Editorial Comments

Insights into potential cellular mechanisms of cisplatin nephrotoxicity and their clinical application

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Introduction

Cisplatin (cis-dichlorodiammine-platinum (II), CP) is an effective antineoplastic agent in the treatment of various solid tumours, whose full clinical utility is limited because of its renal toxicity. Since even vigorous hydration may not eliminate toxicity, discontinuation of CP remains the only option in cases of progressive renal failure. Novel measures to protect kidneys from CP toxicity are currently being investigated. These studies, preferentially using animal or cell culture models, are based upon the increasing knowledge of subcellular mechanisms of CP toxicity.

Proximal tubular uptake and accumulation

The major site of renal injury is the S3 segment of the proximal tubule, located in the outer stripe of the outer medulla of the kidney. Cisplatin concentrations in proximal tubular epithelial cells exceed plasma concentrations by a factor of five. This is at least in part due to active uptake via energy-dependent processes, such as the probenecid inhibitable organic base transport protein [1]. Intracellularly, the highest concentrations of CP are found in the cytosol, mitochondria, nuclei, and microsomes. Low intracellular chloride concentrations favour the replacement of the two chloride ligands of the cisplatin molecul by hydroxyl groups, resulting in even more toxic aquated platinum derivates.

Cisplatin toxicity in proximal tubular epithelial cells is morphologically characterized by tubular necrosis, loss of microvilli, alterations in number and size of lysosomes, and mitochondrial vacuolization. These structural alterations are accompanied by functional disturbance of various cell organelles. Meanwhile, two distinct pathophysiological mechanisms have been recognized as primary promoters of cellular damage, i.e. inhibition of protein synthesis and glutathione depletion; in contrast, mitochondrial damage, inhibition of membranous transport proteins and lipid peroxidation are considered merely as sequelae of established cell damage (Table 1).

Primary effects of platinum compounds

Inhibition of protein synthesis

Cell culture as well as animal studies indicate that inhibition of protein synthesis is the earliest biochemical manifestation of CP toxicity in proximal tubular cells. The detailed mechanisms are unclear. One possibility is disruption of the nucleolus as the site of ribosome biosynthesis. Leibbrandt et al. [2] demonstrated nucleolar condensation and fragmentation in tubular cells treated for as little as 2 h with 100 μM CP, a concentration that is readily reached in patients. These authors argue that interference of CP with nucleolar structure or function might decrease ribosome number and ultimately protein synthesis. In addition, they report on the inhibition of lysosomal protein degradation following CP treatment.

Another possible explanation for inhibition of protein synthesis is CP’s ability to decrease ribosome assembly in vitro by interference with the joining of the 48S and 60S subunits [3], indicating that in non-proliferating cells, for instance renal tubular epithelial cells, inhibition of protein synthesis seems to be a post-transcriptional event. This contrasts with the major

Table 1. Potential subcellular mechanisms of cisplatin toxicity in renal proximal tubular cells

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mechanism of CP cytotoxicity in proliferating cell systems, i.e. inhibition of DNA and RNA synthesis.

Glutathione and protein-SH depletion

Reduced glutathione (GSH) is present in millimolar concentration in mammalian cells. As the major cellular oxidant defence system it is a potent factor in the control of lipid peroxidation. Intracellular glutathione consists of cytosolic (30%) and mitochondrial (70%) pools. Mitochondrial GSH appears to be essential in the regulation of inner membrane permeability by keeping intramitochondrial SH groups in the reduced state.

