Introduction

Is nephropharmacology a common scientific denominator?
The kidney is central to the pharmacology of most drugs and is a target both for therapy and side-effects. The approach of nephropharmacology for improvement in diagnostics and therapy is a multidisciplinary one in which nephrologists and specialized pharmacologists work together.
The Fifth Congress of Nephropharmacology tried to bring all interested groups working in this field together. The fact that there have already been five congresses demonstrates the acceptance of this discipline by the scientific community.
To focus the scope of this congress we decided to ask interested groups to concentrate on five main subjects:
- role of the kidney in pharmacokinetics and pharmacodynamics
- mechanisms of nephrotoxicity
- new strategies in nephroprotection
- transplantation
- what is new?
These extended abstracts will allow a very large package of information to reach both the core group of researchers and the wide periphery of interested nephrologists and pharmacologists.

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Transport mechanisms for cationic drugs and proteins in kidney, liver and intestine: implication for drug interactions and cell-specific drug delivery

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Drug excretion is a concerted action of kidney, liver and intestines [1,2]. One ultimate goal of the study of membrane transport of drugs is to answer the question: which factors determine the relative contribution of excretory organs to the total body clearance of various classes of drugs?
Earlier studies on the excretory profiles of cationic drugs, using 14 organic cations with increasing molecular weight and lipophilicity, revealed that liver, kidney and intestine dispose of such agents differently depending on their relative lipophilicity. The agents with relatively low molecular weight and lipophilicity are predominantly excreted by the kidneys. With increasing molecular weight of the agents, the small intestine and, in particular, the liver, play a more dominant role in their overall excretion [3]. Drug interactions on the biliary excretion of cationic P-glycoprotein (P-gp) substrates and other cationic drugs can be predicted on the basis of their relative lipophilicity [4].
The impact of hydrophobicity on elimination routes can, among others, be explained by the affinity of these
agents for the uptake and secretion carriers involved. The importance of hydrophobic interactions have been demonstrated: for the organic cation uptake carriers transporters OCT1 and OCT2 [5], for the multispecific organic anion transporter peptide uptake carrier (OATP) that also accommodates certain uncharged and cationic drugs [6] and recently for the secretory P-gp (mdr) system [4]. The relative expression and cellular localization of these carriers in the three excretory organs is a major factor in determining the elimination routes. It is of note that a high affinity of a substrate for a carrier does not always imply efficient transport. For instance, the so-called type 2 organic cations strongly inhibit type 1 transport but not vice versa [7]. This may be due to binding to an allosteric binding site on the type 1 carrier [5]. In fact, recent oocyte studies with the OCT1 carrier, that accommodates the type 1 organic cations, indicate that type 2 compounds are bound with high affinity but are not transported [5]. This and other recent observations in various laboratories clearly indicate that the functionally defined type 1 and type 2 organic cation uptake systems, as inferred from earlier studies [7,8], have an apparent molecular basis: OCT1 in liver and intestine as well as OCT1 and OCT2 in the rat kidney accommodate the type 1 compounds, while bulky (type 2) organic cations as well as their inhibitors, (cardiac glycosides), can be transported by various OATP isoforms [9]. The recently cloned OATP2 preferentially accommodates cardiac glycosides, exhibiting a 1000-fold difference in \( k_m \) between ouabain and digoxin [10]. A similar difference was earlier found between these cardiac glycosides with regard to inhibition of type 2 organic cation uptake [8]. We recently detected an interesting stereospecificity in inhibition of hepatic uptake of cardiac glycosides by the (dia)-stereoisomers quinine and quinidine, quinine being a much more potent inhibitor [11]. We are presently studying whether this stereospecific interaction may reflect differences in affinity for the isoforms of OCTP [12]. Interactions between cardiac glycosides and basic drugs at the renal and hepatic level represent one of the clinically relevant drug interactions that have been reported to occur in patients [13].

To establish the role of P-gp in elimination of various cationic drugs more definitely, we investigated transport of the P-gp substrate vinblastine together with the cationic drugs TBuMA, PAEB and vecuronium (Vec) which, in structure, is closely related to rocuronium. We studied transport of the abovementioned compounds in mice with a mdr 1a gene disruption (mdr 1a(−)) [14] or in mdr 1a/1b double gene (mdr 1a/1b(−/−)) knock-out mice [15]. In addition, directional transport in Transwell systems was studied using LLC-PK1 cells that were stably transfected with the various mdr genes in order to check substrate specificity of isoforms of P-gp towards the cationic model compounds [16]. In these cells the mdr gene products are highly expressed at the apical domain. In mdr1a(−) mice, biliary excretion of TBuMA, PAEB and Vec was reduced to ~50% of control (as % of the i.v. dose in 1h). Interestingly, TBuMA, PAEB and Vec secretion into the small intestinal lumen was also largely decreased in mdr 1a(−) mice, while renal excretion was less affected. In the mdr1a/1b(−/−) mice, the biliary excretion of the studied drugs was further decreased: but renal clearance in the complete absence of P-gp was even increased [15]. In the Transwell studies [16] we found that the apical flux of vinblastine was 5-fold higher in cells transfected with mdr cDNAs. Expression of P-gp at the apical domain of the transfected cells also resulted in an increased flux of TBuMA and PAEB. In the liver, uptake of both type 1 and type 2 organic cations can be explained by the presence of OCT1 as well as OATP1 and OATP2. The OATP isoform OATP2, that is particularly highly expressed in liver, could very well represent the earlier defined type 2 carrier since it also accommodates cardiac glycosides.

Small organic cations in the kidney can not only be readily taken up in tubular cells via the OCT1/OCT2 carriers but can also be effectively transported out of the cells in the primary urine through the well defined proton-antiporter system. In contrast to the liver, an ‘outside to inside’ proton-gradient is present in the kidney [17,18]. Larger cationic drugs will have problems entering tubular cells since the OATPs are likely to be located at the apical membrane [6,10] and are not recognized as substrates by OCT1 and OCT2 [5]. If they could enter the tubular cells they may, at least to some extent, be transported into primary urine by P-gp that is expressed in the particular cell type [19,20]. However the \( \text{H}^+ \)-antiporter seems to be quantitatively more important in the renal secretion process. In some cases, organic cations such as choline can undergo significant tubular reabsorption. Substrate specificity for this carrier or other organic cations has been recently reviewed [18]. In view of the above mentioned increased renal clearance in the mdr (1a/1b) knock-out mice, P-gp might play a role in active or passive reabsorption [20].

With regard to the small intestine, only OCT1 and not OATP is present at basolateral domains of the mucosa cells. These cells are important for direct secretion of organic cations from blood into the intestinal lumen [21]. The mdr 1a isoform is certainly present at the brush border domain, and mediates secretion from the mucosal cells into the intestines in addition to \( \text{H}^+ \)-antiport systems that may operate here due to the lumen to cell \( \text{H}^+ \) gradient. These factors may explain why small organic cations are not efficiently excreted in the gut whereas agents with intermediate lipophilicity that are substrates for OCT1 and P-gp are secreted [22,23]. However, for larger and more (bulky) organic cations, OATP uptake systems, as present in liver, are not expressed in the gut. Due to this, no extra intestinal excretion may occur if hydrophobicity of the organic cations reaches higher values [27].

Depending on the relative rates of uptake and secretion, drugs will, to some extent, accumulate in hepatic, intestinal and renal tubular cells. This may lead to potential toxicity if uptake is much more rapid than excretion and if little binding to cytosolic proteins or sequestration in intracellular organelles occurs. Yet
uptake of organic cations in organelles can also lead to perturbation of their function and in particular accumulation in endosomes (lysosomes and mitochondria have been reported [24]). Cytoplasmic accumulation of cationic and anionic drugs can also lead to a high driving force for metabolic conversion: the final body clearance of indomethacin was reported to be due to renal glucuronidation as the consequence of renal accumulation of the compound and futile enterohepatic cycling of the drug and its metabolites [25].

Knowledge of membrane transport processes in the kidney can also be used for the cell-specific delivery of renal prodrugs and drug conjugates. Cationic proteins such as lysozyme were used for the tubular delivery of various drugs, that can be covalently coupled to this low molecular weight protein [26]. After receptor-mediated reabsorption, the protein carrier is degraded and intestinal clearance of amphiphilic cationic drugs in mice in which both mdr 1a and mdr 1b genes have been disrupted. Br J Pharmacol 1998; 124: 416–424

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The area under the effect–time curve as a target parameter for dosage adaptation in renal insufficiency

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Introduction

To predict effects of drugs one needs pharmacodynamic models. Due to the lack of pharmacodynamic knowledge, pharmacokinetic parameters \([C_{\text{peak}}, C_{\text{trough}}, \text{area under the curve (AUC)}]\) are regularly used as a surrogate for the effect [1]. A prediction of an effect only by means of these parameters, might be insufficient.

Theory

We developed a new pharmacodynamic model, based on the sigmoid \(E_{\text{max}}\) function, which integrates pharmacokinetics and pharmacodynamics. We used the open one-compartment model and the sigmoid \(E_{\text{max}}\) model to describe pharmacokinetic and pharmacodynamic characteristics respectively. The resulting function describes the area under the effect–time curve (AUETC).

\[
\text{AUETC}(t) = \frac{E_{\text{max}}}{k\cdot H} \cdot \ln \left( \frac{E_{C_{50}} + C_{\text{peak}}}{E_{C_{50}} + C_{\text{peak}} \cdot e^{-kH}} \right)
\]

It is necessary to use the sigmoid \(E_{\text{max}}\) model rather than the simple \(E_{\text{max}}\) model (i.e. the Michaelis–Menten equation) as some pharmacodynamic characteristics can only be described by an AUETC function based on this model (e.g. advantage of bolus dosage compared with an infusion regimen) [2]. Similar to the pharmacokinetic parameter AUC, as a summary parameter of the concentration–time course, the AUETC provides an integrated parameter of the effect–time course and therefore is a summative effect parameter.

Further extension of the model results in a function which is able to describe the overall clinical effect (total effect, TE) in the course of many dosage intervals.

\[
\text{TE}_{\text{ss}}(t) = \frac{E_{\text{max}}}{(n \cdot \text{AUETC}_{\text{ss}})^{\gamma}} \cdot \left( \frac{E_{C_{50}}}{(n \cdot \text{AUETC}_{\text{ss}})^{\gamma}} + (n \cdot \text{AUETC}_{\text{ss}})^{\gamma} \right)
\]

AUETC_{ss} is the AUETC of one dosage interval \(\tau\) in the steady state (ss).

Results

It is suggested to use the AUETC or the TE parameter as a pharmacodynamic target parameter for the calculation of dosage adaptations in the state of disease-related changes of pharmacokinetic parameters.

We used pharmacokinetic and pharmacodynamic data, which are available from the scientific literature [3,4], in order to derive the model parameter describing aminoglycoside efficacy and nephrotoxicity. A nonlinear regression analysis has been used. The resulting parameter sets provide the basis for simulating the efficacy and toxicity of different dosing schemes of aminoglycosides at various degrees of renal impairment. Depending on the primary goal (e.g. high efficacy vs low nephrotoxicity) different dosing schemes result.

To avoid nephrotoxicity the normal gentamicin dose (240 mg/24 h) should be reduced to a maintenance dose of 40 mg/48 h for a glomerular filtration rate (GFR) \(\leq 5\) ml/h (start dose 60 mg). To maintain a high bactericidal effect the normal dose (280 mg/24 h) should be reduced to a start dose of 160 mg and a maintenance dose of 100 mg/48 h for GFR \(\leq 5\) ml/h (D. Czock et al., submitted).

References

Pharmacokinetics and pharmacodynamics of insulin Lispro compared with regular insulin in haemodialysis patients with diabetes mellitus

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Introduction

Chronic renal failure considerably increases the half-life of insulin due to restricted insulin degradation by the kidney [1]. In more advanced stages of renal insufficiency, this effect is antagonized by insulin resistance caused by circulating inhibitors of glucose uptake [2]. As the result of these antagonistic effects, it is not possible to predict the insulin requirements of diabetic patients with impaired renal function. When receiving conventional insulin treatment, these patients are threatened by hyperinsulinaemia and severe hypoglycaemic episodes [3].

Insulin Lispro (LP; Humalog, Lilly Deutschland GmH), a recombinant monomeric insulin analogue, is identical to human insulin except for the transposition of proline and lysine at positions 28 and 29 in the C-terminus of the B chain. LP shows a faster onset of action, higher peak insulin levels and a shorter duration of action compared with human insulin (HI) [4,5]. In the present study, we tested the hypothesis of whether LP may facilitate blood glucose control in haemodialysis patients with diabetes mellitus.

Study design

Eight haemodialysed diabetes mellitus patients (female/male: 3/5; age: 59±10 years; duration of diabetes: 28±10 years; duration of dialysis: 2.4±3.8 years) of whom two were type 1 diabetics (body mass index (BMI): 22.5±0.5 kg/m²; haemoglobin Alc (HbA1c): 6.7±0.4%) and six were type 2 diabetics (BMI: 27.2±5.5 kg/m², HbA1c: 11.5±3.3%) participated in the study. The patients received comparable doses of either LP (mean 9.4±6.5 IE) or HI (9.6±5.7 IE) 5 min after starting a 4 h haemodialysis procedure. Immediately after subcutaneous injection in the abdominal wall, the patients had breakfast. Blood glucose and serum insulin were measured by specific assays at 0, 20, 40, 60, 90, 120, 180 and 240 min after injection, as recently described [5]. Pharmacokinetic
parameters were calculated using a computer program for non-linear regression analysis [6].

**Results**

The absorption of LP was significantly faster than that of HI. As shown in Figure 1A, maximum plasma insulin concentrations (C_{max}) were reached after 30 (LP) vs 51 min (HI). Peak insulin concentrations were significantly greater with LP (146 μU/ml) than with HI (88 μU/ml). Insulin concentrations returned to baseline values more quickly with LP than with HI. At 120 min after injection, plasma insulin concentrations were 37% of C_{max} (LP) vs 77% (HI). Data obtained by computer-assisted calculation revealed that the absorption half-life of LP was significantly shorter compared with HI (12 ± 8 vs 32 ± 8 min), whereas the elimination half-life and the volume of distribution of LP were not different from HI (43 ± 21 vs 40 ± 9 min; 61 ± 54 vs 75 ± 49 l). Blood glucose levels declined within 20 min after LP injection (Figure 1B), whereas after HI injection blood glucose even increased during the first 40 min. The nadir of blood glucose was reached 3 h after LP injection, whereas with HI glucose levels decreased further.

**Discussion**

In diabetic haemodialysis patients, the rapid changes in insulin and glucose metabolism require fast and short-acting insulin preparations. In these patients, the time-action profile of HI with its delayed onset and prolonged duration of action does not coincide with glycaemic excursions. In contrast, LP is absorbed more rapidly, leading to a faster onset and shorter duration of action compared with HI. The pulsatile pharmacokinetic profile of LP may not only facilitate the correction of hyperglycaemia but may also decrease the risk of late hypoglycaemic episodes which are of particular clinical relevance in haemodialysed diabetic patients. Furthermore, LP offers the advantage of immediate pre-meal injection which is important for treatment satisfaction and may enhance the quality of life.

**References**


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**Pharmacodynamic half-life and effect–time course in renal impairment**

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**Background and objective**

In patients with renal impairment, the elimination of drugs is impaired, and elimination half-life increases [1]. In pharmacokinetics, the kinetic half-life (T_{1/2}) is used to describe the relation between concentration (C) and time (t). To describe the relation between effect (E) and concentration (C) the sigmoid E_{max}-model is used with Hill coefficient (H), and concentration (C_{E50}) producing half-maximum effect [2].

\[ E = \frac{E_{max} \cdot C^H}{C_{E50}^H + C^H} \]

If the term half-life is not reserved to be used for logarithmic first order kinetics, a pharmacodynamic half-life can be derived for the effect–time course that depends on the concentration–time course. Such a parameter might be used to specify the effect–time course in renal impairment.

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Pharmacodynamic half-life

The bisection time \((t_2 - t_1 = t_2 - t_1)\) is required to decrease the effect by one half \((E_2 = \frac{1}{2}E_1)\). For the most simple case, this bisection is due to a mono-exponential decrease in concentrations according to the linear coefficient \((\lambda)\).

\[
C_2 = C_1 \exp(-\lambda (t_2 - t_1))
\]

The bisection time of the effect depends on the linear coefficient \((\lambda)\):

\[
t_2 - t_1 = \ln(C_1/C_2)/\lambda
\]

Transforming the equation of the sigmoid \(E_{\text{max}}\) model, we obtain:

\[
C_2^H = \frac{CE_{50}^H}{(E_{\text{max}}/E_1) - 1}
\]

The concentration \((C_2)\) at effect \((E_2 = \frac{1}{2}E_1)\) can also be stated.

\[
C_2^H = \frac{CE_{50}^H}{(E_{\text{max}}/E_1) - 1}
\]

Since \((E_1 = E_{\text{max}} C_1^H/[CE_{50}^H + C_1^H])\) we can eliminate \(C_2\) in the above equation derived for \(t_2 - t_1\).

\[
t_2 - t_1 = \ln \left[ \frac{C_1}{CE_{50}^H \left[ 2(CE_{50}^H/C_1^H) + 1 \right]^{1/\lambda}} \right] / \lambda
\]

Transformation results in:

\[
t_2 - t_1 = (1/\lambda) \left( 1/H \right) \ln \left[ 2 + C_1^H/CE_{50}^H \right]
\]

The bisection time of the effect \((t_2 - t_1)\) depends on the linear coefficient, and thus on the kinetic half-life \((T_{\text{kin}}^2 = \ln(2)/\lambda)\).

\[
t_2 - t_1 = [T_{\text{kin}}^2 \ln(2)] \left( 1/H \right) \ln \left[ 2 + C_1^H/CE_{50}^H \right]
\]

The bisection time \((t_2 - t_1)\) of the pharmacodynamic effect can be termed pharmacodynamic half-life \((T_{\text{dyn}}^2 = t_2 - t_1)\). The pharmacodynamic half-life \((T_{\text{dyn}}^2)\) is a concentration-dependent parameter and a non-linear function of the kinetic half-life \((T_{\text{kin}}^2)\), where \([1/\ln(2)] = 1.44\).

\[
T_{\text{dyn}}^2 = T_{\text{kin}}^2 \left( 1.44/H \right) \ln \left[ 2 + C_1^H/CE_{50}^H \right]
\]

High drug concentrations \((C_1 \gg CE_{50})\) will lead to prolonged drug action, and increased dynamic half-life [3]. A high Hill coefficient \((H > 1)\) results in a short dynamic half-life \((T_{\text{dyn}}^2 < T_{\text{kin}}^2)\).

