKNOWLEDGEMENTS

This research is the conclusion of my first master's in Pharmaceutical Sciences at Ghent University.

I would like to thank everyone who assisted me throughout this semester in writing this literature search. First of all, I would like to thank Professor Dr. Apr. An Vermeulen for giving me the opportunity to conduct research into the renal clearance and plasma protein binding of uremic toxins. I would like to thank my supervisor, Apr. Jonas Langeraert, for his invaluable help in drawing up this master's thesis.

Finally, thanks to my parents and everyone else who took the time to review my research and provide feedback. Without their contribution and commitment, this master's thesis would simply have been impossible.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AA	Amino acid
ABiC	Binding capacity of albumin
ACE-I	Angiotensin converting enzyme inhibitors
AGEs	Advanced glycation end products
ARBs	Angiotensin receptor blockers
BCRP	Breast cancer resistance protein
Bmax	Binding capacity
C	Concentration
ciPTEC	Conditionally immortalized proximal tubular epithelial cells
CKD	Chronic kidney disease
CLCr	Creatinine clearance
CLr	Renal clearance
CLt	Total clearance
CVD	Cardiovascular disease
CYP	Cytochrome P450 enzymes
CYP2E1	Cytochrome P450 2E1
DC	Drug conjugation
eGFR	Estimated glomerular filtration rate
ESRD	End stage of renal disease
Fe	Excreted fraction
Fu	Unbound fraction
FUR	Furosemide
GFR	Glomerular filtration rate
GI	Gastrointestinal
HSA	Human serum albumin
HD	Hemodialysis
HMA	Mercaptalbumin
HNA-1	Human non-mercaptalbumin 1
HNA-2	Human non-mercaptalbumin 2
HPLC	High-performance liquid chromatography
IC50	Half maximal inhibitory concentration
IS	Indoxyl sulfate

Ka	Binding constant
Kd	Dissociating constant
KDIGO	Kidney Disease: Improving Global Outcomes
LC-MS/MS	Liquid chromatography-mass spectrometry
MATE	Multidrug and toxic compound extrusion
MRP	Multidrug resistance-associated transporter
NICE	National Institute for Health and Care
OAT	Organic anion transporter
OCT	Organic cation transporter
PB%	Percent protein binding
PBUTs	Protein bound uremic toxins
pCS	p-Cresyl sulfate
PTM	Posttranslational modification
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SULT1A1	Sulfotransferase 1A1
UT	Uremic toxin
Vd	Volume of distribution

1. INTRODUCTION

1.1. UREMIC TOXINS

Uremic toxins are organic chemical substances that are generated in the body as a waste product. These substances are always present in the body, but will accumulate in the blood as a result of impaired kidney function, such as chronic kidney disease (CKD) (1). This accumulation can eventually lead to uremia or uremic syndrome. The accumulation of various waste products in blood can lead to different symptoms such as foot edema, pain, itching, nausea and weight loss (2,3).

Koch and Massry first classified uremic toxins. According to the authors, toxin concentrations should be quantifiable in biological fluids and markedly increased while uremia is present. This concentration should be associated with one or more manifestations of uremia. Furthermore, it is important to provide evidence of in vivo cellular toxicity by administering toxins to healthy humans or animals. In addition, it is crucial to establish a plausible pathobiological mechanism that elucidates the connection between the toxin and the manifestation of uremia (3,4).

Uremic toxins are products that arise from a fermentation process. This process is catalyzed by enzymes present in the bacteria that are part of the natural intestinal flora, the microbiome. Substrates of these enzymes are amino acids that originate from proteins in the diet. These products are always present in the body, however toxicity only arises when they accumulate (1,5,6).

A majority of micro-organisms present on and in the body occur in the intestine. The natural intestinal flora is a collection of different genera of bacteria such as *Lactobacillus, Helicobacter, Staphylococcus* and *Enterobacteriaceae* (7–10). The bacteria of the gut-microbiota are mainly involved in various metabolic pathways such as the protein fermentation process in which they ensure the breakdown of proteins to essential amino acids (10). The metabolization products that remain are the building blocks of the uremic toxins. Most protein-bound uremic toxins are hence formed in the intestine from food, such as the Maillard-products (e.g. 3-deoxyglucosone and methylglyoxal), hippurates (e.g. hippuric acid), indoles (e.g. indoxyl sulfate and indole-

3-acetic acid), phenols (e.g. p-cresyl sulfate and 2-methoxyresorcinol) and finally polyamines (e.g. spermidine).

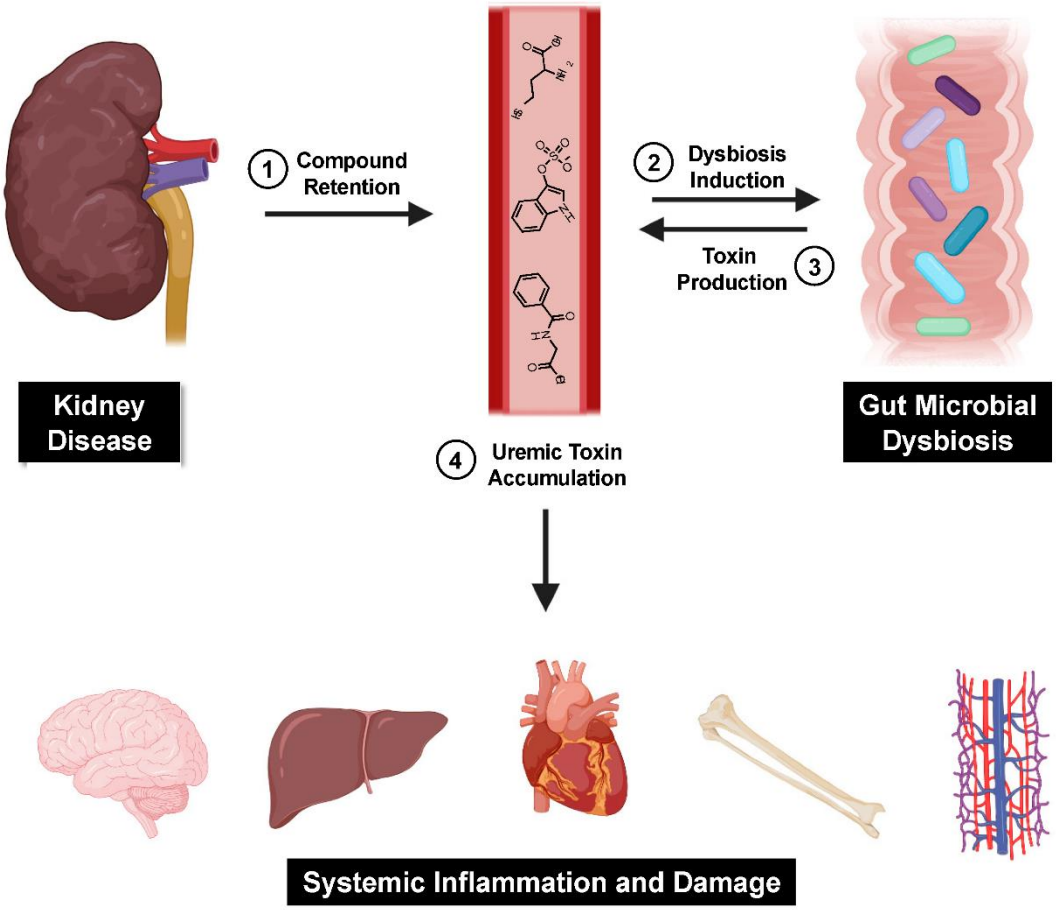

Figure 1.1: Schematic representation of the causes and consequences of uremic toxin accumulation. (11)

During the microbial fermentation process, various precursors are formed that later lead to uremic toxins. It is difficult to classify these different formed uremic toxins based on food ingested in the diet. This is because different precursors are formed that lead to the same end product and because interactions can occur between the precursors (12). These precursors are absorbed and transported to the liver where the building blocks are converted into uremic toxins, which can then be excreted by the kidney **(see below)** (13).

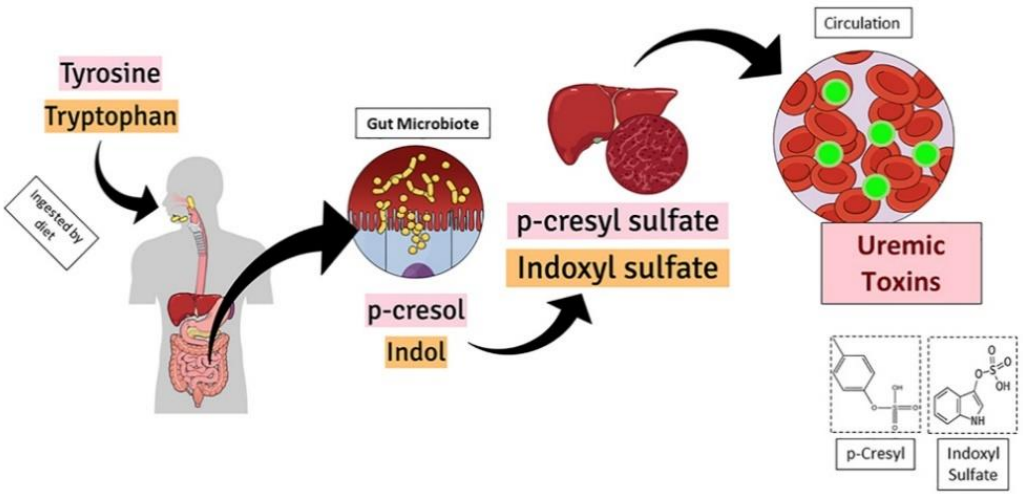

Figure 1.2: Chemical structure and formation of the protein-bound uremic toxins, p-cresyl sulfate and indoxyl sulfate, by the protein fermentation process by the gut microbiome. (3)

The products are filtered out of the blood by the kidney and end up in the urine. This is done through several processes that together form the renal clearance (14). When the kidney function decreases, those substances accumulate in the bloodstream and several tissues. That accumulation can eventually cause damage to various organs and systems in the body (14–16).

There are about 100 uremic toxins that are mainly classified according to their physicochemical properties. On the one hand, there are the water-soluble low molecular weight molecules (e.g. urea, creatinine and uric acid) with molecular weights lower than 500 Da (17) making them easy to remove by dialysis (12). In addition to the water-soluble molecules, there are also the medium-sized molecules, characterized by molecular weights between 500 Da and 60 000 Da (17,18), and the protein-bound molecules (e.g. the Maillard reaction products, hippurates, indoles, phenols and polyamines) (1,6).

The main focus of this thesis lies on the protein-bound uremic toxins and their clearance in patients with CKD. This is because these are difficult to remove by hemodialysis and are the main cause of the toxic effects (19). More specifically, the focus will be on the protein-bound uremic toxins indoxyl sulfate (IS) and p-cresyl sulfate

(pCS), since a metabolomics approach identified both as the most discriminating biomarkers of uremia (20).

1.1.1. Protein bound uremic toxins

Protein-bound uremic toxins (PBUTs) are metabolites produced exclusively through protein fermentation by the gut microbiota. These products are renally cleared through active tubular secretion and thus end up in the urine **(see mechanism below)**. P-Cresyl sulfate (pCS) and indoxyl sulfate (IS) are both PBUTs, pCS belongs to the phenols and IS belongs to the indoles (19). Studies show that IS and pCS in particular play a prominent role in both the development of CKD and the development of cardiovascular disease (CVD). In vitro research has shown the toxic effects of both and they are seen as emerging mortality risk factors (19–22).

Indoxyl sulfate is a metabolic product that is eventually formed by the liver from indole, which is produced from tryptophan by the intestinal microbiome. Indole undergoes hydroxylation in the liver by cytochrome P450 2E1 (CYP2E1) to create 3-hydroxy-indole, which is then sulfated by sulfotransferase 1A1 (SULT1A1) to form IS. The excretion of IS in the urine occurs almost exclusively through proximal tubular secretion via organic anion transporter 1 (OAT1) and OAT3 **(see mechanism below)**. These transporters are located on the basolateral membrane of the cells in the proximal tubule. Due to its strong binding to albumin, IS cannot be effectively eliminated through dialysis. As kidney function deteriorates, the levels of IS in the bloodstream rise, intensifying the advancement of CKD (23).

Tyrosine and phenylalanine, derived from the diet, are metabolized by the gut microbiota. This reaction results in p-cresol, a phenol derivative. p-Cresol is sulphated by SULT1A1 enzymes from the liver, creating p-cresyl sulfate (pCS). Since pCS is largely bound to albumin (90%), it cannot be efficiently eliminated by dialysis. Like IS, the pCS elimination depends mainly on the tubular OAT1 and 3 transporters (23).

1.2. THE KIDNEY

Uremic toxins are renally cleared. First, the anatomy, physiology and functioning of the kidney will be described. The kidney is one of the most important clearance organs in our body, and the nephron makes up its functional unit.

1.2.1. The nephron

A healthy kidney has approximately between 800 000 and 1 million nephrons, which are functional units responsible for purifying blood and producing urine. The nephron consists of a glomerulus and tubules such as the proximal and distal tubule, the loop of Henle and the collection tube (24).

1.2.1.1. The glomerulus

The glomerulus forms, along with Bowman's capsule, the glomerular filtration barrier. Bowman's capsule consists of several different cells that allow filtration. Bowman's capsule consists of an inner and an outer layer of cells, respectively the visceral and parietal layers. Both made up of squamous epithelium. The visceral layer consists of specialized podocytes (25). These are differentiated epithelial cells that cover the basal membrane, which lies just below the visceral cells. Podocytes have a specific morphological structure. They consist of a cell body that carries trabeculae. These are thick, long suckers, with microvilli (26). These are bound to the capillary via various adhesion proteins, such as integrin. They form slits through which the glomerular filtrate enters the nephron (25).

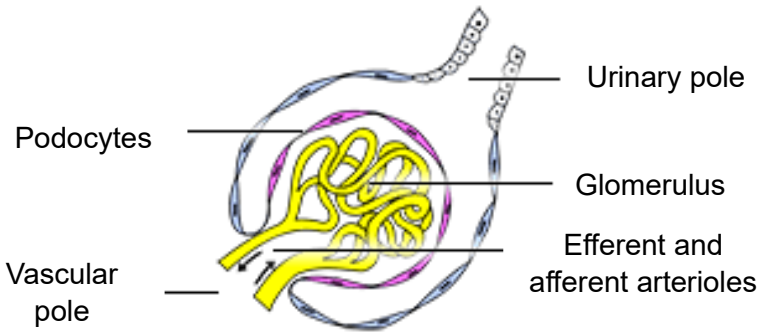

Figure 1.3: The structure of the glomerulus and Bowman's capsule. (29)

In addition to these specialized cells, the glomerular filter also consists of a basal membrane. This is a network of collagen type IV, laminins, nidogene and heparan sulfate proteoglycans. These are structural units on the one hand, while on the other hand they are also responsible for adhesion of endothelial cells and podocytes to the basal membrane (30,31).

This system is responsible for the first step in filtration, namely the renal ultrafiltration. The blood enters the nephron through the afferent arterioles, which are small blood vessels that maintain hydrostatic pressure (32). They ensure that blood flow continues and the blood can be filtered. The filtration pores filter substances by molecule size (the pores allow passage of molecules up to 60 000Da), charge (neutral molecules can be allowed through, a charge hinders the diffusion), polarity and plasma protein binding (33). They enable passive filtration of various solutes such as glucose, amino acids and other compounds that occur unbound in the blood plasma. What remains is called the glomerular filtrate and ends up in Bowman's capsule (34).

The glomerular filtration depends on the pressure difference generated by the afferent and efferent arterioles that have high and low blood pressure, respectively (35).

1.2.1.2. The tubuli

The main function of the renal tubules is to extract important substances, such as glucose and water, from the glomerular filtrate, also called the pre-urine, for reuse. For example, the tubules are responsible for the reabsorption of 99% of electrolytes and water coming from the glomerular filtrate (20).

The nephron, next to the glomerulus and Bowman's capsule, consists of a long series of ducts, which represent the renal tubules. The glomerular filtrate is sent from Bowmans's capsule to the first part of this series, the proximal tubule.

The proximal tubule plays a crucial role in maintaining balance of fluids, electrolytes, and nutrients. This by reclaiming around 60-70% of the water and sodium chloride (NaCl), a larger portion of sodium bicarbonate (NaHCO3), and almost all of the nutrients present in the ultrafiltrate. These substances are reabsorbed back into the peritubular capillaries. From the proximal tubule the filtrate passes into the loop of

Henle. This loop consists of a descending and an ascending part. In the descending part, water is mainly exchanged between the blood and fluid in the loop. In the rising part only salts, such as NaCl, are exchanged. The main function of the loop is to concentrate the pre-urine (36). The loop of Henle flows into the distal tubule, which is a short tube that is very important for homeostasis of various ions such as sodium and potassium (37), which are reabsorbed into the blood circulation. Furthermore, water is also reabsorbed here and excess substances such as ions, medicines, metabolic waste and toxins are secreted (38). Everything that remains ends up in the collection tube.

On the membranes of these different tubules are membrane-bound carriers, transporters, which are necessary to obtain the final resulting urine **(see 1.2.2. The renal clearance)** (39).

What remains is the urine that contains water, metabolic waste products and electrolytes (40).

1.2.2. The renal clearance

Renal clearance is the excretion of metabolites/substances through the kidney and refers to the rate at which the kidneys clear a certain substance from the body. This process is important to discuss as the uremic toxins are exclusively cleared through this route. It is important to know that a large part of these uremic toxins are either too large or protein-bound (14), which makes them more difficult to filter. Their clearance is mainly based on active tubular secretion via renal transporters (41).