The interaction of the platinum compound with sulph-hydryl groups is the second important factor promoting cytotoxicity. Due to the molecule’s high affinity to SH groups, its chloride moieties are replaced by sulph-hydryl-groups, thereby inactivating the platinum compound. Although binding of CP by GSH and protein-SH may primarily appear as a process of detoxification, it actually turns out to be the initial step in a cascade leading to further cell damage. The formation of stable protein-S-CP adducts results in dysfunction of membrane associated and cytoplasmic proteins (e.g. Na+/phosphate and Na+/glucose cotransporters) [4] and decreases the activity of important enzyme systems (e.g. glutathione-S transferase, reductase and peroxidase) [5]. In addition, stable glutathione-CP adds lead to a decrease in the amount of reduced glutathione available to scavenge free reactive oxygen metabolites [6]. This may effectively harm the cellular oxidant defence systems, eventually leading to lipid peroxidation. Nevertheless it appears that cellular GSH content must decline below a critical threshold of 30% from normal before spontaneous lipid peroxidation occurs [7]. Thus the extent of cellular damage seems to be highly dependent on the amount of cellular reactive SH groups. This conclusion is further supported by experimental data showing that depletion of cellular GSH content prior to CP exposition increases toxicity, while administration of SH groups or SH-reducing agents ameliorates CP-induced injury in vitro as well as in vivo.

Secondary effects of platinum compounds

Lipid peroxidation

There is increasing evidence for an important role of free oxygen radicals in the pathophysiology of CP induced cytotoxicity. Reactive oxygen species (ROS) such as hydrogen peroxide, superoxide anion, or hydroxyl radicals are normally generated in renal cells and immediately detoxified by endogenous antioxidants, e.g. GSH, catalase or superoxide dismutase. Unopposed intracellular accumulation of ROS leads to membrane lipid peroxidation and DNA damage. As mentioned above, CP may lead to GSH depletion, thus allowing lipid peroxidation to occur.

Indirect evidence for the participation of ROS in cisplatin toxicity has been derived from studies demonstrating protective effects of various radical scavengers and antioxidants in vitro and in vivo. The protective effects of these substances are probably related to preventing or delaying membraneous lipid peroxidation and not to a repletion of cellular GSH content [8]. Further evidence supporting this hypothesis has been gained from experiments demonstrating a decrease in cellular activity of endogenous antioxidant enzymes following CP exposure. Finally, lipid peroxidation has been documented by direct measurement in various animal and cell culture models of cisplatin toxicity [7].

Mitochondrial damage

Morphological or functional changes of mitochondria have been reported in almost all models of CP nephrotoxicity. These findings include a reduction in mitochondrial Ca2+ uptake, inhibition of Na+ /K+-ATPase, depletion of pyridine nucleotides, lipid peroxidation and collapse of the mitochondrial membrane potential. All these changes can be explained as direct consequences of mitochondrial GSH and protein-SH depletion [7]. The resulting oxidative stress will lead to lipid peroxidation and eventually to the collapse of the mitochondrial membrane potential.

The extent of mitochondrial damage depends strongly on CP concentration. In studies using micromolar CP concentrations, similar to those measured in patients, mitochondrial dysfunction occurs rather late [2] and is preceded by inhibition of protein synthesis and loss of lysosomal function. In contrast, damage to renal tubular mitochondria occurs early in the course of experiments when millimolar CP concentrations are applied [7]. These differences led to a discussion as to whether mitochondria are a primary or secondary target of platinum compounds. In our view, these differences may be explained by high mitochondrial GSH concentrations and the necessity to decrease GSH concentrations below a certain threshold before lipid peroxidation occurs. Thus mitochondrial damage will be observed earlier when intracellular CP concentrations are high.

