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Tacrolimus in acute renal failure: does L-arginine-infusion prevent changes in renal hemodynamics?

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Abstract Nephrotoxicity is one of the main side effects of calcineurin-inhibitors. The influence of tacrolimus on the renal vasculature has not been well described. We have therefore examined the effects of tacrolimus on renal functional parameters as well as the contribution of the NO-system in a model of ischemic acute renal failure (ARF). Induction of ARF was achieved by clamping both renal arteries of female Sprague-Dawley rats. During the experiment, RBF, GFR, MAP, RVR and FENa were determined during infusion of vehicle, TAC, TAC and the NOS-activator L-arginine, and TAC and NOS-inhibition due to L-NMMA. TAC induced a significant rise in RVR with further decrease of RBF and GFR. Simultaneous L-arginine-infusion could reverse these effects during the infusion without complete restoration to preischemic levels. NOS-inhibition increased MAP and RBF without any effect on GFR. FENa did not differ significantly between the groups. Tacrolimus in the situation of ischemic acute renal failure causes vasoconstriction of pre- and postglomerular vessels with a further deterioration of renal function. L-arginine abolishes the functional deterioration, most likely due to increased NO-liberation.

Keywords Tacrolimus · L-arginine · Acute renal failure · NO-system · Nephrotoxicity

Abbreviations *ARF* Acute renal failure · *CNI* Calcineurin inhibitor · *CsA* Cyclosporin A · *FENa* Fractional excretion of sodium · *GFR* Glomerular filtration rate · *L-Arg* L-arginine · *L-NMMA* L-nitro-monomethyl-arginine · *MAP* Mean arterial blood pressure · *NO* Nitric oxide · *NOS* Nitric oxide synthase · *RBF* Renal blood flow · *RPF* Renal plasma flow · *RVR* Renal vascular resistance · *TAC* Tacrolimus

Introduction

One of the main obstacles of immunosuppressive therapy with calcineurin-inhibitors (CNI) like cyclosporine or tacrolimus is nephrotoxicity. After orthotopic liver transplantation, tacrolimus-induced renal impairment was observed in about 40% of all patients [18]. After preimposed injury, for example by ischemia/reperfusion, the renal microvasculature in particular appears to react very sensitively to the administration of this kind of drug. In cell culture systems, ATP-depletion in renal epithelial cells [13], endothelin-1-release of mesangial cells [7] and decreased viability with increased vacuolisation and lipid inclusions in human proximal tubular cells [2] have been demonstrated after addition of TAC. In a model implementing the isolated perfused rat kidney, TAC significantly impaired renal function in form of reduced renal plasma flow (RPF) and glomerular filtration rate (GFR), without affecting renal vascular resistance [3]. In another in vivo-model, acute bolus

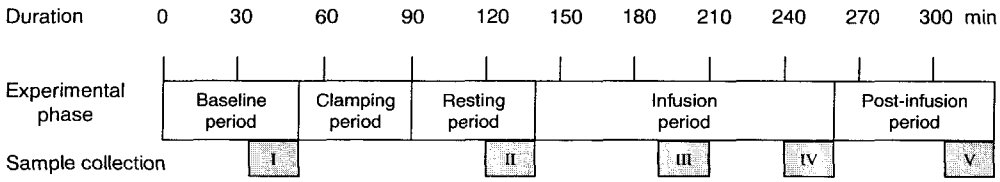

Fig. 1 Experimental protocol

administration of TAC led to a 60% decrease in GFR and a 40% decrease in single nephron-GFR, due to a reduction of glomerular plasma flow [8]. Nielsen et al could demonstrate a predominantly preglomerular effect of tacrolimus with a marked reduction of inulin-clearance as marker for GFR after a four-week administration in rats [16]. Experiments in spontaneous hypertensive rats have pointed out associated increases of plasma renin activity and urinary thromboxane excretion and decreased prostacyclin-metabolite-excretion together with a decrease in creatinine-clearance after CNI-administration [14]. Involvement of serotoninergic pathways has been suspected as one mediator of vasoconstriction and diminished renal blood flow [15]. In liver transplant recipients, decreases of renal blood flow and GFR in a similar manner have been observed during TAC- as well as cyclosporine A-treatment [24], and renal impairment has been shown to be alleviated by administration of nifedipine in tacrolimus-treated liver transplant patients [23], which also points towards a preglomerular vasoconstricting effect of tacrolimus which could be reversed by calcium-channel-blockers.

