Delayed graft function in renal transplantation

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CHAPTER 1

INTRODUCTION AND OUTLINE OF THE THESIS

INTRODUCTION

Acute renal failure (ARF) on the basis of acute tubular necrosis (ATN) was defined in the early days of the 20 *th* century by the German pathologist Hackstadt. His observations were based on the clinical evaluation of soldiers who sustained severe traumatic injury. However its significance was more or less neglected until the second world war, when Bywaters described the crush injury syndrome in victims of the London Blitz (1). Initially the underlying morphological changes in this kind of renal failure was thought to be related to distal tubular injury due to pigment toxicity and the term lower nephron nephrosis was introduced for this clinical condition (2). Later classic micro dissection studies in kidneys of rats showed that the dominant side of injury in ATN was the straight segment of the proximal tubules (S3 segment, pars recta) (3).

Diversities of acute renal failure

The diversity of definitions has hampered the analysis with regard to the incidence of ARF, as it may present with or without oliguria. Some studies defined ARF on the basis of elevated serum creatinine concentration; others referred to the increments above baseline serum creatinine levels or included only patients with ARF who required dialysis. ARF is caused by a variety of different etiologies and occurs in a variety of clinical settings. In daily clinical practice, ARF due to hypo-perfusion is one of the most common forms of ARF, it may account for 40 to 80 per cent of all cases of ARF, and if prolonged, pre-renal ARF may develop into ATN. Postrenal causes of ARF by ureter-, bladder neck or urethral obstruction, are less common causes of ARF and are encountered in only 2 to 10 per cent of all cases. Renal causes of ARF are diseases like acute and crescentic glomerulonephritis, hemolytic uremic syndrome, interstitial nephritis and ATN.

Acute tubular necrosis

Studies on patients with ARF due to ATN illustrate that different insults to renal function can be present simultaneously. The most common predisposing factor in the development of ATN appears to be renal ischemia resulting from prolonged oxygen deprevation resulting clinically in pre renal azotaemia. Sepsis is a major predisposing factor and nephrotoxins account for about 25 per cent of all cases of ATN (amino-glycosides, radiocontrast media, organic solvents, NSAID's and heavy metals). ATN can also be the result of heavy loads of haem pigment to be excreted by the kidney, such as in rhabdomyolysis or haemolysis (4). It has been estimated that some 40 to 60 per cent of cases of ATN occur in a postoperative or trauma related setting, while the remainders emerge in a medical setting. The causes of the renal failure in these circumstances are often multifactorial in origin. High-risk settings are abdominal aortic aneurysm surgery, severe burns, pancreatitis, and advanced chronic liver disease. Mortality associated with ATN was reported to be between 37 % and 79 % (5).

Pathophysiology of renal failure in ATN

One of the possible mechanisms of the decreased GFR in ARF due to ATN is related to tubular damage resulting from ischemia/reperfusion injury. Degeneration, necrosis and exfoliation of tubular epithelium, interstitial edema and interstitial cellular infiltration are usually observed in biopsies in ARF (6). There are two possible explanations for the impaired glomerular filtration rate.
One of the possible mechanisms of decreased GFR is tubular obstruction and tubular backleakage or there is a maladaptive response of the tubuloglomerular feedback loop.
Tubular obstruction and backleakage: In the early phase of ATN, tubular obstruction by exfoliated tubular cells and protein precipitates results in tubular obstruction and elevated proximal and distal intratubular pressures and a low net filtration pressure (7). Although obstruction is morphologically hard to demonstrate, since it can be present on many levels of the nephron, high intratubular pressures can be demonstrated in animal models (8,9). Increased intratubular pressures are always present in cases of ischemic ATN but they do not sustain, as they decline in a period of days to normal or even low levels. Lowering of intratubular pressure is not uniformly associated with recovery of renal function. Another possibility, associated with the obstruction theory, is the existence of backleakage. Backleakage of glomerular filtrate was demonstrated and associated with severe tubular dysfunction in animal models (10,11). Myers et al demonstrated the existence of backleakage in human ATN (12). The underlying pathophysiological mechanism is the disruption of the intercellular tight junctions in damaged tubular cells. Although it explains the impaired glomerular filtration rate in more severe ATN cases (12) it does not explain the decline of GFR in milder cases of ATN.
Decreased tubuloglomerular feedback: Another explanation for the relation between decline in glomerular filtration and tubular damage, is the reduction in renal bloodflow under the influence of decreased sodium and chloride reabsorption. The supply of chloride and sodium to the juxtaglomerular apparatus leads to an afferent and efferent vasoconstriction and diminished renal blood flow and glomerular filtration pressures (13,14). This can be seen as a maladaptive mechanism of a teleologically appropriate mechanism of the kidney to prevent excessive loss of fluid and electrolytes (15).