For the clearance of a drug which is renally eliminated, the following applies:

If: renal clearance (CLr) < unbound fraction (F_u) x creatinine clearance (CLCr)	= Tubular reabsorption / passive back diffusion
If: CLr > fu x CLCr	= Tubular secretion / tubular reabsorption
If: CLr = fu x CLCr	= Glomerular filtration

The blood flow delivered to the kidney is about 25% of the cardiac output, which corresponds to a maximum clearance of 600mL plasma per minute (24). Renal clearance involves several processes: glomerular filtration, active secretion and passive/active reabsorption that take place in the nephron. The filtration by the kidney is a unidirectional passive diffusion process, while the two other processes are bidirectional and are characterized by both passive diffusion and active membrane-bound processes (39).

Renal clearance (CLr) can be calculated by different equations:

$$CLr = rate\ of\ urinary\ excretion/C, \quad (1)$$

Where C is the drug-concentration in plasma and CLr stands for the apparent volume of plasma cleared from a given substance by the kidney per unit of time;

$$CLr = total\ amount\ excreted\ unchanged\ in\ urine/AUC, \quad (2)$$

where AUC is the area under the curve. This shows the plasma concentration curve of the administered drug, over time.

$$CLr = fe\ x\ CLt, \quad (3)$$

Where fe is the fraction of the excreted drug in the urine and CLt is the total body clearance (42).

1.2.2.1. The glomerular filtration

The glomerular filtration is the process by which the blood is passively filtered by the glomeruli of the kidneys. About 20% of the plasma is filtered at the glomerulus, which equates to a filtration rate of 120-130mL per minute. The glomerulus only filters unbound substances with a molecular weight of up to 60kDa. The filtration through the glomerular capillaries depends on the pressure difference arising in the afferent and efferent arterioles (35). If a drug is only filtered, i.e., its clearance/excretion only depends on the glomerular filtration, its filtration rate is equal to the excretion rate.

The rate of filtration is (42):

$$Rate\ of\ filtration = fu\ x\ GFR\ x\ C, \quad (4)$$

Where fu is the free/unbound drug-fraction in the plasma and GFR is the glomerular filtration rate.

As a measure and indicator of renal function, the glomerular filtration rate (GFR) is used. The GFR is:

$$GFR\ =\ [UrineX\ (mg/mL)]\ *\ urine\ flow\ (mL/min)/\ [PlasmaX\ (mg/mL)] \quad (5)$$

Where X is a compound that is totally excreted through glomerular filtration.

To know the GFR, the biochemical marker creatinine is applied. This is a waste product of creatine phosphate that is generated at a constant rate by the skeletal muscles. This product is purified from the blood and enters the urine almost exclusively via glomerular filtration. A small fraction is also secreted by the peritubular capillaries, which makes that the creatinine clearance (CLCr) overestimates the GFR by 10%-20% (43). Despite this slight overestimation, this method is still used in clinical practice because it is a quick method. Moreover, it is also a non-invasive and a budget-proof method. (35). This is therefore an excellent indicator of the GFR in both patients and healthy volunteers. To estimate the CLCr-rate, the Cockcroft-Gault formula is often used. This formula takes into account body weight, sex and age (44):

$$CLCr = \frac{[(140 - Age)\ x\ weight\ (kg)\ x\ 0.85\ if\ female]}{\left[72\ x\ Serum\ Creatinine\ \left(\frac{mg}{dL}\right)\right]} \quad (6)$$

1.2.2.2. Active tubular secretion

As mentioned earlier, most uremic toxins will be eliminated via active tubular secretion rather than filtration, because of their size and their protein-bound character (14). To achieve this, the proximal tubule cells are provide with various transport proteins (41). Through these carrier-mediated transport systems, the uremic toxins are actively secreted into the glomerular filtrate.

The (basolateral) organic anion transporters (OAT) have the ability to transport organic anions against the concentration gradient. This process requires energy, however, research showed that the OATs do not rely directly on ATP hydrolysis. The energy required for this process is extracted from the transport of sodium by the Na/K-ATPase (33,45). This active transport creates a sodium gradient that forms the driving factor for dicarboxylate transport in the cells. The created carboxylate gradient ensures the transport of the OAT1 and OAT3 substrates (46).

In humans, a number of isoforms of this group of transporters are active and occur not only in the kidney but also, for example, in the liver (47). OAT1 (SLC22A6) and OAT3 (SLC22A8) secrete anionic uremic toxins into the lumen of the tubule cell in exchange for alpha-ketoglutarate (34) and other dicarboxylic anions. IS and pCS are examples of uremic toxins transported by OAT1 and OAT3 (48). These are the two main transporters for renal absorption and excretion of various compounds such as drugs, exogenous toxins and endogenous substances such as the uremic toxins (49). Situated on the apical membrane of the proximal tubule cells are the efflux pumps breast cancer resistance protein (BCRP) and multidrug resistance-associated transporters 2 and 4 (MRP2/4), which are responsible for the definitive removal of substances from the cell lumen into the urine (41).

In addition to the OAT family, there is the organic cation transporter family (OCT) (50). These, like the OAT, are active at the basolateral side of the tubule cells. As the name suggests, they ensure the transport of cationic substances such as trimethylamine N-oxide (45).

As mentioned earlier, the 2 prototypes of the protein-bound uremic toxins IS and pCS are removed from the body almost exclusively via active tubular secretion using OAT1 and OAT3, however, the small fraction that occurs unbound can be removed via filtration (23).

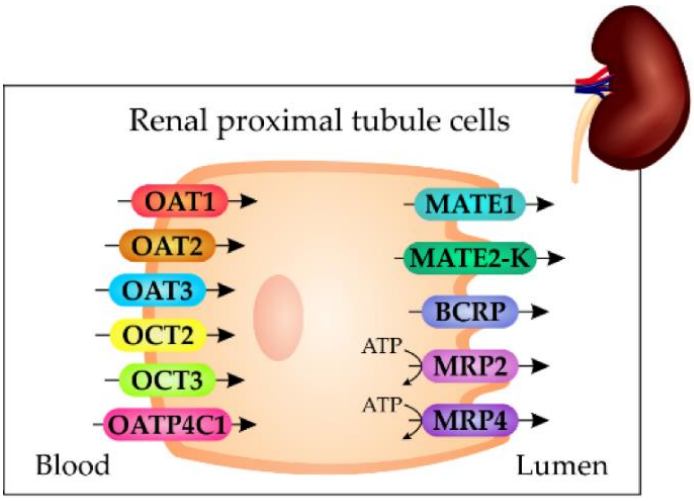

Figure 1.4: Proximal tubule cell with active transporters involved in the secretion of both endogenous and exogenous substances, like the organic anion transporters (OAT) and the efflux pumps breast cancer resistance protein (BCRP) and multidrug resistance-associated transporters 2 and 4 (MRP2/4). (51)
Abbreviations: BCRP = Breast cancer resistance protein; MATE = Multidrug and toxic compound extrusion; OAT = Organic anion transporter; OCT(N) = Organic cation transporter.

1.2.2.3. Active reabsorption

Active reabsorption is less important for uremic toxins. Active reabsorption ensures that various substances, such as glucose, are absorbed back into the body from the pre-urine. This transport is made possible by transporters located on the apical membranes of the tubule cells. These transporters can transport against a concentration gradient. In this way, substances that end up in the pre-urine via glomerular filtration, but are still important in the body, can be brought back into the blood which thus prevents their excretion and various health problems associated with it (52).

A few examples of active transporters are (53):

- SGLT (sodium-glucose cotransporter) for the active reabsorption of glucose in the proximal tubule;
- Na+/K+/2Cl cotransporter for the active reabsorption of sodium, potassium and chloride in Henle's thick ascending loop;
- Na+/Cl cotransporter for the active reabsorption of sodium and chloride in the distal tubule.

1.3. CHRONIC KIDNEY DISEASE

Chronic renal failure, an irreversible condition, results in a progressive loss of kidney function (23). Chronic renal failure is characterized by either kidney damage or a decline in the glomerular filtration rate (GFR) that persists for a minimum of three months. In this condition, the kidneys lose their ability to eliminate waste products from the body in the usual manner (54).

The prevalence of CKD is increasing globally, affecting an estimated 8-16% of the world's population (55,56). Worldwide, about 2% of the health budget in the health system goes to this disease. The main causes for the development of CKD are hypertension and diabetes (57).

1.3.1. Pathophysiology of CKD

The pathophysiology of CKD includes a progressive loss of nephrons, the functional units of the kidneys, which leads to a decrease in GFR and a build-up of waste products in the blood (54).

In advanced stages of CKD, the excretory function of the kidney is compromised causing the accumulation of harmful metabolites which can lead to uremia (5,58). It is suggested that there is a correlation between the accumulation of those uremic toxins and an increased production of uremic toxin precursors by the intestinal microbiome due to dysbiosis (5). Results of numerous studies confirm the different composition of the intestinal microbiota in CKD (58,59). This potentially creates an overproduction of the protein-bound uremic toxins, which could thus accumulate and promote toxicity (60,61).

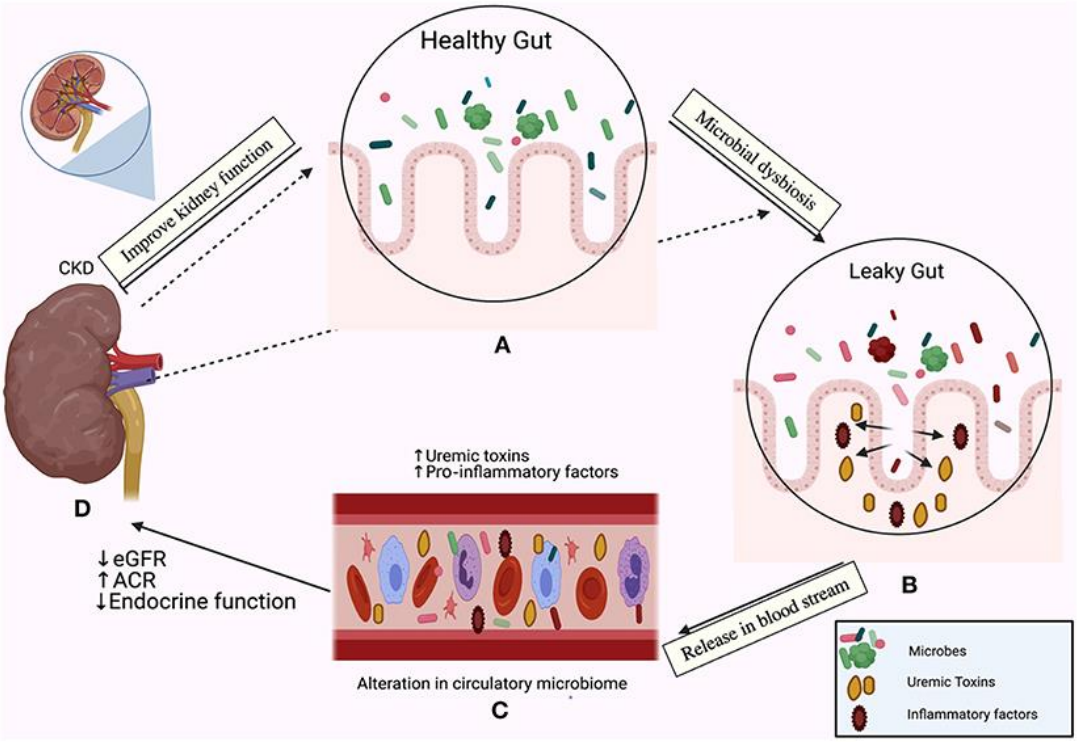

Figure 1.5: Relationship between dysbiotic intestinal microbiome and increased production of uremic toxin precursors in CKD. (61) **Abbreviations: eGFR = Estimated glomerular filtration rate; ACR = Albumin-to-creatinine ratio; CKD = Chronic kidney disease.**

CKD is characterized by a decrease in glomerular filtration rate. As a result, more compounds will accumulate in the blood, such as the unbound PBUTs, that are ideally filtered by the kidney. Their accumulation can lead to various complications and symptoms such as anemia, bone disorders and CVD. CKD is classified into 5 categories according to the KDIGO-guidelines (Kidney Disease: Improving Global Outcomes) based on the GFR (I, II, III, IV and V) (23). Stages 1, 2, 3a, 3b, 4 and 5 are characterized by, respectively, a GFR higher than 90 ml/min/1.73m², a GFR between 60 and 89 ml/min/1.73m² (= mild renal failure), a GFR between 45 and 59 ml/min/1.73m² (= moderate renal failure), a GFR between 30 and 44 ml/min/1.73m² (= moderate renal failure), a GFR between 15 and 29 ml/min/1.73m² (= severe renal failure) and finally a GFR lower than 15 ml/min/1.73m² (54,62) Stage 5 of CKD is also called end-stage renal disease (ESRD). At this stage, there is very serious kidney damage and an almost complete loss of kidney function. Dialysis or a kidney transplant are required to replace kidney function (54).

In addition to a reduced GFR, there are already signs of kidney damage from CKD stadium I. This can manifest itself in, for example, microalbuminuria, proteinuria or hematuria. This damage causes some changes in the nephron, which gives rise to a modified renal clearance of substances.

A reduced GFR, in turn, can give rise to interactions between substances. Altered pharmacokinetics and pharmacodynamics significantly increase the risk of interactions which can lead to toxicity (54).

As mentioned earlier, there will be a loss of glomerular filtration capacity. This leads to more proteins ending up in the urine and fluid retention in the body.

1.3.2. Causes of CKD

The causes of CKD are multifactorial and may include (63):

- Diabetes, in which high blood sugar can damage blood vessels in the kidney;
- Hypertension, in which blood circulation in the kidneys is damaged;
- Polycystic kidney disease, a condition in which cysts form in the kidney;
- Glomerulonephritis, inflammation of the kidney filters;
- Medication;
- Etc.

All these causes can damage the kidney and consequently also the transporters, which are responsible for the renal clearance of various compounds, such as the protein-bound uremic toxins (48). In this way, the waste products in the blood can no longer be transported to the pre-urine and will accumulate in the blood. This also has important consequences for drugs that are normally removed from the blood via these transporters. Like the uremic toxins, they accumulate in the blood and may cause toxicity.

Furthermore, the transport of uremic toxins via OAT transporters could also lead to kidney disease if the functioning of the efflux pumps is reduced. In this situation, the uremic toxins accumulate in the tubule cells, causing kidney damage, which can lead to CKD (48).

1.3.3. Albumin

The plasma protein albumin, with molecular weight of 66 348 Da, ensures on the one hand the transport of various substances, such as drugs and metabolic products, in the plasma. On the other hand, it maintains the osmotic blood pressure. The protein is formed by the hepatocytes (liver) (64–66). Its active form contains 585 amino acids. Most drugs or other substances that bind to albumin are mainly acidic or neutral, while albumin itself is alkaline.

Albumin binds various uremic toxins, such as IS and pCS and only the unbound or free fraction is potentially active. However, in CKD there are altered (lower) albumin concentrations leading to increased circulating concentrations of IS and pCS. In addition to the lower amount of albumin in the blood, the albumin protein is also modified in CKD, for example by post translational modifications. This is the result of the accumulation of uremic toxins that occurs with decreased renal function and could have an influence on the binding capacity of the protein (51). Since both uremic toxins are strongly bound to albumin, the change in both concentration and conformation of the protein could alter the volume of distribution (Vd) of the toxins (67). The volume of distribution (Vd) is (68):

$$Vd = [fup / fuT] \times VT + VP \quad (7)$$

Where fup is the unbound drug-fraction, in the plasma, fuT is the unbound drug-fraction, in the tissue, VT is the volume of the tissue and VP is the volume of the plasma.

In pharmacokinetics, Vd is described as a fictitious quantity. It is the apparent volume in which a substance, such as a drug or toxin compound, is distributed throughout the body. In general, it can be said that a high distribution volume implies that there is a high degree of tissue binding and that the probability of a large free fraction is low. The reverse is true for a small distribution volume (69).

If the volume of distribution is:

= 42L per 70 kg	= There is no binding.
< 42L per 70 kg	= There is more plasma binding than tissue binding.
> 42L per 70 kg	= There is more tissue binding than plasma binding.

$$Vd = amount\ of\ drug\ in\ the\ body\ /\ plasma\ concentration \quad (8)$$

Vd is expressed in L or L/kg.

1.3.4. Drugs used in CKD

There is currently no medicinal cure for CKD. However, it is treated with medicines for the prevention and management of complications and comorbidities related to CKD such as diabetes, hypertension, high cholesterol, anemia, etc. (70). The main groups of medicines used in the management of CKD are discussed in the NICE-guidelines (National Institute for Health an Care Excellent) (25 August 2021) and consist of: statins, ACE-I, ARBs and diuretics. If necessary, anti-coagulants and calcium channel blockers are also used (71).