Example and discussion

Physostigmine has a pharmacokinetic half-life of 0.27 h. The pharmacodynamic effect on plasma butyrylcholinesterase activity decreases with a 5-times longer half-life of 1.4 h [4]. This indicates that the pharmacokinetic–pharmacodynamic relation is located in the right, concave and saturated part of the sigmoid \(E_{\text{max}}\)-model \((C > CE_{50})\). Since for low concentrations the effect near-linearly increases with the concentration, it can be assumed that it holds \((H \approx 1)\). The peak level \((C_{\text{peak}})\) was 12.5 nM, corresponding to 3 ng/ml. According to the above equations, the unknown concentration at half-maximum effect can be calculated \((CE_{50} = 0.42 \text{ nM})\), corresponding to 0.1 ng/ml. Since we do not know the \(E_{\text{max}}\), the value would be read off 12-times higher from the published graph \((CE_{50} = 5 \text{ nM})\), corresponding to 1.2 ng/ml.

We can draw practical inferences on the usually unknown dynamic parameters if we know the kinetic and dynamic half-lives describing drug elimination and effect duration [5]. With constant target concentration \((C_{\text{peak}} = C_1)\), dynamic half-life or duration of drug effect will increase in proportion to elimination half-life in renal failure. Even when the dose is adjusted to
identical target concentrations ($C_{\text{peak}} = \text{constant}$), the effect might be longer lasting with a prolonged dynamic half-life in renal impairment.

References


Cross-talk between activated tubular epithelia of human kidney and monocytes: a basis for target cell-specific pharmacotherapy?

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Changes in the tubulointerstitial compartment govern the progression and outcome in most patients suffering from renal diseases. Under pathological conditions, the influx of monocytes into the kidney and local proliferation of blood-derived macrophages releasing proinflammatory and fibrogenic cytokines contribute to structural and functional deterioration [1]. In addition, cell lesions may result directly from cooperative but maladapted cell interactions between ‘activated tubular cells’ which are capable of recruiting and stimulating monocytes to invade the glomeruli and/or the tubulointerstitium, thus resulting in progressive sublethal injury, necrosis and fibrosis. In the present work, we summarize our experimental and clinical data which support an engaged interaction (cross-talk) of tubular epithelia of proximal and distal origin with monocytes/macrophages in human renal diseases [2–4].

Human renal proximal and distal tubular cells.

Human renal proximal (PTC) and distal (DTC) tubule cells were isolated immunomagnetically as described earlier, applying monoclonal antibodies raised against distinct segments of the human nephron [2]. PTC were strongly positive for aminopeptidase M (CD13); however, DTC were negative for CD13 antigen. Ultrastructural analyses of PTC primary isolates revealed a highly preserved brush border, whereas DTC showed multiple basolateral invaginations and many fewer apical microvilli. Both cell types formed tight junctions and expressed cytokeratin and vimentin, whereas stains for desmin, α-actin and von Willebrand’s factor were negative. A different response after hormonal stimulation [parathyroid hormone (PTH), calcitonin] was found where cAMP production was especially high in DTC after challenge with PTH [2,5].

Activated tubule cells

After incubation of cultured cells with a mix of 25 U/ml interleukin (IL)-1β, 10 ng/ml tumour necrosis factor-α (TNF-α) and 200 U/ml interferon-γ (IFN-γ), the production of RANTES, a chemokine for monocytes, increased dramatically in both PTC and DTC [6]. Compared with basal conditions, the release of RANTES into the supernatant was 107- to 133-fold increased up to 364 pg/48 h/10⁵ cells. In parallel, expression of HLA-DR and interstitial cell adhesion molecule-1 (ICAM-1) increased significantly, as analysed by flow cytometry. Unstimulated PTC and DTC did not express HLA-DR; DTC expressed ICAM-1 constitutively in very small amounts.

Effect of anti-inflammatory drugs

Glucocorticoids such as dexamethasone ($10^{-6}$ M) as well as cyclooxygenase II inhibitors down-regulated...
the synthesis of RANTES in cytokine-stimulated PTC/DTC ($P < 0.05$; Baer et al., submitted for publication). Lipopolysaccharide (LPS) from *Escherichia coli* 128:B12 (5 ng/ml) did not modulate HLA-DR and ICAM-I expression of PTC and DTC.

**Monocytes/macrophages**

In healthy subjects, 92% of circulating blood monocytes (median 336 cells/μl) expressed an endotoxin receptor, the CD14 antigen, at a high rate [7]. A minor population (median 8%) revealed an immunophenotype of CD14+ cells which co-expressed the CD16 antigen, an FcγRIII molecule [3,7,8].

**Activated monocytes and drug response**

In cultured monocytes, membrane CD14 and release of soluble CD14 (sCD14) were dramatically up-regulated in the presence of LPS, where the LPS-binding capacity [of fluorescein isothiocyanate (FITC)-labelled LPS] was correlated directly with monocyte CD14 expression ($r = 0.89$, $P < 10^{-4}$; ref. [7]). Endotoxin-induced stimulation of CD14+ and sCD14 synthesis was markedly (but not completely) abolished by various glucocorticoids following a sigmoid curve dose dependency [7]. In addition, glucocorticoids significantly decreased the secretion of IL-1 of LPS-activated monocytes. Blood monocytes expressing both the CD14+ and CD16+ antigen constituted a proinflammatory subtype, which exhibited features of tissue macrophages. CD14+/CD16+ monocytes and sCD14 were highly increased in patients with infectious and non-infectious inflammatory diseases [8,9]. They also disclosed an augmented HLA-DR expression and phagocytic activity compared with CD14+/CD16− cells [9]. CD14+ (and CD68+) cells accumulated up to 10-fold in kidneys of patients with progressive renal damage and in allografts with chronic rejection, as shown by immunohistochemistry. Glucocorticoid therapy selectively affected the CD14+/CD16− subset of monocytes not only by down-regulating CD14 expression and sCD14 release but also inducing a rapid decline in the amount of the circulating proinflammatory cells [3,7].

**Cross-talk of tubular epithelia and monocytes**

Tubular epithelium may cross-talk with monocytes through mechanisms described above (Figure 1). Various stimuli such as immune complexes, ischaemia, oxygen radicals and cytokines (IL-1, IFN, cytomix as shown) activate tubule cells to synthesize and secrete monocyte-attracting chemokines MCP-1 and RANTES. Cytokines, which stimulate tubule epithelia to overexpress HLA-DR and adhesion molecules (ICAM-1), are released by ‘activated’ monocytes or lymphocytes at an increased rate. In the presence of LPS, oxidized lipoproteins or other stimuli, transform blood monocytes (CD14+/CD16−) into a proinflammatory subtype carrying the CD16 epitope as described [10]. This cell type may pass the endothelial barrier, and accumulate (proliferate) within the glomeruli and the tubulointerstitial space. Experimental and clinical studies reveal a tight association of macrophage accumulation and proliferation with local renal damage. Release of profibrogenic cytokines and growth factors by activated monocytes/macrophages (IL-1, TNF, prostaglandins, etc.) may amplify progressive cell

![Fig. 1. Cross-talk of tubular epithelia of human kidney and monocytes capable of transforming into the proinflammatory macrophage-like subtype (CD14+, CD16+, HLA-DR+) which invades the kidney. Pre-lethally damaged or cytokine-activated tubular cells may present antigens to lymphocytes and release chemokines (RANTES) to attract activated monocytes/macrophages. oLip = oxidized lipoproteins, LPS = lipopolysaccharide (endotoxin), O* = oxygen radicals. For details, see text.](image-url)
injury, interstitial fibrosis, glomerulosclerosis and functional deterioration. Anti-inflammatory drugs positively interfere the cross-talk of both cell types.

References


Role of the protein kinases A and C and of the calcium/calmodulin-dependent protein kinase II in the regulation of the renal basolateral PAH and dicarboxylate transporters

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The kidneys have a major role in the elimination of drugs and their metabolites. The elimination mechanism involves mainly glomerular filtration, but drugs which are organic bases (cations) or acids (anions) may, in addition, be actively secreted by the proximal tubules. The secretory system accepts a wide variety of pharmacologically highly active drugs or their metabolites [e.g. antibiotics, non-steroidal anti-inflammatory drugs, loop and thiazide diuretics, angiotensin-converting enzyme (ACE) inhibitors, AT1 receptor antagonists].

There are two transport systems present in the kidneys by which drugs can be secreted from blood into urine, one for organic anions and another for organic cations. The transport proteins, which recently have been cloned, reside predominantly in the S2 segment of proximal tubules [1]. Regarding the renal transport system for organic anions, of which p-aminohippurate (PAH) is the prototype, the active step in the secretion process is confined to the basolateral membrane. The current model (Figure 1) indicates that the cellular uptake of organic anions across the basolateral cell membrane is mediated by a tertiary active process. The primary event is hydrolysis of ATP.

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Identification by HPLC of uraemic retention solutes decreasing theophylline protein binding

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Substantial changes in protein binding of drugs occur during the progression of renal insufficiency. Protein-bound uraemic solutes may play a role in the inhibition of drug protein binding. Previously we demonstrated that hippuric acid in uraemic ultrafiltrate (UUF) was a theophylline protein binding inhibitor [1]. In this study, we deproteinized uraemic serum to obtain the total amount of uraemic compounds to evaluate their effect on the theophylline protein binding. The ligands were displaced from their binding sites by five deproteinization methods: heat deproteinization, acetonitrile extraction, trichloroacetic acid precipitation, acid precipitation and acetonitrile extraction and bilirubin displacement [2].

Two HPLC procedures were applied: (1) semi-prep HPLC to fractionate and characterize the protein binding inhibiting compounds and (2) analytical HPLC to determine the concentration of the identified compounds. In addition, a uraemic solution was prepared with the identified compounds at the same concentration as in uraemic serum. Two HPLC procedures were applied: (1) semi-prep HPLC to fractionate and characterize the protein binding inhibiting compounds and (2) analytical HPLC to determine the concentration of the identified compounds. In addition, a uraemic solution was prepared with the identified compounds at the same concentration as in uraemic serum.

The effect on the theophylline protein binding of deproteinized uraemic serum (DUS) was compared with UUF. A significant progressive decline in theophylline protein binding vs control serum (C) was found when increasing quantities of lyophilized DUS or UUF were added to C (P < 0.05), whereby inhibition...
was more important for DUS than for UUF. The influence of 30 preparative HPLC fractions from DUS or from UUF on the theophylline protein binding was evaluated. The most important inhibition was observed in HPLC fractions 6, 10 to 13, 15 and 28; resp. 61.5 ± 10.8, 64.5 ± 7.6, 60.9 ± 10.1, 47.5 ± 3.3, 60.0 ± 6.7, 60.7 ± 6.3 and 61.3 ± 6.9 vs 69.1 ± 2.4% protein binding for C (P < 0.05). A lesser decreased protein binding was found in fractions 9 and 20.

To characterize the uraemic compounds with an inhibitory effect on the theophylline protein binding, the elution and the UV or fluorescence detection of compounds in a standard solution were compared with the elution and detection of peaks in the uraemic samples. The standard solution contained: creatinine, pseudouridine, uric acid, hypoxanthine, tyrosine, p-hydroxyhippuric acid, indoxyl sulfate, hippuric acid, tryptophan, indole-3-acetic acid and 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid (CMPF).

Deproteinized uraemic serum was spiked with the standard solution. All compounds, except tyrosine co-eluted with a peak of the deproteinized uraemic serum. The detection wavelength 254 nm or 280 nm/340 nm of the other compounds in the standard solution fitted with the detection of the corresponding peaks in the DUS.

The characterized compounds eluting in the inhibitory fractions were indoxyl sulfate in fraction 11, hippuric acid and tryptophan in fraction 12, indole-3-acetic acid in fraction 20 and CMPF in fraction 28. In addition, the peak height of these compounds in the DUS was increased vs the peak height in UUF, indicating their protein-bound nature.

The total concentration of five characterized compounds in the DUS fractions was for indoxyl sulfate: 2.2 mg/100 ml, hippuric acid: 6.5 mg/100 ml, tryptophan: 0.5 mg/100 ml, indole-3-acetic acid: 0.5 mg/100 ml and CMPF: 5.6 mg/100 ml.

A uraemic solution was prepared with the five substances at the same concentration. This solution was added to C and decreased the theophylline protein binding from 66.7 ± 1.1% to 61.3 ± 1.3%, this was however less important than for genuine uraemic serum (47.4 ± 3.6%) (P < 0.01). Dose–response curves with the identified compounds, revealed that the main role can be attributed to hippuric acid and CMPF, whereas the other solutes were less important, at least at the concentrations found in DUS.

In conclusion, these data suggest that the yield of protein binding-inhibiting compounds was more important with DUS than with UUF. The identified uraemic compounds were not entirely responsible for the decreased protein binding of theophylline, indicating that factors other than those identified in this study affect the protein binding.

References


**Patterns of potassium (K) wasting in response to stepwise combinations of diuretics in nephrotic syndrome**

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The potassium (K) wasting effects of diuretics have been known for a long time, though not yet entirely clarified. We have studied the K wasting action of frusemide administered alone and in combination with chlorthalidone as well as with potassium sparing diuretics (PSD; ‘antikaluuretics’) by investigating K excretion (UKV) and the transtubular potassium gradient (TTKG) as well as other specific renal functions in 11 patients with renal oedema; seven patients suffered from advanced nephrotic syndrome and four patients were classified as ‘forme fruste’. The average basal glomerular filtration rate was 28.52 ± 2.07 ml/min. Frusemide was given in doses of 200–700 mg and chlorthalidone in doses of 50–100 mg daily. PSDs were administered alone in ‘low doses’ (usual dosage) and in ‘high doses’ (i.e. higher than normal dosage and/or in combination with other PSDs). Diuresis and natriuresis were maintained at 2000 ml/24h and 200 mmol/24h, respectively. Surprisingly, in response to frusemide monotherapy UKV hardly increased (from 33 ± 2 to 40 ± 5 mmol/24 h; 20%; NS) and there

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was a significant decrease in TTKG (from 5.90 ± 0.38 to 4.30 ± 0.42; P < 0.05; Figure 1, upper panel). Nevertheless the combination of chlorthalidone with frusemide induced a definite increase in both UKV (to 52 ± 3 mmol/24 h; P < 0.05) and TTKG (at least in comparison with frusemide administered alone; 5.24 ± 0.35; NS; Figure 1, upper panel) either without PSDs or during administration of PSDs (UKV increased from 16 ± 2 to 38 ± 4 mmol/24 h, TTKG from 2.81 ± 0.28 to 4.06 ± 0.29; P < 0.01). Without administration of diuretics (‘baseline’) a normal relationship was found between UKV and TTKG: y = 0.0866x + 3.1053; r = 0.35; P < 0.02. However, during monotherapy with frusemide and in response to the combination of frusemide + chlorthalidone the relationship became distorted: y = 0.0138x + 3.6416; r = 0.07; NS (frusemide); y = 0.0293x + 3.7249; r = 0.28; NS (frusemide + chlorthalidone), supporting the concept of a divergent action of frusemide on TTKG and UKV. In response to antikaluretics TTKG decreased not only on the day of administration during the diuretic effect (Figure 1, middle panel) but also when the effect was over in the post-diuretic period (lower panel). Without PSDs, TTKG increased in the post-diuretic period (Figure 1, lower panel) probably indicating diuretic-induced hyperaldosteronism. Frusemide caused a small K loss, probably because of the enhanced ‘distal volume flow’, and at the same time a decrease of TTKG representing an opposite (‘inhibitory’) influence. The combination of frusemide with chlorthalidone operated in the direction of K loss. On the other hand, inclusion of high doses of antikaluretics into the above combination of diuretics caused a remarkable K retention by inducing a decrease in TTKG. It was concluded that (i) significant K loss was induced only during co-administration of chlorthalidone; (ii) frusemide not only induced K loss, but must also have an opposite (perhaps secondary inhibiting) influence.