ARF in renal transplantation

In renal transplantation a delayed start of graft function (DGF) is found in 10-50% of cases (16-21). Literature on the pathophysiology and morphology of ARF in transplanted kidneys is scarce. Although risk factors for DGF and ARF in native kidneys are different in transplanted kidneys than in native kidneys, the underlying morphological and pathophysiological characteristics are often considered to be similar. The prevailing views are mainly based on studies of acute tubular renal failure in experimental animals (22).
In renal transplantation risk factors for delayed renal function immediately after transplantation are well known. They can be divided into donor linked factors, factors related to the transplantation procedure and factors related to the post-transplant period. These clinical conditions are unique for the transplantation setting and therefore differ substantially from the conditions in which ARF occurs in native kidneys. This makes the comparison with ARF

in native kidneys hazardous. Experimental data on ischemia and reperfusion injury are also difficult to interpret since in these conditions the kidney usually is totally deprived from perfusion by clamping the renal artery. Despite these limitations, the literature on ischemic ARF in native kidneys and on experimental ATN could contribute to our understanding of the pathophysiology of DGF and its meaning for graft outcome and for the development of specific therapeutic interventions.

Definition of DGF

In most studies DGF is defined as the need of dialysis treatment in the first week. This is a criterion that is easy to register objectively and to obtain from large databases. However, dialysis during the first week after transplantation is also performed for other reasons than DGF, like hyperkaliemia and fluid overload. Others define for that reason DGF as a functional parameter distinct from the need of dialysis and use the time needed to achieve an arbitrarily defined creatinine clearance as a marker for DGF. Since, the pathogenesis of DGF is supposed to be of ischemic origin it is relevant to define DGF as a functional abnormality distinct from the need of dialysis treatment. In addition acute rejection should be excluded as a cause of DGF. We propose to use a functional definition using the decrease of serum creatinine of more than 10% per day for at least 3 consecutive days for more than 1 week after transplantation and excluding acute rejection and calcineurin inhibitor toxicity as a possible cause of this DGF. Using this definition, it is possible to analyze the risk factors and consequences of ischemic damage and associated reperfusion injury that is supposed to be the underlying cause of DGF and excluding changes of serum creatinine concentration by other causes like dilution, surgical complications, cyclosporin toxicity and acute rejection.

DGF and long-term graft survival

The effect of DGF on short term and long term patient and graft survival is still controversial. Some authors found a deleterious effect effect of DGF on graft survival (20,23) and others did not or only found this effect when it coincided with acute rejection episodes (24). These discrepancies may be related to the criteria used to define DGF or to differences in data analysis. Most authors used the need for dialysis within the first week as the diagnostic inclusion criterion but this does reflect the various causes of DGF such as ischemia- reperfusion injury, early acute rejection episodes or calcineurin inhibitor toxicity. In the UNOS registry, DGF was defined as the need for dialysis in the first week after transplantation. It had a significant and independent impact on graft half-life. This effect was independent from cold ischemia time, occurrence of acute rejection episodes, donor age and serum creatinine levels (25). Others found a detrimental effect of DGF, also defined as the need for dialysis in the first week, on graft survival only when it was complicated by one or more acute rejection episodes (24,26,27). When the time required to reach a Cockroft renal clearance of more than 10 ml/min was used, DGF lasting for more than 6 days had a deleterious effect on graft survival whereas DGF of shorter duration did not influence graft survival (28).
Looking at the different survival curves it is striking that one year after transplantation the survival curves essentially run parallel. This suggests an effect of DGF on graft survival wit-

hin the first year but no effect beyond. This is supported by studies that analyzed the risk factors on graft survival at different time intervals after transplantation and found that progression of chronic graft failure is mainly associated with donor age, creatinine clearance at 1 year after transplantation, proteinuria and the presence of hypertension in the recipient and not with the occurrence of DGF.

Risk factors of DGF

Risk factors for DGF can be divided in donor-related factors, transplantation-related factors and recipient related risk factors. The cadaveric kidney is subject to damage at every step along the way from procurement to reperfusion whereas kidneys from living donors rarely develop DGF.