New developments in nephroprotection from cisplatin toxicity

From the pathomechanisms discussed above, novel rationales for cytoprotection against CP toxicity have emerged, i.e. prevention of GSH depletion and ROS scavenging. The former can be achieved by the application of SH-containing substances. Meanwhile, various thiols have been tested in a clinical setting for their efficacy to ameliorate CP nephrotoxicity. These substances may protect from CP toxicity not only through intracellular binding of cisplatin, but also by scavenging of free oxygen radicals. Substances tested so far include sodium thiosulphate, amifostine (WR–2721), glutathione, diethyl-dithiocarbamate (DDTC), and...
L-methionine. In a randomized, multicentre, double-blind study, no significant chemoprotection from CP toxicities could be demonstrated for DDTC [9]. In contrast, amifostine, an aminothiol, proved effective in multiple studies and has recently received approval by the American FDA and various European countries as a cytoprotective agent for radio and chemotherapy [10]. Amifostine is rapidly taken up by and accumulates in non-tumour cells after dephosphorylation by membrane-bound alkaline phosphatase. In contrast, uptake into alkaline phosphatase deficient tumour tissue is negligible. Intracellularly, the free thiol can bind directly to, and thus detoxify, the active species of alkylating or platinum drugs, remove preformed DNA-platinum adducts and scavenge oxygen free radicals. Several studies yielded promising results, indicating that amifostine may provide broad-spectrum cytoprotection without attenuating antitumour response.

Summary

Cisplatin preferentially accumulates in cells of the S3 segment of the renal proximal tubule and is toxified intracellularly by hydration. The earliest manifestation of toxicity is inhibition of protein synthesis. GSH depletion is another important mechanism causing CP toxicity. Intracellular binding to SH groups leads to GSH depletion, resulting in lipid peroxidation and eventually mitochondrial damage. New measures to prevent GSH depletion and scavenge intracellular free oxygen radicals have been tried in clinical studies. Promising results indicate that cisplatin nephrotoxicity can be further reduced in the future.

References


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**Oxidative stress and vascular injury—relevant for atherogenesis in uraemic patients?**

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**Introduction**

There is no doubt that uraemic patients suffer from a high incidence of cardiovascular complications such as coronary heart disease, peripheral vascular disease, and stroke, all diseases which are related to atherogenesis. Indeed, two decades ago it has already been suggested that accelerated atherogenesis is the main factor responsible for the high mortality in uraemic patients [1]. However, it is still a matter of debate whether uraemic patients truly have accelerated atherogenesis, or whether the high cardiovascular mortality is a consequence of several risk factors present in these patients already in the predialysis phase. Thus it is important to clearly identify risk factors which are unique to or enhanced in uraemic patients. Beside other well-known risk factors for atherogenesis, such as dyslipidaemia, diabetes mellitus, obesity, hypertension, or smoking, it has become increasingly apparent...
that oxidative stress may play an important role in the pathogenesis of atherosclerosis [2], probably through attenuation of endothelial function. This editorial comment will focus on the role of oxidative stress in the process of atherogenesis and endothelial dysfunction, and evaluate its significance in the context of uraemia.

What is oxidative stress?

Evidence for the relevance of oxidative stress in the pathogenesis of atherosclerosis derives from numerous in vitro and animal studies and from the protective role of antioxidants as described in the majority of the investigations on this subject [3]. It is now well established that free radicals, such as superoxide (O$_2^-$), hydroxyl (OH$^-$), and nitric oxide (NO), and other reactive oxygen species (ROS), such as hydrogen peroxide (H$_2$O$_2$), are continuously formed in vivo [2]. ROS originate from activated macrophages, endothelial cells, smooth-muscle cells, and, in the kidney, various glomerular cells [4]. Under physiological conditions in healthy subjects, there seems to be an approximate balance between the production of ROS and the activity of antioxidant enzymes such as superoxide dismutases (SODs), catalase, and glutathion peroxidase. An imbalance between formation of ROS and antioxidant defences characterizes the state of oxidative stress. The following example demonstrates how fragile this balance can be: once O$_2^-$ is formed, the activity of SOD will transform it to H$_2$O$_2$. H$_2$O$_2$, in case of sufficient catalase activity, will react to harmless H$_2$O and O$_2$. However, too much SOD, in relation to H$_2$O$_2$-removing catalase, can be deleterious, giving rise to the formation of the highly reactive hydroxyl radical in the presence of metal ions [2]. On the other hand, OH$^-$ can be produced in the presence of Fe$^{2+}$ from O$_2^-$, when there is too little SOD activity (Figure 1). Thus, the balance between the various ROSs is delicate and depends strongly on the specific environment.