**Nephrotoxicity: focusing on radiocontrast nephropathy**

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Nephrotoxicity is an important cause of renal failure throughout the world, and its development significantly increases the in-hospital mortality of our patients [1]. Many diagnostic and therapeutic agents are involved. The underlying mechanisms of nephrotoxicity are: post-renal failure induced by crystal formation (acyclovir), immune-mediated glomerular damage (penicillamine), pre-renal vasoconstriction (angiotensin-converting enzyme inhibitors), allergic reactions within the kidney (penicillin), endothelial damage (cyclosporin) and in the majority of cases a tubulo-interstitial
damage (radiocontrast agents). As contrast media are used widely, it is not surprising that radiocontrast-induced nephropathy (RCIN) is one of the major causes of acute renal failure [2]. Kidney damage induced by radiocontrast agents includes a haemodynamic response and a self-standing tubulotoxicity, and RCIN could serve as a human model of nephrotoxicity [3]. After injection of radiocontrast there is a transient increase, followed by a more prolonged decrease, in renal blood flow (RBF) [4,5]. A variety of vasoactive substances may modulate the radiocontrast-induced vasoconstriction [6]. Contrast media induce an intrarenal hypoxia, possibly related to the haemodynamic changes and/or an increased tubular energy expenditure due to osmotic stress [3]. It has been proposed that increased renal adenosine concentrations as a result of enhanced ATP hydrolysis may be a major factor in the development of acute renal failure after radiocontrast application. This is corroborated by the finding that administration of contrast media increases urinary adenosine [7,8] and the observation that dipyridamole, a nucleoside uptake blocker, enhances the renal haemodynamic effects of contrast media [7,8]. In addition, there are some similarities between radiocontrast-induced nephrotoxicity and renal haemodynamic changes induced by adenosine. Sodium depletion is known to potentiate adenosine action in the kidney, and also augments the nephrotoxicity of contrast media [5]. Blockade of the production of vasodilatory prostaglandins by indomethacin increases both the adenosine effect in the kidney [9] and the vasoconstriction induced by contrast media [10–12]. Ischaemia prior to application of contrast media increases their toxicity [13], and renal ischaemia enhances adenosine generation, leading to renal vasoconstriction [14–16]. Contrast media and adenosine both show disparate effects regarding regional blood flow of the kidney with medullary vasodilatation [12,17].

Experimental studies in different animal models of acute renal failure reveal a nephroprotective effect of adenosine antagonism [18–24]. Theophylline, for example, acts as a non-specific adenosine receptor antagonist. Studies in dogs showed a nephroprotective effect of theophylline after radiocontrast [7]. Preliminary results in humans also indicate a nephroprotective effect of theophylline concerning the reduction of the glomerular filtration rate (GFR) after radiocontrast [8,25]. In a follow-up study of patients with renal insufficiency, the incidence of an acute renal dysfunction after radiocontrast was low in both groups (placebo group, 3.4%; theophylline group, 5.7%). In this study, all patients were hydrated (3000 ml per day) starting at least 24 h before administration of contrast medium, which was not done in our first trial. It is known that in a dehydrated state, both RBF and the GFR are decreased and, therefore, the magnitude of the effects of contrast media on these parameters is more marked [26,27]. Thus, it can be argued that the prolonged tubular exposure to contrast media because of low tubular flow rates in combination with a stimulation of the renin—angiotensin system is the main reason for a reduction in the GFR. Under these conditions, the best protective effect of adenosine antagonists could be demonstrated. This is underlined by our experimental results showing that rats under chronic NO blockade are highly sensitive to radiocontrast-induced damage [28], and adenosine antagonists showed favourable effects concerning the prevention of a decline in GFR and RBF in these animals [28]. So far, patients with heart failure or those unable to be hydrated sufficiently due to other conditions and with a higher degree of renal insufficiency have been excluded from trials investigating the effects of theophylline. Clinical trials involving patients with contraindications for hydration should be carried out in order to evaluate clearly the value of theophylline in the prevention of RCIN. Preliminary results obtained from a retrospective study showed that theophylline administration on an ICU produces good results regarding the incidence of acute renal failure in patients with cardiac insufficiency (incidence of acute renal failure without theophylline, 15%; with theophylline, 7%) [29].

References

Prostaglandin E₁ (PGE₁)—prophylaxis against radiocontrast-induced nephrotoxicity in patients with pre-existing renal dysfunction

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Purpose

The administration of intravascular iodinated contrast media to patients with pre-existing renal dysfunction is frequently followed by variable degrees of acute renal failure thought to be due to renal vascular ischaemia and/or direct tubular cell toxicity [1,2]. However, 23% of group 4 patients required discontinuation or reduction of infusion rate due to clinically relevant blood pressure reduction as opposed to a dramatically lower percentage of reductions in groups 1–3. No difference in the creatinine clearance between the four groups was observed.

Methods

Since the prostaglandin PGE₁ is cytoprotective and has vasodilatory effects, we performed a pilot study to evaluate its effect in 117 patients (male/female: 77/40; age 67 ± 10 years) with a baseline creatinine ≥ 1.5 mg/dl (mean: 2.2 ± 0.7 mg/dl) receiving ≥ 75 ml of intravascular contrast. A 6 h infusion of study drug began 1 h prior to contrast application: group 1 (placebo, n = 29); group 2 (10 ng PGE₁/kg/min, n = 33); group 3 (20 ng PGE₁/kg/min, n = 33); group 4 (40 ng PGE₁/kg/min, n = 23).

Results

Increases in serum creatinine at 48 h post-contrast were: group 1 = 0.72 mg/dl; group 2 = 0.3 mg/dl; group 3 = 0.12 mg/dl; and group 4 = 0.29 mg/dl. No patient had a serious adverse event related to the study drug; however, 23% of group 4 patients required discontinuation or reduction of infusion rate due to clinically relevant blood pressure reduction as opposed to a dramatically lower percentage of reductions in groups 1–3. No difference in the creatinine clearance between the four groups was observed.

Conclusions

PGE₁ was well tolerated at all but the highest dosage. The middle dose (20 ng/kg/min) emerged as the most promising [3] expressed by the smallest increase from baseline in peak serum creatinine over 48 h, without affecting blood pressure. Results from this pilot programme suggest that intravascular PGE₁ may be used efficaciously and safely to prevent radiocontrast media-induced renal dysfunction in patients with pre-existing renal insufficiency.

References

Role of serotonin in the development of Chinese herbs nephropathy?

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Chinese herbs nephropathy (CHN) is a rapidly progressive chronic tubulointerstitial nephritis, leading to extensive interstitial fibrosis. Though aristolochic acid has been put forward as the aetiological factor in the development of CHN, a number of arguments plead against its unique pathophysiological role. Since serotonin-receptor-agonists were part of the slimming treatment inducing CHN, the hypothesis was formulated that a serotonin-like substance might contribute to the aetiology of the observed renal lesions.

Rats were injected i.p. weekly with serotonin (20, 30 or 40 mg/kg body weight) or with saline for three consecutive weeks. The animals were sacrificed 35 days after onset of treatment. Renal interstitial fibrosis was evaluated on Masson trichrome-stained histological sections and the proliferation of interstitial macrophages was quantified on double-stained (ED1/PCNA) sections. Additionally, a technique was developed to produce renal vascular casts to visualize serotonin-induced vasoconstriction.

Macroscopically, kidneys of serotonin-treated rats showed a bumpy surface with fibrous retraction.

Reconsideration of some hypotheses on the mechanism of thallium toxicity in rats with special respect to riboflavin and glutathione

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Thallium (Tl)-induced nephrotoxicity in female Wistar rats (Han:Wist) was characterized functionally by oliguria, proteinuria, decreased glomerular filtration rate (GFR), and increased blood urea nitrogen concentration (BUN). Maximal changes occurred on the second day after the administration of 2 mg of TlSO₄/100 g body weight [1].

Several molecular mechanisms of Tl toxicity have been postulated previously: (i) Alteration of K⁺-dependent processes because of chemical similarities; Tl⁺ replaces K⁺ in many processes and enzyme functions such as Na/K-ATPase or enzymes of energy...
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**Table 1.** Influence of 5 mg of riboflavin (B2)/100 g body weight on the activity of glutathione reductase (GR) and the concentrations of GSH and GSSG in renal cortex and medulla.

**Fig. 1.** Influence of ac-cys and BSO on the concentration of GSH and GSSG in renal cortex and medulla as well as its influence on Tl-induced nephrotoxicity. Urinary protein excretion was determined during 5 days after the administration of 2 mg TlSO₄/100 g body weight. N-acetylcysteine: 100 mg/100 g body weight 12 h before, concomitantly with, and 12 h and 24 h after Tl (ac-cys/Tl). Buthionine sulfoximine: 0.4 mmol/100 g body weight, 4 h before and 24 h after Tl (BSO/Tl). *Significantly different from control value at time 0 (n = 6, P ≤ 0.05); + significantly different from Tl group (n = 6, P ≤ 0.05).

*Statistically significant differences from control values (n = 6, P ≤ 0.05).
administration in diuresis experiments) by the determination of GR activity and the concentrations of GSH and GSSG in renal tissue (Table 1). Despite using the effective dose and different time schedules in diuresis experiments, the riboflavin effect on TI induced proteinuria has never been observed (not shown).

Although there was no decrease of GSH concentration in renal tissue in TI-treated rats [3], we tested the effect of N-acetylcyesteine (ac-cys) and buthionine sulfoximine (BSO) on TI-induced proteinuria. At 12 h after ac-cys (corresponding to the time of TI administration in diuresis experiments), GSH concentration was significantly enhanced in renal tissue (see the table in Figure 1). Repeated administrations guarantee continuously high GSH levels for >36 h after the administration of TI. Nevertheless, there was no detectable influence on TI-induced proteinuria (Figure 1).

BSO was effective in decreasing both GSH and GSSG concentrations in renal cortex and medulla (see table in Figure 1) which was followed by significantly decreased proteinuria in comparison with TI-treated rats (Figure 1). Previously, it has been shown that this protective effect is caused by accelerated urinary TI excretion [3].

From our results, it can be concluded (i) that the hypothesis of TI interaction with riboflavin has to be rejected because TI did not decrease GR activity and riboflavin administration did not influence TI-induced nephrotoxicity; and (ii) TI affinity for GSH has to be shown.

Although there was no decrease of GSH concentration in renal tissue and enhanced GSH concentration in renal tissue in TI-treated rats [3], we tested the effect of N-acetylcysteine (ac-cys) and buthionine sulfoximine (BSO) on TI-induced proteinuria. At 12 h after ac-cys (corresponding to the time of TI administration in diuresis experiments), GSH concentration was significantly enhanced in renal tissue (see the table in Figure 1). Repeated administrations guarantee continuously high GSH levels for >36 h after the administration of TI. Nevertheless, there was no detectable influence on TI-induced proteinuria (Figure 1).

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From our results, it can be concluded (i) that the hypothesis of TI interaction with riboflavin has to be rejected because TI did not decrease GR activity and riboflavin administration did not influence TI-induced nephrotoxicity; and (ii) TI affinity for GSH has to be rejected because TI did not decrease GSH concentration in renal tissue and enhanced GSH concentration did not ameliorate TI nephrotoxicity. Decreased GSH concentration did ameliorate TI nephrotoxicity by accelerated TI excretion.

The molecular mechanism which works in TI toxicity remains to be clarified.

References


Unexpected electrophysiological effects of D-19575, a new cytostatic drug

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Objective

As a consequence of renal and urotoxic side effects of the oxazaphosphorines cyclophosphamide and ifosfamide (IFO) [1] D-19575 (β-D-glucosyl-isophosphoramide-mustard) with a presumed transmembrane transport like that of glucose was developed [2,3]. In D-19575 the active metabolite of IFO ifosfamide-mustard (IFM) is coupled to glucose.

Active glucose transport is maintained by means of SGLT1, SGLT2 and perhaps SAAT1 (pSGLT2, SGLT3). Glucose reabsorption in the renal proximal tubule is a Na⁺-coupled process depending on the membrane voltage (V_m) and the membrane conductance (G_m) of differentiated renal proximal tubular cells, LLC-PK1 [4]. Xenopus laevis oocytes were injected with in vitro synthesized cRNA encoding for flounder renal organic anion transport (fROAT) as described [5], and oocytes were examined by means of a two-electrode voltage clamp device.

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showed no significant effect on $V_m$ and $G_m$ ($n=12–16$) (Figures 2 and 3).

Differentiated LLC-PK1 cells have been shown to express SGLT1 as well as SAAT1 [6–8]. Therefore, a depolarization accompanied by an increase of $G_m$ was expected if D-19575 was transported by these systems. Because LLC-PK1 cells are lacking organic anion transport [9], we assayed *X. laevis* oocytes expressing fROAT [5] for possible D-19575-induced currents. D-19575 as well as $\alpha$-D-glucose (1 mmol/l each) elicited barely detectable currents at a clamp potential of $-60$ mV, whereas in the same oocytes, the $p$-aminohippurate (PAH)-induced current (0.1 mmol/l) was $-10.3\pm5.7$ nA ($n=6$).

**Conclusion**

In summary, we show in renal proximal tubular LLC-PK1 cells an effect on $V_m$ and $G_m$ by the cytostatically active metabolite IFM indicating interference with cell metabolism. We were able to demonstrate the electrical equivalents of Na$^+$-coupled glucose transport in LLC-PK1 cells. We could not observe effects by D-19575 on either membrane voltage or ion currents indicating that D-19575 is not transported like glucose, which is in contrast to previous findings [10]. Because D-19575 shows no direct effect on the membrane voltage or ion currents in comparison to IFM we speculate that D-19575 is not directly cytotoxic, which is in contrast to previous findings [2]. From the physiological point of view we doubt the usefulness of cytostatic drugs transported like glucose.

**Results**

Glucose revealed a significant reversible and fast depolarization of $V_m$ by $11\pm2$ mV and an increase in $G_m$ of $11\pm5\%$ ($n=26–38$). IFM showed a significant and slow depolarization of $V_m$ by $9\pm1$ mV and a decrease of $G_m$ by $19\pm8\%$ ($n=13–14$). In contrast D-19575 showed no significant effect on $V_m$ and $G_m$ ($n=12–16$) (Figures 2 and 3).

**Acknowledgements.** This work was supported by grants from ‘Innovative Medizinische Forschung—University of Muenster’ and ‘Deutsche Forschungsgemeinschaft KL 976/7-1’. We are indebted to Dr J. Pohl from ASTA Medica, Frankfurt, Germany, for the supply of D-19575 and ifosfamide mustard.

**Fig. 2.** Summary of effects of glucose (5 mmol/l), ifosfamide-mustard (1 mmol/l) and D-19575 (1 mmol/l) on the membrane voltage in LLC-PK1 cells. Empty bars represent pre- and post-control. * indicate statistical significance between controls and effects. Shown are mean values ±SEM, $n$ refers to the number of experiments.
Abstracts

**Fig. 3.** Summary of effects of glucose (5 mmol/l), ifosfamide-mustard (1 mmol/l) and D-19575 (1 mmol/l) on the change of the membrane conductance in LLC-PK1 cells. * indicate statistical significance between controls and effects. Shown are mean values ± SEM, n refers to the number of experiments.

References


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**Glycosaminoglycan prevents hyperglycemia-induced renal TGF-β1 gene expression**

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Patients with diabetes mellitus account for nearly half of all patients on haemodialysis. Progressive expansion of the mesangial matrix and thickening of the glomerular and tubular basement membranes are hallmarks of human and experimental diabetic nephropathy. These lesions may lead to glomerular fibrosis, a central feature in the development of diabetic nephropathy. We have previously reported that chronic therapy with a low-anticoagulant, heparin-derived glycosaminoglycan preparation (GAG/mH) may prevent diabetic nephropathy, as assessed by albuminuria and histo-

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logical signs, in long-term streptozotocin diabetic rats [1]. Particularly, we could demonstrate that GAG/mH treatment prevents the diabetes-induced increase in renal collagen IV gene expression and deposition [2]. In consideration of the suggested key role of transforming growth factor β (TGF-β) in diabetic nephropathy [3], we wondered whether these agents may be renoprotective by inhibition of the TGF-β cascade. Since our in situ hybridization studies revealed that mesangial cells are a main target of diabetes-induced increase in collagen IV synthesis we used cultured mesangial cells to investigate the molecular mechanism of GAG on TGF-β1 overexpression.

Mesangial cells were isolated from porcine glomeruli and grown in normal and elevated glucose concentrations [4] with and without GAG/mH or dermatan sulfate the same GAGs as were used in the animal studies [1,5]. To evaluate the structure–function relationship dermatan sulfate was included into the study. Both GAG preparations were kindly provided by Dr E. Marchi (Alfa Wassermann SPA, Bologna, Italy). Cellular mRNA levels of TGF-β1 were determined after reverse transcription and PCR amplification (RT–PCR). The results revealed a more than two-fold increase with increasing glucose concentrations which was prevented by addition of 10 µg/ml GAG/mH. Furthermore, the dose-dependent, glucose-induced overexpression of TGF-β1 mRNA was confirmed by in situ hybridization in cultured cells. Quantitative analysis of the grains showed a more than three-fold increase (10 vs 30 mM glucose; P < 0.005) which was prevented by addition of 10 µg/ml GAG/mH. We used 10 µg/ml GAG/mH since preliminary experiments showed significant effects at this concentration. To demonstrate that the heparin structure and possible associated activities (e.g. anticoagulation, growth factor binding) are not necessary for the inhibitory effect a structurally non-related GAG, i.e. dermatan sulfate (GAG/DS) was used. The presence of increasing amounts of this GAG/DS prevented high glucose-induced TGF-β1 mRNA increase dose-dependently, although less efficiently when compared to GAG/mH. Neither GAG changed TGF-β1 mRNA levels.

The effect of GAG/mH and GAG/DS on mesangial TGF-β protein expression was also studied. GAG/mH treatment attenuated high glucose-induced mesangial overproduction of TGF-β protein and formation of bioactive TGF-β without affecting basal levels. To exclude a direct effect of GAG on TGF-β1 bioactivity 10 µg/ml GAG (i.e. more than 1000-fold molar excess) were added to active TGF-β1 (1 ng/ml) and incubated with mesangial cells. TGF-β1 bioactivity was determined by the conventional mink lung proliferation assay. While addition of 1 ng/ml TGF-β1 reduced DNA synthesis by more than 60% the presence of the GAG preparations used in the animal studies had only marginal effects on TGF-β1 bioactivity. It is noteworthy that addition of GAG slightly reduced prolif-

erative effect of TGF-β1 was observed.

To evaluate whether the inhibitory effect of GAG is exerted at the gene activation level TGF-β1 promoter activity studies were performed using a TGF-β1 promoter–luciferase construct. Mesangial cells were transiently transfected with this and a control construct and incubated with glucose for 48 h. Increasing glucose concentration enhanced TGF-β1 promoter activity by 67%. This increase was prevented by the presence of 10 µg/ml GAG/mH indicating that the GAGs are inhibiting TGF-β1 gene transcription.