Donor related risk factors

Well-known donor related risk factors for DGF are donor age over 50 years and an elevated serum creatinine or decreased renal function of the donor. In human adults total metabolism and renal function in terms of glomerular filtration rate and renal blood flow and muscle mass decrease with age. This implies that for the same serum creatinine concentration GFR in the elderly can be severely impaired in comparison to younger adults. Kidneys from older individuals may have several structural and functional changes compared with kidneys from younger donors. Longitudinal studies of elderly individuals have shown a diminution in renal reserve, along with functional constraints on the kidney's ability to respond appropriately to challenges of either excesses or deficits (29). Studies of kidneys obtained at autopsies demonstrated a progressive decrease in the number and size of glomeruli with age, resulting in a progressive decrease of the glomerular filtration volume (30,31). In addition to the loss of glomeruli, there is an age-dependent increase in the cortical interstitial volume as a result of progressive interstitial fibrosis (31,32). Most renal biopsies from kidney donors who are older than 40 yr show intimal fibrosis in the smaller arteries, arteriolar hyalinosis, and interstitial fibrosis (33).

Not only factors intrinsically related to the donor, but also the events prior to brain death and harvest of the kidney contribute to the occurrence of DGF. Before the establishment of brain death of the potential donor, the kidney may be damaged by the underlying disease process, or by the therapeutic maneuvers instituted in an attempt to revive the patient or to maintain circulation after brain death, like the use of dopaminergic medication and resuscitation procedures. Decreasing platelet count and disseminated intravascular coagulation are frequent and suggest that endothelial injury or dysfunction may already be present in the postmortal donor. During episodes of cardiac arrest or prolonged hypotension, the kidney will suffer from warm ischemia and reperfusion injury. Catecholamine release and pharmacological inotropes may contribute to intrarenal vasospasms leading to relative hypoperfusion. The donor will be in a catabolic state, making recovery from ischemic damage more difficult. Finally many donors have evidence of intravascular coagulation, which may be either a cause or a consequence of endothelial injury in the microcirculation. In addition after brain death but before explantation, the patient may not be considered a high priority for surgery at a busy intensive care unit and resuscitation may be delegated to those with limited experience in appropriate care (34).

Risk factors related to the transplantation procedure

Organ procurement also contributes to the development of DGF. This starts with hypoperfusion after circulation of the donor has stopped (warm ischemia time (WIT)). With the multi organ procurement procedures this WIT is limited to several minutes. However, with the raising interest of non-heart beating procedures, WIT becomes a serious contributor to DGF (35,36). During surgery errors in line placement can result in inadequate flushing of blood and cooling, and undue manipulation of renal arteries can induce vascular spasm. The most important independent and robust risk factor is cold ischemia time (CIT) (18,37-42). During preservation the kidney is exposed to ATP depletion probably enhanced by reperfusion induced vasoconstriction, resulting in apoptosis and necrosis of individual cells and leading to severe functional damage (43). The type of preservation fluid also has been recognized as a risk factor for DGF in a study in the Eurotransplant area, in which the preservation fluid developed by the University of Wisconsin (UW) appeared to be superior to Euro Collins (EC) (44). Cold pulsatile perfusion in which organs are perfused with a pulsatile preservation machine are described to have a lower incidence of DGF than organs that are preserved with a cold flush (40).

After perfusion is re-established several mechanisms exist that can damage the renal allograft including the generation of free radicals, mechanical injury to blood vessels from sudden high blood flow, vasomotor derangement from prostaglandins and other regulatory peptide imbalances and cytokine release from inflammatory infiltrates (45)

Recipient related risk factors

Recipient age is a risk factor for DGF especially when kidneys from pediatric donors to adult recipients are involved (20). The relation between the occurrence of DGF and the discrepancy between donor and recipient Body Mass Indexes (BMI) supports this hypothesis (46). Secondary hyperparathyroidism is also associated with a higher incidence of DGF (47,48). The lower occurrence rate of DGF with zero HLA mismatch and low levels of panel reactive antibodies (PRA) (49), suggests that immunology related factors are involved in the development of DGF. Since the studies that describe this effect, used dialysis treatment in the first week as their definition of DGF, it is very likely that early acute rejection activity is the missing link (20,40,50). In addition, the use of calcineurin inhibitors is a riskfactor because their vasoconstrictive properties influence renal perfusion and enhance ischemic damage (51).