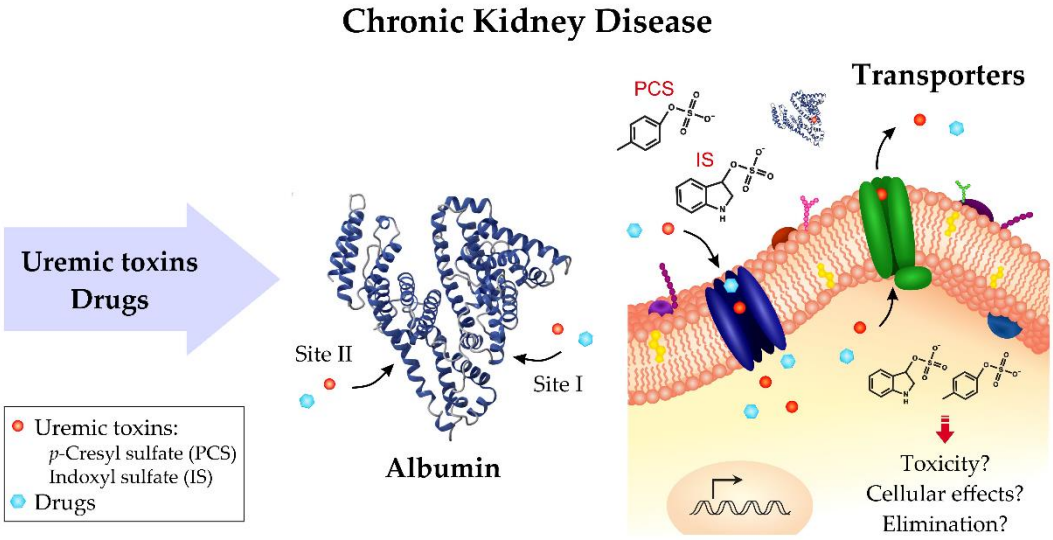

Figure 1.6: The interplay between uremic toxins and drugs for binding to albumin and organic anion transporters. (51)

2. OBJECTIVES

Uremic toxins are, as mentioned in the previous section, metabolites or waste products that are produced in the body. On the one hand, accumulation of these products can potentially occur due to a modified composition of the gut microbiome, which initially ensures the production of these substances. On the other hand, accumulation can also be the result of impaired kidney function, which is the case in chronic kidney disease. In this thesis, we will mainly focus on the uremic toxins indoxyl sulfate and p-cresyl sulfate, since both have been shown to play an important role in disrupting various biological functions. Both uremic toxins are extensively bound to plasma albumin and are cleared from the body almost exclusively via renal tubular transport, using the OAT1 and OAT3 transporters. Drugs, used in the treatment of CKD, can also be substrates for these transporters. Disruption in normal levels of uremic toxins, may alter the pharmacokinetics of these drugs.

It is therefore crucial to gain more insight into this in order to optimize the treatment of kidney patients and to take into account these interactions when determining a suitable dose regimen of the medication.

With this information we asked ourselves the following research question: “What is the influence of indoxyl sulfate and p-cresyl sulfate on the renal clearance through OAT1/3 active transporters and on the plasma albumin binding of drugs, used in CKD?”.

In the further part of this thesis we will investigate various aspects of the renal clearance of the uremic toxins. We are looking for answers to various questions such as: “What are potential changes CKD causes of albumin and transporters?”, “Does the dysbiosis of the microbiome cause altered production in the precursors of IS and pCS?”. We will examine which drugs, used in the management of CKD, bind to albumin and are substrates of the same OATs as IS and pCS. We will investigate whether there are any interactions between these uremic toxins and the drugs for binding with albumin and/or the transporters and how this affects their pharmacokinetics and more specifically, their clearance.

To investigate what is known about this subject, a literature search was performed.

3. METHODS

To gain more insight into the subject, an extensive literature study was done. This was done using various databases, such as PubMed, Embase, Google Scholar and the Cochrane library.

To screen articles relevant to the topic, Rayyan was used. This was done for the articles that resulted from the following search: "uremic toxin*" AND (transport*); it was filtered so that only English articles after the year 2000 were retained. This search produced in 259 results/articles. Through Rayyan, 53 articles based on the following criteria were included:

- Articles including drugs that are significantly protein bound: IS and pCS bind to a large extent with albumin. It seemed interesting to look for substrates that bind with albumin at the same binding sites as these PBUTs. In order to see if there is any interaction at this binding site and what effect this has on the pharmacokinetics of the substances.
- Articles including drugs that are eliminated from the body by the same route as the investigated PBUTs, namely by the OATs.

Since relatively few relevant articles were found, not only human studies were included, but also studies in rats, mice and studies conducted in cell lines. When interesting articles referred to other articles that also seemed relevant for the research topic, they were also included.

The appendix contains a list of drugs that were used in this thesis, based on the various studies searched and that fulfilled two important characteristics (albumin binding and OAT clearance). Based on the data found, the interaction between these drugs and uremic toxins for binding to albumin and interaction with transporters was investigated, and how this affects overall pharmacokinetics including renal clearance **(see appendix A)**. The investigation was made through various searches listed in the appendix **(see appendix B).**

Data for adapted figures, which can be found in the results section, were extracted using WebPlotDigitizer version 4.6. and recreated using R-version 4.2.1 in Rstudio build 576.

The preparation of the graphs and tables, used in the results section, was performed in Excel version 2304.

4. RESULTS

4.1. THE INTESTINAL FLORA AND UREMIC TOXINS

The protein-bound uremic toxins, such as IS and pCS, are products that are generated by the natural gut microbiome. In normal circumstances, the microbiome is composed of different bacterial species whose composition can vary from person to person. In patients with normal gut health, there are a number of bacterial genera responsible for the production of uremic toxins. IS is usually formed from the precursor indole, which is extracted from tryptophan by tryptophanase. This enzyme occurs in the genera *Citrobacter, Proteus* and *Escherichia*. Indole is absorbed and processed into indoxyl by the liver enzyme cytochrome P450-2E1. Subsequently, indoxyl is sulphated by sulfotransferase with IS as the reaction product. Studies dating back to the 60s showed that an increase in tryptophan in the diet causes an increase in production of indoxyl sulfate (12,72). pCS is the reaction product of a conjugation reaction in the intestinal wall between sulfate and p-cresol (73). P-cresol is a phenol formed from tyrosine and phenylalanine by anaerobic commensals such as *Bacteroides, Lactobacillus, Clostridium* and *Bifidobacterium* (12,74,75).

A review on gut microbiota in chronic kidney disease dating back to 2016, released by Guldris et al. states that a dysbiosis of the natural microbiome is developing in patients with CKD. There are changes both qualitatively and quantitatively (75). In CKD there is a secretion of urea in the gastrointestinal (GI) tract giving rise to ammonia, which causes a change in pH in the GI tract. This increased pH could be inducive to dysbiosis (76).

In a study conducted by Saito et al. (77) in which 153 species were screened for the production of phenols and/or p-cresol, of which 152 species occur in the human gut, a higher p-cresol concentration than the background level was observed in 55 strains. In 4 of them, it was significantly higher and produced more than 100 µM p-cresol compared to the remaining 51 strains that produced less than 10 µM: *Blautia hydrogenotrophica, Clostridioides difficile, Olsenalle uli and Romboutsia lituseburensis.* The aforementioned study identified 14 strains, including *Anaerostipes hadrus, Bacteroides caccae, Bacteroides ovatus, Bacteroid vulgatus, Clostridium Celerecrescens, Clostridium clostridioforme, Clostridium cochlearium, Clostridium indolis, Clostridium innocuum, Clostridium saccharolyticum, Clostridium sphenoides,*

Fusobacterium varium, Olsenella uli, and Veillonella parvula, as producers of p-cresol and phenol. To summarize, p-cresol was primarily synthesized by strains belonging to: *Bifidobacteriaceae, Coriobacteriaceae, Bacteroidaceae, Fusobacteriaceae, and Lactobacillaceae*, as well as certain *Clostridium clusters, namely clusters XVI, IX, IV, I, XI, XIII,* and *XIV,* and *Fusobacteriaceae, Coriobacteriaceae, Bacteroidaceae, or Clostridium clusters XVI, IX, I,* and *XIV* (77).

Changes in the natural intestinal flora in CKD patients were listed in Table 4.1, the contents are taken from Guldris et al. (75).

Table 4.1: Differences in composition of the gut microbiome between a healthy person and a patient with CKD. Content taken from Guldris et al. (75) **Abbreviation and characters: CKD = Chronic kidney disease; ↑ = Increased amount of bacteria; ↓ = Decreased amount of bacteria; ↔ = Same amount of bacteria.**

Intestinal tract	Normal	CKD
Stomach	*Lactobacillus*	↔
	Helicobacter	↔
Duodenum	*Staphylococcus*	↑
	Lactobacillus	↑
Jejunum	*Enterococcus*	↑
	Streptococcus	↑
	Lactobacillus	↑
Ileum	*Enterobacteriaceae*	↑
	Bacteroides	↑
	Clostridium	↑
Colon	*Bacteroids*	↑: *Proteobacteria, Enterobacteria, Escherichia coli, Acinetobacter, Proteus spp., Clostridium* (100x) ↓: *Lactobacillus, Bifidobacterium spp.*
	Acenitobacteria	
	Proteus	
	Clostridium	
	Lactobacilli	
	Prevotellaceae	
	Fusobacterium	

A 2020 study by Gryp et al. investigated the contribution of uremic toxin production by the microbiome to the overall increase in blood concentrations in CKD. This was done by collecting feces, blood and urine. This collection was done for 14 controls 141 patients with CKD. All samples were analyzed using ultra-high performance liquid chromatography. The results showed a clear increase in IS and pCS plasma concentrations at the different stages of CKD, **see Table 4.2.** If we look at the amino

acid (AA) concentrations in the feces, from which these uremic toxins are formed , we see that tryptophan (precursor of indole and indoxyl sulfate) shows similar concentrations (nmol/g wet feces) between the controls and the CKD patients. Similar results were reported for tyrosine and phenylalanine, precursors of p-cresol and pCS. However, fecal concentrations of tyrosine, phenylalanine, p-cresol and pCS were significantly higher compared to tryptophan, indole and indoxyl sulfate. The results showed a correlation between the precursors in the stool and their plasma uremic toxin concentration. However, this was not found to be the case for indole and IS. Ex vivo experiments were also performed via anaerobic culturing of fecal samples from the controls, CKD 1 and CKD 5 patients. This study showed that p-cresol concentrations increased after an incubation of 48h; the result was more pronounced at CKD stage 5 compared to the controls and CKD stage 1. However, the indole concentrations were equally pronounced in the three groups. After seven days of incubation, significantly higher amounts of indole were observed at the two stages of CKD compared to the baseline concentration. However, this was not significant for p-cresol, which is probably due to the interindividual differences between the participants of the study. Based on the results of this study, it was concluded that there was little to no difference in the production of the precursors of the studied uremic toxins if we compare the control group with the different groups of CKD patients (78,79).

Table 4.2: Concentrations of the uremic toxins p-cresyl sulfate and isulfate in plasma and their precursors in feces, compared between patients with different stages of CKD and a control group. Content taken from Gryp et al. (78)

Abbreviations: CKD = Chronic kidney disease. Data are presented as median (25^{th}–75^{th} percentile).

Metabolites	Control	CKD 1	CKD 2	CKD 3	CKD 4	CKD 5
Plasma (µM)						
p-Cresyl sulfate	13.2	11.61	19.37	47.97	69.63	121.1
	(6.85-19.71)	*(5.88-19.28)*	*(11.15-24.17)*	*(31.99-69.4)*	*(42.57-93.92)*	*(85.9-215.1)*
Indoxyl sulfate	2.79	3.34	4.57	7.5	12.71	42.51
	(2.05-5.70)	*(2.37-4.11)*	*(2.32-6.71)*	*(5.28-10.74)*	*(9.03-17.93)*	*(19.6-50.49)*
Feces (nmol/g wet feces)						
Tyrosine	347.6	365.1	298.2	320.6	316.3	374.1
	(261.5-500.3)	*(223.0-536.0)*	*(234.1-421.1)*	*(215.2-428.2)*	*(200.7-495.2)*	*(263.3-489.2)*
Phenylalanine	321.7	317.0	261.3	263.9	276.6	349.5
	(218.8–463.4)	*(190.9–494.6)*	*(205.3–396.4)*	*(205.4–397.8)*	*(176.1–469.9)*	*(260.4–443.6)*
Tryptophan	63.3	70.1	54.1	54.9	53.2	61.8
	(36.6–81.5)	*(45.4–101.9)*	*(42.1–80.7)*	*(40.6–74.4)*	*(40.2–74.6)*	*(46.9–96.7)*
p-Cresol	204.9	168.3	257.2	250.2	196.5	240.0
	(129.1–342.0)	*(79.9–305.0)*	*(191.4–429.6)*	*(187.9–433.2)*	*(164.7–288.3)*	*(166.8–565.1)*
Indole	38.5	52.1	51.2	38.9	37.0	44.5
	(23.8–58.7)	*(23.1–124.9)*	*(25.2–115.6)*	*(21.1–72.7)*	*(12.4–82.0)*	*(15.9–122.3)*

4.2. ALBUMIN

Albumin is the most abundant transport protein in humans and makes up about 60% of the total protein. The physiological serum concentrations range between 3.0 g/dL and 5.0 g/dL. More recent studies indicate that the current concentration is more likely to be between 3.5-5.4 g/dL (80,81).

4.2.1. Albuminuria and hypoalbuminemia

With normal kidney function, none to relatively little albumin enters the urine. Albumin in the urine, albuminuria, is therefore also a good predictor for CKD (82–84). The average albumin urinary concentration in a healthy person is less than 30 mg/day. One speaks of micro-albuminuria and macro-albuminuria when the concentration in the urine is 30-300 mg/day and 300-3000 mg/day, respectively. If the albumin content is 3 g or more, there is nephrotic protein loss (78,80). As mentioned earlier in the introduction, the glomerular filtration barriers comprise various cells, including endothelial cells, the basal membrane, and podocytes. These cells are encompassed by a glycocalyx layer with a negative charge, which plays a role in limiting the filtration of negatively charged substances like albumin. In CKD, damage to this glycocalyx layer occurs, resulting in elevated levels of albumin in the urine, a condition known as albuminuria (75). Protein loss can be reduced by treating patients with ACE-I such as enalapril and lisinopril and ARBs such as losartan and valsartan. In addition to their blood pressure lowering effect, these drugs also have a positive effect on the kidney function, in some cases, however, this effect is minimal (85).

Iimori et al. investigated the relationship between serum albumin concentrations and the different stages of CKD. This was investigated by taking blood samples, among other things, from 1138 patients with CKD at different stages between 2 and 5. This involved a Japanese population that was eventually compared with a Western cohort of studies. Results can be seen in **Table 4.3.** If these results are compared with the normal albumin serum concentrations, one can observe that the average values are still in the reference interval but that they are on the low side (88).

Table 4.3: Albumin values linked to glomerular filtration rates plotted per stage of CKD compared to the reference interval for healthy adults. Content taken from Iimori et al. (88) **Abbreviations: CKD = Chronic kidney disease; eGFR = Estimated glomerular filtration rate. Data are presented as mean ± standard deviation and median (25th–75th percentile).**

	CKD 2	CKD 3	CKD 4	CKD 5	Normal
Albumin (g/dL)	4.0 ± 0.6	3.8 ± 0.6	3.5 ± 0.6	3.8 ± 0.6	3.5 - 5.4
	4.1 (3.8 - 4.4)	*3.9 (3.5 - 4.2)*	*3.6 (3.2 - 4.0)*	*4.0 (3.5 - 4.3)*	
eGFR (mL/min per 1.73 m2)	72.1 ± 8.5	43.3 ± 8.2	22.0 ± 4.4	9.9 ± 3.0	>90
	70.6 (64.9 - 79.7)	*43.2 (35.5 - 50.2)*	*21.7 (17.9 - 25.6)*	*9.8 (7.4 - 12.4)*	

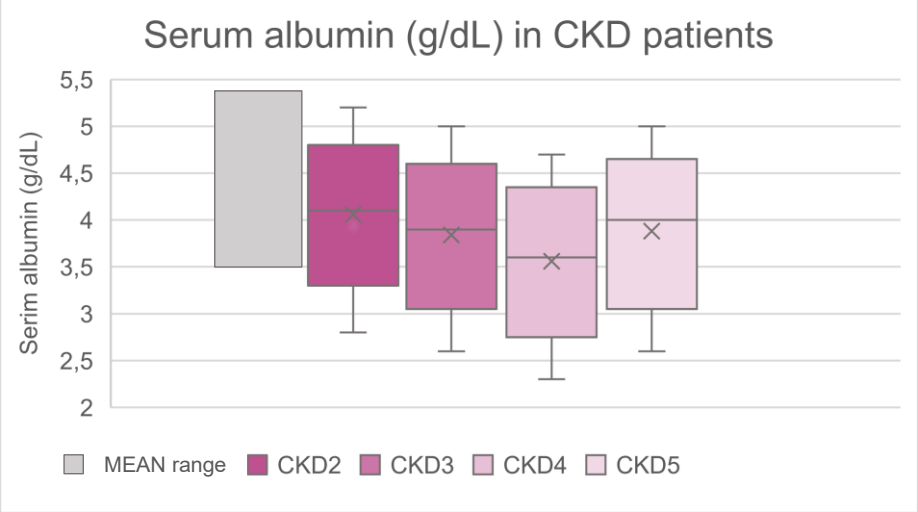

Figure 4.1: Differences in serum albumin values between the different stages of CKD compared to the reference interval for healthy adults, presented in a box plot. Content taken from Iimori et al. (88) **Abbreviation: CKD = chronic kidney disease. Error bars are displayed as well as the mean by 'x'.**

These findings can be compared with a study conducted by Klammt et al. (89), which investigated the reduced binding capacity observed in patients diagnosed with CKD. The study population comprised 120 individuals receiving treatment at the nephrology outpatient department or the dialysis department of the University of Rostock in Denmark. In these patients, the serum albumin concentrations were also measured, results can be found in **Table 4.4.**

Table 4.4: Albumin values linked to glomerular filtration rates plotted per stage of CKD. Content taken from Klammt et al. (89) **Abbreviations: CKD = Chronic kidney disease; eGFR = Estimated glomerular filtration rate. Data are presented as mean ± standard deviation.**

	CKD 1/2	CKD 3	CKD 4	CKD 5
Albumin (g/L)	43.9 ± 2.3	43.5 ± 3.5	42.0 ± 2.8	41.2 ± 3.3
eGFR (mL/min per 1.73 m2)	>60	30-59	15-29	<15

Another article on hypoalbuminemia in renal failure, published in 2006, concluded that hypoalbuminemia is more common in patients with end-stage renal disease (ESRD). However, this hypoalbuminemia is mainly caused by systemic inflammation (90). In addition, serum albumin concentrations vary greatly between individuals and depend on interindividual factors such as gender, age, CKD stages, etc. (90).

