Cyclosporin: A double edged sword

The dual role of cyclosporin as a nephrotoxicant and protecting agent against ischemia-reperfusion injury

Bachelor thesis

Bianca Sibering

Student number: s2939908

Date: 22-04-2018

Superviser: I.A.M. de Graaf

Abstract

Since cyclosporin was successfully tested to prevent graft rejection in 1978 the field of organ transplants became increasingly important. Cyclosporin works as an immunosuppressant by reducing T-cell function. Multiple studies have shown that cyclosporin also has a protective effect on kidneys against ischemia injury and that this positive effect is concentration dependent. When cyclosporin is used in high concentrations it has a nephrotoxic effect. In this thesis we will explain how ischemia injury is caused and determine how the protective effect and the toxic effect are caused by cyclosporin. We will examine via which pathways this is caused and why it is a concentration dependent process. Cyclosporin causes nephrotoxicity via an imbalance between vasodilation and vasoconstriction. Cyclosporin leads to a protective effect against ischemia-reperfusion injury (IRI) by binding to cyclophilin D with peptidyl prolyl *cis trans* isomerase activity and eventually blocking the mPTPs (mitochondrial Permeability Transition Pores). The toxic and protective effect are caused via 2 different mechanisms and is concentration dependent because of the high affinity of cyclosporin for cyclophilin D. Together with other protective compounds cyclosporin could be a successful drug against IRI.

Table of Contents

Introduction

Transplantation of a kidney is often the only possible solution for acute kidney failure. Unfortunately, the number of people on the waiting list for an organ transplantation becomes much larger every year. As of August 2017 there were over 116,000 people on the national transplant waiting list in de United States[1]. Only 33,611 transplants were performed in the United Stated in 2016. This has as a result that 20 people die each day waiting for a transplant.

Because of this there is an increasing use of suboptimal deceased donors[2]. Organs from these donors have more damage and show a reduced chance for a proper function. Because of the donor shortage there came a need to increase preservation time and to improve graft function. This can be done by optimizing the preservation solutions and techniques and by adding certain drugs to the preservation solution or during reperfusion. This all intends to limit the damage of ischemic-reperfusion injury (IRI).

Ischemia is the process in which the kidney gets removed from the donor and the blood supply gets interrupted. This process causes a sequence of chemical events which lead to cellular dysfunction and eventually cell death[3]. Oxygen is crucial to maintain a regular cell function, but because of ischemia there is a lack of oxygen. The cells can't maintain homeostasis because of the exhaustion of the cellular energy stores and this leads to a loss of ion gradients across the cell membranes.[4].

During ischemia the kidney can be stored under hypothermic conditions, this is called Cold Storage (CS)[5]. This induces a lower metabolism in the cells, so the oxygen consumption decreases. If the cells are stored under hypothermic conditions they use less ATP and the Na^+/K^+ ATPase works less hard. Hypothermia is used to reduce the kinetics of metabolic activities that would otherwise lead to cellular degradation when oxygen is removed from the donor organ[5].

After CS preservation the blood flow to the kidney must be restored. This has two beneficial consequences for ischemic tissue: the energy supply is restored and toxic metabolites can be removed[3]. Reperfusion is essential for recovery from ischemic injury, but it may also cause metabolic consequences by bringing the toxic metabolites to the systemic circulation[6]. This could lead to further local tissue injury[7]. Paradoxically, reperfusion after ischemia leads to more damage. Cellular damage after reperfusion of ischemic tissue is defined as ischemia-reperfusion injury (IRI)[8]. During IRI damage the repair and regeneration processes occur together with cellular apoptosis, autophagy and necrosis[2]. The fate of the organ depends on whether cell death or regeneration comes out on top. It has a fundamentally influence on both the early and late function of a transplanted kidney.

A lot of the improvements to reduce the damage of IRI were discovered during the 20th century. In 1969 the EuroCollins' (EC) solution was developed and because of that the Cold Storage (CS) preservation time for kidneys was extended to 30 hours[9]. Since the 1980s the Belzer's University of Wisconsin (UW) solution has been used as the standard solution for organ transplants. The UW solution has a protective effect on renal vascular components[10]. The preservation time for kidneys was also extended from 24 hours in EC solution to 72 hours in UW solution[11].

The damage of IRI can also be reduced by adding drugs to the preservation solution or during reperfusion. An interesting drug to use is cyclosporin. This compound was successfully tested in transplant patients to prevent graft rejection in 1978[12]. This discovery had a huge impact in the field

of organ transplants and from then on the field became really important. Cyclosporin was discovered in the soil fungus *Tolypocladium inflatum* and is identified as a cyclic peptide which contains 11 amino acid peptide products.[13] Because of the use of cyclosporin and the improvement of preservation techniques the one-year graft survival for patients receiving cadaver kidneys was raised from 50% to 80-85%[14]. The one-year graft survival for patients receiving kidneys from living related donors raised from 75% to 95%. Many studies have shown that cyclosporin does not only works as an immunosuppressant, but it can also reduce the IRI damage[15]. Nevertheless it is also known that cyclosporin can lead to nephrotoxicity. The effect of cyclosporin on the IRI damage works via another pathway then the immunosuppressant effect, as shown by this study[16].