![Fig. 1. Possible interaction of superoxide anion (O$_2^-$) with nitric oxide (NO) and potential formation of hydroxyl radical (OH$^-$). O$_2^-$ inactivates nitric oxide, thereby producing peroxynitrite (ONOO$^-$). The acid of peroxynitrite favours formation of hydroxyl radical (OH$^-$), which is a potent cytotoxic agent. OH$^-$ is also a reaction product of NO and H$_2$O$_2$. (Adapted from Galle J, Wanner C. Impact of nitric oxide on renal haemodynamics and glomerular function — modulation by atherogenic lipoproteins? Kidney Blood Press Res 1996; 19: 2–15.)](image)

Consequences of an excess production of reactive oxygen species for vascular biology

In general, formation of ROS is part of the non-specific defence system of an organism against e.g. bacteria and other microbes. However, the organism’s own cells can also be targets of ROS, e.g. at sites of acute ‘controlled’ inflammation. There is a variety of renal disease entities in which oxidants are considered to be pathogenic, such as glomerular diseases, progressive renal failure, acute renal failure, or pyelonephritis [4]. With regard to the vascular system, the interaction of O$_2^-$ with NO seems to be of major importance, particularly in the setting of hypercholesterolaemia and atherosclerosis. It has been known for more than 10 years that the so-called endothelium-derived relaxing factor (EDRF), meanwhile identified as NO, is inactivated by O$_2^-$ [5]. NO reacts with O$_2^-$ to yield peroxynitrite (ONOO$^-$), resulting in NO scavenging [6]. Peroxynitrite itself is rather stable, but it is in equilibrium with its acid, HOONO, which rearranges to form nitrate and the highly reactive OH$^-$; These reactions affect the vascular system at least in two ways: First, scavenging of NO leads to attenuation of a major function of the arterial endothelium, the endothelium-dependent dilatation. Attenuation of endothelium-dependent dilatations results in disturbed organ perfusion and systemic hypertension [7]. Second, formation of OH$^-$ can cause cellular damage and may contribute to inflammation [2]. There is evidence that these reactions indeed take place in vivo: Hypercholesterolaemia and atherosclerosis are strongly associated with impairment of endothelium-dependent dilatations and arteries obtained from hypercholesterolaemic animals produce significantly higher rates of hydroxyl radical in the presence of metal ions [2]. On the other hand, OH$^-$ can be produced in the presence of Fe$^{2+}$ from O$_2^-$, when there is too little SOD activity (Figure 1). Thus, the balance between the various ROSs is delicate and depends strongly on the specific environment.
Oxidative stress in uraemia—a matter of disturbed pro- and antioxidant balance?

Is there any evidence that the balance between pro- and antioxidant mechanisms is disturbed in uraemia? Unfortunately, data are lacking that directly demonstrate that oxidative stress, contributing to endothelial dysfunction and atherogenesis, is enhanced in the arteries of uraemic patients. However, there are some arguments supporting the hypothesis of enhanced oxidative stress in uraemia. Haemodialysis-membrane-induced activation of oxidative metabolism has been described. Presumably it results from activation of macrophages on the surface of dialysis membranes during the dialysis session. Loss or deficiency of antioxidant activity, e.g. vitamin E deficiency, could also contribute to enhanced oxidative stress in uraemia. Furthermore, in uraemic patients undergoing dialysis, antibodies against oxidatively modified LDL have been found [13], providing indirect evidence for a stimulation of lipid peroxidation processes. Another indirect proof is the observation that lipoproteins from haemodialysis patients are oxidized more easily [14].