It has previously been suggested that the therapeutic effect and efficacy of a drug depend on the concentrations of albumin in the blood when that drug binds significantly to albumin (91). Hypoalbuminemia would therefore have a detrimental effect on the efficacy of a medicine. When a drug is highly bound to albumin and serum albumin concentrations drop, a larger free fraction of the drug will occur. The serum concentration of the drug will increase, so it can be suspected that the effect (possibly adverse) of the medicine will be much greater. However, this only appears to be the case for drugs with a high degree of drug conjugation (DC) (91), this is the case for antibacterial drugs and antiviral drugs.

4.2.2. Difference in structure

Albumin possesses a structure resembling a heart shape. High-resolution X-ray crystallography studies have revealed that this transport protein comprises three homologous domains. Domain I spans the first 195 amino acids, domain II encompasses amino acids 196 to 383, and domain III consists of amino acids 384 to 585. Each of these domains is further divided into subdomains A and B. In human albumin, approximately 67% of the protein structure consists of alpha-helices, 10% comprises turns, 23% constitutes random coils, while there are no beta sheets. The protein's stability primarily arises from the presence of 17 intramolecular disulfide bonds, predominantly situated between the alpha helices (64,92).

Sudlow et al. defined two major binding sites on the protein: Sudlow site I and Sudlow site II (93). Several endogenous and exogenous ligands bind to these binding sites. These binding sites are located in subdomain IIA and IIIA respectively (65), where Sudlow site I mainly prefers voluminous heterocyclic compounds and Sudlow site II prefers aromatic compounds (51).

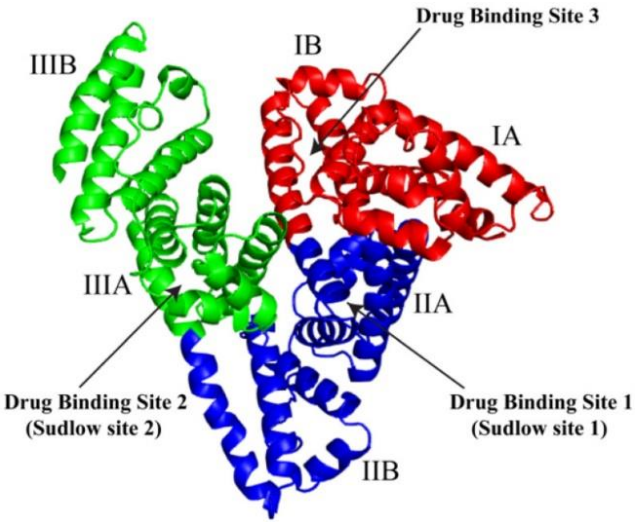

Figure 4.2: Schematic representation of an X-ray structure of human serum albumin. (64)

Sudlow site I is predominantly apolar but contains a few polar residual groups. This binding site includes tyrosine 150, histidine 242 and arginine 257, which are amino acids with polar residual groups located completely at the bottom of this Sudlow site I binding site. At the opening of the binding site lysine 195, lysine 199, arginine 218 and arginine 222 are situated (64). Examples of ligands that bind to the Sudlow site I binding site are furosemide, warfarin, phenylbutazone, amantadine, azapropazone, azidothymidine, indomethacin, jodipamide, oxyfenbutazone, 2' indole sulfate and 3' diflunisal (64). Sudlow site II is mainly hydrophobic with the following amino acids in its binding site: arginine 410, serine 489 and lysine 414 (65). Watanabe et al. showed in 2000 that arginine 410 and tyrosine 411 play an important role in the binding of a ligand to the Sudlow site II (94). Examples of drugs that bind here are: ibuprofen, digitoxin, benzodiazepine, halothane, propofol and nonsteroidal anti-inflammatory drugs (64).

A more recent review summarizes all the major amino acids involved in binding to a ligand with high affinity, see **Table 4.5.**

Table 4.5: Summary of the most commonly involved amino acids at the 2 most important binding sites, that bind with high affinity, on the human serum albumin (HAS). Content taken from Bteich et al. (95)

Sudlow-site I	Sudlow-site II
Asp187	Pro384
Lys190	Leu387
Lys195	Ile388
Lys199	Asn391
Phe211	Cys392
Trp214	Phe395
Ala215	Arg410
Arg218	Tyr411
Leu219	Lys414
Arg222	Leu430
Phe223	Val433
Leu234	Cys438
Leu238	Ala449
Arg257	Glu450
Leu260	Leu453
Ala261	
Ile264	
Ile290	
Ala291	

Studies have shown that posttranslational modifications (PTM) of the plasma protein play a role in the progression of CKD (66). On the other hand, the oxidative stress that arises in patients with CKD would also give rise to the same PTM of the protein (97). These modified proteins can then act as a biomarker of CKD (72). The most commonly reported PTMs linked to CKD are carbamylation, glycation and oxidation. Other PTMs such as nitrosylation and cysteinylation also occur, but very little is known about these changes. The link between these modifications and modified binding between the protein and ligands requires further research. It has been suggested that albuminuria promotes carbamylation of the protein, leading to a change in the binding site and changes in affinity for different drugs (65,98).

Shi et al. (2019) investigated the effect of the ionic strength and pH on the binding between uremic toxins and albumin. It was observed that an increase in pH from 6.0

to 8.5 brought about little or no change in the binding character. However, a decrease in percent protein binding (PB%) was observed with an increase in ionic strength (99).

4.2.2.1. Glycation

Glycation is a non-enzymatic reaction in which sugars are bound to albumin. This has consequences for the structure and functionality of the protein. In this process glycation products, the advanced glycation end products (AGEs), are oxidatively created. Glycated albumin loses its biological functionality (96). Research shows that glycation mainly occurs at the level of the following amino acid residues: lysine 525, lysine 199, lysine 233, lysine 281 and Lysine 438 (60).

Another study on the modification and glycation adduct quantitation using liquid chromatography-mass spectrometry (LC-MS/MS) looked at the altered molecular characteristics of human and bovine serum albumin at low and large amounts of methylglyoxal, this is an organic compound that ensures the glycation of albumin in physiological conditions. The presence of glycation was investigated by looking at the loss of lysine and arginine and quantifying it. This study mainly showed that arginine residues are affected by glycation, namely: arginine 114, arginine 218 and arginine 428, as well as lysine 186 (65,100).

4.2.2.2. Oxidation

Oxidation is described as an important one for the progression of CKD due to the antioxidative properties of albumin. Albumin consists of a monomer chain of AA , each of which contains a residual group that can possibly be oxidized. Oxidation of these residue groups can occur through the loss of electrons, the addition of oxygen, or the removal of water molecules. Albumin is known for its significant antioxidant properties, primarily attributed to the abundance of reduced sulfhydryl groups. These sulfhydryl groups play a crucial role in capturing reactive oxygen species (ROS) and reactive nitrogen species (RNS). When present in high concentrations, these reactive ROS molecules can cause oxidative damage. Examples of such species are hydroxyl radicals (which are preferred for the AA methionine and cysteine residues), monoxide radicals, alkoxyl radicals, aldehydes, hypochlorous acid, etc (87,101,102).

Albumin can also bind with free copper (Cu2+) which will cause a reduction in the production of these reactive species (51). As mentioned earlier, oxidative stress causes the progression of CKD. Oxidative stress can cause oxidative modifications of the protein such as glycation, disulfide bridge formation and carbonylation. Cysteine 34 is the only cysteine of the 35 residues present in the HSA that does not attach via 1 of the 17 intramolecular disulfide bridge bonds. Consequently, this thiol group is free and redox-active. Furthermore, Annibal et al. (103) showed that cysteine, tryptophan, tyrosine and methionine residues are most sensitive to oxidation

The advanced oxidation protein products, which are the oxidized forms of phenylalanine and tyrosine, are used as a biomarker for the oxidation of albumin. The irreversible oxidation of the cysteine 34 residue is, together with albumin carbonylation, associated with the stage of CKD (96). The protein can occur in three different isoforms, depending on its redox state (87,101):

1) Mercaptalbumin (HMA): In this isoform, the thiol group, belonging to cysteine 34, occurs in its reduced form,
2) Human non-mercaptalbumin 1 (HNA-1): In this isoform the thiol group is reversibly oxidized,
3) Human non-mercaptalbumin 2 (HNA-2): this is the isoform in which the thiol group is irreversibly oxidized, with the result that the antioxidant function disappears completely.

4.2.2.3. Carbamylation

Carbamylation is a non-enzymatically catalyzed reaction between isocyanate with a primary amine or a free sulfhydryl group, in which the carbamoyl group (-CONH2) is added to functional parts of the protein. Mainly lysine residue 549 is carbamylated, which is provoked by urea (51,104).

All these modifications, which give a change in the conformation of the binding sites, cause a modification of the protein. This could be a possible explanation for the altered binding capacity of albumin that occurs in CKD (89).

4.2.3. Binding of ligands with albumin

Meijers et al. summarized binding characteristics of albumin in CKD in a narrative review. The binding between a ligand and albumin can be described in two ways, by a covalent way on the one hand and by a non-covalent way on the other hand. A covalent bond indicates the formation of a disulfide bridge. A free cysteine group binds with cysteine 34. Carbonylation is another example of covalent binding. Regarding non-covalent binding, one indicates the existence of Van der Waals forces and hydrophobic interactions between the ligand and albumin. However, a covalent bond does not necessarily mean that it is stronger than the non-covalent bond. Both are important, the non-covalent bonds are reversible and will provide some flexibility. This facilitates binding and allows the ligands to easily dissociate from and bind to the protein. The covalent bonds provide more stability for the bonded complex. Often this binding is irreversible. Indoxyl sulfate is known to bind non-covalently to the Sudlow site II of albumin (105). **Table 4.6.** summarizes the extent of plasma protein binding of the drugs (classes) typically used in CKD.

Table 4.6: Summary of the extent of albumin binding of the most commonly used drug(classes) in CKD. Content taken from Meijers et al. (105) **Abbreviations: ACE-I = Angiotensin converting enzyme inhibitors; ARBs = Angiotensin receptor blockers.**

Drug (class)	ACE-I	ARBs	Verapamil	Furosemide	Statins
Protein binding (%)	0-97	>90	90	95	43-98

As mentioned earlier, albumin can be modified, however, this happens much more in patients with CKD. Since patients with CKD are constantly exposed to oxidative stress, the albumin protein is oxidized. This oxidation causes a reduction in the binding capacity of the protein, mainly at the Sudlow site II. Glycation can also take place, in which mainly the lysine residues are affected. Further oxidation of the obtained glycation products ensures the formation of the advanced glycation end products. However, the binding characteristics of glycated albumin are limitedly characterized. Finally, carbamylation causes an average decrease of 67% in the binding capacity of the protein. This is a substantial reduction, however, this study was conducted in vitro with a carbamylation degree of 80% of the protein. This is relatively high, but in order to demonstrate significance, it would have to be compared with observations in relevant physiological conditions. All these modifications of the primary structure of

albumin are common. However, it is still not fully clarified what influence these have on the binding characteristics (105).

Binding of ligands to albumin is described with the Ka which is the binding constant. A higher binding constant implies a stronger binding of the ligand with albumin and therefore also a smaller free fraction. As a result, for ligands with a higher binding constant, more fluctuations will show at the altered albumin concentrations in CKD (105). The binding constant is:

$$Ka = \frac{[LP]}{[L][P]} \tag{9}$$

Where $[LP]$ is the concentration of ligand-protein complexes, $[L]$ is the unbound ligand concentration and $[P]$ the concentration of unbound free binding sites of the protein.

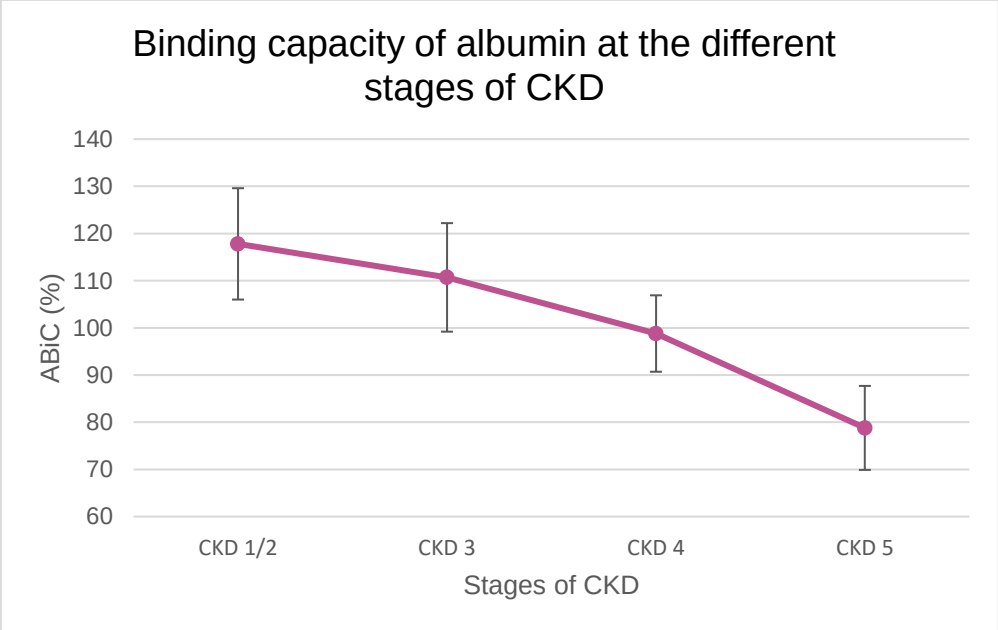

Figure 4.3: Binding capacity of albumin by stage of CKD. Content taken from Meijers et al. (105) **Abbreviations: ABiC = Binding capacity albumin; CKD = Chronic kidney disease. Data are presented as mean ± standard deviation**

4.2.3.1. Binding of IS and PCS with albumin

P-cresyl sulfate and indoxyl sulfate, both more than 90% bound to albumin, are difficult to remove via dialysis because albumin is too large (41). Research showed that albumin contains two important binding sites for the binding to ligands, where one

binding site has a high affinity and another has a low affinity (71). The most significant binding site, which constitutes over 90% of the overall binding, holds utmost importance. By engaging in competition at this particular site, it becomes feasible to ascertain the proportion of unbound uremic toxins (106). IS binds on both Sudlow site I and Sudlow site II, with a preference (higher affinity) for site II. pCS, on the other hand, only binds to Sudlow site II (51). There are several observations about the binding of pCS with albumin. In vitro spiking experiments using serum from hemodialysis patients showed that an increase in IS greatly increased the free fraction of PCS and vice versa, which indicates a common binding site (71), which suggests that IS and PCS are bound to the same binding site. Research by Watanabe et al. from 2012 reveals more information about the interaction between the two uremic toxins during binding to HSA (72). Both organic compounds share 1 binding site on the protein, Sudlow site II. Both uremic toxins therefore compete with each other for binding to the protein (107,108).

Deltombe et al. (109) investigated the binding characteristics and related competition of different uremic toxins. Binding curves were established in three different conditions (healthy serum, blank hemodialysis (HD) serum (free of uremic toxins) and HD serum) and were compared for 4 protein-bound uremic toxins (PBUTs) (hippuric acid (HA), Indole-3 acetic acid (IAA), IS and PCS). Serum was taken from patients with CKD with varying stages between 1 and 5. The bound and unbound fraction were determined through equilibrium analysis. The total protein concentration in the healthy serum was found to be 74.2 g/L while that in the HD serum was reduced to 54.2 g/L. Of these, 48.1 g/L (64.82%) and 40.1 g/L (61.60%) consisted of albumin in the healthy and HD serum, respectively. In the healthy serum, a total concentration of 4.54 µM IS and 11.6 µM pCS was detected, both of which were starkly increased to 111 µM IS and 196 µM pCS in the HD serum. These data were used to construct the binding curves and extract the binding characteristics such as: dissociating constant (Kd) and binding capacity (Bmax). Since IS binds at 2 binding sites on the serum albumin, a two binding model provided the best fit to the data. Since pCS binds to the protein via 1 binding site, a one site binding model was chosen for this uremic toxin (UT). Consequently, Kd1 and a Kd2 were determined for IS, as well as Bmax1 and Bmax2. see **Table 4.7.**

Table 4.7: Results on the binding characteristics of IS and pCS for albumin. Content taken from Deltombe et al. (109) **Abbreviations: IS = Indoxyl sulfate; pCS = p-Cresyl sulfate; HD = Hemodialysis; K_d = Dissociation constant; Bmax = Binding capacity. Presentation of the data not mentioned in the article.**

		Healthy serum	Blank HD	HD serum
IS	Kd1/Kd2 (µM)	3.10×10^{-5} / 1.11×10^{-3}	1.01×10^{-3}	6.60×10^{-4}
	Bmax1/Bmax2	0.69 / 5.85	5.81	4.64
pCS	Kd (µM)	3.07×10^{-4}	2.94×10^{-4}	3.02×10^{-4}
	Bmax	5.00	3.69	3.8

Both uremic toxins bind with a high affinity to the protein. There is a minimal difference in affinity between the different situations. However, differences in the binding capacity are observed. The same study also examined the competition between the two uremic toxins for binding to albumin. In this study, it was demonstrated that adding a maximum concentration of 200 mM of pCS and IS to blank HD serum that initially was spiked with 100 mM of IS or pCS, there was a significant and rapid reduction in the protein-bound fraction of pCS or IS (109).