The aim of this thesis is to investigate if cyclosporin could be used as a drug to prevent kidneys from IRI damage after transplantation. We will do this by answering the following questions:

- Via what mechanisms is IRI caused?
- Via what mechanism protects cyclosporin the kidney from IRI?
- Via what mechanism causes cyclosporin nephrotoxicity?

Injury

Ischemic injury

Ischemia is defined as the stop of blood flow with immediate oxygen deprivation of cells. The lack of oxygen causes the cells to switch to the anaerobic glucose metabolism pathway[2]. This occurs within minutes after the stop of blood flow. Anaerobic metabolism does not generate enough energy for aerobic tissues like the kidney. In addition ischemia also causes metabolic substrate starvation, this can be observed by fewer glycogen granules in ischemic tissue. The glycolysis pathway can also not work properly because of the lack of ATP to phosphorylate fructose-6-phosphate. Because of the substrate starvation and the lack of ATP the anaerobic glycolysis is immediately brought to an arrest.

Anaerobic glycolysis also results in another process called acidosis. The cells will start with the fermentation of pyruvate to lactic acid to regenerate NAD^+. Because there is no blood flow to remove the lactic acid the cytoplasm becomes strongly acidic. The acidic environment also inhibits the ATP production and the energy production is brought to an arrest[2]. A lot of intracellular enzymes and regulator proteins get damaged because of the acidic environment and this leads to more cellular dysfunction[17].

During ischemia products are not washed out or eliminated and get a chance to accumulate. The ADP gets catabolized into adenosine, inosine and eventually hypoxanthine. The longer the duration of the ischemia, the more accumulation of hypoxanthine[18]. Hypoxanthine plays an important role during reperfusion injury. Even when there would suddenly be enough blood flow with enough oxygen there is no more ADP left in the cells to synthesize ATP.

Because of the ATP depletion the Na^+/K^+ membrane phosphatase gets inhibited. The cell cannot maintain membrane potential and cell excitability, because these processes need the gradient-driven K+ and Na+ ion transport. Besides, inhibition of Na+/K+ can lead to edema. Because sodium isn't dependent on a gradient-driven pump it can just enter the cells. It enters the cytoplasm and takes a large amount of water with it. This causes cell swelling. In addition, the lack of energy in the cells eventually stops the glycolysis. Because of this a lot of intermediates such as glucose-6-phosphate and α-glycerol phosphate accumulate. Together with the dephosphorylation of ATP into inorganic phosphate and adenosine this causes an enormous increase in the intracellular osmolarity. Hyperosmolarity causes water to become intracellular via diffusion, aquaporins and chloride channels. The last pathway is thought to be the main pathway responsible for ischemic edema. Especially when a perfusion solution is used with a low osmolarity during machine perfusion the intracellular hyperosmolarity becomes even bigger and the formation of edema gets accelerated. Edema leads to a disruption of the outer cellular membrane, but also of the endoplasmatic reticulum, Golgi apparatus, mitochondrial membranes and cytoskeletal microtubules.

Because sodium is accumulating in the cell and the Na-K-ATPase cannot remove this, the Na^+Ca2^+ starts pumping calcium into the cell. [19].This creates an intracellular calcium overload. The influx of calcium causes translocation of the Na/K-ATPase to the cytoplasm[20]. This translocation increases the shortage of the Na/K-ATPase. It is necessary to maintain Ca^{2+} in the mitochondrial matrix to preserve the mitochondrial membrane potential[21]. Intracellular calcium overload induces a whole cascade of reactions which eventually lead to permeating of cytochrome c into the cytosol. Cytochrome C activates caspase 3 leading to apoptosis.

High concentrations of cytosolic calcium lead to an activation of cytoplasmic phospholipases and proteases. Calcium-dependent cysteine proteases (calpains) cleave proteins, such as protein kinase C, and they can also convert xanthine dehydrogenase (XD) into xanthine oxidase (XO)[17]. XO is an enzyme that produces Reactive Oxygen Species (ROS) and induces damage during reperfusion. It is showed that phospholipase A2 plays an important role in ischemic injury[22]. Myocardial IRI was decreased in phospholipase A2 knock out mice partly through the suppression of neutrophil cytotoxic activities. Phospholipase A2 leads to degradation of membrane phospholipids to free fatty acids. This leads to intracellular and cell membrane structural alterations.

During ischemia nuclear factor-kappaβ (NFκβ) gets activated because of cellular stress[23]. This leads to the production of inflammatory mediators. NFKβ activates platelet-activating factors as well as inflammatory cytokines and their receptors. Because of this the neutrophils can pass through the vascular endothelium. Because of changes of the cell membrane and the high amount of neutrophils, capillaries may become obstruct by neutrophils[24].

Reperfusion

Reperfusion injury develops during hours and days after the initial insult. When the organ is again provided from blood and oxygen a lot of processes start. The organ can start to recover and regenerate, but there will also be additional injury. Reperfusion may augment the tissue injury that is produced during ischemia.