Morphological characteristics of ATN in native kidneys and grafts

Changes in renal morphology that accompany ARF in native kidneys are subtle (52,53). In renal biopsies from patients suffering ARF, regeneration and necrosis coexist, suggesting that the injury and repair process coincide. Solez studied 57 biopsies from patients with ARF and defined twelve characteristic morphological items for ATN (52,53). These items represent the morphological consequences of necrosis and regeneration respectively. Markers for necrosis were: the presence of individual necrosis, loss of brush border, tubular dilatation and the presence of tubular casts, signs of interstitial inflammation and edema, hyperplasia of the juxta glomerular apparatus, dilatation of Bowman's space, tubularization of the parietal epithelial cells of Bowman's capsule. As signs of recovery the presence of mitotic figures and signs of tubular regeneration were studied. Also the accumulation of

leukocytes in the vasa recta were scored as signs of ATN. All items were scored on a 4 point scale from score 0 representing the absence of the item to score 3+, representing abundant presence of the item. Only the presence of necrosis of individual tubular cells and the loss of the brush border were specific for oliguric ARF compared to biopsies from patients that had recovered recently from ARF (52). ARF after renal transplantation has a distinctly different etiology than ARF in native kidneys. Therefore the same group of investigators compared the above mentioned 57 biopsies with 13 allograft biopsies from patients with DGF and 5 biopsies from grafts with a stable function. The most striking difference between the graft biopsies with DGF and the biopsies from native kidneys with ARF due to ATN was that the non-replacement phenomenon was seen more often in the grafts. They also had larger interstitial infiltrates than the native kidneys with ATN. Furthermore grafts with DGF showed slightly less frequent disappearance of the tubular brush border, fewer tubular casts, less dilation of the Bowman's space but a greater number polarizable oxalate crystals (6,54). Another striking feature in biopsies of ATN is the presence of an interstitial infiltrate. It is supposed to be related of the process of brain death (55) and ischemia/reperfusion injury leading to a local inflammatory reaction which is possibly associated with the production of free oxygen radicals (56,57). Ischemia and reperfusion injury is associated with an upregulation of pro-inflammatory cytokines like interleukines (IL-1, IL-6, IL-8 and IL-10) and monocyte chemoattractant protein 1 (MCP-1) (58-60). As a consequence adhesion molecules that are important in the migration of leucocytes, like intercellular adhesion molecule-1 (ICAM-1), vascular adhesion molecule (VCAM) and endothelial leukocyte adhesion molecule-1 (ELAM-1) (61,62) also are upregulated.
Marcussen concluded there were no differences between biopsies with ATN of native and transplanted kidneys, as far as the composition of the interstitial infiltrates is concerned (6) but that in contrast to acute rejection episodes the infiltrate in ATN consisted mainly of granulocytes. Haug and Dragun showed in animal models that the detrimental effects of ischemia and reperfusion could be minimized with neutropenia and the use of blocking anti-bodies against ICAM-1, LFA-1 and P-selectin (63) or ICAM-1 antisense oligodesoxynucleotides (64).

Pathogenesis of acute ischemic renal injury and subsequent recovery

In the pathogenesis of acute ischemic failure 3 stages can be recognized. The first stage is the **ischemic phase** in which ischemic and reperfusion injury takes place and in which renal epithelial and endothelial cells are subjected to lethal insults leading to apoptosis and /or necrosis (65). The **maintenance phase** represents a phase of stabilization of injury by intrinsic or upregulated defense mechanisms. During this phase events leading to cellular repair, proliferation and redifferentiation. lead to the **recovery phase** in which epithelial en endothelial function improve, leading to the recovery of renal function.