A recent review by da Cunha et al. provided more insight into the levels of uremic toxins found in patients with CKD. Patients with CKD had a reported concentration of up to 500 µM IS while in healthy patients it was 0.1-2.39 µM. In patients with mild stage of CKD, the observed concentrations of pCS ranged from 2.8 ± 1.7 mg/L to 6.6 ± 3.7 mg/L. On the other hand, patients with a severe stage of CKD exhibited concentrations ranging from 21.8 ± 12.4 mg/L to 107 ± 44.6 mg/L. These measurements were conducted in serum using high-performance liquid chromatography (HPLC) and in plasma using liquid chromatography with tandem mass spectrometry (LC-MS/MS) (51).

4.2.3.2. Drugs used in CKD and competition with PBUTs for binding to albumin

Furosemide, a loop diuretic, is often used in patients with CKD. Furosemide is known to bind strongly to albumin, 95%-99%. More recently, furosemide has been found to bind to Sudlow site I of albumin via hydrophobic interactions and hydrogen bond formation. However, interactions between furosemide and other amino acids were also found, suggesting that this is a binding site close to Sudlow site II. The binding to site I occurs with a higher affinity ($K_a \sim 10^4$) than to the binding site close to

site II ($K_a \sim 10^3$). Displacement experiments show that furosemide directly competes with uremic toxins for binding to Arg410, Lys414, and Ser489, belonging to Sudlow site II, and that the drug enters into direct competition for Sudlow site I with uremic toxins that bind to it, as shown in **Figure 4.4**. Conformational changes of albumin affect the binding of the drug to its binding sites. This results in a higher free fraction of the drug (110,111). This is observed when looking at the volume of distribution. For furosemide, the mean Vd is 0.11 L/kg, while in patients with renal failure it averages 0.18 L/kg. This result suggests that a larger free fraction of the drug is most likely present in a CKD patient than in a patient with healthy kidneys (112).

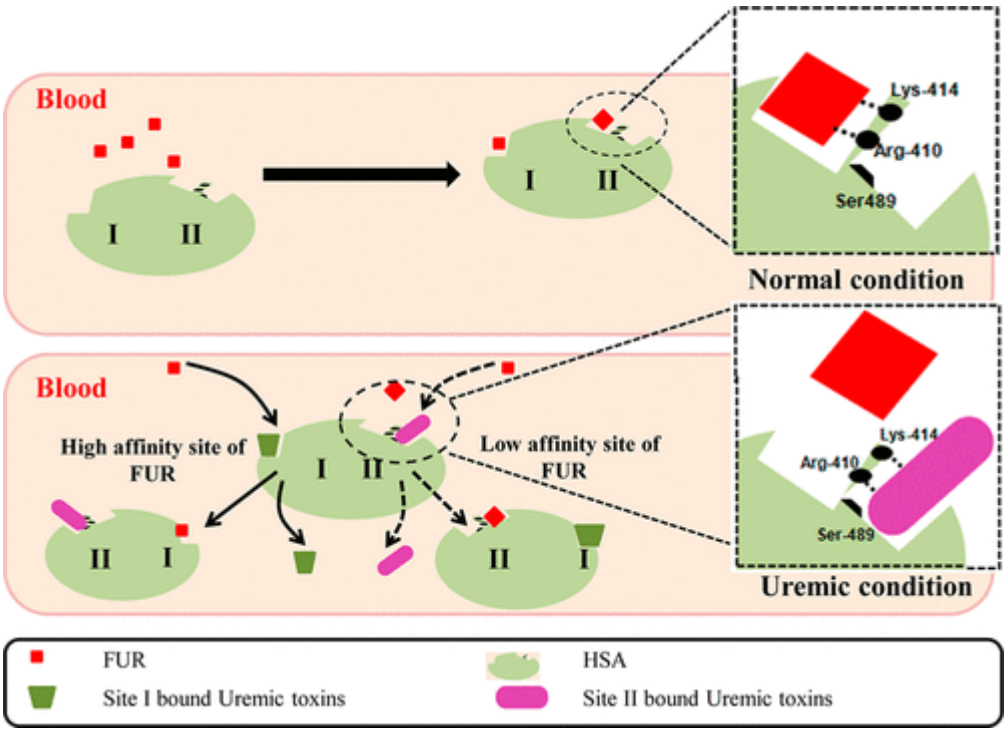

Figure 4.4: Schematic representation of the interaction between furosemide and uremic toxins for binding site Sudlow site I and a site near to Sudlow site II. (111)

Abbreviations: HSA = Human serum albumin; FUR = Furosemide.

Shi et al. investigated the effect of drugs (ibuprofen, warfarin, indomethacin and furosemide) on the albumin binding of uremic toxins. However, all these aforementioned drugs also bind to the Sudlow site I and/or II, just like IS (I and II) and pCS (II). The results show that the drugs significantly reduce the %PB these uremic toxins (99).

4.3. ACTIVE TUBULAR TRANSPORT

The nephron, which serves as the functional unit of the kidney, plays a crucial role in the elimination of diverse uremic toxins. As discussed in the introduction, IS and pCS, protein bound uremic toxins, are largely removed by active tubular secretion. Transporters responsible for this process are located on the proximal tubule cells. Given the importance and contribution of tubular secretion by the proximal tubule cells in the clearance of various substances such as drugs and uremic toxins, CKD would imply a modification of clearance efficiency.

In the past, few studies and experiments have been conducted to investigate the influence and effects of CKD on the active transport of drugs, so little data have been obtained (98).

4.3.1. Transport of ligands for the transporter

4.3.1.1. Transport of IS and pCS

Both uremic toxins are highly protein bound, as already demonstrated. This implies that only a small fraction, which is not protein bound, is subject to glomerular filtration. The bound fraction is consequently removed from the body via tubular secretion, more specifically by OAT1 and 3. For these substances, which have a small unbound fraction, renal clearance is represented as a near-linear function of the GFR (113,114). A study by Poesen et al. (113) found that the estimated GFR is an acceptable way to estimate the renal clearance of pCS and IS. In other words, the clearance of these uremic toxins will decrease as a function of the increasing stages of CKD characterized by a decrease in GFR.

A study by Suchy-Dicey et al. (115) investigated tubular secretion in CKD patients. This was done by using LC-MS/MS on blood and urine samples from 298 participants. Using this technique, IS and pCS concentrations were quantified. Based on the results obtained, tubular secretion (clearance mL/min) was estimated. In this study, no subdivision was made into the different stages of CKD. However, the median and interquartile range of the eGFR was calculated, which was 41.1 (28.1–63.1) (115). Renal clearance for IS obtained in this study was 20.2 mL/min, with the proportion of tubular secretion reported to be 88%. This result can be compared with the renal

clearance of IS in healthy patients, with normal renal function, obtained in a study by Rivara et al. (116). The renal clearance of IS averaged 53 mL/min, the proportion of tubular secretion there was 93%.

Table 4.8: Renal clearance, serum and urine concentrations of the protein-bound uremic toxins IS and pCS in CKD patients. Content taken from Suchy-Dicey et al. (115) **Abbreviations: IS = Indoxyl sulfate: pCS = p-Cresyl sulfate. Data are presented as median (interquartile range).**

pCS		
Clearance (mL/min)	Serum concentration (µg/mL)	Urine concentration (µg/mL)
8.3 (5.3-12.8)	12.1 (6.5-20.9)	69.0 (32.5-110.6)
IS		
Clearance (mL/min)	Serum concentration (µg/mL)	Urine concentration (µg/mL)
20.2 (12.3-34.8)	3.2 (1.9-4.9)	43.3 (23.7-69.7)

An in vitro study by van der Made et al. (117) investigated the effect of the modification of albumin occurring in CKD on the tubular secretion of IS. Immortalized human proximal tubule cells were incubated with varying concentrations of IS, ranging from 5µM to 200µM. This experiment was conducted using three types of mediums: a HSA-free medium (with an K_m IS value of 29.3 µM), a HSA-rich medium (with an K_m IS value of 14.4 µM), and a medium containing modified HSA resembling the conditions found in CKD. The affinity of IS for the transporter present in the albumin medium was greater than in the albumin-free medium. A difference in free fraction (f_u) was observed from 1 to 0.1 respectively without and with HSA. Furthermore, it was also compared with the modified HSA medium. In addition, there was a free fraction of IS of 0.26. The affinity for the OAT1 transporter has been reduced fourfold and the V_{max} was also found to be reduced. Results of this study can be seen in **Table 4.9.** The $CL_{int,u,scaled}$ was obtained by multiplying the $CL_{int,u}$ by several factors (REF_{OAT1} = 1; PTCPGK = 60 × 10^6 cells/g kidney; KW_{cortex} = 169 g). The obtained value was used to predict clearance.

Table 4.9: Different renal clearance parameters listed per medium used. Content taken from K. van der Made et al. (117) **Abbreviations: V_{max} = Maximum velocity; K_m = Michaelis-Menten constant; F_u = Free fraction; IS = Indoxyl sulfate; $CL_{int,u}$ = Unbound intrinsic clearance; CL_R = Renal clearance.**

Medium used	Vmax (pmol/min/10^6 cells)	K_m (µM)	F_u IS	$K_{m,u}$ (µM)	CLint,u (µL/min/10^6 cells)	$CL_{int,u,scaled}$ (mL/min)	Predicted CL_R (mL/min)
HSA-free	26.5 ± 2.2	29.3 ± 7.5	1	29.3	0.9	9.2	2.9
HSA	48.1 ± 2.5	14.4 ± 2.9	0.1	1.4	33.7	342.5	11
Modified HSA	27.7 ± 1.0	21.0 ± 2.5	0.26	5.4	5.1	52	4.4

4.3.1.2. Drugs used in CKD and competition with PBUTs for transport

A study (74) from the year 2020 investigated the influence of drugs commonly used in CKD on the effect of renal active tubular secretion of uremic toxins. In this experiment, conditionally immortalized proximal tubular epithelial cells (ciPTEC) cell lines were used. Using these cells, the proximal tubular cells are mimicked to qualify drug interactions. The study only examined the interaction between the substances at the level of the OAT1, so the cells were equipped with OAT1. Drugs studied were ACE-I (captopril, enalaprilat and lisinopril), ARBs (losartan and valsartan), statins (pravastatin and simvastatin) and furosemide. The drug interactions were then evaluated using an OAT1-mediated fluorescein assay. First, the effect of these drugs on the transport of fluorescein was investigated. This is a model substrate for OAT1 that has been shown to be transported only via OAT1. If we assume that the uptake of fluorescein is 100%, then the ARBs and furosemide provided the most potent interactions. They caused a decrease of about 50% in fluorescein uptake at their highest therapeutic concentrations. This was 20% when statins were added and no difference in fluorescein uptake was observed for the ACE-I. This study revealed different IC50 values for these drugs that can be found in **Table 4.10.** Furthermore, the effect of IS and pCS on the inhibitory effect of the drugs on fluorescein was tested, which were added at concentrations of 110 µM and 125 µM, respectively. If we look at the results of the increase in IS, we see that the fluorescein uptake is greatly reduced. For the ARBs, this reduction progressed with increasing concentrations of the drug, while for statins and furosemide, we only saw a decrease in fluorescein uptake at increased therapeutic concentrations of the drugs. This was also concluded from the

results of adding pCS (74). The interaction between valsartan and the PBUTs is shown in **Figure 4.5.**

Table 4.10: Listed IC50 values for the medicinal products for OAT1. Content taken from Mihaila et al. (74) **Abbreviations: IS = indoxyl sulfate; ACE-I = angiotensin converting enzyme inhibitors; ARBs = angiotensin receptor blockers; pCS = p-cresyl sulfate; NA = not available; IC50 = half maximal inhibitory concentration. Data are presented as mean ± standard deviation. *Further testing for interaction between the ACE-I and IS/pCS did not occur because these interactions are supratherapeutical and clinically irrelevant.**

Drug class	Drugs	IC50 (µM)	+ 110 µM IS IC50 (µM)	+ 125 µM pCS IC50 (µM)
ACE-I	Captopril	2022 ± 465	-*	-*
	Enalaprilate	1853 ± 370	-*	-*
	Lisinopril	NA	-*	-*
ARBs	Losartan	8.6 ± 2.5	13.9 ± 5.90	15.97 ± 3.90
	Valsartan	11.5 ± 3.5	16.1 ± 3.60	17.97 ± 3.80
Diuretics	Furosemide	28.1 ± 9.1	44.7 ± 12.4	60.2 ± 1.00
Statins	Pravastatin	13.8 ± 8.5	40.9 ± 9.20	19.1 ± 3.20
	Simvastatin	21.3 ± 3.8	71.8 ± 27.3	32.8 ± 7.60

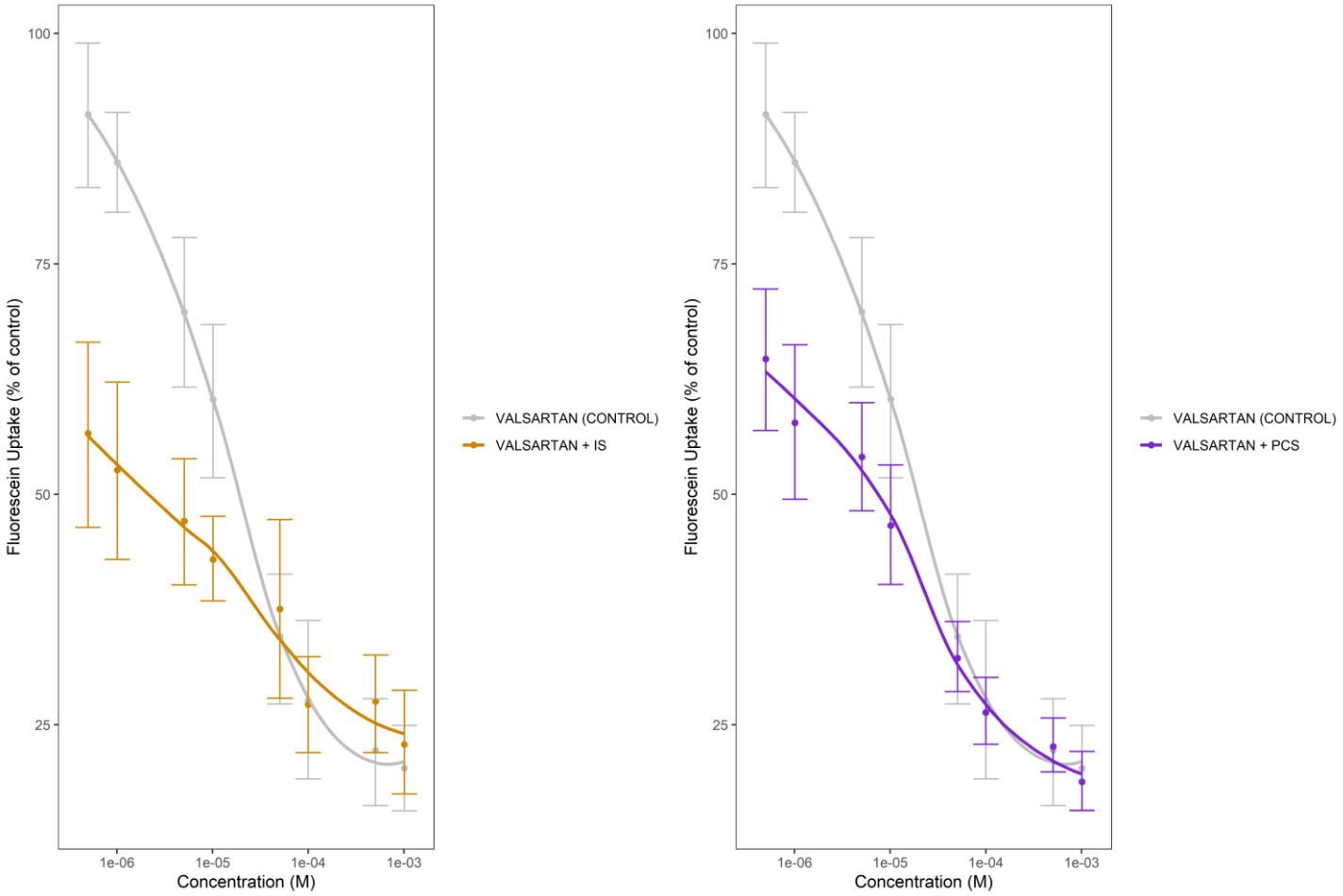

Figure 4.5: Graphical representation of the interaction between PBUTs and valsartan for binding to OAT1. **Abbreviations: IS = indoxyl sulfate; pCS = p-cresyl sulfate. Data are presented as mean ± standard deviation.**

5. DISCUSSION

The renal clearance of protein-bound uremic toxins in CKD patients remains a highly relevant topic within society. These toxins accumulate in the blood of these patients and have various toxic and harmful effects. Especially the patients with more advanced stages of CKD have an extensive accumulation of these toxins. Often, patients with CKD stage 5 or end- stage renal disease (ESRD) are completely dependent on renal dialysis or undergo a renal transplant, which has a major impact on the quality of life for these patients. It is predicted that by the year 2030, worldwide, about 5 million patients will be dependent on renal replacement therapy (41). Patients suffering from CKD are mainly treated with drugs to manage the risk factors associated with the condition. To this day, this is a topic that requires a lot of research. The existing literature on this subject gives only a small view of the possible interactions, often via in vitro and in vivo (rat) experiments. Because the human data is limited, we don't know how well these results are transferable to the human situation.