When the organ is provided from a sufficient amount of blood and oxygen it can start the aerobic metabolism again. This way the cells get provided with enough ATP. The cells need enough energy to induce apoptosis, so this process will start after reperfusion. During the ischemic injury there is a lot of cytochrome c released and there are many caspases activated. Apoptosis gets induced because of reduced mitochondrial activity and loss of electric potential. Only 5 minutes after reperfusion the cytochrome release and caspase activation can be measured in cardiomyocytes[25]. Normally ATP[26] will prevent the mitochondrial Permeability Transition Pores from opening, but since there is a calcium overload and not enough ATP the mPTPs open and this results in depolarization of the mitochondrial membrane[27]. During reperfusion the XO will combine with H_20 and the accumulated hypoxanthine to create uric acid and superoxide. A burst of free radical generation will occur within 10 seconds after reperfusion[28].

The cells are able to recover from the calcium overload if the cytosolic calcium levels return to normal after reperfusion[29]. In case the cytosolic calcium levels do not return to normal the injury is irreversible. This could be because of the use of a damaged organ from a suboptimal donor or because the ischemia period was too long. Beyond this time limit there is too much mitochondrial dysfunction and the damage becomes irreversible[16].

Because of decreased sodium reabsorption, distal segments of the tubule are activated to release signals that induce constriction of the vasa afferentia to prevent the loss of extracellular volume and sodium[30].

The hypoxanthine that is accumulated during ischemia provides a large amount of substrate for superoxide production during reperfusion. Superoxide radicals get converted into hydrogen peroxide. The large amounts of superoxide and hydrogen peroxide overwhelm the antioxidant

defense mechanisms. This results in the production of hydroxyl radicals and this leads to oxidant-induced tissue injury (figure 1)[18]. The oxidants induce the generation of leukotriene B4, complement C5a, and platelet-activating factor. These promote chemotaxis and the adherence of neutrophils to endothelial cells[17]. Even after reperfusion, the redistribution of blood to affected areas may not produce enough force to clear the blood vessels from the accumulated neutrophils[24].

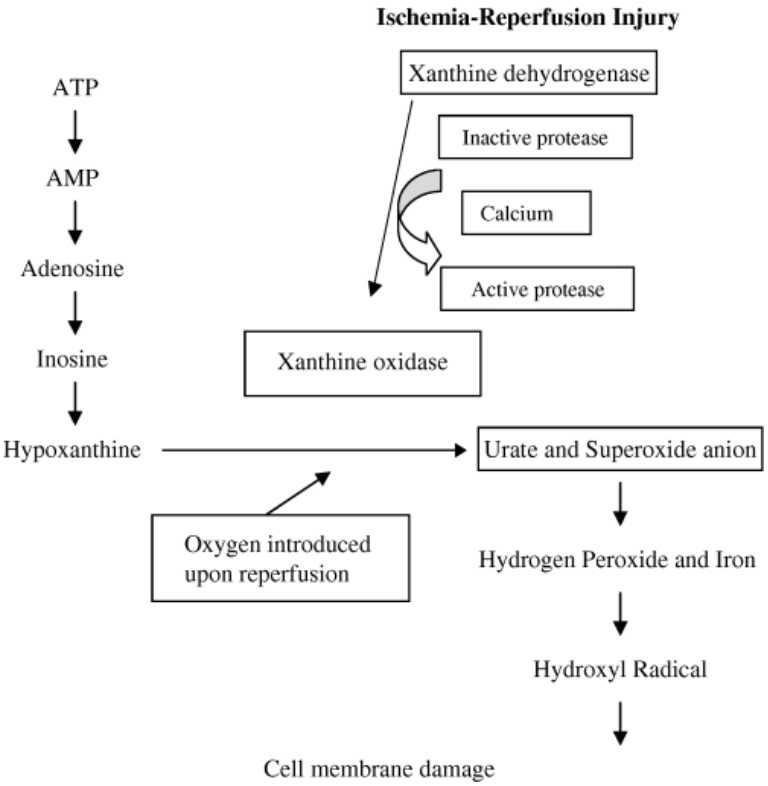

Figure 1: How the degradation of ATP during IRI leads to cell membrane damage via the formation of ROS.[31]

After reperfusion there is an influx of neutrophils and leukocyte function associated molecule-1 (LFA-1)[31]. Cell adhesion molecules increase and are especially localized on the endothelium elastic vessels. After 24 hours till 1 week there are Very Late Antigen-4 (VLA-4)-positive monocytes infiltrate in vessels expressing Vascular Cell Adhesion Molecule-1 (VCAM-1). Matrix proteins, fibronectin and laminin also play important roles to facilitate leukocyte migration across vessels[2].

During reperfusion the damaged endoplasmatic reticulum fragments increase and this causes the formation of autophagosomes[32].

Cyclosporin as nephrotoxic agent

Cyclosporin leads to nephrotoxicity by causing an imbalance between renal vasoconstriction and renal vasodilatation. There is no specific mediator of cyclosporin toxicity, but it is rather caused by an imbalance between several regulatory mechanisms[55]. Cyclosporin also induces fibrosis in the kidneys[33].