In conclusion, our results demonstrate that in mesangial cells an increased, hyperglycaemia-induced expression of TGF-β1 mRNA occurs, and that this effect is prevented by GAG treatment with both GAG preparations indicating that the heparin structure is not essential for the inhibitory effects. The promoter activity studies suggest that GAGs exert their activity at or proximal to transcription factors activating TGF-β1 promoter. Together with previous results including experimental animals—and preliminary human studies [6]—our data indicate that chronic administration of chemically modified, low-anticoagulant GAG preparations could prevent both functional and structural glomerular and in particular mesangial alterations occurring in diabetes mellitus.

References

Targeting TGF-β overexpression: maximizing the antifibrotic actions of angiotensin II blockade in anti-Thy1 glomerulonephritis

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Introduction

Progressive accumulation of pathological extracellular matrix in the kidney is the main cause of end-stage renal disease in patients with hypertension, diabetes mellitus or glomerulonephritis. Overexpression of the profibrotic cytokine transforming growth factor (TGF)-β has been identified as key mediator of matrix accumulation in a number of human and experimental renal diseases [1]. In rats following induction of anti-Thy1 glomerulonephritis, we used reduction in TGF-β overexpression as a novel target to evaluate several strategies to maximize the antifibrotic action of angiotensin (Ang) II blockade [2]. We asked whether TGF-β expression can be reduced more effectively or even normalized by increasing the doses of Ang II-blocking drugs or by combining angiotensin-converting enzyme (ACE) and angiotensin type 1 (AT1) receptor antagonism.

Methods

Anti-Thy1 glomerulonephritis was induced in male Sprague–Dawley rats (225–280 g) fed a normal protein diet by the i.v. injection of OX-7 antibody (1.5 mg/kg). At 24 h after disease induction, rats were given the ACE inhibitor enalapril (0, 10–1000 mg/l, set 1) or the AT1 antagonist losartan (0, 50–2000 mg/l, set 2) in their drinking water. In set 3, rats were treated with enalapril (100 mg/l), losartan (500 mg/l) or a combination of both. The doses selected for enalapril and losartan had shown maximal efficacy in set 1 or 2. The animals were sacrificed 6 days after disease induction. Glomerular TGF-β1 synthesis was measured by enzyme-linked immunosorbent assay (ELISA) in the supernatant of cultured glomeruli isolated from individual rats. Matrix accumulation was estimated using periodic acid–Schiff (PAS)-stained kidney tissue by a blinded observer.

Results

Following induction of anti-Thy1 glomerulonephritis, increasing doses of both enalapril and losartan reduced glomerular TGF-β1 production in a dose-dependent manner (Figure 1A and B). A moderate decrease in pathological TGF-β overproduction was seen in rats treated with 10–20 mg of enalapril or 50–100 mg of losartan per litre of drinking water. With increasing enalapril or losartan dose, a maximal reduction in TGF-β overproduction was seen starting with 100 mg of enalapril or 500 mg of losartan in the drinking water. A further increase in enalapril or losartan dose did not decrease glomerular TGF-β production further. Side by side comparison of maximal effective doses of enalapril or losartan showed a comparable reduction in TGF-β overexpression (enalapril −46%, losartan −45%). Combined therapy with both modes of Ang II blockade did not result in additional beneficial effects (−45%). In all experiments, changes in TGF-β expression were closely correlated to the glomerular matrix accumulation (r = 0.96, P < 0.001). No therapy entirely normalized TGF-β and matrix protein overproduction.

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Conclusions

The present study shows that (i) TGF-β expression is a valid target in the management of fibrotic renal diseases and (ii) Ang II blockade reduces pathological TGF-β expression and matrix accumulation following tissue injury more effectively at higher doses. The data suggest that (i) ACE inhibition and AT1 receptor antagonism act through very similar pathways and (ii) Ang II blockade must be combined with other agents, which act through different pathways, in order to halt renal fibrosis more effectively.

References


Adenosine receptor antagonism in the prevention of acute cyclosporine A-nephrotoxicity in normal, diabetic and hypertensive rats

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Centre of Clinical Pharmacology Tübingen–Stuttgart, ¹Department of Internal Medicine, Section of Nephrology and Hypertension and ²Department of Surgery, University of Tübingen, Germany

Cyclosporine A (CsA) is a principle component of immunosuppressive regimens applied in organ transplantation and autoimmune disorders. Although having considerably improved patient outcome in these diseases, acute and chronic CsA nephrotoxicity limits clinical application in certain patients. Acute CsA nephrotoxicity is characterized by renal haemodynamic and tubular alterations, resulting in a marked decline in renal function. Although generally reversible upon dose reduction or withdrawal of the drug, overt acute renal failure may result in patients where additional risk factors such as volume depletion, diabetes mellitus and arterial hypertension are present. For these patients, specific therapeutic strategies have to be developed. The present experiments were designed to elucidate the role of adenosine in acute CsA nephrotoxicity and to evaluate the nephroprotective potential of different adenosine receptor antagonists.

In standard clearance experiments, CsA (Sandimmune®; 20 mg/kg i.v.)-induced alterations in renal haemodynamics were investigated in streptozotocin diabetic (60 mg/kg i.p.), l-NAME hypertensive (N-nitro-L-arginine methylster, 50 mg/l, added to the drinking water for 8 weeks) and age-matched control rats. Glomerular filtration rate (GFR) was

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calculated as the clearance of $[^3]$H]inulin, and renal blood flow (RBF) was measured by means of an electromagnetic flowmeter. In subgroups, the effects of the subtype-selective adenosine A$_3$ receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX, 100 µg/kg followed by 10 µg/kg/h) and the combined adenosine A$_1$/A$_2$ receptor antagonist theophylline (6 µg/kg/h) were assessed. Differences between means of groups were compared by ANOVA and respective post-tests for paired or unpaired comparison.

In control animals, CsA resulted in a significant decline in GFR and RBF and an increase in renovascular resistance (RVR), without changes in systemic blood pressure (cf. Table 1). Diabetic animals demonstrated glomerular hyperfiltration, whereas l-NAME hypertensive animals exhibited significantly lower GFR and RBF and an increase in RVR under baseline conditions. Renal haemodynamic changes in response to CsA were more pronounced in both diabetic and hypertensive animals.

DPCPX, selectively inhibiting adenosine A$_1$ receptor-mediated afferent arteriolar vasoconstriction, significantly attenuated the decrease in RBF by 57, 28 and 38% in control, diabetic and l-NAME hypertensive animals, respectively; however could not fully prevent the fall in GFR. Theophylline, by additionally inhibiting adenosine A$_3$ receptor-mediated efferent arteriolar vasoconstriction, completely abolished the renal haemodynamic response to CsA in all groups.

The results of the present study demonstrate adenosine to be a principle mediator of CsA-induced acute renal haemodynamic alterations. Adenosine receptor antagonists may therefore constitute a specific therapeutic approach in the prevention of CsA-induced acute renal failure, especially in patients with additional risk factors, e.g. diabetes mellitus or arterial hypertension.

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>GFR (ml/min/100 g)</th>
<th>RBF (ml/min/100 g)</th>
<th>RVR (dynes.s.cm$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>CsA</td>
<td>Baseline</td>
</tr>
<tr>
<td>Control</td>
<td>0.33 ± 0.02</td>
<td>0.28 ± 0.02*</td>
<td>2.20 ± 0.22</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0.36 ± 0.04**</td>
<td>0.28 ± 0.04*</td>
<td>2.15 ± 0.14</td>
</tr>
<tr>
<td>l-NAME</td>
<td>0.28 ± 0.04**</td>
<td>0.25 ± 0.04*</td>
<td>1.69 ± 0.14**</td>
</tr>
</tbody>
</table>

Values are means ± SEM.

*P < 0.05 vs baseline; **P < 0.05 vs CON, n = 8–12.

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**Dopamine D$_3$ receptors in the rat kidney: glomerular and tubular actions**

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Dopamine receptors play a role in the regulation of cardiovascular and renal function. At least five distinct dopamine receptors have been identified which were divided into two subfamilies: the D$_1$-like subgroup including the D$_1$ and D$_5$ receptors and the D$_2$-like subgroup in which the D$_2$, D$_3$ and D$_4$ receptors are included [1]. Dopamine D$_3$ receptor mRNA was recently demonstrated in the kidney of Wistar–Kyoto and spontaneously hypertensive rats [2] but, up to now, no data are available describing the effects of pharmacological D$_3$ receptor activation on kidney function. Therefore, we investigated the renal effects of an acute administration of R(+)-7-hydroxy-dipropylaminotetraline (7-OH-DPAT), a D$_3$ receptor agonist [3].

Standard clearance experiments were performed in thiopental-anaesthetized Sprague–Dawley rats. 7-OH-DPAT infusion (0.01, 0.03, 0.1, 0.3 and 1.0 µg/kg/min) increased glomerular filtration rate (GFR) dose-dependently by 4 ± 2, 5 ± 2, 13 ± 3, 17 ± 4 and 20 ± 2%, respectively, whereas mean arterial blood pressure (MAP) was not affected. The increase in GFR due to 7-OH-DPAT was accompanied by a significant diuresis (from 16.2 ± 5.4 at baseline to 27.7 ± 4.3 µl/min/100 g) and natriuresis (from 0.95 ± 0.26 to 3.30 ± 0.58 µmol/min/100 g). Since fractional sodium excretion was elevated as well (from 0.79 ± 0.19 to 2.48 ± 0.32%) 7-OH-DPAT, besides its glomerular action, apparently affects tubular sodium handling. In agreement with these results, the presence of D$_3$ receptor message was demonstrated by reverse transcription–polymerase chain reaction (RT–PCR) both in tubular and glomerular fractions of kidneys taken from Sprague–Dawley rats. Specificity of the amplified PCR products was confirmed.
by specific techniques. Specific probe derived from rat brain mRNA. The renal effects of 7-OH-DPAT were not influenced by the selective D3 receptor antagonist S(-)-sulpiride but abolished by pre-treatment with the D3 antagonist 5,6-dimethoxy-2-(di-n-propylamino)indane (U-99194A). Taken these results together, 7-OH-DPAT appears to affect glomerular and tubular function by specific D3 receptor activation.

In a second set of experiments renal blood flow (RBF) was measured by an electromagnetic flow transducer placed on the left renal artery. Also in this setting, 7-OH-DPAT (1.0 μg/kg/min) increased GFR by 19±3%. Interestingly, RBF was significantly reduced by 26±3% compared with baseline. Renal vascular resistance was significantly elevated by 25±4% due to 7-OH-DPAT infusion. Haemodynamic changes of the kidney were not influenced by pre-treatment with S(-)-sulpiride but were completely abolished by pretreatment with U-99194A. The hypothesis that 7-OH-DPAT infusion might cause vasoconstriction of postglomerular vessels was tested employing micropuncture experiments. Stop flow pressure (SFP) was measured in the early proximal tubule as an indicator of the glomerular capillary pressure. Compared with infusion of isotonic saline, 7-OH-DPAT (1.0 μg/kg/min) significantly increased SFP while MAP was not altered. Furthermore, hydrostatic pressure in the efferent arteriole was reduced during 7-OH-DPAT infusion. Taken together, the results of the micropuncture experiments support the hypothesis of a 7-OH-DPAT-induced postglomerular vasoconstriction.

The role of D3 receptors in the regulation of kidney function under pathophysiological conditions is unclear. Asico et al. [4] described that transgenic mice lacking both D3 receptors developed systemic hypertension and had an impaired ability to excrete an acute saline load. In addition, renal renin activity was higher in the homozygous than in wild-type mice. Due to its expression in juxtaglomerular cells the D3 receptor may negatively affect renin secretion [5]. From these data it was suggested that D3 receptors—possibly by impairment of the renal excretory capacity for sodium or increased renin excretion—may play a pathogenetic role in some forms of hypertension. However, 7-OH-DPAT induced a similar diuresis and natriuresis in spontaneously hypertensive and Wistar-Kyoto rats [6].

In summary, pharmacological activation of dopamine D3 receptors affects tubular function and renal haemodynamics in anaesthetized rats, the latter possibly by post-glomerular vasoconstriction. Whether D3 receptors are involved in the pathophysiology of systemic or renal haemodynamics remains to be determined.

Acknowledgements. These studies were supported by grants from the Federal Ministry of Education, Science, Research and Technology (Fo. 01KS9602) and the Interdisciplinary Clinical Research Center (IKFZ) and by the Deutsche Forschungsgemeinschaft (DFG, Förderkennzeichen Mu 1297/1-1).

References


Treatment with the angiotensin II antagonist valsartan in patients with chronic renal failure and hypertension

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Introduction

Angiotensin II (Ang II) type 1 receptor antagonists are a class of drugs derived from imidazole-5-acetic acid. Losartan is the most widely studied receptor antagonist up to now and comparable with angiotensin-converting enzyme inhibitors (ACEIs) in its blood pressure lowering effect in patients with essential hypertension [1,2]. Direct blocking of the Ang II type 1 receptor-binding site by Ang II antagonists may provide the advantage of a more specific blocking of Ang II action by additionally inhibiting the Ang II generation caused
by tissue enzymes (e.g. chymases or CAGE) [3]. While there are an increasing number of reports about the potency of Ang II receptor antagonists to lower blood pressure in essential hypertension [4] and benefits in cardiovascular disease [5], there is not much information about their antihypertensive and antiproteinuric (‘nephroprotective’) effects in patients with arterial hypertension and impaired renal function.

Patients and methods

The effects of the Ang II antagonist valsartan (80 mg/day) on proteinuria and glomerular permselectivity were studied in patients with chronic renal failure during a 6-month treatment period. We followed a double-blind, randomized, placebo-controlled study [treatment group (V-group); n = 5, age: 57 ± 7 years, serum creatinine: 365 ± 122 μmol/l; placebo group (P-group); n = 4, age: 62 ± 11 years, serum creatinine 346 ± 61 μmol/l]. Study parameters included blood pressure, 24 h proteinuria, glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) as determined by inulin and PAH clearance. Changes in glomerular permselectivity were assessed by measuring the fractional clearances of neutral dextrans by HPLC gel permeation chromatography.

Fig. 1. (A) Proteinuria and (B) albuminuria during 6 months of treatment with valsartan 80 mg/day (V-group, n = 5) or placebo (P-group, n = 4) in patients with renal failure. The relative changes vs the run-in phase (averaged, set to 100%) are shown and, additionally, the absolute daily excretion (mg/24 h) during run-in and after 6 months of treatment is indicated. Values are given as mean ± SEM. Changes vs the run-in phase are indicated by asterisks, *P < 0.05. Differences between both study groups during the treatment phase are tested by MANOVA and indicated by |———|.

Fig. 2. Change of the glomerular sieving coefficients for neutral dextrans of different Einstein stokes radii (fractional dextran clearances) after 6 months of treatment with (A) valsartan 80 mg/day (V-group, n = 5) or (B) placebo (P-group, n = 4) in patients with renal failure. Reference values of healthy controls (n = 10) are shown. Data are given as mean ± SEM. Differences within the study groups before and after treatment (valsartan/placebo) are tested by Student t-test, *P < 0.05, **P < 0.01. The differences between baseline values in the study groups and the sieving coefficients of healthy controls are tested by i-test for independent samples, *P < 0.05, **P < 0.01.
Results

Valsartan lowered the mean arterial pressure on average by $13.6 \pm 3.7$ mmHg during 6 months treatment ($P<0.05$). Average blood pressure reduction over 6 months was significantly different between the V-group and the P-group.

Baseline serum potassium concentrations in the V-group moderately increased from $4.4 \pm 0.4$ mmol/l to $4.9 \pm 0.5$ mmol/l after 3 months of valsartan treatment ($P<0.05$) and remained almost unchanged thereafter.

In the V-group, baseline GFR was $20 \pm 7$ ml/min in the run-in period and averaged $18 \pm 6$ ml/min for the three measurements during the treatment phase (NS). In the P-group, GFR changed from $19 \pm 5$ ml/min to $21 \pm 8$ ml/min (NS). RBF decreased slightly over time from $152 \pm 47$ to $140 \pm 47$ ml/min in the V-group and from $143 \pm 53$ to $129 \pm 55$ ml/min in the P-group. The trend between the groups concerning ERPF and RBF was not significantly different with regard to the whole observation period.

After 6 months of valsartan treatment, proteinuria was reduced by $396 \pm 323$ mg/24 h ($26 \pm 18\%$) and albuminuria by $531 \pm 499$ mg/24 h ($41 \pm 21\%$) with respect to baseline values ($P<0.05$). In the P-group, both proteinuria and albuminuria increased slightly with time (by $30 \pm 43\%$ and $30 \pm 54\%$, respectively, NS) (Figure 1).

The fractional clearances of high molecular weight dextrans $>66 \text{ Å}$ were significantly reduced after 6 months of valsartan treatment ($P<0.05$), indicating a reduction of the glomerular shunt volume by $54 \pm 20\%$ ($P<0.05$) according to the model of Deen et al. [6].

Conclusions

Our findings with the Ang II antagonist valsartan show a sustained reduction in blood pressure and proteinuria even in patients with advanced renal failure. While GFR and ERPF remained nearly stable, this effect could be attributed to an improvement in glomerular permselectivity. A preserved excretory renal function together with functional benefits in glomerular permselectivity may recommend this class of antihypertensives as a 'nephroprotective alternative' to ACEIs. This will require further long-term studies.

References


A randomized, double-blind, parallel study on the safety and antihypertensive efficacy of losartan compared to captopril in patients with mild to moderate hypertension and impaired renal function

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The angiotensin II (Ang II) pathway plays an important role in the progression of renal disease. The renal effects of Ang II are also crucially involved in maintaining blood pressure (BP) in hypertension.