1. Ischemia and reperfusion in ATN

a. Molecular biological characteristics

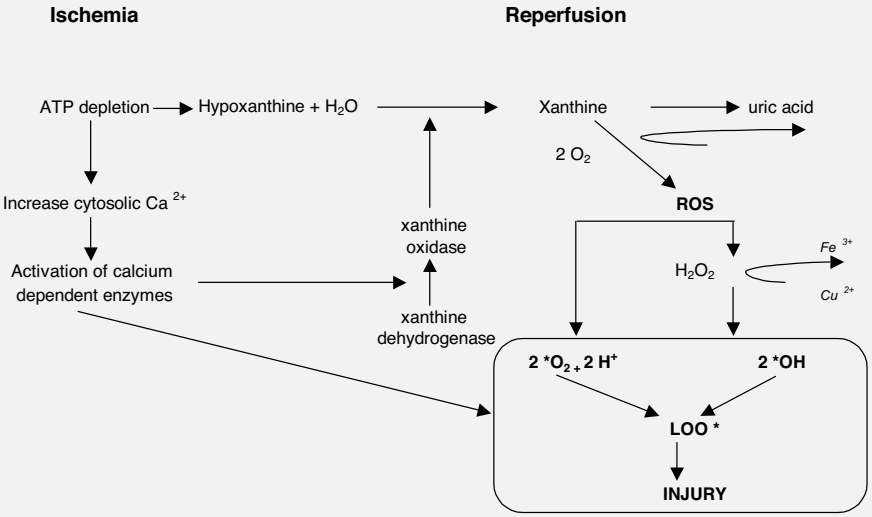
Ischemic phase

During the ischemic phase, renal tissue suffers from the lack of oxygen and nutritional substrates whereas cell metabolism continues, resulting in decreased adenosine triphosphate (ATP) levels and a decreased supply of adenosine diphosphate (ADP) to the mitochondria (66). The shift to anaerobic metabolism results in the accumulation of lactic acid and a decrease in pH. One of the most energy intensive cell functions is sodium and water homeostasis via the sodium–potassium pumps (67). When these pumps fail cellular, mitochondrial and nuclear swelling occurs. Increased extracellular potassium stimulates calcium ion channels, increasing cytosolic and mitochondrial calcium concentrations (68). Cytosolic calcium levels activate calcium dependent enzymes like cystein proteases, the phospholipases and endothelial nitric oxide synthetase (e-NOS) (69). Calpain is an example of a calcium dependent cystein protease capable of disrupting the cellular polarity by breaking down the integrity of the organizational proteins spectrin and ankyrin whereas caspase-3 is a calcium dependent cystein protease playing a major role in programmed cell death. Phospholipase-2 is able to change tubular cell polarity by breaking down cytoskeleton matrix and it plays a role in the synthesis of reactive oxygen species (ROS). The role of e-NOS is dual since it has been proven to be tubulotoxic (70), but also causes vascular dilation (71).
In this ischemic condition, hypoxanthine is the most damaging degradation product. It accumulates in the cell as a byproduct of the conversion of ATP to inosine. Under aerobic conditions hypoxanthine is metabolized via the production of xanthine to uric acid but in anaerobic conditions hypoxanthine accumulates in the endothelial and tubular cells. Xanthine oxidase is produced by proteolysis of the xanthine dehydrogenase under the influence of calcium dependent proteases (72).

Reperfusion phase

After the reinstitution of perfusion several factors contribute to the damage. The sudden increase of perfusion pressure causes endothelial damage and inflammation. Because of unequal distribution of perfusion, ischemia may persist in underperfused areas. Two types of molecules have been widely studied and have been implicated in ischemia and reperfusion injury; i.e. endothelin and Nitric Oxide (NO), which both modulate vascular tone. During ischemia systemic endothelin levels are elevated whereas anti-endothelin antibodies or endothelin receptor antagonists protect against ischemia and reperfusion injury (73,74). Many of the actions of endothelin are counteracted by constitutively expressed and endothelin induced Nitric Oxide (NO). NO causes vasodilatation which protects against ischemic renal injury (75), but on the other hand it also is toxic for tubular cells (70,71). This toxicity is probably caused by one of its metabolites, peroxynitrite, , which is a highly reactive oxidant resulting from the interaction between NO and the superoxide anion. During reperfusion molecular oxygen (O_2) is reintroduced (fig.1). O_2 reacts with the hypoxanthine that has accumulated in the cells during the ischemia phase. This reaction is mediated by xanthine oxidase and produces xanthine and reactive oxygen species (ROS). ROS refers to any compound derived from molecular oxygen that has acquired less than 4 electrons. The free radical members of ROS are Superoxide ($^{\cdot}O_2$) and the hydroxyl radical ($^{\cdot}OH$). They are

Figure 1. Schematic representation of the ischemia and reperfusion injury cascade



A period of oxygen deprivation results in a deprivation of cellular ATP. Hypoxanthine accumulates in the cell as a byproduct of the anaerobic conversion of ATP to inosine. Xanthine oxidase catalyses the reaction of hypoxanthine and oxygen to form reactive oxygen species (ROS). The free radical members of ROS, $^{\cdot}O_2$ and $^{\cdot}OH$ are the most harmful ones. They lead to lipid peroxidation in the cellular membrane, eventually leading to cell death by destruction of the cellular walls.

probably the most important biologically active free radicals. $^{\cdot}O_2$ is formed by transfer of a single electron to O_2 and $^{\cdot}OH$ is formed from hydrogen peroxide (H_2O_2). The latter reaction is spontaneous but is accelerated in the presence of a catalyzing transitional metal ion, for example Iron (Fe^{3+}) or Copper (Cu^{2+}).