Induction of Vasoconstriction systems

Cyclosporine can induce vasocontriction in the kidney via several pathways. Cyclosporin stimulates endothelin synthesis and its release from renal cells. In this[34] study it is shown that anti-endothelin antibodies or endothelin receptor antagonists reduced this release. Cyclosporin also leads to an increase in endothelin-1 levels in the liver and kidneys during rat liver allograft rejection[35]. Endothelin-1 gets cleaved after translation into proendothelin. Endothelin can lead to hypertension and progressive renal injury via the activation of the ET_A-receptor.[36]

This[37] study shows that Cyclosporin leads to an increase in sympathic nervous system activity. The excitatory response of the sympathic nervous system depends on baroreceptor afferent input. This response leads to an upward resetting of the arterial baroreflex and thus renal vasoconstriction.

This[38] study shows that cyclosporin leads to microvascular constriction and hypoperfusion. Antioxidants are able to diminish the effect of cyclosporin on the microvascular constriction. This suggests that cyclosporin induces the formation of ODFR and causes microvascular constriction and hypoperfusion via this pathway.

It has been demonstrated that adenosine plasma levels are increased in cyclosporine treated kidney transplant recipients and that these adenosine plasma levels correlate with the cyclosporin blood levels[39]. Adenosine is an immunosuppressor via a transformation into deoxyadenosine. Deoxyadenosine gets converted into the toxic compound deoxyadenosine triphosphate that inhibits T-cell function. Adenosine can also work via another pathway that may be involved in cyclosporin induced renal toxicity. Adenosine can bind to high affinity A1 receptors inducing renal vasoconstriction and with low affinity A2 receptors inducing vasodilation[40]. The high adenosine levels are caused by the mechanism in which cyclosporin inhibits the uptake of adenosine by red blood cells. High plasma levels of adenosine caused by cyclosporin induce vasoconstriction.

Inhibition of Vasodilation systems

Cyclosporine creates such a strong vasoconstriction that the Nitric oxide (NO)-dependent counter-regulatory mechanism is overwhelmed[55]. NO normally induces vasodilation, but this mechanism is impaired during cyclosporin treatment. The nephrotoxic effect of cyclosporin increases when rats are treated with a NO synthase inhibitor[41]. The nephrotoxic effect of cyclosporin decreases if rats are treated with a substrate for the NO synthase[42]. This suggests that vasorelaxation mediated by nitric oxide in renal vessels is impaired because of cyclosporin treatment. However, cyclosporin causes an increase in NO production. It seems that NO is an important protective mechanism against the nephrotoxic effect of cyclosporin. Despite the increase in NO production this mechanism is not capable of protecting the kidneys from toxicity, because the vasoconstriction overwhelms it.

Cyclosporin leads to an increase in expression of the kallikrein-kinin system[43]. This system is involved in vasodilation and blood pressure regulation. Tissue kallikrein cleaves kininogen to release the vasoactive bradykinin peptide. Kinins bind to bradykinin B1 and B2 receptors and induce a lot of

biological effects, including vasodilation, blood pressure reduction and increased renal blood flow. When rats are treated with cyclosporin the immunoreactive renal kallikrein and urinary excretion of tissue kallikrein levels increase. Kallikrein-releasable kininogens in sera increased because of the cyclosporin treatment. A chronic treatment with cyclosporin led to an increase in renal kallikrein, bradykinin B2 receptor and hepatic kininogen mRNA levels. There was also an increase in urine excretion and water intake after chronic cyclosporin treatment. This data implies that the activity of the kallikrein-kinin system is increased to induce vasodilatation and prevent from hypertension caused by cyclosporin.

Renal fibrosis

Cyclosporin leads to an increase in expression of Transforming Growth Factor-β (TGF-β)[33]. TGF-β has multiple actions in tissue repair and can bind to 3 different TGF-β membrane receptors. TGF-β can strongly increase the deposition of extracellular matrix by creating an increase in the synthesis of matrix molecules like fibronectin, collagen and proteoglycans. TGF-β can also inhibit the degradation of extracellular matrix by decreasing the secretion of proteases and activating the production of protease inhibitors. The integrin matrix receptors on cells, which are able to facilitate cell matrix adhesion and matrix deposition are also modulated by TGF-β. Via these 4 pathways TGF-β is able to strongly increase the deposition of extracellular matrix. In addition, TGF-β auto induces its own production (figure 3). This way its effects become even greater and the renal fibrosis will lead to a decreased renal function.