Conclusion

Oxidative stress emerges as an important cofactor for the development of endothelial dysfunction and atherogenesis. Although until now no study directly investigated the level of oxidative stress in arteries of uraemic patients, in vitro data strengthen the hypothesis that oxygen radicals contribute to atherogenesis in uraemia. Future studies, particularly intervention studies with antioxidants, are required to prove whether the concept holds true.

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Towards cost-effective dialysis therapy in Europe: the need for a multidisciplinary approach

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Introduction

European countries spend different proportions of their gross domestic product on health care and different amounts of resources are spent on specific services [1]. There is an urgent need to measure the quality and cost of such health care interventions in different European countries, particularly in highly resource intensive areas of health care such as renal replacement therapy.

Effectiveness and efficiency

In the face of increasing costs of health care interventions and financial constraints, health care providers, policy makers and consumers of health services are confronted with the need to rationalize the use of resources. Two important conditions need to be met in order to help decision making. Firstly, robust evidence of effectiveness (does the treatment work?) is needed, preferably from results of randomized control trials or systematic literature reviews. Secondly, the resources used and costs must be accurately known to evaluate efficiency (is the cost of treatment justified in the light of evidence of effectiveness?).

End-stage renal disease—the public health perspective

Public health physicians and policy makers evaluate many clinical interventions for their cost-effectiveness; few are as effective as renal replacement therapy (RRT), without which patients with ESRD will certainly die. Although this is well recognized, the cost of treatment makes automatic allocation of resources for the increasing number of patients difficult. This is compounded by the fact that data on the costs of RRT in Europe are scant and there are none linking outcome measures such as survival, quality of life and intermediate outcome measures (e.g. haemoglobin, phosphate) with costs and resource use.

Existing data on costs

Renal replacement therapy is now very advanced and safe and hence older patients and those with comorbid illnesses can be treated successfully. As a result the number of patients receiving RRT in Europe has more than trebled in the last decade (from 71 000 in 1981 to 218 694 in 1994). This latter figure is likely to be an underestimate by about 30–40% as several centres fail to report to the European Renal Registry because there is no special resource available to renal units to provide such data and staff are having to devote more and more of their time to clinical management of the increasing patient load [2,3]. Current estimates of cost of hospital haemodialysis (by far the commonest mode of RRT in Europe) vary from 13 000 ECUs to 35 000 ECUs per patient per year, depending upon which source one consults [4,5].

The studies do not describe their methodologies in detail, and therefore are unlikely to be comparable; some costs (e.g. drug prescriptions from family practitioners) are included in one but not in the other. The above studies also did not consider the influence of case-mix on costs. Moreover, the estimated costs are global for each mode of treatment and hence of limited use for future service planning where some components of the cost may increase more than others. Furthermore, such costings do not take account of...
hospital admissions for intercurrent illness (e.g. cardiac failure, sepsicaemia).

The acceptance rate for RRT is rising rapidly and will continue to do so with technological improvement and the ageing population profile of most European countries. It is likely that on a conservative basis the total number of patients will exceed 300,000 by 1999. In addition in some eastern European countries many children and young adults are dying of renal failure because of lack of facilities [6]. As public awareness of the ease and safety of modern dialysis therapy grows, lack of treatment will increasingly be seen as unacceptable.

It has been estimated that a ‘steady state’ will be reached and that the eventual stock of patients from a typical Caucasian population will be 800 per million, overall objective of this multicentre European study is and higher... study are consuming up to 4% of the money spent on all health budget. Thus 0.08% of the population would reach and that the eventual stock of patients from a BIOMED 2 programme of research in Europe. The variations in outcomes in end-stage renal disease in Europe... their influence on outcomes in RRT, criteria for selection of patients for renal transplantation, frequency and duration of haemodialysis, use of synthetic membranes, and approaches to treatment of anaemia and bone disease; (b) patient-related factors such as the presence of comorbidity; and (c) differences in resources and resource use. While many studies have examined the influence of treatment-related and patient-related factors on outcomes in RRT, none has addressed the impact of resource use on outcomes—there is therefore the need for such a study.