The microbiome is responsible for the production of IS and pCS, two of the protein-bound uremic toxins. Several studies show that the composition of the microbiome in CKD has changed (58,59,118,119). In CKD, accumulation of these toxins is observed in the blood. When looking at the results obtained in the study from Gryp et al. (78) **(see section 4.1.**). There was an increase in serum concentrations of IS and pCS, with increasing stages of CKD. Remarkable in the results is that there was a higher serum concentration of pCS in the controls (mean = 13.2 µM) compared to CKD stage 1 (mean = 11.6 µM). However, there is a strong increase in pCS serum concentrations when looking at the other CKD stages where it can be suggested that the decrease is probably related to bias such as interindividual differences or errors in measurement (78). A cause of this accumulation could be increased production due to the changed composition of the microbiome observed in CKD patients. However, this hypothesis is not confirmed. Another factor that could play a role in the accumulation of protein-bound uremic toxins is an altered kidney function, which occurs in CKD. No significant difference was observed in absolute levels between the different amino acids and the precursors formed by the microbiome. Also, no significant differences were seen in the ex vivo study looking at the presence and quantity of precursors in fecal samples. From these results, it can be said that the increased serum concentrations of the uremic toxins IS and pCS, present at different stages of CKD, are probably not due to the

bacterial formation of the precursors by the microbiome present in CKD. It can be assumed that the accumulation in the serum is largely due to the altered kidney function in patients with CKD (78,79). However, the study by Gryp et al has several shortcomings, such as insufficient data on the protein intake of the different participants, as well as the presence of diabetes and its influence on the composition of the microbiome. Both have an effect on the observed data. Nevertheless, restoring the composition of the disturbed microbiome remains an part of the therapy and prevention of CKD. This can be made possible by a fiber diet, pre-, pro- and symbiotics and adsorptive therapies (73,78).

Looking at the results **(see Table 4.3.)** of the study by Iimori et al. (88) measuring albumin concentrations in CKD patients, there is a significant decrease in serum concentrations of the transport protein. However, the study consisted of a Japanese population, so the decrease may be due to interpopulation differences, like the Japanese diet. The Japanese diet is somewhat Westernized but still differs enough with that of the Western world. The Japanese population also has a lower body size as well as a lower BMI, both of which can also bias the results. The results of the same study show that patients with CKD 5 (mean of the serum albumin concentration: 3.8 g/dL) have a slightly higher concentration of serum albumin than patients with CKD 4 (mean of the serum albumin concentration: 3.5 g/dL). However, the difference is minimal and both values are still on the low end of the serum albumin range of healthy patients. A study by Klammt et al. (89), which initially investigated the altered binding capacity of albumin in CKD, also measured serum albumin concentrations in different CKD patients **(see Table 4.4.)**. When these are compared with the results obtained in the previous study, we also see a decrease compared to healthy volunteers, but it is much less pronounced. The average values are between 41.2g/L (CKD 5) and 43.9g/L (CKD 1/2). Both studies do show a decrease, which confirms the hypothesis of hypoalbuminemia in CKD, although it is most pronounced at the most advanced stages. This can have a number of consequences. On the one hand, this could mean that the free fraction of these toxins increases, which could lead to increased toxicity. On the other hand, hypoalbuminemia may also imply altered tissue distribution, which can also lead to undesirable effects.

Both uremic toxins bind significantly to albumin. In the literature, a decreased binding of these uremic toxins with serum albumin was seen in patients with uremia. It has been suggested that the decreased binding may be caused by post-translational modifications, which alter the primary structure of the protein. The most common are oxidation, glycation and carbamylation. On the other hand, it could also be the result of competition between the different PBUTs, which occur in increased concentrations in CKD. However, it is not yet clear what the share of both causes is on the altered binding (109). Meijers et al (105) mapped the binding characteristics of albumin with drugs from the most commonly used drug classes in CKD **(see Table 4.6.)**. However, it is still not entirely clear what effect these changes have on the binding characteristics of albumin.

Various studies (96,105) showed that there are significantly more post-translational modifications of the protein during CKD, which leads to conformational changes to the albumin protein. This could be a possible explanation for the altered binding capacity of albumin that occurs in CKD (89,105,108,109) **(see Figure 4.3.)**. The modifications could probably lead to altered free fractions of ligands that bind with the protein. In other words, it is highly likely that the pharmacokinetics of these ligands will change.

In recent years, studies have been conducted on the competitive behavior of pCS and IS (109). However, these were in vitro experiments. The binding characteristics of IS and pCS are shown in **Table 4.7.** The data found in the literature seem to concur that there is indeed competition between the two uremic toxins. However, there is talk of mutual competition. The Bmax (binding capacity) values found in the study by Deltombe et al. (109) differ from Bmax values found in other studies (106,108,120), which are lower. This is because saturation of the binding sites was taken into account in this study, as well as working under physiological and uremic conditions that are as close as possible to the actual situation. A limitation of this study, however, is that the protein concentration of the blank HD serum used was slightly reduced. Nevertheless, the impact of this factor was negligible since all the tests were carried out using the same source of blank HD serum, which enabled appropriate adjustments and corrections.

Another aspect to consider when considering the binding character of compounds with serum proteins is their binding to α_1-acid glycoprotein. This is next to albumin the

most abundant serum protein and like albumin, it binds to various compounds. Lisowska-Myjak et al. (121) state that the individual binding character of IS with both albumin and α_1-acid glycoprotein is not known. It is suggested that both will play an important role in the disposition of both uremic toxins and drugs (92,121). Mainly tryptophan, tyrosine, lysine and histidine residues play a role in the binding of drugs with α_1-acid glycoprotein. This can, as is the case with albumin, be disturbed by the increased levels of uremic toxins in CKD. This aspect must be taken into account, and therefore complicates the prediction of the total renal clearance of drugs in combination with uremic toxins (92).

Given the suspicion of important interactions in CKD between the uremic toxins and drugs, as well as the harmful effects associated with the accumulation of these uremic toxins, the uremic toxins must be removed from the body to prevent such interaction. Dialysis techniques for removing these toxins from the body, are an option. However, this technique is detrimental to the patient's quality of life and has only brought about mild improvements. Using drugs that compete with uremic toxins for binding with albumin is another way to remove uremic toxins. Drugs with a higher affinity for albumin and/or the OATs are used, with the aim of removing the uremic toxins from their binding site through competitive binding. As a result, the free fraction of PBUTs increases (122). As a result, the concentration gradient across the dialysis membrane rises again, so that this driving force ensures the removal of the UT (123). This technique has been tested in vivo and has been shown to be effective for removing UT from the body (122). Tao et al. (124) investigated the binding affinity for the human serum albumin for various drugs by means of equilibrium dialysis. ibuprofen came out as the frontrunner with the highest affinity for the protein. This led to increased free fractions of both IS and pCS. However, this was studied in vitro. This result gives a strong indication that it would cause an interaction in humans, but the degree of interaction is debatable and requires further research in humans.

In the results section, the interaction between furosemide and uremic toxins for binding to albumin was cited (110,111). In this example **(see section 4.2.3.2.)**, it is suggested that the uremic toxins cause conformational alterations of the protein and therefore albumin will bind less to the drug. Although relatively few examples of the interaction between uremic toxins and drugs used in CKD for binding to the protein were found in the literature, the interaction can be illustrated for other drug classes.

Nishi et al. (125) mapped this for the binding of the antipsychotic drug aripiprazole with albumin. It is bound for 98.3% and 97.8% respectively in the absence and in the presence of normal uremic toxin serum concentrations, which indicates that normal concentrations hardly affect the binding capacity of albumin for aripiprazole. However, this is not the case for supra-normal concentrations of uremic toxins. At these concentrations, pCS and IS produced a noticeably increased free fraction of the drug. Fluorescent probes were used to investigate the binding site of this medicine. Results of this study suggested that this drug binds at the same binding site as the uremic toxins, namely Sudlow site II.

For pCS and IS, lower fractional renal clearance was seen in several studies in later stages of CKD (78,122). These uremic toxins are largely cleared via tubular secretion, while a small fraction is subject to glomerular filtration. This decrease in fractional renal clearance would be mainly due to a disruption of tubular secretion, which occur in CKD.

When looking at the results of a study by Suchy-Dicey et al. (115) **(see Table 4.8)** a tubular renal clearance of pCS of 8.3 mL/min and of IS of 20.2 mL/min was observed. However, in this study, tubular transport was generalized and no distinction was made between OAT1 and OAT3. The eGFR of all participants was also measured, and the median of these measurements was 41.8 ml/min per 1.73m^2. If this value is compared with a study by Klammt et al. (89) **(see Table 4.4)**, the median of the eGFR belongs to CKD stage 3. Van der Made et al. further investigated different clearance parameters of IS for transport via OAT1, and what the effect of (modified) albumin binding was on this. Clear differences were observed based on affinity for the receptor and free fraction, where the affinity of IS for the receptor was higher in the presence of normal albumin compared to modified albumin. Based on these values, the renal clearance was estimated using a mathematical calculation. In this study, ciPTEC cell lines were used that were incubated with different concentrations of IS. However, there are some reservations about this method, such as to what extent this result is extrapolatable to humans. Important to note that this method was able to accurately predict the percentual difference between clearance with normal and modified albumin, but that the absolute values of these clearances were underpredicted. This is helpful in the sense that it cannot only predict whether the clearance will increase or decrease, but

also by how much. A downside of this thesis is that it only focused on the OAT transporters for the renal clearance of these PBUTs. However, it was mentioned earlier, in the introduction, that the efflux transporters (BCRP and MDR2/4) also contribute significantly to the elimination (41), since they ensure the final removal from the body. It would therefore have been interesting to find out what the accumulation of these uremic toxins have on these efflux transporters.

It is important to realize that uremic toxins do not only affect the renal clearance of drugs, but also the non-renal clearance or the metabolism of drugs. This has to be taken into account when looking at medicines that are partly or completely cleared via this route. IS could affect the activity of various Cytochrome P450 enzymes (CYP) when present in high concentrations. This was demonstrated by Naud et al. (98). IS has been observed to reduce the activity of CYP2A and CYP3A, in rat microsomes, in high concentrations, thereby exerting an inhibitory effect on the metabolism of the substrates of these enzymes, such as losartan. This also applies to erythromycin (98). High concentrations of IS inhibit the n-demethylation catalyzed by CYP3A, also in the microsomes of a rat. Another study also proved that p-cresol, the precursor of pCS, present in the uremic serum provides an inhibition of the hepatic uptake of digoxin by 25% both in the hepatocytes from human and rats (126). This might indicate that this uremic toxin affects the metabolism of the drug.

However, since few human studies have been done on this subject, it is not possible to say with 100% certainty what the impact of the accumulation of the PBUTs on renal clearance of the drugs used in CKD truly is. However, some studies have already been carried out that measure the interaction between UT and drugs in animals (127). An example of this is the study by Fujita et al., which looked at the interaction of IS and probenecid in rats. A decrease in renal clearance of IS was observed from 1.4 mL x min^{-1} x kg^{-1} to 0.2 mL x min^{-1} x kg^{-1} with the addition of probenecid. This was also done for the interaction with quinapril, where there was a decrease in renal clearance from 1.2 mL x min^{-1} x kg^{-1} to 0.6 mL x min^{-1} x kg^{-1} (127). In an in vivo study by Bovée et al., it was seen that higher serum concentrations of IS and decreased eGFR, which is the case with CKD, are associated with lower clearance of various diuretics such as hydrochlorothiazide. This diuretic is transported into the urine trough proximal tubule

transporters, after which its exerts its effect in the distal tubule and is then excreted trough the kidney (128).

Based on the results that were found from various experiments with cell lines and animals, It is reasonable to anticipate that there will indeed be interactions in which the renal clearance of the substances is altered. The expected clearance for drugs, given the expected competition with uremic toxins, is difficult to predict as it depends on many different things such as the binding affinity of both substances which compete for the binding of both albumin and the OAT. However, in order to be able to make concrete statements about these interactions and the influence on clearance, further studies will have to be carried out in humans. The results showed potential interactions, but this has not been verified. However, some predictions can be made. At the level of albumin, competition can be expected between the increased concentration of uremic toxins found in CKD and drugs that also bind significantly with the protein. Modified pharmacokinetics can be expected for these drugs. This will lead to the need to monitor these medicines in the treatment of CKD so that a sufficiently effective therapeutic concentration appears in the blood. An example of a drug showing altered pharmacokinetics in patients with CKD is vildagliptin. He et al. (129) reported changes in the pharmacokinetic of the drug in patients with renal impairment. A separate study conducted by Guo et al. (130) in rats posed that inhibition of the OAT transporter by uremic toxins decreased the clearance of M20.7 (metabolite of the drug), while the reduced clearance of vildagliptin stemmed from reduced GFR.

For years, dosage adjustments and recommendations have been made based on the fact that the pharmacokinetics of the drugs, which are taken by CKD patients, change. The hypoalbuminemia and the fact that the accumulation of uremic toxins, which occurs in CKD, cause modifications at the level of albumin, ensure that there will be differences in the distribution of drugs and thus also in the volume of distribution of these drugs. As previously reported, albumin will primarily bind with acidic drugs, mainly affecting and modifying the pharmacokinetics of these drugs. Competition for the binding sites between medicinal products on the one hand or between medicinal products and uremic toxins on the other hand may lead to an increased free fraction (72,131). However, a study by Benet et al. (132) investigated the clinical relevance of the altered protein binding. This study showed that no dose adjustments were needed

for most drugs, as these changes usually have no impact. However, it is necessary to take this into account for the development of new drugs. One might question the extent to which it is necessary to make dose adjustments, as patients with the most advanced stage of CKD are most likely to undergo a kidney transplant in order to maintain quality of life.

6. CONCLUSION

In conclusion, it can be established that the renal clearance of uremic toxins and their interaction with drugs used for the treatment of CKD is complex. The aim of this thesis was to use an extensive literature study to find answers the renal clearance of these uremic toxins and the possible interaction between these toxins and drugs used in CKD patients. After studying the literature, it became clear that very little data can be found obtained from in vivo (human) studies. Based on the studies found with cell lines (in vitro) and animals, it is reasonable to anticipate that there will indeed be interactions/competition in which the pharmacokinetics and the renal clearance of the substances is altered.

Interaction for the binding at the level of albumin can be expected. The effect of the posttranslational modifications, which occur to a greater extent with CKD, on the binding with the ligands of albumin is less clear. As well as the effect of the decreased binding capacity of albumin in CKD. However, a higher volume of distribution was observed for furosemide, in the presence of larger concentrations of IS. Which can possibly be explained by the alteration of the albumin that occur with CKD.

What exactly happens at the level of the OAT transporters is more unclear. It was possible to establish, however, from the results of an in vitro study, that the renal clearance of IS in CKD decreases as well as the proportion of tubular secretion. It also has been shown, however, again in an in vitro study, that the inhibitory effect of certain medicinal products on the OAT1 transporter in the presence of significant concentrations of PBUTs, is enhanced.

It is clear that this topic requires further in vivo research, in humans, in order to paint a better picture of the effective interaction between the drugs and PBUTS and its effect on the pharmacokinetics of these drugs.

7. REFERENCES

1. Glassock RJ, Massry SG. Chapter 6 - Uremic toxins: an integrated overview of classification and pathobiology. In: Kopple JD, Massry SG, Kalantar-Zadeh K, Fouque D, editors. Nutritional Management of Renal Disease (Fourth Edition). Academic Press; 2022 [cited 2023 Mar 3]. p. 77–89. Available from: https://www.sciencedirect.com/science/article/pii/B978012818540700015X

2. Smith M. Uremia and Uremic Syndrome. WebMD. [cited 2023 Mar 9]. Available from: https://www.webmd.com/a-to-z-guides/uremia-uremic-syndrome

3. Falconi CA, Junho CV da C, Fogaça-Ruiz F, Vernier ICS, da Cunha RS, Stinghen AEM, et al. Uremic Toxins: An Alarming Danger Concerning the Cardiovascular System. Front Physiol. 2021 [cited 2023 Mar 9];12. Available from: https://www.frontiersin.org/articles/10.3389/fphys.2021.686249

4. Uremic Toxicity - ScienceDirect. [cited 2023 Mar 3]. Available from: https://www.sciencedirect.com/science/article/pii/B9780123919342000047

5. Rysz J, Franczyk B, Ławiński J, Olszewski R, Ciałkowska-Rysz A, Gluba-Brzózka A. The Impact of CKD on Uremic Toxins and Gut Microbiota. Toxins. 2021 Mar 31;13(4):252.

6. Chao CT, Lin SH. Uremic Toxins and Frailty in Patients with Chronic Kidney Disease: A Molecular Insight. Int J Mol Sci. 2021 Jan;22(12):6270.

7. Wołyniec W, Kasprowicz K, Giebułtowicz J, Korytowska N, Zorena K, Bartoszewicz M, et al. Changes in Water Soluble Uremic Toxins and Urinary Acute Kidney Injury Biomarkers After 10- and 100-km Runs. Int J Environ Res Public Health. 2019 Nov;16(21):4153.

8. Practitioners TRAC of general. The gut microbiome. Australian Family Physician. The Royal Australian College of general Practitioners; [cited 2023 Mar 2]. Available from: https://www.racgp.org.au/afp/2017/april/the-gut-microbiome

9. Regunathan-Shenk R, Shah NB, Raj DS. Chapter 11 - The gut microbiome and the kidney. In: Kopple JD, Massry SG, Kalantar-Zadeh K, Fouque D, editors. Nutritional Management of Renal Disease (Fourth Edition). Academic Press; 2022 [cited 2023 Mar 3]. p. 147–61. Available from: https://www.sciencedirect.com/science/article/pii/B9780128185407000318

10. Bibbò S, Ianiro G, Giorgio V, Scaldaferri F, Masucci L, Gasbarrini A, et al. The role of diet on gut microbiota composition.