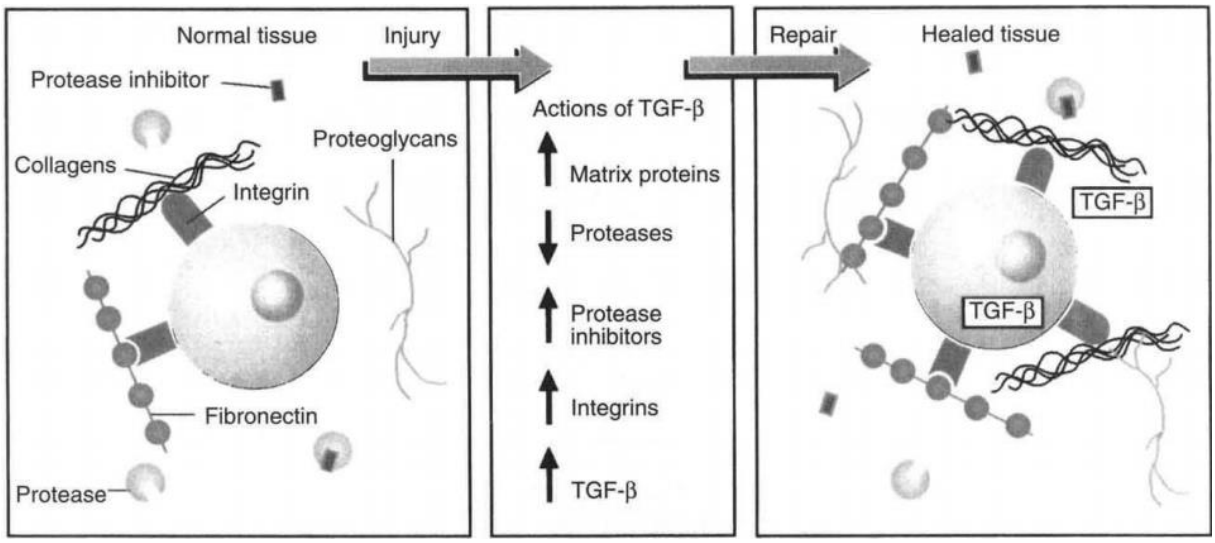

Figure 2: The mechanism of TGF-β which leads to extracellular matrix deposition and eventually fibrosis[44]

Cyclosporin as protecting agent

Cyclosporin may lead to a protective effect on kidneys via a mechanism that has an effect on apoptosis[45]. During reperfusion ROS species are formed. These ROS species activate the mitochondrial permeability transition pore and release cytochrome C into the cytosol. This leads to an activation of caspases and eventually cell death.

This[46] research shows that cyclosporin can inhibit ROS induced apoptosis. Apoptosis is characterized by a cascade of events which eventually will lead to DNA fragmentation. Pre-treatment of the fibroblasts with cyclosporin reduced the amount of DNA fragmentation caused by ROS. The effect of cyclosporin on the physiological state of the mitochondria was also assessed by measuring the MTT reduction. Pre-treatment with cyclosporin reversed the decrease in mitochondrial function caused by ROS. Because a disruption in the mitochondria leads to the release of cytochrome C into the cytosol the amount of it in the cytosolic fraction was also measured. Pre-incubation with cyclosporin suppressed the release of cytochrome C. Pre-incubation with cyclosporin also leads to a lower amount of caspase activity. This study shows that ROS causes dysfunction of the mitochondria, which leads to the release of cytochrome C in the cytosol and eventually apoptosis. The most crucial part of this research is that the MTT reduction measurements proved that cyclosporin reversed the decrease in mitochondrial function caused by ROS. Thus, cyclosporin inhibits apoptosis early in the pathway and the protective effect of cyclosporin seems to involve an influence on the mitochondria itself.

Cyclosporin inhibits Cyclophilin D with a high affinity and this leads to desensitization of the mitochondrial permeability transition pore (mPTP)[47]. The mPTPs are an important regulator of mitochondrial Ca^{2+} exchange. Cyclosporin inhibits opening of the mPTP via binding to Cyclophilin D and thus prevents depolarization of the mitochondrial inner membrane.[48-49].

This[50] study showed that Cyclophilin is a protein with peptidyl prolyl *cis trans* isomerase (PPIase) activity and that inhibition of PPI by cyclosporin leads to inhibition of the opening of the mPTPs. According to multiple studies[16-51] cyclosporin inhibits the opening of mPTPs via binding to PPI and then displacing it from the membrane protein. The Cyclophilin translocation to the inner membrane of the mitochondria is blocked. This leads to a decrease in sensibility of the mPTPs for calcium. As explained earlier the calcium overload and lack of oxygen cause the mPTPs to open during reperfusion. This leads to dysfunction of the mitochondria and eventually apoptosis. Cyclosporin affects this process during IRI by decreasing the sensibility of the mPTPs for calcium. This way there will be less mitochondrial dysfunction and apoptosis.

Cyclosporin should be administered right before the start of reperfusion and during reperfusion. When it is administered during the ischemia period alone it will not have an effect, because the mPTPs are not opened during ischemia. During reperfusion the mPTPs open and when cyclosporin is administered it can prevent the mPTPs from opening[27]. This way there will be less cytochrome C released into the cytosol and eventually less apoptosis. This study shows that cyclosporin is the most effective when administered during ischemia and reperfusion[16].

Conclusion

To conclude, literature provides enough information to know which effects are caused by cyclosporin and via which action mechanisms they work. The first damage during a kidney transplantation is caused by ischemic injury. Because of the interrupted blood flow the cells aren't provided with enough oxygen to maintain the aerobic metabolism. ATP gets catabolized into adenosine, inosine and eventually hypoxanthine. Sodium entering the cytoplasm together with large amounts of water and accumulation of many intermediates which increase intracellular osmolarity lead to cell swelling. Because of the fermentation of pyruvate into lactic acid the cytoplasm becomes strongly acidic and this causes more cellular dysfunction. The mitochondrial membrane potential can't be maintained and cytochrome C will be released into the cytosol. This leads to an activation of caspases and eventually cell death. Because of the high concentrations of cytosolic calcium the calpains get activated en xanthine dehydrogenase gets converted into xanthine oxidase. NFκB also gets activated and results in the production of inflammatory mediators.