It has been shown by our group and others that the intrarenal NO-system is involved in the regulation of renal perfusion in different models of acute renal failure (ARF) as for example in toxic [21] and in postischemic ARF [22]. Warm ischemia/reperfusion after renal transplantation is one reason for rapid and sometimes irreversible deterioration of graft function. In most cases CNI-therapy continues during the insult.

In this study, we examined the effects of intravenous tacrolimus administration on renal hemodynamics during and after warm ischemic renal failure. Furthermore, we were interested if the beneficial effects of L-arginine, which we have seen in other models of ARF [21, 22], could also be observed in this study, and if they depend on activation of the NO-system.

Materials and methods

Drugs

L-arginine (L-Arg) and L-nitromonomethylarginine (L-NMMA) were purchased from Sigma (Deisenhofen, Germany), FITC-inulin was ordered from Bioflor (Uppsala, Sweden), sodium-para-aminohippurate (PAH) from Merck, Sharpe & Dohme (West Point, USA), Tacrolimus in a preparation for intravenous use was kindly provided by Fujisawa (Munich, Germany).

Four groups of animals, consisting of 8 rats each, were investigated: Control animals were treated with Ringer saline as vehicle, the TAC-group with tacrolimus 0.05 mg/kg × h, the TAC/L-Arg-group with tacrolimus 0.05 mg/kg × h combined with L-Arg 500 mg/kg × h and the TAC/L-NMMA-group with tacrolimus 0.05 mg/kg × h in combination with L-NMMA 1.0 mg/kg × h. The tacrolimus dose used was in accordance to the manufacturer's instructions for intravenous TAC-administration in humans. The dosage of L-arginine was chosen according to previous dose finding experiments in clamping-induced acute renal failure where a positive effect on renal function without detrimental influences on blood pressure regulation could be shown with slightly lower dosages. Because we expected rather more pronounced renal functional impairment we decided to use a higher dosage of L-arginine (500 mg/kg × h instead of 300 mg/kg × h). The L-NMMA-dose used was chosen to obtain renovascular impairment without systemic effects [22].

Experimental procedure

Female Sprague-Dawley rats (n = 32, weight 235 ± 12 g) with free access to water and standard rat chow prior to the experiments have been used. Animals were housed with a regular 12-h-light-dark-cycle and under steady temperature-, pressure- and humidity conditions, according to the regulations of the German Tierschutzgesetz as well as the NIH-principles of laboratory animal care.

After anaesthesia with 100 mg/kg i.p. thiobutabarbital, which lasted for the whole duration of the experiment, the animals were placed on a thermoregulated heating table to maintain the body temperature at 37.5 °C. A tracheostomy with insertion of an endotracheal tube allowing spontaneous breathing was performed. Thereafter, the left femoral vein was cannulated with a PE 50-catheter (Portex, Hythe, UK) for constant infusion of ringer lactate 2 ml/h for replacement of fluid and electrolyte losses and for administration of drugs. Another PE 50-catheter was placed in the left femoral artery for continuous measurement of the arterial blood pressure via a pressure-transducer (Hellige, Freiburg, Germany) and for blood collections. Through a suprapubic incision, a PE 10-catheter was inserted in the urinary bladder for monitoring of urinary flow and collecting urine samples. Thereafter, a bilateral horizontal flank incision was performed, and both renal pedicles were prepared, renal arteries were divided from the Vv. renales. After completion of all surgical manipulations, a bolus of 3 mg fluorescence-marked inulin together with 2 mg paraaminohippurate was administered intravenously, followed by a continous infusion of both substances in saline. After an equilibration period of 30 min, the experimental protocol was initiated as shown in Fig. 1. Directly after the baseline sampling-period, both renal arteries were clamped for exactly 40 min, venous outflow was not inhibited. A subsequent resting period was then followed by 120 min of drug infusion.