This international multicentre study was conducted to compare the effects on blood pressure, creatinine clearance, proteinuria and lipids of the Ang II AT1 receptor antagonists losartan (LOS) and captopril (CAP) in patients with mild to moderate hypertension and impaired renal function. Another aim of the present study was to evaluate the safety and tolerability of LOS in this special group of patients which are not yet well documented.

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One hundred and two of the total of 129 patients recruited from 18 centres in 10 countries (mean age 55.2 years) completed this 16-week double-blind, parallel two-arm, randomized study. The initial 4-week placebo period was followed by 12 weeks of active, double-blind treatment with 50 mg LOS, possibly titrated to 100 mg, once daily (group 1, LOS; n = 64) or 25 mg CAP, possibly titrated to 50 mg, twice daily (group 2, CAP; n = 65). Mild hypertension was defined as 95 to 105, moderate hypertension as 106 to 115 mmHg sitting diastolic blood pressure (SiDBP), while creatinine clearance was required to be in a range of 20 to 60 ml/min/1.73 m². Antihypertensive efficacy was evaluated by the reduction of BP after 12 weeks. The main efficacy measurement was the SiDBP. Secondary endpoints included sitting systolic and standing BP, creatinine clearance (CCI), total proteinuria (TP), total cholesterol, triglycerides and HDL-cholesterol. Safety was assessed by the incidence of adverse events, clinical and laboratory safety measurements.

After 12 weeks administration of LOS a reduction of 12.2 ± 10.2 mmHg in SiDBP and 15.5 ± 18.0 mmHg in SiSBP was observed, compared with 11.2 ± 11.4 mmHg and 15.6 ± 20.6 mmHg in CAP-treated patients. There was thus no statistically significant difference between the two groups, which was also consistent with the other parameters recorded. In group 1 (LOS) TP was significantly decreased at the end of the study. Creatinine clearance and lipids showed no remarkable changes at all in the two groups. No significant differences were observed between the two groups with respect to the proportion of patients with clinical adverse experiences, although adverse experiences related to the respiratory system appeared significantly more often in CAP-treated patients.

To summarize, after 12 weeks’ treatment both drugs demonstrated similar effects on BP, CCI, and lipid profiles. LOS but not CAP showed a significant decrease in TP. LOS and CAP were equally well tolerated, leading to the conclusion that LOS is as suitable as CAP as an antihypertensive agent in cases where renal function is impaired.

Blood pressure reduction is necessary for the reduction of proteinuria in diabetic nephropathy—comparison of different antihypertensive agents

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As is well known, besides blood glucose concentrations, proteinuria and hypertension are the main factors in the progression of diabetic nephropathy. We performed a randomized, double-blind and placebo-controlled study to answer the following questions: do different antihypertensive agents—especially β-receptor blockers with vasodilating activity—influence renal haemodynamics and proteinuria in patients with diabetic nephropathy and is there a relationship to blood pressure reduction?

The following drugs were used: the β1-receptor antagonist metoprolol (95 mg/day), the β1-antagonist and β2-agonist celiprolol (200 mg/day) and the angiotensin-converting enzyme (ACE) inhibitor benazepril (5 mg/day). Each drug, in addition to placebo, was applied over a period of 4 weeks with a wash-out period of 2 weeks in between.

Twelve patients (age 63 ± 3 years) with diabetes mellitus IIb took part in the study. To guarantee a homogeneous group, the participants had to fulfil the following inclusion criteria: proteinuria of 300 mg–3.5 g/day, serum creatinine <1.8 mg/dl and a mild hyper-tension after the initial wash-out period of 2 weeks. The patients were allowed to continue the diet they were used to. During the whole study, they remained on an out-patient basis. At the end of each medication period, blood pressure was measured continuously over 24 h. Proteinuria was determined as the mean of a 2 day collecting period. The glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were measured by inulin and PAH clearance. The results are given in Table 1.

The measured hormone concentrations showed the expected significant changes under the ACE inhibitor treatment: an increase of plasma renin activity and a decrease of ACE. These observations can act as the control of the compliance of our patients.

Summing up our results, there were no significant changes in renal haemodynamics either with the ACE inhibitor or with the vasodilating β-blocker celiprolol in doses not affecting systemic blood pressure. No significant reduction of proteinuria could be observed with the β-receptor blockers and ACE inhibitor within the 4 weeks of treatment despite the significant reduction of blood pressure by metoprolol.

We were surprised that no significant changes in our
Table 1. Effects of antihypertensive agents

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Metoprolol</th>
<th>Benazepril</th>
<th>Celiprolol</th>
</tr>
</thead>
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<tr>
<td>MAP (mmHg)</td>
<td>99 ± 3</td>
<td>93 ± 2*</td>
<td>97 ± 3</td>
<td>100 ± 3</td>
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<tr>
<td>Heart rate (min⁻¹)</td>
<td>78 ± 3</td>
<td>67 ± 3*</td>
<td>79 ± 2</td>
<td>75 ± 3</td>
</tr>
<tr>
<td>GFR (ml/min/1.73m²)</td>
<td>56 ± 7</td>
<td>62 ± 4</td>
<td>73 ± 9</td>
<td>67 ± 6</td>
</tr>
<tr>
<td>ERPF (ml/min/1.73m²)</td>
<td>302 ± 27</td>
<td>376 ± 47</td>
<td>374 ± 48</td>
<td>317 ± 23</td>
</tr>
<tr>
<td>Proteinuria (mg/24h)</td>
<td>984 ± 321</td>
<td>759 ± 245</td>
<td>895 ± 259</td>
<td>1036 ± 489</td>
</tr>
</tbody>
</table>

*P < 0.01 vs placebo according to a standard least squares test.

main parameter proteinuria occurred, because in a previous study [1] with 11 patients with biopsy proven chronic glomerulonephritis, which fulfilled comparable inclusion criteria and were treated according to the same study scheme, there was a significant reduction of blood pressure and proteinuria under each antihypertensive drug.

According to our results, no recommendation for one of the agents used in these doses can be made, because in this placebo-controlled study no antiproteinuric effect could be observed, even under the ACE inhibitor treatment. Thus we conclude that in patients with diabetic nephropathy, stronger antihypertensive medication has to be used to achieve a significant reduction of blood pressure and perhaps the desired antiproteinuric effect and nephroprotection.

Reference


Protective effects of endothelin antagonists in chronic renal failure

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Introduction

Cardiovascular complications remain the leading cause of death and one of the most important reasons for morbidity in patients suffering from chronic renal failure. In particular, the prevalence of severe left ventricular hypertrophy (LVH) is a very important factor for survival and morbidity in uremic patients. In these patients, LVH seems to be a negative independent predictor. In chronic uraemia, there is no clear correlation between blood pressure and left ventricular mass. This finding implies that further pathophysiological factors are associated with uraemia that maintain hypertrophy.

Endothelin-1 (ET-1) is a circulatory and local active hormone that plays an important role in the development of cardiovascular diseases in uraemia. ET-1 is not only a potent vasoconstrictor but also has growth-promoting properties. Recent studies indicated that ET-1 might be involved in LVH.

Since clinical data have documented that plasma ET-1 is elevated in patients with chronic renal failure, we were interested in evaluating the role of ET-1 in the pathogenesis of cardiac hypertrophy in a model of experimental renal failure.

Therefore, the aim of our study was to investigate the influence of the selective ET₁ receptor antagonist in comparison with the unselective ET₂ receptor antagonist on systolic blood pressure, renal function, protein excretion and total heart weight, and to assess the role of the renin–angiotensin system.

Experimental design

Male Sprague–Dawley rats (Charles River, Germany), with a body weight of 200–230 g at the start of the experiment, were used in these studies. The animals were divided randomly into four groups. Group 1 (Co) was sham-operated (decapsulation of the kidney) and untreated, taking special care that the adrenal glands were not damaged. All animals were caged individually.

Groups 2–4 underwent a two-step 5/6 subtotal nephrectomy (SNX). First the right kidney was removed. After 1 week, the lower and upper poles of the kidney were dissected under anaesthesia. Following surgery, group 2 (SNX) received no treatment, group 3 (ET-A) was treated with a selective ET₁ receptor antagonist (LU 302146, Knoll, Germany) and group 4 (ET-AB) was given an unselective ET₂ receptor antagonist (LU...
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302872, Knoll, Germany) both in a dose of 30 mg/kg/day orally in the drinking water for 12 weeks (n = 5–7). The administration of the ET receptor antagonists was started 2 days after nephrectomy. Body weight, food and water intake and systolic blood pressure (SBP) were determined prior to operation and at 2 week intervals during the experiment. Blood pressure measurements were made in conscious animals by tail plethysmography. Blood was obtained from the tail vein to determine haematocrit, serum creatinine, serum urea, plasma ET-1 and aldosterone. At the same time intervals, the rats were placed into individual metabolic cages and 24 h urine collections were performed to determine urine volume, proteinuria and immunoreactive ET-1 concentration. The experiment was terminated after 12 weeks. The weight of the kidney and the heart was measured. Data are expressed as means ± SEM and were analysed statistically using Student’s Newman Keul test. P-values <0.05 were considered to denote statistically significant differences.

Results

SNX animals showed a significant increase of serum creatinine (P<0.01) and urea 12 weeks after nephrectomy as compared with sham-operated animals. During the 12 weeks of the experiment, there was a continuous increase in ET-1 excretion in the urine (2- to 3-fold) in SNX animals. A maximum was reached after 8 weeks without any further significant increase compared with controls (P<0.01). The ET\textsubscript{A} and ET\textsubscript{AB} receptor antagonist-treated rats showed a delayed elevation of urinary ET excretion. In contrast, the plasma ET was not elevated in untreated SNX rats, whereas the treated SNX animals showed a marked increase. There was only a slight elevation in SBP in untreated SNX animals after 12 weeks compared with sham-operated controls. The ET\textsubscript{A} and the ET\textsubscript{AB} receptor antagonists did not reduce SBP significantly (Co, 133 ± 5; SNX, 144 ± 8; ET-A, 132 ± 8; ET-AB, 140 ± 6 mmHg; NS). Urinary protein excretion was increased 20-fold in untreated SNX animals after 12 weeks compared with controls (P<0.01). Both ET receptor antagonists were able to reduce proteinuria in SNX rats, whereas the selective ET\textsubscript{A} receptor antagonist reduced proteinuria to a greater extent than the combined ET\textsubscript{AB} receptor antagonist (proteinuria: CO, 6 ± 2; SNX, 92 ± 9; ET-A, 27 ± 2; ET-AB, 43 ± 6 mg/24 h; P<0.01). The hypertrophy of the heart in untreated SNX rats was prevented significantly by both ET receptor antagonists. The plasma aldosterone showed an activation in untreated SNX rats, being completely antagonized by ET\textsubscript{AB} but not by ET\textsubscript{A} receptor antagonist.

Summary

The present study suggests that ET-1 is involved in the pathogenesis of uraemic cardiac hypertrophy and in the progression of renal failure in rats with subtotal nephrectomy examined after an intermediate period of 12 weeks of renal failure. Furthermore, proteinuria is reduced by the selective ET\textsubscript{A} receptor antagonist more than by the unselective ET\textsubscript{AB} receptor antagonist, without reducing the blood pressure. ET receptor blockade might preserve renal function by reduction of protein excretion. In addition, ET receptor antagonists influence the aldosterone system. In our animal studies, the medication was well tolerated. Our study results provide a possible therapeutic approach using ET receptor antagonists for cardiac hypertrophy and renal protein excretion by blockade of endogenous ET-1.

Further human studies are needed to show whether this protection of the heart and kidney might influence the survival and life expectancy of patients suffering from chronic renal failure, of patients on dialysis or after kidney transplantation.


Decreased diurnal blood pressure variability and low dehydroepiandrosterone sulfate levels in patients with renal hypertension, and after kidney transplantation

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The decrease in diurnal rhythm of blood pressure and low plasma dehydroepiandrosterone sulfate (DHEAS) levels are strong predictors of cardiovascular morbidity and mortality [1,2]. In our earlier study, we found a close correlation between serum DHEAS levels and diurnal indices in normotensive volunteers and in patients with essential hypertension [3]. The aim of the study was to determine the relationship between the level of DHEAS and the diurnal blood pressure variability in patients with renal hypertension (RH)

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and after kidney transplantation (TPX). The ambulatory blood pressure monitoring and assessment of diurnal variability were performed with a Meditech-02 ABPM device; blood pressure and heart rate were measured every 20 min during the day and every 30 min at night. Each patient kept a log on the events of the day, the times of drug intake, if any, and the time of getting up and going to bed. The determination of DHEAS was done by a radioimmunoassay technique [4]. The prevalence of reversed systolic/diastolic diurnal indices was 2/2% in normotension, 25/25% in RH and 48/33% in TPX patients ($P<0.001$ with $\chi^2$ test). We found significant correlations between systolic and diastolic indices, and serum DHEAS levels in the total population. These relationships were also significant when analysing normotensive subjects, RH subjects and TPX patients separately (Table 1).

Mean diurnal systolic/diastolic indices (SI/DI) were 12/17% for normotensives, 8/12% in RH, and 3/5% after TPX. The mean DHEAS was significantly different between normotensive, RH and TPX patients ($4.7 \text{ vs } 3.0 \text{ vs } 1.5 \mu\text{mol/l}$), respectively ($P<0.001$) (Figure 1).

These findings indicate that low DHEAS and decreased SI/DI are often associated with each other, indicating increased cardiovascular risk, which is particularly dominant in patients after renal transplantation.

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>$n$</th>
<th>DHEAS/SI</th>
<th></th>
<th>DHEAS/DI</th>
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<td></td>
<td></td>
<td>$r$</td>
<td>$P$</td>
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<td>0.0001</td>
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<td>0.01</td>
<td>0.350</td>
<td>0.01</td>
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<tr>
<td>After transplantation</td>
<td>32</td>
<td>0.370</td>
<td>0.05</td>
<td>0.340</td>
<td>0.05</td>
</tr>
</tbody>
</table>

### References

Prostaglandin E\(_1\) reduces the risk of delayed graft function after cadaveric renal transplantation

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**Objective**

There is an association between delayed graft function (DGF) and reduced graft survival of cadaveric renal transplants [1]. Several approaches were made in the past to reduce DGF [2]. Prostaglandin E\(_1\) (PGE\(_1\)) is known to be cytoprotective; it inhibits platelet aggregation and production of superoxide anions by polymorphonuclear leukocytes [3]. We have found that PGE\(_1\) reduces the frequency of acute renal failure after prolonged warm ischaemia in dogs [4] and after surgery of renal arteries in humans by 30\% [5]. Because there has been no controlled trial of the influence of PGE\(_1\) on DGF and graft survival after cadaveric renal transplantation, we carried out a pilot study between September 1993 and November 1995.

**Patients and methods**

A 500 µg dose of PGE\(_1\) (Minprog päd®, Upjohn Pharma) was given i.v. at random to 30 of 58 cadaveric organ donors 2 min before clamping the aorta and starting cold perfusion. Clinical data were available from 58 patients transplanted with a PGE\(_1\)-pre-treated renal allograft and from 53 patients with a non-treated renal allograft. Forty-five renal transplantations were performed in our centre and 66 allografts were sent to other transplant centres within the Eurotransplant region.

DGF was diagnosed in all patients requiring dialysis treatment within the first 9 days after renal transplantation. Differences between groups were tested with Student’s \(t\)-test and \(\chi^2\) tests. The time course of prevalence of DGF requiring dialysis treatment was compared by the Kaplan–Meier method and difference was estimated by the log rank test. Clinical data of donors and recipients and the ischaemic times are displayed in Table 1.

**Results**

DGF was seen in 36.2\% of patients with PGE\(_1\)-treated and 49.1\% of patients with untreated renal allografts (\(P=0.1659\)). After the first week, 74.1 and 69.8\%, respectively, of patients were without dialysis. Survival analysis showed a beneficial trend for patients transplanted with a PGE\(_1\)-pre-treated kidney (\(P=0.18\), Figure 1). In patients who recovered renal function between day 0 and day 9, the dialysis dependency during this time was significantly less frequent (\(P<0.02\)) in patients transplanted with a PGE\(_1\)-pre-treated kidney (Figure 2).

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with our observations in patients after kidney transplantation. PGE1 lessened the risk of DGF in our patients by 10–15%.

Recently, Polyak and co-workers found in extended criteria donor kidneys, that PGE1 reduces the incidence of DGF and enhances perfusion characteristics during continuous hypothermic pulsatile perfusion [6]. In this group with a very high risk of DGF, PGE1 was superior to trifluoperazine and verapamil. The PGE1 effect on DGF reached significance in this high risk group with 25 patients in each group.

Regarding our data, we would need a prospective study with 239 patients in each group with a statistical power of 90% to show a significant effect for non-selected kidney donors [7]. Furthermore, the PGE1 effect on long-term graft survival has to be determined.

**Fig. 2.** Time course of patients requiring dialysis treatment after kidney transplantation. Subgroup of patients who recover renal function until the end of the observation period of 9 days.

### Discussion

This pilot study shows an encouraging benefit of a single dose of PGE1 given to the cadaveric donor to reduce DGF. This effect could be clearly shown in the subgroup of patients who were independent of dialysis until day 9 after transplantation. In the whole group, analysis was more difficult because the time of observation finished at day 9. This short observation time was chosen in order to improve the rate of reply of the centres to whom we sent kidneys.

In previous studies of patients with normal renal function, PGE1 was shown to decrease the incidence of acute renal failure (serum creatinine > 2.0 mg/dl) from 37.5% to 11.5% after ischaemia during surgery of suprarenal or renal arteries. Dialysis treatment was necessary in 12.5% of controls and in none of the PGE1-treated patients [3]. This effect is comparable to our observations in patients after kidney transplantation. PGE1 lessened the risk of DGF in our patients by 10–15%.