11. Graboski AL, Redinbo MR. Gut-Derived Protein-Bound Uremic Toxins. Toxins. 2020 Sep;12(9):590.

12. Lauriola M, Farré R, Evenepoel P, Overbeek SA, Meijers B. Food-Derived Uremic Toxins in Chronic Kidney Disease. Toxins. 2023 Feb;15(2):116.

13. Uremische toxines | UZ Gent. [cited 2023 Feb 20]. Available from: https://www.uzgent.be/patient/zoek-een-arts-of-dienst/nefrologie-kinderen/wetenschappelijk-onderzoek/utopaed/uremische-toxines

14. Masereeuw R, Mutsaers HAM, Toyohara T, Abe T, Jhawar S, Sweet DH, et al. The kidney and uremic toxin removal: glomerulus or tubule? Semin Nephrol. 2014 Mar;34(2):191–208.

15. Renal Clearance - an overview | ScienceDirect Topics. [cited 2023 Mar 3]. Available from: https://www.sciencedirect.com/topics/engineering/renal-clearance

16. Dhondt A, Vanholder R, Van Biesen W, Lameire N. The removal of uremic toxins. Kidney Int Suppl. 2000 Aug;76:S47-59.

17. Vanholder R, De Smet R, Glorieux G, Argilés A, Baurmeister U, Brunet P, et al. Review on uremic toxins: Classification, concentration, and interindividual variability. Kidney Int. 2003 May 1;63(5):1934–43.

18. Cheung KWK, Hsueh CH, Zhao P, Meyer TW, Zhang L, Huang SM, et al. The Effect of Uremic Solutes on the Organic Cation Transporter 2. J Pharm Sci. 2017 Sep 1;106(9):2551–7.

19. Liabeuf S, Drüeke TB, Massy ZA. Protein-Bound Uremic Toxins: New Insight from Clinical Studies. Toxins. 2011 Jul 20;3(7):911–9.

20. Dou L, Bertrand E, Cerini C, Faure V, Sampol J, Vanholder R, et al. The uremic solutes p-cresol and indoxyl sulfate inhibit endothelial proliferation and wound repair. Kidney Int. 2004 Feb;65(2):442–51.

21. Liabeuf S, Barreto DV, Barreto FC, Meert N, Glorieux G, Schepers E, et al. Free p-cresylsulphate is a predictor of mortality in patients at different stages of chronic kidney disease. Nephrol Dial Transplant Off Publ Eur Dial Transpl Assoc - Eur Ren Assoc. 2010 Apr;25(4):1183–91.

22. Barreto FC, Barreto DV, Liabeuf S, Meert N, Glorieux G, Temmar M, et al. Serum Indoxyl Sulfate Is Associated with Vascular Disease and Mortality in Chronic Kidney Disease Patients. Clin J Am Soc Nephrol CJASN. 2009 Oct;4(10):1551–8.

23. Lim YJ, Sidor NA, Tonial NC, Che A, Urquhart BL. Uremic Toxins in the Progression of Chronic Kidney Disease and Cardiovascular Disease: Mechanisms and Therapeutic Targets. Toxins. 2021 Feb 13;13(2):142.

24. Zhuo JL, Li XC. Proximal Nephron. Compr Physiol. 2013 Jul 1;3(3):1079–123.

25. Hausmann R, Kuppe C, Egger H, Schweda F, Knecht V, Elger M, et al. Electrical Forces Determine Glomerular Permeability. J Am Soc Nephrol JASN. 2010 Dec;21(12):2053–8.

26. Gabri MS, Butler RD. The ultrastructure of the renal corpuscle of a lizard. Tissue Cell. 1984 Jan 1;16(4):627–34.

27. Structure Of A Nephron And Formation Of The Urine Stockvectorkunst en meer beelden van Nefron - Nefron, Nier - Inwendig orgaan, Activiteit - iStock]. [cited 2023 Mar 3]. Available from: https://www.istockphoto.com/nl/vector/structure-of-a-nephron-and-formation-of-the-urine-gm1353206903-428371831

28. Podocytdisfunctie en proteïnurie | NTvG [Internet]. 2004 [cited 2023 Mar 3]. Available from: https://www.ntvg.nl/artikelen/podocytdisfunctie-en-proteinurie

29. Kidney Glomerulus (SEM) | Urinary System. [cited 2023 May 12]. Available from: http://histologyguide.com/EM-view/EM-225-kidney-glomerulus/16-photo-1.html

30. Faul C, Asanuma K, Yanagida-Asanuma E, Kim K, Mundel P. Actin up: regulation of podocyte structure and function by components of the actin cytoskeleton. Trends Cell Biol. 2007 Sep;17(9):428–37.

31. Glomerulus and nephron: MedlinePlus Medical Encyclopedia Image. [cited 2023 Mar 6]. Available from: https://medlineplus.gov/ency/imagepages/19932.htm

32. Persson BE. Dynamics of glomerular ultrafiltration in Amphiuma means. Pflugers Arch. 1981 Aug;391(2):135–40.

33. VanWert AL, Gionfriddo MR, Sweet DH. Organic anion transporters: discovery, pharmacology, regulation and roles in pathophysiology. Biopharm Drug Dispos. 2010;31(1):1–71.

34. Falkson SR, Bordoni B. Anatomy, Abdomen and Pelvis: Bowman Capsule. In: StatPearls . StatPearls Publishing; 2022 [cited 2023 May 12]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK554474/

35. Shahbaz H, Gupta M. Creatinine Clearance [Internet]. StatPearls. StatPearls Publishing; 2022 [cited 2023 Mar 6]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK544228/

36. The countercurrent multiplier function of the loop of Henle. [cited 2023 Mar 4]. Available from: https://www.studysmartwithchris.com/nl/samenvattingen/physiology-of-domestic-animals-sjaastad/gedeelte-lis-vloeistof/

37. Subramanya AR, Ellison DH. Distal convoluted tubule. Clin J Am Soc Nephrol CJASN. 2014 Dec 5;9(12):2147–63.

38. Reabsorptie in het vroege segment van de distale tubulus. Klinisch Redeneren. [cited 2023 Mar 5]. Available from: https://www.klinischredeneren.nl/animatie/reabsorptie-in-het-vroege-segment-van-de-distale-tubulus/

39. Bendayan R. Renal Drug Transport: A Review. Pharmacother J Hum Pharmacol Drug Ther. 1996;16(6):971–85.

40. Glomerular filtration (glomerulus) | Renal physiology (article) | Khan Academy [Internet]. [cited 2023 Mar 4]. Available from: https://www.khanacademy.org/_render

41. Masereeuw R. The Dual Roles of Protein-Bound Solutes as Toxins and Signaling Molecules in Uremia. Toxins. 2022 Jun 11;14(6):402.

42. Morrissey KM, Stocker SL, Wittwer MB, Xu L, Giacomini KM. Renal transporters in drug development. Annu Rev Pharmacol Toxicol. 2013;53:503–29.

43. Stevens LA, Coresh J, Greene T, Levey AS. Assessing kidney function--measured and estimated glomerular filtration rate. N Engl J Med. 2006 Jun 8;354(23):2473–83.

44. Michels WM, Grootendorst DC, Verduijn M, Elliott EG, Dekker FW, Krediet RT. Performance of the Cockcroft-Gault, MDRD, and New CKD-EPI Formulas in Relation to GFR, Age, and Body Size. Clin J Am Soc Nephrol CJASN. 2010 Jun;5(6):1003–9.

45. Mair RD, Sirich TL, Meyer TW. Uremic Toxin Clearance and Cardiovascular Toxicities. Toxins. 2018 Jun 2;10(6):226.

46. Zhu L, Lu L, Wang S, Wu J, Shi J, Yan T, et al. Chapter 11 - Oral Absorption Basics: Pathways and Physicochemical and Biological Factors Affecting Absorption. In: Qiu Y, Chen Y, Zhang GGZ, Yu L, Mantri RV, editors. Developing Solid Oral Dosage Forms (Second Edition). Boston: Academic Press; 2017 [cited 2023 May 25]. p. 297–329. Available from: https://www.sciencedirect.com/science/article/pii/B978012802447800011X

47. Lai Y. 6 - Organic anion-transporting polypeptides (OATPs/SLCOs). In: Lai Y, editor. Transporters in Drug Discovery and Development [Internet]. Woodhead Publishing; 2013 [cited 2023 Mar 10]. p. 353–454. (Woodhead Publishing Series in Biomedicine). Available from: https://www.sciencedirect.com/science/article/pii/B9781907568213500063

48. Bush KT, Singh P, Nigam SK. Gut-derived uremic toxin handling in vivo requires OAT-mediated tubular secretion in chronic kidney disease. JCI Insight. 2020 Apr 9 [cited 2023 Mar 10];5(7). Available from: https://insight.jci.org/articles/view/133817

49. Wu W, Bush KT, Nigam SK. Key Role for the Organic Anion Transporters, OAT1 and OAT3, in the in vivo Handling of Uremic Toxins and Solutes. Sci Rep. 2017 Jul 10;7:4939.

50. Robertson S, Penzak SR, Huang SM. Chapter 15 - Drug Interactions. In: Atkinson AJ, Huang SM, Lertora JJL, Markey SP, editors. Principles of Clinical Pharmacology (Third Edition). Academic Press; 2012 [cited 2023 Mar 10]. p. 239–57. Available from: https://www.sciencedirect.com/science/article/pii/B9780123854711000155

51. da Cunha RS, Azevedo CAB, Falconi CA, Ruiz FF, Liabeuf S, Carneiro-Ramos MS, et al. The Interplay between Uremic Toxins and Albumin, Membrane Transporters and Drug Interaction. Toxins. 2022 Feb 26;14(3):177.

52. Tubular reabsorption article (article) | Khan Academy]. [cited 2023 May 12]. Available from: https://www.khanacademy.org/_render

53. Active transport - Definition and Examples - Biology Online Dictionary [Internet]. Biology Articles, Tutorials & Dictionary Online. 2019 [cited 2023 May 12]. Available from: https://www.biologyonline.com/dictionary/active-transport

54. Chapter 1: Definition and classification of CKD. Kidney Int Suppl. 2013 Jan;3(1):19–62.

55. Hill NR, Fatoba ST, Oke JL, Hirst JA, O'Callaghan CA, Lasserson DS, et al. Global Prevalence of Chronic Kidney Disease – A Systematic Review and Meta-Analysis. Remuzzi G, editor. PLOS ONE. 2016 Jul 6;11(7):e0158765.

56. Khamis SS, Zahran AM, Hegazy NN, Kasem HE, El-Fiky HK. Prevalence of Chronic Kidney Disease in Patients with Cardiovascular Disease. Open J Nephrol. 2020;10(03):227–40.

57. López-Novoa JM, Martínez-Salgado C, Rodríguez-Peña AB, Hernández FJL. Common pathophysiological mechanisms of chronic kidney disease: Therapeutic perspectives. Pharmacol Ther. 2010 Oct;128(1):61–81.

58. Chronic kidney disease alters intestinal microbial flora. Kidney Int. 2013 Feb 1;83(2):308–15.

59. Wong J, Piceno YM, DeSantis TZ, Pahl M, Andersen GL, Vaziri ND. Expansion of urease- and uricase-containing, indole- and p-cresol-forming and contraction of short chain fatty acid-producing intestinal microbiota in ESRD. Am J Nephrol. 2014;39(3):230–7.

60. Rocchetti MT, Cosola C, Ranieri E, Gesualdo L. Protein-Bound Uremic Toxins and Immunity. In: Gigante M, Ranieri E, editors. Cytotoxic T-Cells: Methods and Protocols. New York, NY: Springer US; 2021 [cited 2023 Mar 14]. p. 215–27. (Methods in Molecular Biology (MIMB, volume 2325)). Available from: https://doi.org/10.1007/978-1-0716-1507-2_15

61. Wehedy E, Shatat IF, Al Khodor S. The Human Microbiome in Chronic Kidney Disease: A Double-Edged Sword. Front Med. 2022 [cited 2023 Mar 18];8. Available from: https://www.frontiersin.org/articles/10.3389/fmed.2021.790783

62. Estimated Glomerular Filtration Rate (eGFR). National Kidney Foundation. 2015 [cited 2023 May 12]. Available from: https://www.kidney.org/atoz/content/gfr

63. Johnson M. Brenner & Rector's the kidney. Can J Surg. 1996 Dec;39(6):515–6.

64. Structural and Biochemical Features of Human Serum Albumin Essential for Eukaryotic Cell Culture - PMC. [cited 2023 Apr 12]. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8395139/

65. Lee P, Wu X. Review: Modifications of Human Serum Albumin and Their Binding Effect. Curr Pharm Des. 2015;21(14):1862–5.

66. Kidney Failure Risk Factor: Serum Albumin [Internet]. National Kidney Foundation. 2020 [cited 2023 Apr 11]. Available from: https://www.kidney.org/content/kidney-failure-risk-factor-serum-albumin

67. Eloot S, Schneditz D, Cornelis T, Van Biesen W, Glorieux G, Dhondt A, et al. Protein-Bound Uremic Toxin Profiling as a Tool to Optimize Hemodialysis. PLoS ONE. 2016 Jan 22;11(1):e0147159.

68. McGinnity DF, Grime K. 4.02 - ADME Optimization in Drug Discovery. In: Chackalamannil S, Rotella D, Ward SE, editors. Comprehensive Medicinal Chemistry III. Oxford: Elsevier; 2017 [cited 2023

May 25]. p. 34–44. Available from: https://www.sciencedirect.com/science/article/pii/B9780124095472123650

69. Volume of Distribution - StatPearls - NCBI Bookshelf. [cited 2023 May 25]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK545280/

70. Medicines to manage kidney disease. [cited 2023 Apr 11]. Available from: https://www.kidneyfund.org/treatments/medicines-manage-kidney-disease

71. Recommendations | Chronic kidney disease: assessment and management | Guidance | NICE . NICE; 2021 [cited 2023 Apr 17]. Available from: https://www.nice.org.uk/guidance/ng203/chapter/Recommendations#pharmacotherapy-for-ckd-in-adults-children-and-young-people-with-related-persistent-proteinuria

72. Banoglu E, Jha GG, King RS. Hepatic microsomal metabolism of indole to indoxyl, a precursor of indoxyl sulfate. Eur J Drug Metab Pharmacokinet. 2001 Dec 1;26(4):235–40.

73. Gut microbiota in chronic kidney disease. [cited 2023 Apr 16]. Available from: https://www.revistanefrologia.com/en-pdf-S2013251417300202

74. Mihaila SM, Faria J, Stefens MFJ, Stamatialis D, Verhaar MC, Gerritsen KGF, et al. Drugs Commonly Applied to Kidney Patients May Compromise Renal Tubular Uremic Toxins Excretion. Toxins. 2020 Jun 12;12(6):391.

75. Cigarran Guldris S, González Parra E, Cases Amenós A. Gut microbiota in chronic kidney disease. Nefrol Publicacion Of Soc Espanola Nefrol. 2017;37(1):9–19.

76. Vaziri ND, Zhao YY, Pahl MV. Altered intestinal microbial flora and impaired epithelial barrier structure and function in CKD: the nature, mechanisms, consequences and potential treatment. Nephrol Dial Transplant Off Publ Eur Dial Transpl Assoc - Eur Ren Assoc. 2016 May;31(5):737–46.

77. Saito Y, Sato T, Nomoto K, Tsuji H. Identification of phenol- and p-cresol-producing intestinal bacteria by using media supplemented with tyrosine and its metabolites. FEMS Microbiol Ecol. 2018 Jun 22;94(9):fiy125.

78. Gryp T, De Paepe K, Vanholder R, Kerckhof FM, Van Biesen W, Van de Wiele T, et al. Gut microbiota generation of protein-bound uremic toxins and related metabolites is not altered at different stages of chronic kidney disease. Kidney Int. 2020 Jun;97(6):1230–42.

79. Popkov VA, Zharikova AA, Demchenko EA, Andrianova NV, Zorov DB, Plotnikov EY. Gut Microbiota as a Source of Uremic Toxins. Int J Mol Sci. 2022 Jan 1;23(1):483.

80. Busher JT. Serum Albumin and Globulin. Clinical Methods: The History, Physical, and Laboratory Examinations. 3rd edition. Butterworths; 1990 [cited 2023 Apr 22]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK204/

81. Moman RN, Gupta N, Varacallo M. Physiology, Albumin. StatPearls. StatPearls Publishing; 2022 [cited 2023 Apr 22]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK459198/

82. Risso MA, Sallustio S, Sueiro V, Bertoni V, Gonzalez-Torres H, Musso CG. The Importance of Tubular Function in Chronic Kidney Disease. Int J Nephrol Renov Dis. 2019 Dec 12;12:257–62.

83. Watanabe H, Miyamoto Y, Otagiri M, Maruyama T. Update on the pharmacokinetics and redox properties of protein-bound uremic toxins. J Pharm Sci. 2011 Sep;100(9):3682–95.