Every urine collecting period lasted for 20 min, a corresponding blood sample (300 µl each) was taken after 10 min and immediately centrifuged. For all collecting periods inulin-clearances and

Table 1 All results expressed as mean ± SEM

	Groups	Baseline	Post Clamping	Infusion period	Post infusion period I	Post infusion period II
Urinary volume in µl/min	Control	6.1 ± 1.6	55.7 ± 19.2	33.2 ± 8.8 *	22.1 ± 5.6 *	17.3 ± 7.2 *
	TAC	6.4 ± 1.7	48.1 ± 23.5	27.0 ± 15.1 *	20.8 ± 10.9 *	7.9 ± 6.3 *
	TAC/L-Arg	6.7 ± 1.8	75.6 ± 31.6	69.3 ± 12.3	50.6 ± 10.7	33.6 ± 7.8
	TAC/NMMA	5.6 ± 0.9	63.3 ± 23.0	42.5 ± 19.8 *	31.4 ± 13.3 *	16.1 ± 11.8 *
MAP in mmHg	Control	91 ± 7.7	104 ± 9.3	96 ± 11.3	86 ± 10.8	79 ± 12.1
	TAC	88 ± 11.6	105 ± 8.8	101 ± 15.1	93 ± 16.9	84 ± 19.1
	TAC/L-Arg	89 ± 11.3	91 ± 8.3	100 ± 8.2	97 ± 9.3	88 ± 14.4
	TAC/NMMA	89 ± 10.9	103 ± 9.3	107 ± 14.4	105 ± 18.1 *	88 ± 17.4
GFR in ml/min	Control	1.37 ± 0.26	0.15 ± 0.10	0.10 ± 0.05 *	0.10 ± 0.06 *	0.08 ± 0.07 *
	TAC	1.39 ± 0.17	0.12 ± 0.05	0.09 ± 0.04 *	0.07 ± 0.03 *	0.04 ± 0.04 *
	TAC/L-Arg	1.45 ± 0.27	0.29 ± 0.15	0.36 ± 0.17	0.32 ± 0.16	0.19 ± 0.10
	TAC/NMMA	1.26 ± 0.19	0.19 ± 0.06	0.13 ± 0.06 *	0.13 ± 0.07 *	0.08 ± 0.07 *
RBF in ml/min	Control	5.50 ± 1.43	0.22 ± 0.14	0.15 ± 0.11 *	0.16 ± 0.12 *	0.19 ± 0.17
	TAC	5.75 ± 1.41	0.18 ± 0.07	0.09 ± 0.04 *	0.07 ± 0.03 *	0.05 ± 0.04 *
	TAC/L-Arg	6.16 ± 2.10	0.30 ± 0.08	0.81 ± 0.66	0.98 ± 0.73	0.82 ± 0.71
	TAC/NMMA	6.11 ± 1.61	0.43 ± 0.19	0.36 ± 0.26	0.36 ± 0.33	0.11 ± 0.10
RVR in kPa × sec/ml	Control	98 ± 38	3051 ± 1466	4637 ± 1866	6308 ± 4709	8005 ± 5542
	TAC	128 ± 74	3660 ± 900	8085 ± 4286 *	10192 ± 5734 *	21010 ± 12733 *
	TAC/L-Arg	83 ± 28	2120 ± 930	1663 ± 683	1740 ± 640	2092 ± 1431
	TAC/NMMA	90 ± 30	2490 ± 1503	3444 ± 1958	6360 ± 2856	7192 ± 5253
FENa in %	Control	0.3 ± 0.1	46.3 ± 18.7	38.1 ± 20.0	24,6 ± 12.3	25.4 ± 10.9
	TAC	0.3 ± 0.1	39.7 ± 14.6	28.2 ± 7.5	32.4 ± 13.3	22.1 ± 8.2
	TAC/L-Arg	0.3 ± 0.2	25.8 ± 14.4	21.9 ± 9.4	18.2 ± 7.2	24.2 ± 14.5
	TAC/NMMA	0.3 ± 0.2	29.0 ± 8.6	30.6 ± 14.0	27.0 ± 11.6	18.7 ± 4.7

Statistical significance with $P \leq 0.05$ in ANOVA is depicted as: * compared to TAC/L-Arg

PAH-clearances were calculated for estimation of GFR and RPF, respectively. At the same time, mean arterial blood pressure was recorded. RBF was calculated in accordance to hematocrit: RBF = RPF/(1-Hc). RVR was computed by the formula RVR = MAP/RBF. Hematocrit was measured with Technicon H1-analyser (Bayer Diagnostics, Wiesbaden, Germany).