During reperfusion the kidney gets again provided from enough blood and oxygen and can restart the aerobic metabolism. Apoptosis can only be induces if there is enough ATP in the cells. During ischemic injury there are a lot of caspases activated and during reperfusion this will induce apoptosis. The mitochondrial Permeability Transition Pores will open and cause desensitization of the mitochondrial membrane. The hypoxanthine that is accumulated during ischemic injury gets converted into superoxide radicals during reperfusion. The antioxidants can't protect against these large amounts of radicals and it will result in oxidant-induced tissue injury.

Cyclosporin causes nephrotoxicity via a misbalance between vasoconstriction and vasodilation and via inducing fibrosis. Vasoconstriction is caused via active renin, endothelin, sympathic nervous system, oxygen derived free radicals and adenosine. Vasodilation is caused via an increase in nitric oxide production and activation of the kallikrein-kinin system. Cyclosporin also leads to an increase in TGF-beta. This results in an increased deposition of extracellular matrix and decreased degradation of extracellular matrix. This eventually leads to renal fibrosis.

Cyclosporin protects the kidneys from apoptosis via the mitochondria. The binding of cyclosporin to cyclophilin with a PPIase activity leads to a block of its translocation to the inner membrane of the mitochondria. This leads to desensitization of the mitochondrial permeability transition pores. The pores are inhibited from opening and thus there is less release from cytochrome C. Eventually this will lead to less activation of caspases and apoptosis.

Cyclosporin does not lead to a full recovery of ischemic-reperfusion injury because it only inhibits the mPTPs from opening. As explained in the chapter of IRI there are many pathways via which damage is caused. Cyclosporin balances between the protective effects caused by the binding to Cyclophilin and the toxic effect caused by an imbalance between vasoconstriction and vasodilation. This[16] study shows that the protective effect of cyclosporin prevails when used in low concentrations (2-5 nm), but the toxic effect prevails when used in high concentrations (1 µM). This study[52] shows that when a concentration of 1.25-10 mg cyclosporin/kg body weight in gerbils is used it leads to the protective effect. For the immunosuppressive effect that helps preventing against graft survival there are concentration of 5 mg cyclosporin/10 kg body weight used in rats[53]. Thus, the protective effect of cyclosporin is concentration dependent. This can be explained by the fact that cyclosporin binds with a high affinity to cyclophilin D. So in low concentrations cyclosporin will only work via the mechanism of inhibiting the mitochondrial permeability transition pores. When used in high concentrations not all the cyclosporin will bind to cyclophilin D, but a lot of the cyclosporin will bind on the parts that

cause vasoconstriction and renal fibrosis, thus nephrotoxicity. Although differences between species should be taken account it can be concluded that is concentration of around 5 mg/kg are used there will be both an immunosuppressive and protective effect of cyclosporin on graft survival.

To conclude this thesis cyclosporin could be used as a drug to prevent kidneys from IRI damage. Cyclosporin should be administered during ischemia and reperfusion in low concentrations. As long as cyclosporin is administered in low concentrations there will not be a nephrotoxic effect, because the protective and toxic effects are caused via 2 different mechanism and these are concentration dependent. Cyclosporin alone cannot protect the kidney from all the damage induced during ischemia and reperfusion. It could be that if the patients are treated with a combination of cyclosporin and other protective compounds like free radical scavengers, adenosine and calcium antagonist the kidney will show less IRI[16]. The use of glycine and an acidotic pH during ischemia could also help in preventing the kidneys from IRI[54]. More research is necessary to investigate cyclosporin as a drug against IRI damage. It may not conquer the damage on its own, but with help of some other compounds it could definitely be a successful drug against IRI.

References

[1] U.S. Department of Healh&Human Services. (2016). *Organ Donation Statisics*. U.S. Government Information on Organ Donation and Tranplantation. U.S. Department of Healh&Human Services.

[2] Kosieradzki, M., Rowinski, W. (2008).*TransplantProc Ischemia/reperfusion injury inkidney transplantation: mechanisms and prevention.* 40(10):3279-88

[3] Grace, P.A. (1994). *Ischaemia-reperfusion injury*. British Journal of Surgery. 81, 637-647

[4] McCord, J.M. (1985). *Oxygen-derived free radicals in postischemic tissue injury*. N Engl J Med. 7;312(3):159-63

[5] Lee, C.Y., Mangino, M.J. (2009) *Preservation methods or kidney and liver*. OrganoGenesis. 5(3): 105-112

[6] Himovici, H. (1979). *Muscular, renal, and metabolic complication of acut arteriol occlusions: monphropathic-metabolic syndrom.* Surgery ; 85(4):461-8

[7] Parks, D.A., Granger, D.N. (1986). *Contributions of ischemia and reperfusion to mucosal lesion formation*. Am J Physiol. 250(6 Pt 1):G749-53

[8] De Rosa, S., Antonelli, M., Ronco, C. (2017). *Hypohermia and kidney: a focus on ischaemia-reperfusion injury*. Nephrology Dilysis Transplanation. 32 (2): 241-247.