The free radicals lead to an oxidative reduction of the poly unsaturated fatty acids (PUFA's) in the plasma membranes, known as lipid peroxidation. This lipid peroxidation leads to the production of fatty acid radicals and by reacting with oxygen to fatty acid peroxyl radicals ($LOO^{\cdot}$) These $LOO^{\cdot}$'s are the central players in a chain reaction that leads to further lipid peroxidation and the formation of reactive aldehydes. The harmful effect of the production of free radicals lies in the extensive damage to the cell membrane, leading to decreased function and/or cellular apoptosis or cell death (76-79)

b. Cell biological characteristics

Ischemia and reperfusion eventually lead to tubular cell death. Two types of cellular death can be distinguished: cells may die either by necrosis or by apoptosis. The morphological characteristics of apoptosis and necrosis are both quite distinct and remarkably constant among all kind of different cell types.

Apoptosis

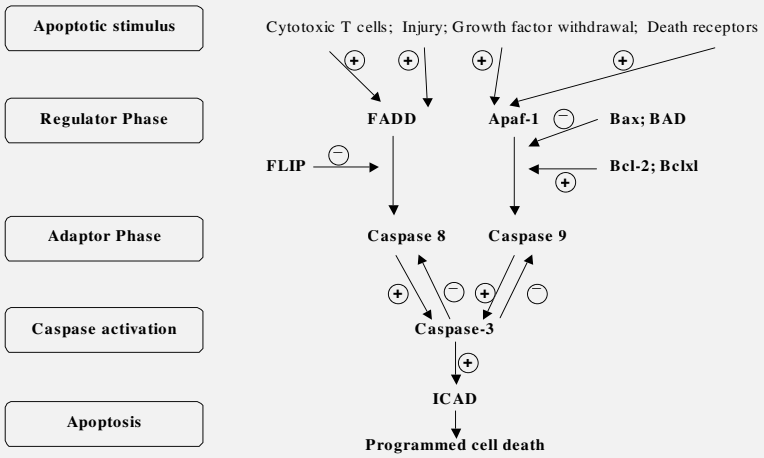
In contrast to necrosis, apoptosis is an active, energy dependent process with morphological characteristics that differ markedly from necrosis. Epithelial cells dying by apoptosis detach early from the underlying matrix and from each other. They become progressively smaller in size and their nuclear chromatin becomes condensed and fragmented. The plasma membrane remains intact and undergoes a process called blebbing. Ultimately, the apoptotic cell disintegrates into many membrane-bound vesicles, some of which contain fragments of condensed chromatin, the so-called apoptotic bodies. Apoptosis is a form of programmed cell death which is used physiologically to remove unwanted cells. The essence of apoptosis is a process of cellular auto-digestion.

Three stages can be recognized in the process of apoptosis (fig.2).

The *first stage* is the regulator stage. Regulator molecules control adaptor proteins by directly interacting with them. For example FADD (Fas associated death domain) activation can be inhibited by cellular FLIP (FLICE (Fas associated death domain like IL-1 beta converting enzyme) inhibitory protein). Apaf-1 (apoptotic protease activating factor 1) activation can be prevented by binding to the anti-apoptotic members of the Bcl-2 families. Furthermore inhibitors of apoptosis proteins (IAP) can directly prevent caspase activation.

The *second stage* in the apoptosis process is the adaptor stage. The adaptor molecules are able to activate the caspases by binding to specific sides and therefore leading to proteolysis of the pro-enzyme. Adaptor molecules are up-stream caspases, like caspase 8 and caspase 9, that activate caspase 3 as the final common caspase. Caspase-8 becomes activated by binding to the FADD adaptor protein and caspase-9 is activated by it's adaptor protein Apaf-1.

Figure 2. Schematic representation of the apoptotic cascade



Apoptosis can be initiated by multiple signals. The strength of the apoptotic signal is evaluated by specific control proteins which can either inhibit or promote cell death. When the apoptotic signal is strong enough, caspase activation degrades cytoskeletal and nuclear proteins. This results in a cascade of intracellular degradation, eventually leading to the formation of apoptotic bodies and the engulfment of apoptotic material by phagocytic cells.