Such a study has recently been proposed and has been approved for funding by the EC through its BIOMED 2 programme of research in Europe. The overall objective of this multicentre European study is to assess the potential to improve the quality of care for a group of patients with ESRD in Europe within existing budgetary constraints. Specific aims of this study are:

- To compare the nature, volume and costs of the resources devoted to the provision of chronic dialysis treatment in different centres in Europe.
- To establish how the resources are used in the context of the financing and planning systems of the countries taking part.
- To quantify the relationship of the resources and costs for chronic dialysis with the patient outcomes, including quality of life and survival corrected for case-mix, in all centres.
- To use the above data along with those from previous studies and national renal registries to model longer-term scenarios for costs, outcomes, and cost-effectiveness of service provision for ESRD patients.

Studies of this kind can only be achieved by a multidisciplinary team—in this instance health economists, nurses, statisticians, and clinical nephrologists. Such an approach should ensure accurate and relevant data collection and allow application of methods of economic appraisal to explore the potential for quality improvement and efficiency gains in providing chronic dialysis services in Europe.

Variation in outcomes in end-stage renal disease in Europe

In a study supported by the BIOMED 1 initiative of the Commission of European Communities we have found that survival in 1407 patients with ESRD was significantly influenced by comorbid illness. Furthermore we showed, for the first time in Europe, that even after adjusting for comorbidity and age there were significant differences in patient survival among the centres [8]. In this retrospective study we showed, using multivariate analysis, including the influence of case-mix, that the probability of survival on RRT was 60% higher in the French and German centres when compared with centres in the UK and the Netherlands. In addition, as part of the same study we collected data prospectively on intermediate outcome measures such as haemoglobin and phosphate concentrations before and after implementing guidelines agreed by consensus among participating nephrologists in a cohort of 829 dialysis patients. Preliminary analysis of these data similarly show variation in both pre- and post-guideline outcomes between centres. It is unclear whether a better outcome is associated with increased resource invested or the way in which resource is used. In the absence of data from randomised trials, it is important to evaluate this by comparing outcome and cost in different centres following adjustment for case-mix.

Can differences in outcomes in renal replacement therapy be explained by variations in resource use?

There are three main reasons why outcomes for patients with ESRD in different countries may vary:

(a) variations in practice including different attitudes towards modality selection in RRT, criteria for selection of patients for renal transplantation, frequency and duration of haemodialysis, use of synthetic membranes, and approaches to treatment of anaemia and bone disease; (b) patient-related factors such as the presence of comorbidity; and (c) differences in resources and resource use. While many studies have examined the influence of treatment-related and patient-related factors on outcomes in RRT, none has addressed the impact of resource use on outcomes—there is therefore the need for such a study.

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The role of adhesion molecules in ischaemia–reperfusion injury of renal transplants

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Introduction

Ischaemia–reperfusion injury is of major importance in kidney transplantation. It can result in delayed graft function (DGF), and according to large scale clinical analyses there is consensus that DGF has a significant impact on long-term graft survival [1]. Experimental data are now exploring the link between the early lesions of graft reperfusion, the occurrence of acute rejection and the late onset of graft atherosclerosis [2]. Ischaemia and reperfusion induce the development of inflammation and adhesion molecules are essential intermediates between activated endothelial cells and circulating leukocytes. Much work has been done in the past years evaluating antiadhesion strategies in animal models. Beyond their interest in giving information about the in vivo relevance of several adhesion molecules, they have therapeutic implications and indeed, monoclonal antibodies (Moab) directed against ICAM-1 [3] and LFA-1 [4] have already been tested in human transplantation.