Analytical procedures

Inulin concentrations were determined by fluorescence-spectrometry using a LS-50 luminescence-spectrometer (Perkin-Elmer, Überlingen, Germany), PAH concentrations by spectrometry at 550 nm, using a microplate reader (Molecular devices, Crawley, UK). Sodium concentrations in serum and urine were measured by flame photometry (Autoanalyzer FCM 6341, Eppendorf, Hamburg, Germany).

Statistical analyses

Results are presented as mean ± SEM. A two-way ranked ANOVA with repeated measurements and a two-sided Student's T-test, if appropriate, were used for statistical analysis, a P-value ≤ 0.05 was considered as statistically significant. Computing was done using SPSS 6.1 for Windows (SPSS GmbH, München, Germany).

Results

All results are summarized in Table 1. The course of mean arterial blood pressure showed no differences between the four experimental groups. Clamping induced a distinct rise in MAP which was significant in control, TAC and TAC/L-NMMA, compared to baseline. During the following experimental periods MAP decresed in groups control, TAC and TAC/L-Arg until the end of the experiment, whereas L-NMMA-administration was shown to sustain this decline during the infusion period ($P < 0.05$ compared to control).

Urine volume (Fig. 2) rose in all groups, due to the clamping procedere (i.e. induction of acute polyuric renal failure) without a significant difference between the different groups. Immediately after clamping urinary volumes of 50–75 µl/min could be observed, followed by a slow decrement in urine excretion until the end of the experimental procedure in all groups. In control and TAC urine volume nearly returned to baseline levels of about 10 µl/min, concomitant L-NMMA-infusion increased urinary volumes slightly but not significantly, whereas L-Arg with tacrolimus significantly sustained the decline especially during drug infusion.

Glomerular function was determined by FITC-inulin-clearance. As shown in Fig. 3, baseline glomerular filtration rate (GFR) during the equilibration period of

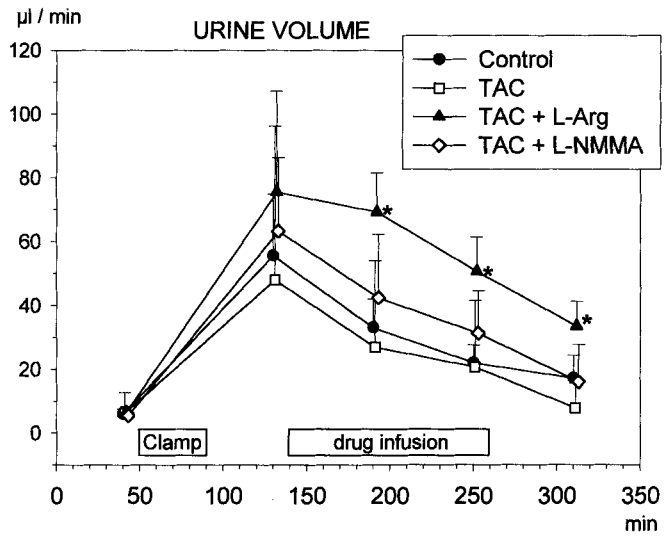

Fig. 2 Urine volume in µl/min, mean + SEM. *Asterixes* depict significant differences of the TAC/L-Arg-group compared to all other groups