Recently, Polyak and co-workers found in extended criteria donor kidneys, that PGE1 reduces the incidence of DGF and enhances perfusion characteristics during continuous hypothermic pulsatile perfusion [6]. In this group with a very high risk of DGF, PGE1 was superior to trifluoperazine and verapamil. The PGE1 effect on DGF reached significance in this high risk group with 25 patients in each group.

Regarding our data, we would need a prospective study with 239 patients in each group with a statistical power of 90% to show a significant effect for non-selected kidney donors [7]. Furthermore, the PGE1 effect on long-term graft survival has to be determined.

### References

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**Pharmacokinetics of mycophenolic acid (MPA) and free MPA in paediatric renal transplant recipients—a multicentre study**

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### Background

Mycophenolate mofetil (MMF), an ester prodrug of the immunosuppressant mycophenolic acid (MPA), has been approved for maintenance immunosuppressive therapy in paediatric renal transplant recipients. MPA acts as a potent, reversible, uncompetitive inhibitor of inosine monophosphate dehydrogenase (IMPDH), the key enzyme in the *de novo* purine biosynthesis of proliferating T and B lymphocytes. Although MMF is widely used in children post-
transplant, pharmacokinetic data for MPA in this patient population are scarce and dosage guidelines are therefore preliminary.

Methods

We investigated the pharmacokinetics of MPA, free MPA and the renal metabolite MPA glucuronide (MPAG) in the initial (sampling at 1 and 3 weeks) and stable phase (3 and 6 months) post-transplant in 17 children (age 12.0 ± 0.77 years; range 5.9–15.8 years) receiving the currently recommended dose of 600 mg MMF/m² body surface area twice a day. Blood samples were collected at 0, 20, 40 and 75 min and at 2, 4, 6, 8 and 12 h after dosing. Plasma concentrations of MPA and MPAG were measured by reverse-phase HPLC. Since MPA is bound extensively to serum albumin and only the free drug is presumed to be pharmacologically active, we also analyzed the free MPA fraction by HPLC after separation through ultrafiltration. Pharmacokinetic parameters were calculated with the biostatistical program BIAS®.

Results

The intraindividual variability of the area under the concentration–time curves (AUC₀₋₁₂) of MPA throughout the 12 h dosing interval was high in the initial phase (correlation of 1 and 3 week AUC values: r = 0.17, P = 0.51), but declined in the stable phase post-transplant (correlation of 3 and 6 month AUC values: r = 0.52, P < 0.05), while the interindividual variability even increased in the stable phase (range at 3 months, 81.4 mg × h/l; 6 months, 64.2 mg × h/l) compared with the initial phase (range at 1 week, 36.4 mg × h/l; 3 weeks, 43.1 mg × h/l). The MPA-AUC₀₋₁₂ values increased 2-fold from 32.4 (range 13.9–57.0) mg × h/l at 3 weeks to 65.1 (range 32.6–114) mg × h/l at 3 months after grafting, whereas the AUC₀₋₁₂ values of free MPA did not change over time. This discrepancy can be attributed to a 35% decline (P < 0.01) of the free MPA fraction from 1.4% (range 0.6–5.1%) in the initial phase post-transplant to 0.9% (range 0.5–1.9%) in the stable phase. The decrease of the free MPA fraction is probably caused by several factors. First, the MPA-binding protein serum albumin increased into the normal range during the study period, paralleled by a 40% decrease of the AUC₀₋₁₂ values of the renal metabolite MPAG, might have contributed to the decline of the free MPA fraction, because high MPAG plasma concentrations have been shown to displace MPA from albumin-binding sites. The mechanism of the increase of total MPA-AUC₀₋₁₂ values is probably multifactorial and may involve decreased MPA metabolism as a consequence of the 35% decline of the free MPA fraction, because it is primarily the free drug that is available for metabolism. This hypothesis is supported by the observation of an inverse correlation (r = −0.61, P < 0.02) between the decrease of the free MPA fraction and the increase of the respective MPA-AUC₀₋₁₂ values in the stable vs initial phase post-transplant.

Conclusions

Paediatric renal transplant recipients on a fixed MMF dose exhibit a 2-fold increase of the AUC₀₋₁₂ of total MPA in the stable phase post-transplant and a 35% decrease of the free MPA fraction, whereas the AUC₀₋₁₂ of free MPA remains stable over time. Because the latter pharmacokinetic variable is theoretically best predictive of the clinical immunosuppressive efficacy of MMF, these findings may have consequences for the dosing recommendations of MMF in renal transplant recipients.

Therapeutic drug monitoring of total and free mycophenolic acid (MPA) and limited sampling strategy for determination of MPA-AUC in paediatric renal transplant recipients

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¹University Children’s Hospitals Heidelberg and ³Freiburg and ³Department of Clinical Chemistry, Göttingen, Germany

Background

Mycophenolate mofetil (MMF) is widely used for immunosuppressive therapy in paediatric renal transplant recipients. Currently, a fixed dose regimen (600 mg of MMF/m² body surface area twice a day) is recommended. However, in view of the considerable interindividual variability of mycophenolic acid (MPA) pharmacokinetics, it is currently being debated as to whether there is a need for a therapeutic drug monitoring of MPA to increase the clinical efficacy and safety of its use by individualized MMF dosing.

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Methods

We analysed the pharmacokinetics of MPA in 40 children on a fixed dose of MMF (600 mg/m² body surface area twice a day) in the initial (1 and 3 weeks) and stable (3 and 6 months) phase after renal transplantation. Blood (EDTA) samples were collected at 0, 20, 40 and 75 min and at 2, 4, 6, 8 and 12 h after dosing. Plasma concentrations of MPA were measured by reverse-phase HPLC, and free (not albumin-bound) MPA, which is assumed to be the pharmacologically active compound, after separation by a protein filter. The area under the concentration–time curves (AUC0–12) values were calculated by the linear trapezoidal rule. Receiver operating calculation (ROC) curves were generated from sensitivities and specificities of both MPA-AUC0–12 and pre-dose MPA concentrations, adjusted to the time period after grafting by calculation of Z values. AUC0–12 values for total and free MPA and MPA trough levels were correlated with defined clinical events, e.g. biopsy-proven acute rejection episodes and adverse events, defined as leucopenia, gastrointestinal and/or infectiological side effects.

Results

There was an obvious association between MPA-AUC0–12 values and acute rejection episodes. Patients with acute rejection episodes tended to have lower AUC0–12 values (median AUC0–12 = 28.7; range 15.3–57.0 mg × h/l) than those without rejection (median AUC0–12 = 38.3; range 3.1–134 mg × h/l). The relative risk for acute rejection episodes in patients with MPA-AUC0–12 values < 30 mg × h/l in the first 3 weeks after renal transplantation (n = 11) was 45% compared with 20% in those above this threshold (n = 20). There was also an obvious association between MPA trough levels and acute rejection episodes: an MPA trough level > 1 mg/l (n = 16) was associated with a relative risk for rejection of 13% compared with 50% in those below this threshold (n = 14). The ROC curve analysis showed that the pharmacokinetic variable MPA-AUC0–12 is superior to trough levels for assessment of the relative risk of acute rejection episodes in the first 6 months post-transplant. According to these data, a therapeutic drug monitoring strategy of total MPA could have the potential to increase the efficacy of MMF in paediatric renal transplant recipients. We therefore sought to validate an individual MPA serum concentration or a limited sampling strategy (abbreviated AUC) to facilitate therapeutic drug monitoring of MPA. MPA pre-dose trough levels obtained at 12 h after dosing correlated only moderately with full time MPA-AUC0–12 values. Furthermore, these coefficients of correlation varied with time after grafting: 1 week after renal transplantation, r = 0.64 (n = 30 patients); 3 weeks, r = 0.73 (n = 30); and 6 months, r = 0.50 (n = 18). In contrast, a calculated MPA-AUC0–12, derived from a limited sampling strategy (sampling at 0 min, 40 min and 2 h after dosing) corresponded reasonably well (r = 0.86, P < 0.001, n = 55) with the respective full time AUC0–12 and may, therefore, be the method of choice for monitoring MPA in paediatric patients. The respective formula was: calculated MPA-AUC0–12 = 12.9 + 5.99 *C0min + 0.528 *C40min + 2.4 *C2h.

Conclusions

There was a clear association between total MPA-AUC0–12 values and acute rejection episodes in paediatric renal transplant recipients. A therapeutic drug monitoring strategy and consecutive individualized MMF dosing in order to target a defined therapeutic range of MPA-AUC values (concentration–control study) may have the potential to increase the efficacy and safety of MMF in this patient population.
The volume and pressure sensors and their effector mechanisms

Volume is measured in the low pressure system (atria) by stretch receptors which communicate via the vagal nerve to the medulla oblongata. Stretch leads to diuresis via inhibition of arginine vasopressin \([\text{AVP} = \text{antidiuretic hormone (ADH)}] [1]\). Stretch receptors at the same site control the secretion of atrial natriuretic peptide (ANP), which acts natriuretically on the kidney [2]. Underfilling has the opposite effects. In addition sympathetic discharge caused by volume contraction has all the well known effects on heart, vascular resistance, renin release and secretion of adrenaline.

The pressure receptors are localized in the ‘high pressure’ system \(\text{sinus aorticus}\) and \(\text{carotid sinus}\). Their afferent fibres again go to the medulla oblongata. High pressure is responded to by a decrease in sympathetic discharge, low pressure has the opposite effect [3]. It is also well known that pressure is measured in the afferent arterioles of the glomeruli, with low pressure increasing renin secretion [4].

The sensors for osmolytes and ions and their effector mechanisms

Osmotic pressure is probably monitored at several levels in the gastrointestinal tract, the liver and, most importantly, in the supraoptical and paraventricular nuclei of the hypothalamus [5]. The sensor cells respond to an increase in ambient osmolality by the opening of non-selective cation currents [6]. The ensuing depolarization probably leads to the activation of magnocellular neurons secreting AVP. Whether there are specific Na\(^+\) sensors in these hypothalamic regions is not quite clear [7].

Na\(^+\) is measured already at the intake site by epithelial Na\(^+\) channels. The sensitivity of salt taste, the salt appetite, is adjusted to requirements by aldosterone. High aldosterone, as a sign of volume depletion, enhances salt appetite [8].

The plasma K\(^+\) concentration is measured by every cell of the body in the sense that the cell voltage is largely determined by extracellular K\(^+\). In this context the glomerulosa cells of the adrenal gland play an important role because the plasma K\(^+\) concentration directly regulates the secretion of aldosterone. Hyperkalaemia enhances the secretion of aldosterone. Hypokalaemia has the opposite effect.

Cl\(^-\) is measured in the luminal fluid at the level of the macula densa cells. The sensor is the frusemide-inhibited Na\(^+\)2Cl\(^-\)K\(^+\) co-transporter. High Cl\(^-\) inhibits renin secretion and reduces the glomerular filtration rate (GFR) by the so-called tubuloglomerular feedback (TGF). Low Cl\(^-\) has the opposite effect. Inasmuch as frusemide inhibits this sensor it also paralyses the TGF and enhances renin secretion. This is remarkable because obviously the Cl\(^-\) concentration in the luminal fluid at the level of the macula densa is increased in the presence of loop diuretics [9]. The additional sensors for Ca\(^{2+}\) and pH will not be discussed further here.

The ‘programmes’ of volume contraction and expansion

Acute programmes usually involve long and short circulation–circulation loops. For example, local factors (H\(^+\), K\(^+\), ATP, NO and others) adjust the perfusion depending on needs (short loops) [10]. Volume depletion leads to vasoconstriction and sympathergic activation of the heart. Volume expansion has the opposite effect (long loops).

The kidney is involved in circulation–kidney loops. However, its response takes some time. In volume contraction salt- and water-saving programmes are called into play: sympathergic increase in proximal tubular absorption; amplification of this effect also in other nephron segments via circulating adrenaline; secretion of renin caused by three major factors (fall in perfusion pressure; sympathergic discharge; and low Cl\(^-\) at the level of the macula densa); AVP effects not only on the vasculature but also at the level of NaCl absorption in the thick ascending limb and most importantly at the level of the collecting duct via activation of aquaporin 2 channels [11].

The renin-induced ‘programme’ activates angiotensin II, and the latter represents the other important stimulus to enhance secretion of aldosterone and also of adrenalin. Hence, the kidney amplifies the emergency responses. Angiotensin II acts systemically on the vasculature and the brain to increase thirst and AVP secretion, and it also acts on the kidney with a vasoconstrictive response on efferent (but also afferent) arterioles [4]. In addition angiotensin II enhances NaCl and H\(_2\)O absorption by the proximal tubule [12].

The aldosterone response is also far reaching. Not only does aldosterone enhance the absorption of Na\(^+\) and the secretion of K\(^+\) and H\(^+\) in the collecting duct, but it also resets the salt appetite and tunes several epithelia for maximal Na\(^+\) saving. These comprise: the colon; the salivary glands; and the sweat glands [4]. In addition, under these circumstances, even the final portions of the proximal tubule appear to enhance NaCl absorption via luminal Na\(^+\) channels [13]. Other (non-genomic) effects of aldosterone, e.g. on the endothelium are currently under investigation.

In volume expansion these processes are inactivated and further mechanisms come into play: the ANP and prostaglandin effects and may be those of other factors such as the ouabain-like (natriuretic) factors [14]. High blood pressure by itself, and in spite of almost perfect autoregulation, produces natriuresis. This probably is caused by an inhibition of proximal tubule NaCl absorption [15].

Heart and kidney failure

Independently of volume status the kidney requires a sufficient perfusion pressure in order to function norm-
ally. If the heart fails to perfuse the kidney adequately, prerenal acute kidney failure threatens the organism. This may occur in states of acute underfilling of the circulation as well as with initially perfectly normal volume status if the heart fails, or, even with overfilling and oedematous states if the heart fails to adapt [16]. Irrespective of what is the pre-renal cause, the acute response of the kidney is a precipitous fall in GFR and acute damage mostly of the proximal tubule but also of the thick ascending limb of the loop of Henle. The key role of tubule obstruction in this state is well known, but medullary blood congestion also contributes [17]. With urine production approaching zero, acute renal failure produces acute volume overload and also circulatory failure. The same complications will obviously occur when the cause of the acute renal failure is intrarenal (e.g. acute glomerular disease, tubule damage) or post-renal.

A specific class of states of overhydration is represented by liver cirrhosis, pre-eclampsia and severe cardiac oedema. These states are characterized by maximal activation of volume- and salt-conserving mechanisms in spite of severe overhydration [16]. The pathophysiological concept postulates that volume sensing is fooled in this state, inasmuch as volume contraction is perceived.

It is well known from clinical experience that in any of these states the response to loop diuretics will most likely be blunted or completely absent. This is due to at least two factors: (i) The secretion of diuretics by the proximal tubule, which involves secondary and maybe even primary active steps is sharply reduced in states of tubule damage, irrespective of their cause. Hence, little diuretic is secreted and little reaches the thick ascending limb of the loop of Henle [18]. (ii) Usually these states are characterized by activation of the renin–angiotensin system. Angiotensin II activates proximal tubule absorption so that very little filtrate is delivered to the thick ascending limb [18]. The first factor can partially be compensated for by the use of higher doses of the diuretic. The second factor may be counterbalanced by the application of angiotensin-converting enzyme inhibitors and co-administration of other diuretics (acetazolamide etc.).

Chronic renal failure causes characteristic complications for the circulation and the heart, which are beyond the scope of this brief review, and will therefore only be mentioned here. Erythropoietin deficiency puts an extra burden on the heart to compensate for the reduced haemoglobin. This may cause cardiac failure. On the other hand, erythropoietin therapy may cause hypertension. The major cause for both, the development of cardiac failure and progression of renal disease is, in fact, hypertension [19,20]. Finally, uraemic pericarditis is another important cause of cardiac failure. These important interrelations between circulation and kidney alert an integrated diagnostic and therapeutic approach to the patient.

References


Fifth Congress of Nephropharmacology
Interaction of the autonomic nervous and the renin–angiotensin system in heart and kidney

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Introduction

Congestive heart failure is characterized by overactivity of the sympathetic nervous system and of the renin–angiotensin system. When plasma noradrenaline is elevated, patient survival is extremely poor [1] due to the high incidence of progressive heart failure and sudden cardiac death. There is substantial evidence to suggest that angiotensin (Ang) II may in part be responsible for an increase in noradrenaline release in this clinical setting [2]. Thus, activation of facilitatory Ang II receptors, located presynaptically on sympathetic nerve endings, leads to enhanced release of noradrenaline per nerve impulse. Chronic renal failure is associated with increased sympathetic tone [3], and sudden cardiac death is the most common cause of death in end-stage renal failure. Angiotensin-converting enzyme (ACE) inhibitor therapy is standard in chronic heart and kidney failure. Today, Ang II (AT1) receptor blockers as a new class of drugs are available. The ELITE study showed that heart failure patients treated with an AT1 receptor blocker had a lower mortality than those treated with an ACE inhibitor [4]. Now we investigate the relative contribution of AT1 and AT2 receptors to Ang II modulation of cardiac and renal transmitter release.

Methods

After loading with [3H]noradrenaline (atria, kidney) or [3H]hemicholine (atria), the nerves were stimulated electrically at 5 Hz, according to methods described earlier [5,6]. The effects of Ang II, the AT1 receptor blocker, EXP3174, and the AT2 receptor blockers, PD123319 and CGP42112A, were tested at different concentrations.