The *last stage* is the effector stage. The key effector molecules are proteases named caspases of which the caspase-3 is the effector enzyme. Caspases are present in an inactivated form. Once they are activated the target of these activated caspases is ICAD (inhibitor of caspase activated D-nase (CAD). CAD is an endogenous endo-nuclease that fragments DNA of cells. Once activated CAD leads to fragmentation of the cellular DNA. Hence DNA fragmentation can be used as a marker of apoptosis.

Necrosis

Frank necrosis is seen in experimental animal models but only in a minority of the human cases. It is usually patchy involving individual cells or small clusters of cells sometimes resulting in small areas of denuded basement membrane (non-replacement phenomenon). Tubular cell necrosis is associated with a rapid metabolic collapse, cell swelling and early loss of plasma membrane integrity and the loss of polarity. Integrity of their tight junctions is disrupted, perhaps as a consequence of alterations in the cytoskeletal network (67,80). Because of the redistribution of the Na/K-ATPases, tubular function is disturbed and cells die. This in turn leads to the release of proteolytic enzymes and other injurious cytosolic components into the extracellular space that not only directly damage surrounding cells but also incites an inflammatory response. More subtle changes include loss of brush border, flattening of the epithelium, detachment of cells, intra-tubular cast formation and dilation of the tubules (fig.3).

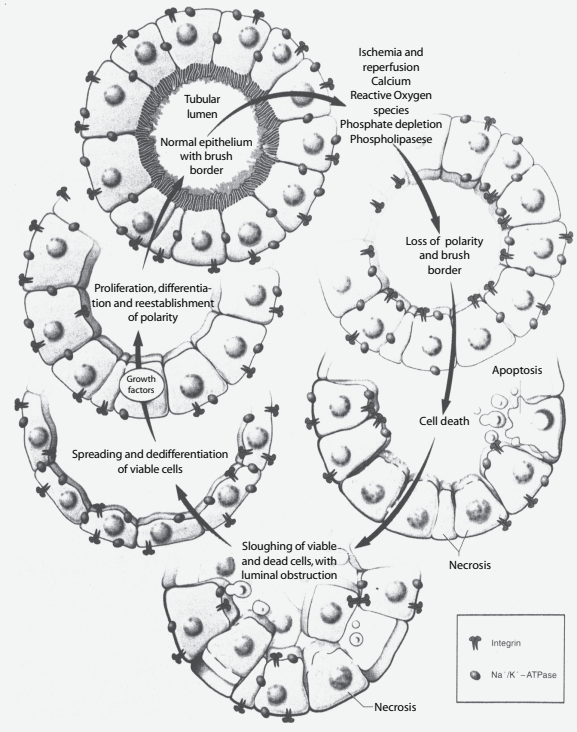


Figure 3.
Cell biological characteristics of acute tubular necrosis

Cells undergoing acute tubular necrosis are morphologically characterized by loss of brush border, flattening of the epithelium (distalisation of proximal tubular cells) and detachment of necrotic cells leading to intratubular cast formation and dilation of the tubules. This makes distal tubules hard to be discriminated from the proximal ones on morphological grounds. [Reprinted with permission (96)]

2. Maintenance phase of ATN

The maintenance phase represents a phase of stabilization of injury in which lethal factors, characteristic for the ischemic stage, on the one hand and repair and survival factors, characteristic for the regeneration phase, on the other hand, are interacting and subsequent leading to cellular repair, division and redifferentiation.

a. Molecular biological characteristics

The kidney has naturally occurring anti-oxidant enzymes to counteract the effects of the free radicals. The catalases and gluthathion peroxidase act by safely decomposing the peroxides. Catalase is located mainly in the peroxisomes of the cells and therefore acts mainly upon hydrogen peroxidase whereas gluthation peroxidase is located in the cellular cytosol and therefore acts mainly on the hydroperoxides, that are derived from membrane's fatty acids. The superoxide dismutases act by scavenging the free radicals especially the $\cdot O_2$. In humans it is present in at least two forms, the cytoplasmatic copper/zinc (CuZn)-SOD and the mitochondrial manganese (Mn)-SOD. Superoxide dismutases are enzymes that catalyze the dismutation of $\cdot O_2$ to hydrogen peroxide (H_2O_2), which is decomposed, by other enzymes like catalase and gluthation peroxidase (76). The presence and the (down regulated) activity of these SOD's seem to be related to the amount of damage induced by ROS in rat kidneys (81,82). However, the clinical use of human recombinant superoxide dismutase, did not protect against DGF in human kidneys (83,84), although a decrease of acute rejection episodes (ARE) and chronic rejection was observed during follow up (85).

b. Cell biological characteristics

During the maintenance phase tubular cells share characteristics of the ischemic phase in which necrosis, apoptosis and the interstitial infiltrate are present and characteristics of the recovery and regeneration phase in which proliferation and redifferentiation are present.