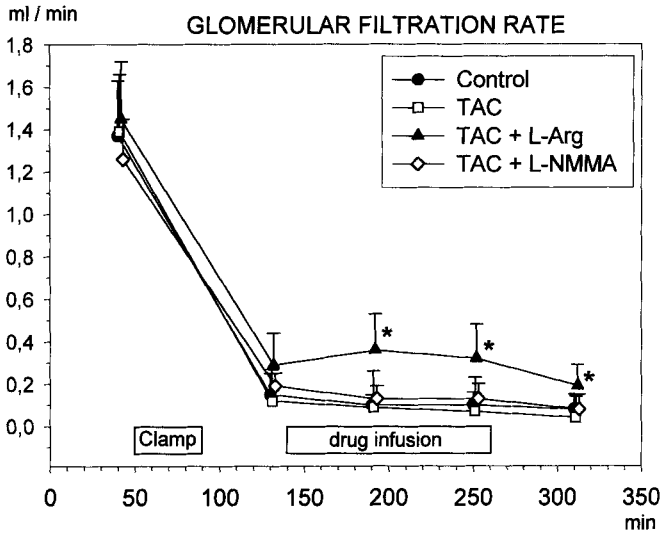

Fig. 3 Glomerular filtration rate in ml/min, mean + SEM. Asterixes depict significant differences of the TAC/L-Arg-group compared to all other groups

50 min after the surgical procedure was about 1.4 ml/min in all groups. Clamping induced a steep decline in GFR to 15% of baseline values without differences between experimental groups. L-Arg-infusion together with tacrolimus led to a slight but significant improvement in glomerular function, compared to the other groups, followed by a decline after cessation of the drug administration. TAC alone, as well as coadministration of L-NMMA, did not induce a further decrement of GFR, compared to control. Sixty minutes after the end of the infusion period, GFR in the TAC/L-Arg-group nearly reached the level observed in the other groups.

Renal blood flow (RBF) was calculated from measured RPF by PAH-clearance and hematocrit. Similar to GFR, RBF also rapidly decreased after induction of renal ischemia from baseline values of 5.5 ml/min to very low levels between 0.15 (TAC) to 1.1 ml/min (TAC/L-Arg). Between the groups at this time point, no significant difference could be detected (however with high SEM in the TAC/L-Arg-group). Drug infusion did not induce major effects in one of the groups, RBF did not show any improvement and stabilized at very low level. However, statistical analysis shows a significant difference between the TAC/L-Arg-group compared to TAC and control during the infusion period until the end of the experiment as demonstrated in Fig. 4.

Renal vascular resistance (RVR) was calculated from MAP and RBF, results are depicted in Fig. 5. At baseline, RVR was about 80–130 kPa x sec/cm^3 in all groups without any statistical difference. Clamping lead to an increase in RVR about 40-fold compared to baseline. During drug infusion, RVR rose further in all groups, statistically higher in the TAC-group until the end of the experiment. TAC-administration alone led to a RVR of about 21000 kPa x sec/cm^3 60 min after the end of the infusion period ($P < 0.05$ compared to all other groups). L-Arg-coadministration with TAC significantly prevented this increase, but also in the L-NMMA-group RVR increased only about twentyfold. Compared to control there was no difference in the TAC/L-Arg- and the TAC/L-NMMA-group.

The fractional sodium excretion FENa was calculated as a marker parameter for tubular function. Clamping induced a sudden rise in FENa in all groups between 26% in the TAC/L-ARG-group up to 45% in the control group. During the experimental procedure, FENa decreased again to about half of the maximal values until the end of the experiment. There were no significant differences between the groups neither during nor after the drug infusion period.

Discussion

The aim of this study was to describe the hemodynamic effects resulting from the intravenous administration of tacrolimus on ischemic renal tissue. As a model for acute ischemic renal failure, clamping of both renal arteries for 40 min was chosen, renal dysfunction is characterized by an acute and persistent fall in renal blood flow measured by PAH-clearance, a concordant depression of glomerular filtration rate, an increase of filtration fraction pointing to the involvement of postglomerular vasoconstriction in this form of ARF, and a rise in renal vascular resistance without significant changes in