[9] Collins, G.M., bravo-Shugarman, M., Terasaki, P.I. (1969*). Kidney Preservation for transportation: initial perfusion and 30 hours ice storage*. The Lancet. 94(7632): 1219-1222

[10] Ueda, Y., Todo, S., Imventarza, O., Furukawa, H., Oks, A., Wu, Y.M., Oguma, S., Starzi, T.E. (1990*). Specific Effect of University of Wisconsin Solution on Renal Hemodynamics and Microvasculature in Canine Kidney Preservation*. Computer File

[11] Ploeg, R.J., Goossens, D., McAnulty, J.F., Southard, J.H., Belzer, F.O. (1988). *Successful 72-hour cold storage of dog kidneys with uW solution.* Transplantation, 46(2), 191-6.

[12] Rang, H. (2015). *R*ang and Dale's Pharmacology (8th edition). Elsevier Health Sciences.

[13] Watts, R. (2009) *Oxford Desk Reference Rheumatology.* Oxford University Press

[14] Sigal NH, Dumont FJ ((1992). *Cyclosporin A, FK-506, and rapamycin: Pharmacoloic probes of lymphocyte signal transduction.* Annual Rev. Immunol. 10:519-560

[15] Agnès M, Arnaud B, Michel V, Bruno B. (2004). *Cyclosporin A but not estradiol can protect endothelial cells against etoposide-induced apoptosis*. Endothelium. 11 (3/4): 141-149.

[16] Griffiths EJ, Halestrap AP (1993). *Protection by Cyclosporin A of ischemia/reperfusion-induced damage in isolated rat hearts.* Journal of molecular and cellular cardiology.25(12):1461-1469

[17] Moore, R.M., Muir, W.W., Granger, D.N. (1995) *Mechanisms of Gastrointestinal Ischemia-Reperfusion Injury and Potential Therapeutic inventions: a review and its implications in the horse*. J Vet InternMed. 9:115-132

[18].McMichael, M., Moore, R.M. (2004*). Ischemia-reperfusion injury pathophysiology, part 1*. Journal of veterinary Emergency and Critical Care. 14(4): 231-241.

[19] Schumacher, C. A., Baartscheer, A., Coronel, R., Fiolet, J.W.T. (1998). *Energy-dependent transport of calcium to the extracellular space during acute ischemia of the rat heart*. Journal of Molecular and Cellular Cardiology. 30 (8): 1631-1642.

[20] Dunbar, L.A., Caplan, M.J. (2001*). Ion Pump in Polarized Cells: sorting and regulation of the Na+, K+- and H+, K+-ATPases.* Journal of biological chemistry. 276, 28617-29620

[21]Chen, Q., Lesnefsky, E.J. (2007). *Modulation of electron transport protects cardiac mitochondria and decreases myocardial injury during ischemia and reperfusion*. Cell Physiology. 292 (1); C137-147

[22] Fujioka, D. Kugiyama, K. (2008). *Reduction in myocardial ischemia/reperfusion injury in group X secretory phospholipase A2-deficient mice*. Molecular Cardiology, Circulation. 117:2977-2985

[23] Breton, A. (2010). *Ischemia and Reperfusion Injury: When Cells Almost Die* Veterinary Technician. 31;7

[24] Ambrosio, G., Tritto, I. (2002). *Myocardial reperfusion injury*. Eur Heart J Supll; 4(suppl B):B28-B30

[25] Vanden Hoek, T.L., Qin, Y. (2003). *Reperfusion, not simulated ischemia, initiates intrinsic apoptosis injury in chick cardiomyocytes*. Heart and Circulatory Physiology. 284 (1): H141-H150.

[26] MCGuinnes O, Crompton M (1990). *CyclosporinA and mitochrondrial dysfunction.* Biochem Soc Trans. 19:883-884.

[27] Hausenloy DJ, Boston-Griffiths EA, Yellon DM. (2012). *Cyclosporin A and cardioprotection: from investigative tool to therapeutic agent.* British Journal of Pharmacology. 165 (5): 1235-1245

[28] Zweier JL, Flaherty JT, Weisfeldt ML. (1987) *Direct measurement of free radical generation following reperfusion of ischemic myocardium.* Proc Natl Acad Sci USA. 84(5);1404-7

[29]Lee, J.A., Allen, D.G. (1992*). Changes in intracellular free calcium concentration during long exposures to simulated ischemia in isolated mammalian ventricular muscle*. Circulation research. 71:58-69

[30] Kellum, J.A. Ronco, C., Vincent, J.L. Karger. (2011). *Controversies in Acute Kidney Injury*. 174: 12-21.