Results

Electrical nerve stimulation elicited noradrenaline release. Ang II (0.01–0.1 μM) increased noradrenaline outflow by ~80% in kidney and in heart atrium (Figure 1). EXP3174 (0.01 μM) shifted the concentration response curve for Ang II to the right (data not shown), whereas a 10 times greater concentration of EXP3174 (0.1 μM) abolished the Ang II-induced noradrenaline outflow (Figure 1). Interestingly, the AT2 receptor blockers PD123319 (1 μM) (Figure 1) and CGP42112A (1 μM) (data not shown) reduced the Ang II-induced increase of noradrenaline by ~50% over the whole concentration range of Ang II in atria. A combination of EXP3174 (0.01 μmol/l) and PD123319 (1 μmol/l) did not inhibit noradrenaline release to a greater extent than EXP3174 alone. An inhibition with PD123319 was not observed in renal cortex (Figure 1). Furthermore, acetylcholine release was increased by Ang II (0.01–1 μM) in atria.

Discussion

Sympathetic nerve endings in human heart and kidney possess Ang II receptors of the AT1 subtype which, when activated by Ang II, mediate an increase of noradrenaline outflow. This facilitatory effect of Ang II is prevented by EXP3174, the in vitro active form of the AT1 receptor blocker losartan. Furthermore, EXP3174, but not the ACE inhibitor captopril, totally blocked Ang I-induced noradrenaline release in human atria [7] and kidney [8]. Therefore, it was suggested that even in the presence of ACE inhibition, substantial amounts of Ang II can be formed from Ang I locally in the heart by enzymes other than ACE to enhance noradrenaline release. In contrast, EXP3174 had abolished Ang I effects on noradrenaline release [5]; therefore, these experimental findings may help to explain the results observed in the ELITE study.

However, especially in the heart, the situation may be far more complex. We found that AT2 receptor blockers attenuated the facilitatory effect of Ang II on noradrenaline release. This may be a non-specific effect. Nevertheless, an effect involving the parasym pathetic nervous system is possible. This idea is supported by the fact that a parasympathetic innervation is lacking in the kidney, where AT2 receptor blockade was without an effect. Acetylcholine is known to inhibit noradrenaline release in the heart of animal models [6], acting through muscarinic receptors on sympathetic nerves. We have shown that Ang II increases acetylcholine release. We speculate that activation of AT1 receptors enhances and activation of AT2 receptors inhibits cardiac acetylcholine release. Thus, AT2 receptor blockade would enhance release of acetylcholine, activating inhibitory muscarinic receptors on sympath-
**Fig. 1.** Effects of AT1 and AT2 receptor antagonists on Ang II-mediated modulation of noradrenaline release in (A) human atria and (B) renal cortex. An asterisk indicates significant effect of Ang II as compared with control. (Student’s unpaired t-test; \( P < 0.05 \)). + indicates significant inhibition by antagonists (ANOVA, \( P < 0.05 \)).

**References**


**Diuretic therapy and diuretic resistance in cardiac failure**

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Thiazides and loop diuretics have been the mainstay of treatment for symptomatic cardiac failure for the last 30–40 years. Diuretic therapy in congestive heart failure (CHF) is effective in relieving symptoms and improving cardiovascular haemodynamics [1–7]. The aim of the present report is briefly to review diuretic treatment in CHF, and to discuss underlying mechanisms of diuretic resistance (reviewed in more detail in [8]).

**Diuretic therapy**

Diuretic therapy in acute cardiac failure with loop diuretics i.v. reduces wedge pressure and increases...
venous capacitance within a few minutes before any measurable increase in urinary output can be seen [1,2]. This rapid haemodynamic improvement is thought to be due to the release of vasodilatory prostaglandins [3]. However, in a study in patients with severe oedema, i.v. piretanide did not cause a decrease in wedge pressure within 120 min (local oedema preventing venous dilation?) [4]. Acute haemodynamic improvements often are sustained during chronic treatment [2,5]. For example, an improvement of NYHA functional class (71 vs 10 NYHA III, 80 vs 112 NYHA II, 0 vs 29 NYHA I at baseline vs 4 weeks) was demonstrated in 151 patients with CHF on 5–10 mg of torasemide for 4 weeks [6,7]. Recent randomized trials have dealt with the treatment of CHF in 17,825 patients as given in Table 1. In patients with advanced heart failure (NYHA III/IV), up to 100% are treated with a (loop) diuretic, whereas in patients with less advanced heart failure the percentage of patients on a diuretic is lower (Table 1). Despite the widespread use of diuretics in the treatment of heart failure, a survival benefit for patients on a diuretic has never been proven, though this appears to be very likely, at least in severe pulmonary oedema. Sharpe et al. randomized 60 patients with Q wave infarction and asymptomatic left ventricular dysfunction to treatment with either 40 mg of frusemide or 75 mg of captopril or placebo for 12 months [9]. Left ventricular volumes increased and ejection fraction decreased slightly in both the frusemide and placebo groups, whereas during captopril treatment end-systolic volume decreased and ejection fraction increased. Thus, in patients with asymptomatic left ventricular dysfunction, diuretic monotherapy may not be sufficient to preserve cardiac function.

Diuretic administration in heart failure

A thiazide may be used in less severe CHF; however, in most patients with advanced CHF, a loop diuretic will be necessary [6,7,10]. Hypokalaemia and hypomagnesaemia caused by a loop diuretic or a thiazide may be prevented by combination with a potassium-sparing diuretic (e.g. amiloride, triamterene or spironolactone) [10]. Most heart failure patients are on an angiotensin-converting enzyme (ACE) inhibitor, thereby necessitating careful dose titration and control of serum potassium levels, because of the considerable risk of hyperkalaemia with such a combination therapy [10,11].

Diuretic resistance

In patients started on a diuretic, there is an initial reduction in body weight and total body sodium. However, a new steady state with equal sodium intake and sodium excretion is soon reached. This physiological diuretic resistance has been termed the ‘braking phenomenon’, which has evolved to prevent excessive fluid and salt losses. Diuretic resistance in a patient with oedema is defined as a clinical state where the braking phenomenon occurs before the therapeutic goal is reached.

Causes and mechanisms of diuretic resistance

Impaired kidney function. Pre-renal azotaemia is very often present in patients with severe CHF either treated or untreated [12,13]. In renal failure, tubular delivery of loop diuretics is impaired due to diminished renal blood flow and to reduced activity of the proximal tubular carrier system caused by competition for the co-transporter from accumulated organic anions [14]. Loop diuretic doses have to be increased considerably to achieve sufficient drug concentrations within the tubular lumen.

Hyponatraemia. Hyponatraemia has been shown frequently to be associated with reduced diuretic efficacy. Hyponatraemia may be caused by thiazides, but is most often due to CHF with stimulation of thirst, and a non-osmotically stimulated vasopressin system that impairs excretion of free water [15]. This is particularly problematic when patients are not compliant with restrictions in free water intake.

Pharmacokinetics. The pharmacokinetics of loop diuretics are altered in CHF (reduced peak concentration, prolonged time to peak concentration, but with no significant reduction of the total amount of frusemide absorbed). These differences are not marked and are overcome by a moderate increase in dose [16]. Furthermore, proximal tubular secretion of the diuretic may be impaired by organic anions that use the same transport pathway (probenecid, penicillins, endogenous organic acids in uraemia) [14].

Sodium and fluid retention. In severe CHF, proximal and distal tubular reabsorption of sodium is stimulated, due to direct (proximal) tubular effects of angiotensin II and catecholamines, to facilitation of passive sodium reabsorption in the proximal tubule and to aldosterone-mediated sodium reabsorption in the collecting duct [8,13,17]. Furthermore, resistance to the natriuretic action of atrial natriuretic peptide contributes to sodium retention in CHF [18,19]. Even in asymptomatic heart failure, a defective adaptation of sodium reabsorption in the proximal nephron in response to salt intake is present [20]. One main cause for the activation of sodium retention in CHF is a reduction in ‘effective’ arterial blood volume (reduced cardiac output and/or reduced peripheral vascular resistance) [21]. When patients with severe CHF with increased proximal tubular sodium reabsorption are treated with a loop diuretic, the ensuing natriuresis is reduced further by stimulation of sodium reabsorption at more distal sites of the nephron. In animals treated chronically with a loop diuretic, a marked hypertrophy of the distal tubule was observed [22]. The above mechanisms all contribute to the well-known rightward shift of frusemide dose–response curves in patients with CHF [23].
Table 1. Management of diuretic resistance in cardiac failure

<table>
<thead>
<tr>
<th></th>
<th>Diuretic (%)</th>
<th>Diuretic</th>
<th>Dose (mg/day)</th>
<th>NYHA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Consensus I 1987</td>
<td>98/50-55/10-14</td>
<td>Furos./Spirono./Other</td>
<td>200-210/80/-</td>
<td>IV</td>
</tr>
<tr>
<td>PRAISE 1996</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>III/IV</td>
</tr>
<tr>
<td>RALES 1996</td>
<td>100</td>
<td>Furosemide</td>
<td>59-85</td>
<td>II-IV</td>
</tr>
<tr>
<td>US Carvedilol 1996</td>
<td>95</td>
<td>Loop Diuretic</td>
<td>-</td>
<td>II/III/IV</td>
</tr>
<tr>
<td>DIG 1997</td>
<td>82</td>
<td>-</td>
<td>-</td>
<td>I-II/III/IV</td>
</tr>
<tr>
<td>MDC 1993</td>
<td>75</td>
<td>Furosemide</td>
<td>62</td>
<td>(II)/III/IV</td>
</tr>
<tr>
<td>SOLVD Treatment 1991</td>
<td>85/9</td>
<td>Diuretic/K+-Sparing</td>
<td>-</td>
<td>II/III</td>
</tr>
<tr>
<td>Can En vs Dig 1991</td>
<td>73/100</td>
<td>Furosemide</td>
<td>46</td>
<td>II/III</td>
</tr>
<tr>
<td>SOLVD Prevention 1992</td>
<td>17/4</td>
<td>Diuretic/K+-Sparing</td>
<td>-</td>
<td>I/II</td>
</tr>
</tbody>
</table>

Fig. 1. Overview of diuretic treatment in cardiac failure according to recent large, randomized trials

Table 1. Management of diuretic resistance in cardiac failure

- Restriction of daily fluid (1.0–1.5 l) and salt intake
- Avoid NSAIDs, and overly aggressive vasodilatory therapy
- Start on an ACE inhibitor (careful dose titration; e.g. 6.25 mg of captopril t.i.d.)
- Short-acting loop diuretic p.o. in several divided (and increasing) doses (e.g. 40–80 mg of furosemide b.i.d. or t.i.d)/i.v. administration of the diuretic/continuous infusion of the diuretic
- Sequential nephron blockade by combination of a loop diuretic and, for example, a thiazide
- Addition of low doses of spironolactone (12.5–25 mg/day) with concomitant ACE inhibition

Therapeutic measures

Control of sodium and fluid intake. A major cause of apparent diuretic resistance in clinical practice is non-compliance with prescribed sodium/fluid intake. A marked increase in sodium absorption 6–24 h after administration of furosemide to healthy subjects on a high sodium diet, thereby abolishing any natriuretic effect of a single daily dose of furosemide, has been shown [24]. In contrast, during a low sodium diet, such a compensatory increase in sodium reabsorption did not occur, because sodium reabsorption had been near maximal already at baseline, thus causing a negative sodium balance during a low-sodium diet, only [2]. Brater et al. have shown also in patients with CHF that depending on the mode of administration (bolus vs infusion) of the loop diuretic bumetanide, retention of an i.v. sodium load occurs [25]. Fluid intake should therefore be limited to 1.0–1.5 l/day and salt intake should be restricted in patients with CHF [10].

Concomitant ACE inhibition. ACE inhibition has been proven successful for the treatment of refractory oedema in patients with diuretic resistance [12]. These beneficial effects may be due to improvement of cardiac performance and suppression of angiotensin II-mediated effects (stimulation of thirst, vasopressin release, tubular sodium reabsorption). However, administration of an ACE inhibitor without a loop diuretic was not effective [26]. It is suggested to institute ACE inhibition in patients with severe CHF on a loop diuretic with small starting doses [10]. However, standard (‘standard’ as defined in clinical survival trials) doses of ACE inhibitors should be given to all patients with CHF during long-term therapy [10]. Diuretic combination therapy. Because of the known stimulation of proximal and distal tubular sodium reabsorption in patients with CHF on a loop diuretic, blockade of sodium reabsorption at different sites in the nephron was suggested. The addition of hydrochlorothiazide, metolazone or acetazolamide in order to inhibit sodium reabsorption distal and/or proximal to the thick ascending limb of the loop of Henle has proven effective in patients with heart and renal failure (acetazolamide use is limited due to development of acidosis) [27,28]. Combination of a loop diuretic with a thiazide results in (super)additive effects on diuresis and natriuresis even in advanced renal failure [27,28]. Such diuretic combinations have a considerable potential for side effects (e.g. hypokalaemia, hypovolaemia). The addition of spironolactone to a regimen including a loop diuretic and an ACE inhibitor was also suggested to be effective in refractory heart failure [11]. The reason for this combination is that during long-term ACE inhibition aldosterone secretion is no longer
sufficiently suppressed (‘aldosterone escape’). In the RALES study, small doses of spironolactone effectively blocked aldosterone action when given in addition to a loop diuretic and an ACE inhibitor [11]. In the pivotal RALES study, a survival benefit of 27% in 1660 patients randomized to spironolactone (mean dose 27 mg/day) vs placebo in addition to ACE inhibition/loop diuretic was shown (B. Pitt; Annual Meeting American Heart Association, November 1998).

**Mode of diuretic administration.** The route of diuretic administration may need to be varied to overcome diuretic resistance; i.e., administration of a loop diuretic avoids uncertainties regarding intestinal resorption of the diuretic, while a continuous diuretic infusion, multiple boli or a long-acting diuretic all prevent a rebound in post-diuretic sodium reabsorption [29–31]. Furthermore, the use of prostaglandin synthase inhibitors may contribute to diuretic resistance by impairing renal function [32]. Finally, the management of diuretic resistance is summarized in Table 1.

Future treatment approaches in the patient with heart failure and diuretic resistance may include blockade of the neutral endopeptidase or blockade of the V2 vasopressin receptor [33,34].

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Treatment of cardiovascular changes in renal failure—ACE inhibition, endothelin receptor blockade or a combination of both strategies?

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Background

Cardiovascular complications are a major problem in patients with renal failure, and cardiac death is the leading cause of death in these patients. Clinical and experimental data have documented a pathogenetic role for the local renin–angiotensin system.

Recently, a potential role for endothelin-1 (ET-1) in the development of cardiovascular changes in renal failure was also postulated, i.e. increased ET-1 mRNA and protein excretion were found in the heart of uraemic patients and of subtotally nephrectomized rats with chronic renal failure. In addition, a close correlation between thickening of the wall of elastic arteries and plasma ET-1 was documented in uraemic patients. 2.08 ± 0.57% wall thickness of intramyocardial arteries and of the aorta were analysed by morphometry. The data argue for a potential role of the local renin–angiotensin system as well as of the ET system in the pathogenesis of cardiovascular changes in renal failure.

Materials and methods

At 24 h after subtotally nephrectomy (SNX), male Sprague–Dawley rats (200 g) were left untreated or started on treatment with the selective ET₁ receptor antagonist LU 135252 (20 mg/kg/day), the ACE-I trandolapril (0.3 mg/kg/day) or a combination of both therapies. The animals were compared with sham-operated control rats (sham) and followed for 15 weeks. Blood pressure was monitored by telemetry in several animals per group during the experiment. Left ventricular weight (LVW), volume density of cardiac interstitial tissue, volume density and length density of myocardial capillaries, and wall thickness of intramyocardial arteries and of the aorta were analysed by morphometry.

Results

Mean arterial blood pressure was significantly greater in untreated SNX (136 ± 0.76 mmHg) than in sham-treated (105 ± 0.52 mmHg) and all treated SNX groups. It was lowest in the combination therapy group. Serum creatinine (75 ± 5.9 µmol/l) and proteinuria (235 ± 93 mg/kg/day) were significantly increased after SNX and were lower with ACE-I (60 ± 2.74 µmol/l and 118 ± 46 mg/kg/day, respectively) than with ET receptor blockade (90 ± 23 µmol/l and 216 ± 112 mg/kg/day, respectively). In untreated SNX animals, a significant increase in relative LVW (2.27 ± 0.15 vs 1.82 ± 0.15 mg/g), cardiac interstitial tissue (3.12 ± 0.63 vs 2.08 ± 0.57%), wall thickness of intramyocardial arteries (5.52 ± 1.69 vs 3.79 ± 0.67 µm) and of the aorta (0.68 ± 0.11 vs 0.52 ± 0.05 mm²) was seen compared with sham-operated control rats.

These structural changes were completely and comparably prevented by all three treatments. In contrast, the decrease in myocardial capillary supply after SNX (3307 ± 534 vs 3995 ± 471 mm²) was only completely prevented by the ET₁ receptor blocker.

Summary and conclusion

ACE-I and specific ET₁ receptor blockade comparably prevented the development of structural cardiovascular alterations such as LVH, myocardial interstitial expansion and wall thickening of intramyocardial and extra-cardiac arteries in experimental renal failure. However, the decrease in myocardial capillary supply and the concomitant increase in intercapillary distance could only be prevented by ET₁ receptor antagonism. These capillary changes which have been shown to occur in experimental renal failure as well as in uraemic patients play an important role in the pathogenesis of reduced cardiac ischaemia tolerance in renal failure.

The data argue for a potential role of the local renin–angiotensin system as well as of the ET system in the pathogenesis of cardiovascular changes in renal failure and for ET receptor blockade as a new therapeutic option in the treatment of these alterations.

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In particular, myocardial capillary supply, which is particularly important for ischaemia tolerance in renal failure, seems to be modulated and regulated predominantly by ET-1.

In contrast to what was found with respect to structural and functional changes of the kidney in various experimental models of renal damage, a combination therapy—at least in the doses used—does not seem to provide additional benefit in the prevention of cardiovascular changes compared with the respective monotherapies.