Adhesion molecules in ischaemia–reperfusion injury

Reperfusion injury is a cascade of events, initiated by tissue ischaemia and the production of oxygen free radicals during the reperfusion process, leading to the development of inflammation, through activation of endothelial cells in the transplant and recruitment of circulating leukocytes. The four families of adhesion molecules, selectins and their carbohydrate ligands, integrins and the molecules of the immunoglobulin superfamily, are involved in all the steps of adhesion of leukocytes to the activated endothelium. The neutrophil, among leukocytes, has been incriminated as the mediator of reperfusion injury in the classical models of arterial occlusion. This evidence is based on histological analysis, showing its presence in the intertubular spaces of the outer medulla, where the tubular lesions generally predominate, and in vivo studies of neutrophil depletion by antisera, that has been found to protect the kidney from renal failure. However, studies reporting the absence of prevention of reperfusion injury by the antineutrophil serum also exist. The variable degree of neutrophil depletion has been suggested as an explanation for these discordant results, since few neutrophils are necessary to provoke reperfusion lesions (see [5] for discussion). It has also been shown that neutrophils have to be primed to cause renal failure in ischaemic kidneys, where they are retained and activated to release oxygen metabolites [6]. Many adhesion molecules are expressed in an ischaemic kidney but ICAM-1 appears to be the most important one. On one hand, ischaemia and reperfusion cause its upregulation on endothelial cells and it may be viewed as a marker of its activation. On the other hand, ICAM-1 is the ligand of CD11a/CD18 and CD11b/CD18, the two major integrins expressed by the neutrophil, that mediate, through interaction with ICAM-1 the phase of strong adhesion process. The rolling of neutrophils on the endothelium is mainly mediated, by L selectin. Their endothelial transmigration is dependent on the ICAM-1/LFA-1 and VCAM-1/VLA-4 pairs. Most recent studies have implicated PECAM 1/CD 31 in this process [7].

Tubular obstruction by casts of desquamated tubular cells is a second mechanism involved in ischaemic acute renal failure. Goligorski et al. [8] have shown that oxidative stress and hypoxia induce a loss in epithelial cell polarity and the redistribution of integrins from the basal to the apical cell membrane. This results in the detachment of tubular cells from the underlying matrix, their desquamation into the tubular lumen, and their organization into aggregates through homophilic and heterophilic interactions between adhesion molecules. The use of labelled synthetic RGD peptides, binding to the basolateral and apical faces of tubular cells and the surface of detached cells, confirmed this hypothesis. β1 and αv integrins recognizing RGD motives on detached cells or matrix fragments have been incriminated in these interactions and reorganiza-
Adhesion molecule expression has been analysed in renal reperfusion injury is better demonstrated by clamping of the renal artery, and most of the results are certainly relevant for transplantation. Adhesion molecule expression has been analysed in the human transplant as well [9]. In the normal kidney, only endothelial cells exhibit strong expression of ICAM-1. This expression does not seem modified after flushing and graft revascularization [10]. However, in post-transplantation biopsies, many proximal tubular cells and infiltrating leukocytes also stain for ICAM-1. VCAM-1 expression, is restricted to glomerular parietal epithelial cells in a normal kidney. After revascularization, however, it is detected on large vessels and capillaries as well as on the proximal tubules. The expression of the other adhesion molecules does not change pre- vs post-transplantation. Finally, no pattern of adhesion molecule expression is specific for reperfusion injury or delayed graft function. In addition, in the animal model of arterial occlusion [11], as well as in the human renal grafts examined in the postoperative period [12], reperfusion injury induces an extensive cytokine response and tubular expression of HLA class I and II, constituting a microenvironment able to induce acute rejection. When pre- and postanastomotic biopsies of kidney transplants were compared, glomerular and interstitial influx of neutrophils was found as an expression of reperfusion injury and significantly correlated with cold ischaemia time and serum creatinine concentration at 3 months (Koo D. Communication at the Basic Sciences meeting, Amsterdam, 1996). The majority of the infiltrating cells were identified as monocyte/macrophages, however, that were already present in preanastomosis biopsies and persisted until day 4, when the infiltrate becomes predominantly lymphocytic. A clear relationship between E and P selectin expression and the presence of neutrophils was demonstrated. Such an expression was not found in living related transplants, suggesting that it is the result of donor condition and cold storage.