[31] Luft, F.C., Haller, H. (2000). *Ischemia-reperfusion injury in renal transplantation is independent of the immunologic background*. Kidney International. 58 (5): 2166-2177

[32] Kirini, T, Sano, K. (1984). *Fine structural Nature of Delayed Neuronal Death Following Ischemia in the Gerbil Hippocampus*. 62: 209-218

[33] Mohamed MAS, Robertson H, Booth TA, Balupuri S, Gerstenkorn C, Kirby JA, Talbot D. (2000). *Active TGF-β1 expression in kidney transplantation: a comparative study of cyclosporin-A (CyA) and tacrolimus (FK506)*. Transpl Int. 13:S295-S298

[34] Kohan, D.E. (1997). *Endothelins in the normal and diseased kidney*. Am J Kidnet Dis 29:2-26

[35] Shiraishi M., Kusano T, Hara J, Hiroyou A,, Miyaguni T., Muto Y. (1997). *The effect of cyclosporin on endothelin levels after orthotopic liver transplantation in rats*. Transplant Int; 10:207-211

[36] Granger J, Abram S, Stec D, Chandler D, Speed J, LaMarca B. (2006) *Endothelin, the Kidney and Hypertension.*Current hypertension reports. 8:298-303

[37] Ryuzaki M, Stahl LK, Lyson T, Victor RG, Bishop VS. (1997*). Sympathoexcitatory Response to Cyclosporin A and baroreflex resetting*. Hypertension;29:576-582

[38] Krysztopik, Richard J, Bentley, Frederick R, Spain, David A, Wilson, Mark A, Garrison, R. Neal. (1997*). Lazaroids prevent acute cyclosporine-induced renal vasoconstriction*. Transplantation. 63(9), 1215-1220

[39] Guieu R, Dussol B,Devaux C, Sampol J, Brunet P, Rochat H, Bechis G, Berland YF. (1998). *Interactions between cyclosporine A and adenosine in kidney transplant recipients*. Kidney international. 53 (1L): 200-204

[40] Murray, BM, Churchill PC. (1985). *Concentration dependency of the renal vascular and renin secretory responses to adenosine receptors agonists*. J Pharmacol Exp Ther. 231L: 189-193

[41] Bobadilla NA, Gamba G, Tapia E, Garcia-Torres R, Bolio A, Lopez-Zetina P. (1998). *Role of NO in cyclosporin nephrotoxicity: effectsof chronic NO inhibition and NO synthases gene expression*. Am J Physiol 274:7791-798

[42] Assis SMA, Monteiro JL, Seguro AC. (1997). *L-arginine and allopurinol protect against cyclosporine nephrotoxicity.* Transplantation. 63:1070-1073

[43] Wang C, Ford P, Chao C, Chao L, Chao J. (1997). *Effect of cyclosporin A on the expression of tissue kallikrein, kininogen and bradykinin receptor in rat.* Am J of Physiology, Renal Physiology. 273 (5): F783-F789

[44] Border WA, Noble NA. (1997). *TGF-β in kidney fibrosis: a target for gene therapy*. Kidney International. 51: 1388-1396

[45] Walter D.H., Haendler J., Galle J., Zeiher A.M., and Dimmeler S. (1998). *Cyclosporin A inhibits apoptosis of human endothelial cells by preventing release of cytochrome C from mitochondria*. Circulation, 98, 1153– 1157.

[46] Sugano, N., Ito, K., Murai, S. (1999). *Cyclosporin A inhibits H202-induced apoptosisof human fibroblasts*. FEBS letters 447: 274-276

[47] Broekemeier, K.M., Pfeiffer, D.R. (1995*). Inhibition of the mitochondrial permeability transition by cyclosporin A during long time frame experiments: relationship between pore opening and the activity of mitochondrial phospholipases.* Biochemistry, 34;16440-16449

[48] Leytin V, Gyulkhandanyan A, Mutlu A, Mykhaylov S, Allen D, Freedman J. (2012). *Co248 mPTP-dependent and – independent mechanisms of platelet apoptosis: Effects of cyclosporin A*. Thrombosis Research. 130 (1): S198-199

[49] Elrod J.W., Wong R., Mishra S., Vagnozzi R.J., Sakthievel B., Goonasekera S.A. et al. (2010). *Cyclophilin D controls mitochondrial pore-dependent Ca^{2+} exchange, metabolicflexibilit, andpropensity for heart failure in mice.* J. Clin. Invest. 120, 3680–3687

[50] Fischer G, Wittman-Liebold B, Lang K, Kiefhaber T, Schmid FX (1989). *Cyclophilin and peptidyl-prolyl cis-trans isomerase are probably identical proteins. Nature 337:476-478.*

[51] Fauvel, H, Marchetti, P, Obert, G. (2002). *Protective effects of cyclosporin A from endotoxin-induced myocardial dysfunction and apoptosis in rats*. Am J Respir Crit Care Med. 165: 449–455.

[52] Domañska-Janik K, Buzañska L, Dluzniewska J (2004). *Neuroprotection by cyclosporin A following transient brain ischemia correlates with the inhibition of the early efflux of cytochrome C to cytoplasm.* Molecular Brain Research. 121(1-2):50-59

[53] Gulati AK, Zalewski AA (1982). *Muscle allograft survival after cyclosporin A immunosuppression.* Experimental Neurology. 77(2):378-385

[54] Nishimura Y, Romer LH, Lemasters JJ (1998). *Mitochondrial dysfunction and cytoskeletal disruption during chemical hypoxia to cultured rat hepatic sinusoidal endothelial cells: the pH paradox and cytoprotection by glucose, acidotic PH and glycine.* Hepatology. 27(4):1039-1049

55 Ader JL, Rostaing L. (1998). *Cyclosporin nephrotoxicity: pathophysiology and comparison with FK-506*. Nephrology and Hypertension;7:539-545