3. Recovery phase of ATN

a. Molecular biological characteristics

This process is regulated by the expression of a number of transcription factors, structural proteins and growth factors and recapitulates many aspects of renal organo-genesis in respect to the high rate of DNA synthesis, like PCNA, the expression of apoptosis and the expression of genes that encode for processes during renal organogenesis like keratin and vimentin (86-88). The latter is a filament protein expressed in mesenchymal cells but not in mature nephrons. Whether these cells are derived by regression of surviving epithelial cells or by the activation of resident, but quiescent renal stem cells is not clear yet. The role of the induction of this gene expression after acute renal injury lies probably in their involvement in determining cell fate (survival versus apoptosis or necrosis) and the transcription of growth factors and their receptors that ultimately mediate tubular cell division and proliferation. Upon ischemia and reperfusion injury growth factors like the hepatic growth factor, insulin like growth factors and fibroblast growth factors are upregulated (89,90). Others like epidermal growth factors are down regulated in injured proximal tubules (91). Treatment with these growth factors in

animal models were promising (89,92,93) but the use in humans is still limited (94).

b. Cell biological characteristics

During recovery from ischemia and reperfusion injury, surviving tubular epithelial or renal stem cells, differentiate and proliferate, eventually replacing the irreversibly injured tubular epithelial cells and restoring tubular integrity (56). Morphologically this is characterized by the presence of mitotic figures and signs of cell proliferation. This process enables the replacement of the damaged epithelium and is maximal at 24 to 48 hours after ischemic injury in the rat (95). Initially proximal tubules reappear in a flattened fashion covering the tubular basement membrane, followed by the reappearance of the brush border. As the number of cells increase the tubular cells become more cubic in shape.

Aim of the thesis

The purpose of this thesis is to evaluate the mechanisms behind DGF and their impact on long-term graft function and survival. Since the main cause of DGF is ischemia and reperfusion injury, we use a functional definition of DGF, excluding other causes for DGF, like acute rejection, cyclosporin toxicity and surgical complications.
In **chapter 2** we analyze the risk factors for DGF in a cohort of patients receiving a cadaveric transplant between 1983 and 1996, using this functional definition. Furthermore we study the impact of DGF on long-term events like graft function and graft loss.
Why some grafts develop DGF and others do not is unclear and needs to be elucidated. The mechanisms how DGF develops and therefore how DGF influences long-term graft faith also is unclear. To answer this question, there is need for a marker of functional renal mass that is easy assessable and can be repeated frequently.
In **chapter 3** we use a parameter developed in the ^{99m}Technetium-mercaptoacetyltriglycine (MAG-3) renal scintigraphy, the Tubular Function Slope (TFS), as a marker of this functional renal mass. The differences in TFS between grafts reacting with DGF or not are analyzed, immediately after transplantation and during follow up.
The response of a graft on ischemic reperfusion injury depends on its protective and regenerative capacities. Some factors like donor age and donor gender are determinants of the functional renal mass and can not be manipulated. Others risk factors, however can. There is no doubt that cold ischemia time (CIT) and warm ischemia time (WIT) influence the occurrence of DGF and can be manipulated. Other factors like serum PTH levels and calcium levels and the use of calcium channel blockers (CCBs) are more controversial with regard to this aspect. However, the influence of serum calcium levels and the use of CCBs on the development of DGF has regained interest since many processes that take place during ischemia and reperfusion injury and subsequent processes like necrosis, apoptosis and cyclosporin toxicity, are thought to be directed by calcium dependent processes. Elucidating the role of serum calcium and PTH levels on the occurrence of DGF, may have clinical implications for the management of potential allograft recipients.
In **Chapter 4** the impact of serum calcium levels and the use of CCBs on the development of DGF are analyzed. Serum calcium levels are not only correlated with the occurrence of DGF but also with the presence of calcium deposits in renal biopsies taken within the first week after transplantation. Furthermore the effect of CCBs is taken into consideration.

In **chapter 5** we describe a study in which the expression of protective enzymes is correlated with the occurrence of DGF and the presence of ATN in biopsies taken within the first week after transplantation.
Chapter 6 reviews the literature on DGF in renal transplantation.
Finally a summary is given in **chapter 7.**

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