Antiadhesion strategies

The functional importance of the above adhesion molecules in renal reperfusion injury is better demonstrated in studies using antiadhesion agents. It has to be emphasized that the adhesion molecule requirement can be different from one tissue to the other and hence the efficiency of the monoclonal antibodies used for their targeting. The administration of an anti-ICAM-1 Moab in rats exposed to ischaemic injury by bilateral clamping of the renal artery prevents renal functional impairment and the typical histological lesions of acute renal failure [13]. The Moab is still effective when it is administered shortly after the ischaemic period. In the same model, antisense oligonucleotides for ICAM-1 also attenuate reperfusion injury in the rat [14] and ICAM-1-deficient mice are protected against acute renal failure [15]. A similar beneficial effect on the acute renal failure of post-transplant reperfusion injury is demonstrated with a murine anti-ICAM-1 Moab, administered to kidney transplanted Cynomolgus monkeys [16].

An anti-LFA-1 (CD11a) Moab alone, injected into rats at the time of renal artery occlusion, provides modest protection at best, but it prevents renal failure when it is combined with a low dose of anti-ICAM-1 Moab, that in itself is non-protective [13]. When an anti-CD11a and an anti-CD11b Moab are administered together, a significant but only moderate reduction in serum creatinine increase and in histologic tubular necrosis is also obtained [17]. These data suggest that blocking of ICAM-1 ligands is less effective than blocking of ICAM-1 itself, at least in animal models. However, the anti-ICAM-1 Moab might activate endothelial cells; consequently the use of ICAM-1 anti-sense oligonucleotides may be a better approach.

The effect of selectin inhibition has not been extensively explored. However, it has been shown that L-selectin-deficient mice are not protected against acute renal failure. In human transplantation, studies using monoclonal antibodies against adhesion molecules have mainly targeted ICAM-1 and LFA-1. An anti-ICAM-1 Moab has been administered in human transplantation during the first 2 weeks post-transplantation, associated with cyclosporine A, in 18 recipients at high risk of delayed graft function [4]. When efficient Moab serum levels were reached, significantly less delayed graft function and rejection were seen. Our team evaluated an anti-LFA-1 Moab, Odulimomab, as induction treatment, in 50 recipients of a first kidney transplant [5]. These patients were also found to be less susceptible to delayed graft function, when compared to 50 patients who received antithymocyte globulin (respectively 19 and 35% of the patients requiring dialysis post-transplantation). This difference was not statistically significant. Nevertheless, in this study only few patients were at high risk of developing delayed graft function. It is still sensible to test a potential protective effect of the anti-LFA-1 Moab in more sensitive situations (aged donors, hyperimmunization, long cold-ischaemia time).

The importance of tubular obstruction in ischaemia reperfusion injury should not be neglected. In rats submitted to renal ischaemia by clamping of the renal artery, the administration of synthetic RGD peptides or antisense oligonucleotides to RGD has been demon-
stratified to improve renal function recovery and histological evidence of tubular dilatation and obstruction [8]. Their association with an anti-ICAM-1 Moab is currently under evaluation.

Finally, according to experimental in vivo studies on the prevention of reperfusion injury, blockade of ICAM-1 seems to be more effective than that of any other molecule. The association of anti-adhesion agents, directed against the same or different pairs of molecules, is also expected to be more successful. Furthermore, many other mediators, such as oxygen radicals, play a role in the genesis of reperfusion injury and their inhibition can also be achieved. Effective prevention of reperfusion injury will probably require the combination of strategies targeting several of the underlying physiopathological mechanisms.

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