Role of sympathetic nerves in the differential effects of T-type and L-type calcium channel blockers on renin secretion and renin gene expression

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Introduction

One major side effect of calcium channel blockers (CCBs) is the stimulation of the renin system [1,2] which, at least in part, counteracts the desired effects of treatment with CCBs. The mechanisms underlying the stimulation of the renin system are probably multifactorial and are still not completely understood. They involve systemic effects, such as the fall in blood pressure and activation of sympathetic outflow, and direct effects on the level of renin-secreting juxtaglomerular cells. Classical CCBs act primarily on L-type (long-lasting, high voltage-activated) calcium channels, whereas mibebradil selectively blocks T-type (transient, low voltage-activated) over L-type calcium channels [3,4]. Wagner and co-workers previously have shown that this pharmacological difference seems to translate into different effects in vivo. They could demonstrate that the T-type CCB mibebradil and the L-type CCB amiodipine have opposite effects on renin secretion and renin gene expression [5]. In the present investigation, we aimed to investigate the role of renal sympathetic nerves in the differential effects of T- and L-type CCB on renin secretion and gene expression.

Parameters assessed

Systolic blood pressure was measured by the tail cuff method. Plasma renin activity (PRA) was determined by using a commercially available radioimmunoassay. Renin gene expression was determined as the ratio of renin mRNA to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA. Renin and GAPDH mRNA in the kidneys were measured by RNase protection, as described previously [5].

Statistics

Results are expressed as mean ± SEM and were compared using Student’s t-test. Differences were considered to be statistically significant when P-values were <0.05.

Results

Effects on blood pressure

Left renal artery clipping led to an increase in systolic blood pressure from 126 ± 3 mmHg to 193 ± 3 mmHg (P < 0.05). Left renal denervation attenuated the increase in blood pressure to 179 ± 12 mmHg (NS vs clipping). Treatment with amiodipine and mibebradil decreased blood pressure to 127 ± 14 and 130 ± 11 mmHg, respectively (P < 0.05 vs clipping + denervation).
Effects on renin secretion and gene expression

Left renal artery clipping increased PRA by +375% (P<0.05 vs control). Left renal denervation reduced PRA by −45% as compared with the clipped control (P<0.05). Treatment with amlodipine stimulated PRA by +190% (P=0.07 vs clipping + denervation). Mibefradil left PRA levels almost unchanged (+3.5%; NS vs clipping + denervation). Renal denervation restored renin mRNA in the left clipped kidney back to normal values as compared with healthy controls. Treatment of denervated 2K-1C rats with amlodipine increased the renin mRNA in both kidneys [left clipped +211% (P<0.05), right contralateral +311% (P=0.07)] as compared with vehicle-treated denervated 2K-1C rats. Mibefradil treatment of denervated 2K-1C rats had no systematic effect on renin mRNA expression [left clipped −6% (NS), right contralateral +23% (NS)].

Discussion

The present investigation shows that in rats carrying a left renal artery clip (2K-1C rats), left renal denervation substantially decreased renin secretion and gene expression. Treatment of left denervated 2K-1C rats with 15 mg/kg amlodipine or mibefradil was able to restore blood pressure values back to those of healthy control rats. Despite similar effects on blood pressure, treatment with amlodipine increased PRA by 190%, whereas treatment with mibefradil left PRA levels unchanged (+3.5%). These differences in PRA were paralleled at the gene expression level, where amlodipine strongly stimulated renin mRNA expression. Mibefradil treatment showed an inconsistent pattern, with a small increase in the left kidney and a small decrease in the right kidney. Previous investigations in 2K-1C rats with intact renal denervation demonstrated an inhibitory effect of mibefradil on renin secretion and gene expression [5]. In contrast, the present study shows that left renal denervation abolished the inhibitory effect of mibefradil on renin secretion and gene expression. Nevertheless, the stimulatory effect of amlodipine on renin secretion and gene expression was unaffected by renal denervation.

In summary, these findings suggest that the inhibitory effects of the T-type CCB mibefradil on renin secretion and renin gene expression in rats are, at least in part, mediated by renal sympathetic nerves.

References


Anticoagulation with r-hirudin in a patient with acute renal failure and heparin-induced thrombocytopenia

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Introduction

Recombinant hirudin (r-hirudin) is a strongly selective thrombin antagonist [1,2] and therefore a potent agent in alternative anticoagulation during heparin-induced thrombocytopenia.

Intravenous application of r-hirudin leads to a quick distribution into the extracellular space. It is mainly eliminated by the kidneys. The dose-dependent elimination half-life is 1 to 2 h [3]. An antidote is not yet available. Only a few pharmacological data are known in patients with renal impairment. In 1992 Nowak et al. [4] showed that the elimination half-life in patients with chronic renal failure was increased up to 15 to 41 h. In two bilaterally nephrectomized patients the elimination half-life was prolonged up to 100 h.

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Therefore a strong accumulation of r-hirudin in patients with acute renal failure is possible accompanied by the risk of acute haemorrhage.

**Case report**

A 37-year-old female patient was admitted to our unit after a Caesarean section because of placental insufficiency. Due to an infected subcutaneous haematoma she developed a systemic infection with fever (39.5°C), an elevation of the white blood cell count (16 700/μl) and C-reactive protein (27 mg/dl). In parallel to this infection the platelet count declined to 40 000/μl, haemoglobin concentration declined to 6 g/dl and acute renal failure developed. One of the possible diagnoses seemed to be haemolytic uraemic syndrome but there was no evidence of schistocytes in the blood smear. After anti-heparin-platelet factor 4 antibodies (Asserachrom® HPIA, Diagnostika Stago) were detected the diagnosis of heparin-induced thrombocytopenia Type II as the cause of the thrombocytopenia was made. Because of the anuric renal failure haemodialysis treatment had to be started with an alternative anticoagulation. A choice could be made between danaparoid-sodium — a low molecular weight heparinoid and lepirudin (Refludan®)—a recombinant hirudin. Referring to the literature there exists a cross-reactivity between danaparoid-sodium and standard heparin in about 10% of cases [5]. Therefore the choice for anticoagulation during haemodialysis treatment was lepirudin, a strongly selective direct thrombin inhibitor with a molecular weight of 7000 Da. To monitor the r-hirudin treatment activated partial thromboplastin time (aPTT) and lepirudin plasma concentrations were determined. After cessation of heparin treatment the platelet count returned to normal within 9 days. The renal function remained impaired. Percutaneous biopsy showed severe tubulo-interstitial damage with no hint of glomerulonephritis.

**Laboratory monitoring during lepirudin treatment**

To monitor the lepirudin therapy activated partial thromboplastin time (aPTT) and lepirudin plasma concentration were determined. To determine the lepirudin plasma concentration the Ecarin clotting time (ECT) and a chromogen factor IIa test were used (Figure 1). Lepirudin treatment was started at 0.1 mg/kg body weight. Under these conditions aPTT was elevated up to 75 s which is in the recommended range of 1.5- to 2.5-fold elevation of aPTT (Figure 2). After the second haemodialysis session vaginal bleeding occurred caused by a known myoma of the uterus. Therefore, the lepirudin dose was reduced to 0.05 mg/kg body weight. Haemodialysis sessions could be performed without any clotting problems. Further vaginal bleeding did not occur.

After the first application of 10 mg lepirudin equivalent to a dose of 0.1 mg/kg body weight the lepirudin plasma concentration was 0.5 μg/ml (Figure 3). After a cumulative dose of 30 mg lepirudin the plasma concentration rose linearly to 1.5 μg/ml. At this plasma concentration the vaginal bleeding occurred which made a dose reduction necessary. It has to be stated that the recommended lepirudin plasma concentration for haemodialysis treatment is 0.6 to 1 μg/ml (Nowak, personal communication). After a dose reduction the lepirudin levels fell between 0.25 μg/ml and 0.46 μg/ml which was the lower border of our measurement setting. It has to be emphasized that clotting problems did not occur during haemodialysis treatments although the plasma lepirudin was well below the recommended range. Thus according to this report it seems to be possible to perform intermittent haemodialysis treatments at very low plasma lepirudin levels.

**Summary**

After a Caesarean section subcutaneous bleeding with a secondary infection occurred accompanied by acute renal failure. Additionally the diagnosis of a heparin-induced thrombocytopenia type II could be made. Therefore, haemodialysis treatments were performed...
Fig. 2. aPTT change during lepirudin treatment in intermittent haemodialysis. The $x$-axis shows the number of haemodialysis treatments. The left $y$-axis shows the aPTT in seconds. The right $y$-axis shows the lepirudin dose in mg/kg body weight. Lepirudin was applied as a bolus injection during haemodialysis treatment. On dialysis-free days there was no additional application of lepirudin except the day after the first dialysis when 5 mg lepirudin were applied subcutaneously twice daily.

Fig. 3. Lepirudin plasma concentrations during lepirudin therapy in haemodialysis treatment. The $x$-axis shows the number of haemodialysis treatments. The left $y$-axis shows the lepirudin plasma concentration in μg/ml. The right $y$-axis shows the lepirudin dose in mg/kg body weight. Lepirudin was administered as in Figure 2. For haemodialysis treatments a polysulfone low-flux dialyser (Hemoflow F7 HPS, Fresenius AG, Bad Homburg, Germany) was used.

with r-hirudin as an alternative anticoagulation. Even in dialysis dependent acute renal failure a safe anticoagulation with r-hirudin is possible if aPTT and plasma r-hirudin are closely monitored.

References

ATP release and degradation in the kidney: modulatory role of neuropeptide Y (NPY)

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Introduction

The sympathetic nervous system controls blood pressure and renal function under physiological and pathophysiological conditions. Noradrenaline is the major neurotransmitter at the sympathetic neuroeffector junction. However, other neurotransmitters such as neuropeptide Y (NPY) and ATP are released along with noradrenaline [1,2]. In particular, NPY may play an as yet underestimated role in situations of enhanced sympathetic activity such as hypertension [3] and renal failure [4]. We investigated ATP-mediated (purinergic) vasoconstriction and its interaction with NPY in isolated rat kidney. Pressor responses to renal nerve stimulation (RNS), ATP release and ATPase activity in the renal effluent were measured in 1 min fractions.

Results

RNS (1 Hz for 30 s) in the presence of the α-adrenoceptor blocker phentolamine (1 μM) induced pressor responses of ~50 mmHg and release of 3 pmol of ATP (Figure 1). α-Adrenoceptor blockade-resistant responses were abolished by the P2 purinoceptor blocker suramin (300 μM). Furthermore, these purinergic pressor responses could be reduced to 50% by the selective Y₁-NPY receptor blocker BIBP 3226 (1 μM). Repetitive RNS induced a progressive increase in pressor responses, which was also prevented by BIBP 3226. Exogenous NPY (0.1 μM) by itself did not increase perfusion pressure; however, it potentiated purinergic pressor responses and ATP release almost 3-fold (Figure 1). This NPY potentiation was accompanied by a significant 25% increase in the ATPase activity in the effluent (Figure 1). All effects of NPY were prevented by BIBP 3226 (Figure 1). Ultracentrifugation of the effluent with a 30 kDa membrane or boiling removed the ATPase activity.

Discussion

NPY by itself appears to be only a weak renal vasconstrictor, but purinergic renal vasoconstriction is potentiated by NPY.

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tiated markedly by NPY in rat kidney. Endogenous NPY acts as a master hormone to modulate the vascular effects of its co-transmitter ATP. This NPY involvement takes place already at physiological rates of renal nerve firing. Even more importantly, our data may be interpreted as evidence for a role for endogenous NPY potentiating vascular effects of ATP in situations of sympathetic overactivity. Neuronally released ATP is broken down by concomitantly released soluble ATPase in the rat mesenteric vascular bed [5]. We have shown that ATP, whether released from neuronal or extraneuronal sources in the kidney, is also subject to rapid degradation by a soluble ATPase. Our findings may shed some light on previous experiments pursuing the idea of a purinergic neurotransmission and its modulation by prejunctional receptors in rat kidney and possibly other isolated tissues by simply measuring ATP release. The functional evidence favoured a role for ATP as a fast neurotransmitter at lower stimulation frequencies (0.3–4 Hz) [6], whereas ATP release was measurable reliably only at higher frequencies (>4 Hz) [7]. This obvious discrepancy may be explained by the rapid breakdown of neurotransmitter ATP by soluble ATPases. Moreover, one has to keep in mind that drugs or endogenous substances such as NPY may modulate ATPase activity. It is therefore advisable to assess drug effects on ATPase activity to prevent misinterpretations.

Acknowledgements.

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References


Immunophenotyping and LDL receptor activity analysis in monocytes/macrophages during low- and high-flux haemodialysis

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Introduction

The aim of the study was to investigate the influence of membrane characteristics on factors involved in the atherogenic progression in chronic renal failure.

Study design and sampling

Twenty-three patients were treated in four 3-month periods as indicated in Table 1. Cuprophan membrane (CUM) was taken as a model in which changes in lipid metabolism could be expected.

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Membrane | Period 1: Low-flux | Period 2: High-flux | Period 3: High-flux | Period 4: Low-flux
---|---|---|---|---
Cuprophan | Polyamide | Polyamide | Cuprophan
Heparin dosage | 6600E | 6600E | 4800E | 6600E
LRA, CD marker: EDTA | $t = 0$ | $t = 0$ | $t = 0$ | $t = 0$
Lipids: citrate | $t = 0$ | $t = 0$ | $t = 0$ | $t = 0$
Plasmatic systems: | $t = 0, 15, 240$ min | $t = 0, 15, 240$ min | $t = 0, 15, 240$ min | $t = 0, 15, 240$ min
EDTA and citrate | | | | |

Time units: $4 \times 3$-month periods. Blood samples for the assessment of LRA and CD marker (EDTA, citrate and heparin) were taken at the end of P1 and P3 before starting the HD; samples for the analyses of plasmatic systems were taken during HD treatment, as indicated.

Table 2. Lipid status of haemodialysis patients ($n = 23$), mean $\pm$ SD in mg/dl

<table>
<thead>
<tr>
<th></th>
<th>Total cholesterol</th>
<th>TG</th>
<th>HDL</th>
<th>LDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>272.9 $\pm$ 41.1</td>
<td>295.4 $\pm$ 136.4</td>
<td>42.4 $\pm$ 15.2</td>
<td>164.3 $\pm$ 46.0</td>
</tr>
<tr>
<td>P3</td>
<td>258.9 $\pm$ 42.0</td>
<td>217.9 $\pm$ 122.2</td>
<td>41.5 $\pm$ 13.1</td>
<td>182.2 $\pm$ 44.7</td>
</tr>
<tr>
<td>P1 vs P3</td>
<td>NS</td>
<td>$P &lt; 0.05$</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Table 3. Apolipoproteins and LRA of haemodialysis patients ($n = 23$), mean $\pm$ SD in mg/dl

<table>
<thead>
<tr>
<th></th>
<th>TG</th>
<th>apoAI</th>
<th>apoB</th>
<th>LRA</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>295.4 $\pm$ 136.4</td>
<td>127.9 $\pm$ 25.1</td>
<td>103.7 $\pm$ 17.3</td>
<td>36.8 $\pm$ 14.0</td>
</tr>
<tr>
<td>P3</td>
<td>217.9 $\pm$ 122.2</td>
<td>157.5 $\pm$ 32.1</td>
<td>164.8 $\pm$ 42.3</td>
<td>49.2 $\pm$ 8.6</td>
</tr>
<tr>
<td>P1 vs P3</td>
<td>$P &lt; 0.05$</td>
<td>$P &lt; 0.01$</td>
<td>$P &lt; 0.001$</td>
<td>NS</td>
</tr>
</tbody>
</table>

genetic disposition. Then the same method was used to assess LRA, i.e. the variability of LRE within the genetic limits. LRE and LRA are independent of food intake. TAT, TCC, PAP and factor XIIa as soluble plasmatic parameters were also measured.

Results

Metabolism of triglycerides

The TG concentration declined significantly in P3 ($P < 0.05$) accompanied by a significant increase in apoAI. Because apoAI is an ampholytic protein which can be found in plasma also without a lipid moiety, we judged this increase to reflect a higher protein synthesis.

The decrease of TG suggests an improved elimination of CM and VLDL. As seen in our previous studies, HD with polyamine (PAM) resulted in a 40–50% reduction of apoCIII (240 mg/dl vs 180 mg/dl) together with an amelioration of the lipid profile within 4 months.

Apolipoproteins and LDL receptor function

After improvement of the metabolism of TG-rich lipoproteins, an increase of LDL must be the correct consequence because of the displacement due to the law of mass action. To explain the increase of apoB in P3 using PAM and reduced heparin dosage while LDL and HDL remained constant, other overlapping effects have to be considered. First, the reactivation of LPL succeeded the formation of HDL and the course of the receptor-mediated apoB cascade. Second, in dys-

Surface markers

The significant increase in the activation and proliferation markers HLA-DR and CD71 ($P < 0.05$ each) on monocytes, as well as the occurrence of macrophages (CD68) and pre-macrophages (CD14/CD16, $P < 0.05$) in P1 (CUM) vs P3 (PAM) demonstrate the higher immunomodulating activity of CUM in comparison...
with high-flux membrane. This was accompanied by reduced TCC production (114.1 vs 33.8, \( P < 0.01 \)) and the normalizing expression of CD11b, therefore signalling a reduction of complement activation under treatment with PAM. The significant decrease in CD23 expression with PAM (34.5 vs 19.0, \( P < 0.01 \)) means an abolition of the blocking of interleukin-2 receptors and, therefore, an improvement of the immunocompetence of the HD patients.

**Conclusions**

Low numbers of activated monocytes and the increase in LRA with PAM may interrupt the cycle of tissue activation and tissue rearrangement in HD patients. The applied methodology of the parallel assessment of LRA and surface markers provides a useful tool to control therapeutic interventions and individual cardiovascular risks.