84. Albuminuria: Albumin in the Urine - NIDDK]. National Institute of Diabetes and Digestive and Kidney Diseases. [cited 2023 Apr 22]. Available from: https://www.niddk.nih.gov/health-information/kidney-disease/chronic-kidney-disease-ckd/tests-diagnosis/albuminuria-albumin-urine

85. Eiwit in de urine | UMC Groningen]. umcg.nl. [cited 2023 Apr 22]. Available from: https://www.umcg.nl/-/eiwitverlies-in-de-urine

86. Shafi T, Coresh J. Chapter 1 - Chronic Kidney Disease: Definition, Epidemiology, Cost, and Outcomes. In: Himmelfarb J, Sayegh MH, editors. Chronic Kidney Disease, Dialysis, and Transplantation (Third Edition). Philadelphia: W.B. Saunders; 2010 [cited 2023 Apr 18]. p. 3–21. Available from: https://www.sciencedirect.com/science/article/pii/B9781437709872000017

87. Figueroa SM, Araos P, Reyes J, Gravez B, Barrera-Chimal J, Amador CA. Oxidized Albumin as a Mediator of Kidney Disease. Antioxidants. 2021 Mar 8;10(3):404.

88. Iimori S, Noda Y, Okado T, Naito S, Toda T, Chida Y, et al. Baseline characteristics and prevalence of cardiovascular disease in newly visiting or referred chronic kidney disease patients to nephrology centers in Japan: a prospective cohort study. BMC Nephrol. 2013 Jul 17;14(1):152.

89. Klammt S, Wojak HJ, Mitzner A, Koball S, Rychly J, Reisinger EC, et al. Albumin-binding capacity (ABiC) is reduced in patients with chronic kidney disease along with an accumulation of protein-bound uraemic toxins. Nephrol Dial Transplant Off Publ Eur Dial Transpl Assoc - Eur Ren Assoc. 2012 Jun;27(6):2377–83.

90. Haller C. Hypoalbuminemia in Renal Failure: Pathogenesis and Therapeutic Considerations. Kidney Blood Press Res. 2005;28(5–6):307–10.

91. Gurevich KG. Effect of blood protein concentrations on drug-dosing regimes: practical guidance. Theor Biol Med Model. 2013 Mar 18;10:20.

92. Otagiri M. A molecular functional study on the interactions of drugs with plasma proteins. Drug Metab Pharmacokinet. 2005 Oct;20(5):309–23.

93. Sudlow G, Birkett DJ, Wade DN. Further Characterization of Specific Drug Binding Sites on Human Serum Albumin. Mol Pharmacol. 1976 Nov 1;12(6):1052–61.

94. Role of Arg-410 and Tyr-411 in human serum albumin for ligand binding and esterase-like activity | Biochemical Journal | Portland Press. [cited 2023 Apr 14]. Available from:

https://portlandpress.com/biochemj/article-abstract/349/3/813/38479/Role-of-Arg-410-and-Tyr-411-in-human-serum-albumin

95. Bteich M. An overview of albumin and alpha-1-acid glycoprotein main characteristics: highlighting the roles of amino acids in binding kinetics and molecular interactions. Heliyon. 2019 Nov 21;5(11):e02879.

96. Gajjala PR, Fliser D, Speer T, Jankowski V, Jankowski J. Emerging role of post-translational modifications in chronic kidney disease and cardiovascular disease. Nephrol Dial Transplant Off Publ Eur Dial Transpl Assoc - Eur Ren Assoc. 2015 Nov;30(11):1814–24.

97. Laget J, Duranton F, Argilés À, Gayrard N. Renal insufficiency and chronic kidney disease – Promotor or consequence of pathological post-translational modifications. Mol Aspects Med. 2022 Aug 1;86:101082.

98. Naud J, Nolin TD, Leblond FA, Pichette V. Current understanding of drug disposition in kidney disease. J Clin Pharmacol. 2012 Jan;52(1 Suppl):10S-22S.

99. Shi Y, Tian H, Wang Y, Shen Y, Zhu Q, Ding F. Effect of Ionic Strength, pH and Chemical Displacers on the Percentage Protein Binding of Protein-Bound Uremic Toxins. Blood Purif. 2019;47(4):351–60.

100. Peptide Mapping Identifies Hotspot Site of Modification in Human Serum Albumin by Methylglyoxal Involved in Ligand Binding and Esterase Activity* | Elsevier Enhanced Reader [Internet]. [cited 2023 Apr 12].

101. Tabata F, Wada Y, Kawakami S, Miyaji K. Serum Albumin Redox States: More Than Oxidative Stress Biomarker. Antioxidants. 2021 Apr [cited 2023 Apr 18];10(4). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8063786/

102. Bruschi M, Candiano G, Santucci L, Ghiggeri GM. Oxidized albumin. The long way of a protein of uncertain function. Biochim Biophys Acta. 2013 Dec;1830(12):5473–9.

103. Annibal A, Colombo G, Milzani A, Dalle-Donne I, Fedorova M, Hoffmann R. Identification of dityrosine cross-linked sites in oxidized human serum albumin. J Chromatogr B. 2016 Apr 15;1019:147–55.

104. Berg AH, Drechsler C, Wenger J, Buccafusca R, Hod T, Kalim S, et al. Carbamylation of Serum Albumin as a Risk Factor for Mortality in Patients with Kidney Failure. Sci Transl Med. 2013 Mar 6;5(175):175ra29.

105. Meijers BKI, Bammens B, Verbeke K, Evenepoel P. A review of albumin binding in CKD. Am J Kidney Dis Off J Natl Kidney Found. 2008 May;51(5):839–50.

106. Viaene L, Annaert P, de Loor H, Poesen R, Evenepoel P, Meijers B. Albumin is the main plasma binding protein for indoxyl sulfate and p-cresyl sulfate. Biopharm Drug Dispos. 2013 Apr;34(3):165–75.

107. Meijers BKI, De Loor H, Bammens B, Verbeke K, Vanrenterghem Y, Evenepoel P. p-Cresyl Sulfate and Indoxyl Sulfate in Hemodialysis Patients. Clin J Am Soc Nephrol. 2009 Dec;4(12):1932.

108. Watanabe H, Noguchi T, Miyamoto Y, Kadowaki D, Kotani S, Nakajima M, et al. Interaction between Two Sulfate-Conjugated Uremic Toxins, p-Cresyl Sulfate and Indoxyl Sulfate, during Binding with Human Serum Albumin. Drug Metab Dispos. 2012 Jul 1;40(7):1423–8.

109. Deltombe O, de Loor H, Glorieux G, Dhondt A, Van Biesen W, Meijers B, et al. Exploring binding characteristics and the related competition of different protein-bound uremic toxins. Biochimie. 2017 Aug 1;139:20–6.

110. Klinkmann G, Klammt S, Jäschke M, Henschel J, Gloger M, Reuter DA, et al. Impact of Albumin Binding Function on Pharmacokinetics and Pharmacodynamics of Furosemide. Medicina (Mex). 2022 Dec;58(12):1780.

111. Zaidi N, Ahmad E, Rehan M, Rabbani G, Ajmal MR, Zaidi Y, et al. Biophysical Insight into Furosemide Binding to Human Serum Albumin: A Study To Unveil Its Impaired Albumin Binding in Uremia. J Phys Chem B. 2013 Mar 7;117(9):2595–604.

112. Lam YW, Banerji S, Hatfield C, Talbert RL. Principles of drug administration in renal insufficiency. Clin Pharmacokinet. 1997 Jan;32(1):30–57.

113. Poesen R, Viaene L, Verbeke K, Claes K, Bammens B, Sprangers B, et al. Renal Clearance and Intestinal Generation of p-Cresyl Sulfate and Indoxyl Sulfate in CKD. Clin J Am Soc Nephrol CJASN. 2013 Sep 6;8(9):1508–14.

114. Janků I. Physiological modelling of renal drug clearance. Eur J Clin Pharmacol. 1993 Jul 1;44(6):513–9.

115. Suchy-Dicey AM, Laha T, Hoofnagle A, Newitt R, Sirich TL, Meyer TW, et al. Tubular Secretion in CKD. J Am Soc Nephrol. 2016 Jul;27(7):2148.

116. Rivara MB, Zelnick LR, Hoofnagle AN, Newitt R, Tracy RP, Kratz M, et al. Diurnal and Long-term Variation in Plasma Concentrations and Renal Clearances of Circulating Markers of Kidney Proximal Tubular Secretion. Clin Chem. 2017 Apr;63(4):915–23.

117. Quantitative Translation of Microfluidic Transporter in Vitro Data to in Vivo Reveals Impaired Albumin-Facilitated Indoxyl Sulfate Secretion in Chronic Kidney Disease | Molecular Pharmaceutics. [cited 2023 May 21].

118. Gut Bacterial Translocation May Aggravate Microinflammation in Hemodialysis Patients | SpringerLink. [cited 2023 May 19].

119. Bacterial Populations of the Small Intestine in Uremia | Nephron | Karger Publishers [cited 2023 May 19].

120. Bertuzzi A, Mingrone G, Gandolfi A, Greco AV, Ringoir S, Vanholder R. Binding of indole-3-acetic acid to human serum albumin and competition with l-tryptophan. Clin Chim Acta. 1997 Sep 30;265(2):183–92.

121. Lisowska-Myjak B, Zborowska H, Jaźwiec R, Karlińska M, Skarżyńska E. Serum indoxyl sulphate and its relation to albumin and α1-acid glycoprotein as a potential biomarkers of maternal intestinal metabolism during pregnancy and postpartum. PLOS ONE. 2021 Nov 5;16(11):e0259501.

122. Maheshwari V, Tao X, Thijssen S, Kotanko P. Removal of Protein-Bound Uremic Toxins Using Binding Competitors in Hemodialysis: A Narrative Review. Toxins. 2021 Sep 4;13(9):622.

123. Maheshwari V, Thijssen S, Tao X, Fuertinger D, Kappel F, Kotanko P. A novel mathematical model of protein-bound uremic toxin kinetics during hemodialysis. Sci Rep. 2017 Sep 4;7(1):10371.

124. Tao X, Thijssen S, Kotanko P, Ho CH, Henrie M, Stroup E, et al. Improved dialytic removal of protein-bound uraemic toxins with use of albumin binding competitors: an in vitro human whole blood study. Sci Rep. 2016 Mar 22;6(1):23389.

125. Nishi K, Sakurama K, Watanabe H, Maruyama T, Yamasaki K, Otagiri M. Effects of Uremic Toxins on the Binding of Aripiprazole to Human Serum Albumin. Biol Pharm Bull. 2021;44(3):437–41.

126. Tsujimoto M, Kinoshita Y, Hirata S, Otagiri M, Ohtani H, Sawada Y. Effects of uremic serum and uremic toxins on hepatic uptake of digoxin. Ther Drug Monit. 2008 Oct;30(5):576–82.

127. Fujita T, Ishihara K, Yasuda S, Nakamura T, Maeda M, Kobayashi M, et al. In Vivo Kinetics of Indoxyl Sulfate in Humans and Its Renal Interaction with Angiotensin-Converting Enzyme Inhibitor Quinapril in Rats. J Pharmacol Exp Ther. 2012 Jun;341(3):626–33.

128. A Randomized Trial of Distal Diuretics versus Dietary Sodium... : Journal of the American Society of Nephrology. [cited 2023 May 21].

129. He YL, Kulmatycki K, Zhang Y, Zhou W, Reynolds C, Ligueros-Saylan M, et al. Pharmacokinetics of vildagliptin in patients with varying degrees of renal impairment. Int J Clin Pharmacol Ther. 2013 Sep 1;51(9):693–703.

130. Guo Z, Kong F, Xie N, Chen Z, Hu J, Chen X. Mechanistic Study on the Effect of Renal Impairment on the Pharmacokinetics of Vildagliptin and its Carboxylic Acid Metabolite. Pharm Res. 2022 Sep;39(9):2147–62.

131. Gabardi S, Abramson S. Drug dosing in chronic kidney disease. Med Clin North Am. 2005 May;89(3):649–87.

132. Benet LZ, Hoener BA. Changes in plasma protein binding have little clinical relevance. Clin Pharmacol Ther. 2002;71(3):115–21.

8. APPENDIX

APENDIX A

Table prepared with the intention of including different medicines selected from the most commonly used drug classes in CKD. This was done on the basis of albumin binding and dependence on the organic anion transporters for its clearance.

Drug	Albumin binding	Substrate (S) or inhibitor (I) for OAT1 and/or 3	In- or exclude
Benazepril	77-93%	/	
Captopril	30%	S OAT1	
Enalapril	Little	OAT1/OAT3	
Lisinopril	3-10%	OAT1/OAT3	
Candesartan	Highly bound	?	
Eprosartan	Highly bound	?	
Losartan	Bound to albumin	OAT1/OAT3	
Olmesartan	Highly bound	I OAT1	
Valsartan	92%	I OAT1 and I OAT3	
Atorvastatin	98,60%	/	
Pravastatin	Less than 50%	OAT3	
Simvastatin	94%-98%	/	
Chlorthalidone	75%	?	
Indapamide	76-79%	?	
Metolazone	95%	/	
Furosemide	95%	OAT1/OAT3	
Spironolactone	88%	?	?
Indomethacin	96%	I OAT1/ I OAT3	
Methotrexaat	87.3%	?	
Probenecide	85%-95%	IOAT1 / IOAT3	
Ibuprofen	99%	SOAT1 / SOAT3	
Warfarine	>95%	/	

APPENDIX B

Searches (with results) with the included medicines.

*("p-cresyl sulfate" OR "indoxyl sulfate") AND pharmacokinetic**

- Effects of Uremic Toxins on the Binding of Aripiprazole to Human Serum Albumin)
- A Randomized Trial of Distal Diuretics versus Dietary Sodium Restriction for Hypertension in Chronic Kidney Disease

- Quantitative Translation of Microfluidic Transporter in Vitro Data to in Vivo Reveals Impaired Albumin-Facilitated Indoxyl Sulfate Secretion in Chronic Kidney Disease
- Transporter-mediated interaction of indican and methotrexate in rats
- In Vivo Kinetics of Indoxyl Sulfate in Humans and Its Renal Interaction with Angiotensin-Converting Enzyme Inhibitor Quinapril in Rats
- Characterization of uremic toxin transport by organic anion transporters in the kidney
- Major role of organic anion transporter 3 in the transport of indoxyl sulfate in the kidney
- Interaction mechanism between indoxyl sulfate, a typical uremic toxin bound to site II, and ligands bound to site I of human serum albumin

("p-cresyl sulfate" OR "indoxyl sulfate") AND "organic anion transporter" AND kidney

- Inhibitory effects of indoxyl sulfate and creatinine on the renal transport of meropenem and biapenem in rats
- Increased Plasma Exposures of Conjugated Metabolites of Morinidazole in Renal Failure Patients: A Critical Role of Uremic Toxins

("p-cresyl sulfate" OR "indoxyl sulfate") AND benazepril

("p-cresyl sulfate" OR "indoxyl sulfate") AND captopril

("p-cresyl sulfate" OR "indoxyl sulfate") AND enalapril

("p-cresyl sulfate" OR "indoxyl sulfate") AND lisinopril

("p-cresyl sulfate" OR "indoxyl sulfate") AND candesartan

("p-cresyl sulfate" OR "indoxyl sulfate") AND eprosartan

("p-cresyl sulfate" OR "indoxyl sulfate") AND losartan

("p-cresyl sulfate" OR "indoxyl sulfate") AND olmesartan

("p-cresyl sulfate" OR "indoxyl sulfate") AND valsartan

("p-cresyl sulfate" OR "indoxyl sulfate") AND atorvastatin

("p-cresyl sulfate" OR "indoxyl sulfate") AND pravastatin

("p-cresyl sulfate" OR "indoxyl sulfate") AND simvastatin

("p-cresyl sulfate" OR "indoxyl sulfate") AND Chlorthalidone

("p-cresyl sulfate" OR "indoxyl sulfate") AND Indapamide

("p-cresyl sulfate" OR "indoxyl sulfate") AND Metolazone

("p-cresyl sulfate" OR "indoxyl sulfate") AND Furosemide

("p-cresyl sulfate" OR "indoxyl sulfate") AND Spironolactone

("p-cresyl sulfate" OR "indoxyl sulfate") AND Indomethacin

- Major role of organic anion transporter 3 in the transport of indoxyl sulfate in the kidney

("p-cresyl sulfate" OR "indoxyl sulfate") AND Methotrexate

- Transporter-mediated interaction of indican and methotrexate in rats

("p-cresyl sulfate" OR "indoxyl sulfate") AND Probenecid

- Key Role for the Organic Anion Transporters, OAT1 and OAT3, in the in vivo Handling of Uremic Toxins and Solutes
- Human organic anion transporters function as a high-capacity transporter for p-cresyl sulfate, a uremic toxin
- In Vivo Kinetics of Indoxyl Sulfate in Humans and Its Renal Interaction with Angiotensin-Converting Enzyme Inhibitor Quinapril in Rats
- Organic anion transporters play an important role in the uptake of p-cresyl sulfate, a uremic toxin, in the kidney
- Major role of organic anion transporter 3 in the transport of indoxyl sulfate in the kidney

("p-cresyl sulfate" OR "indoxyl sulfate") AND ibuprofen

("p-cresyl sulfate" OR "indoxyl sulfate") AND warfarin

- Interaction mechanism between indoxyl sulfate, a typical uremic toxin bound to site II, and ligands bound to site I of human serum albumin

Master dissertation submitted to the faculty of Pharmaceutical Sciences, performed in collaboration with the Laboratory Medical Biochemistry and Clinical Analysis.

Promotor: Prof. dr. An Vermeulen

Commissioners:

Prof. dr. Evelien Snauwaert

Prof. dr. Griet Glorieux

This master dissertation is an examination document that not necessarily has been corrected for eventual mistakes. The information, conclusions and points of view in this master dissertation are those of the author and do not necessarily represent the opinion of the promoter or his/her research group.