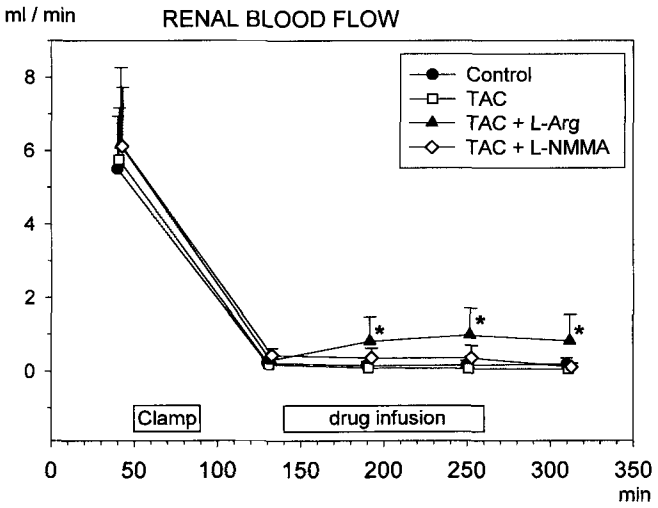

Fig. 4 Renal blood flow in ml/min, mean + SEM. *Asterixes* depict significant differences of the TAC/L-Arg-group compared to Control and TAC-group

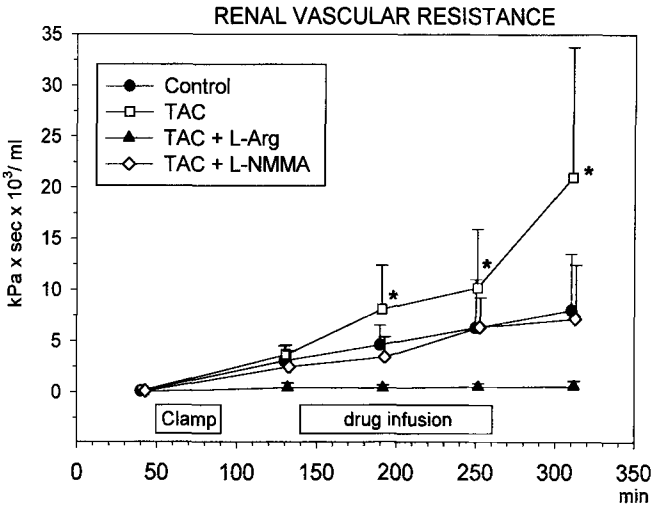

Fig. 5 Renal vascular resistance in kPa x sec/cm^3, mean + SEM. *Asterixes* depict significant differences of the TAC-group compared to all other groups

systemic arterial blood pressure. After releasing the clamps, urine volume increases, most likely due to ischemic tubular changes with loss of proximal and distal tubular reabsorption capacity. This pattern of acute renal insufficiency was described before by our group [22] in a similar experimental protocol.

Nearly all published clinical trials of tacrolimus in kidney transplantation demonstrated a comparable rate of nephrotoxicity of TAC compared to CsA [25]. However, the acute hemodynamic mechanism of TAC-induced vascular changes have not been exactly described in vivo, with the exception of one model of the isolated in-situ-perfused rat kidney [3]. Therefore, we examined the effects of tacrolimus given intravenously 50 min after induction of complete renal (warm) ischemia and release of the clamps. The calcineurin inhibitor caused a further, however not significant depression of RBF and GFR from the already very low levels seen in the control group, the concomitant rise in filtration fraction, more pronounced than in the control group, refers again to a more pronounced postglomerular vasoconstriction, compared to the action on preglomerular arteries. This result is obviously different to the effects described by Bagnis et al (with only minor preimposed ischemic changes due to the operative procedure) of TAC-infusion compared to CsA [3]. While in the later study acute TAC-administration leads to only moderate changes in the renal functional parameters, our results show strong hemodynamic effects when TAC is superimposed on already preinjured renal microvasculature. Furthermore, no changes in RVR due to TAC have been reported in the latter study. However, after complete renal ischemia in our model, also TAC leads to a significant deterioration in renal vascular resistance. For the judgement of the described renal hemodynamic effects it must be emphasized that the applied tacrolimus-dosage was low enough not to cause any rise of mean arterial blood pressure compared to control animals.

Renal perfusion is strongly autoregulated with a tight balance between vasoconstrictors and vasodilating substances. Nitric oxide (NO), continuously produced by endothelial cells and secreted in a paracrine fashion, is one of the most important counterparts of the constrictive effects of, for example, endothelins or angiotensin II [10]. However it has to be mentioned that NO can also exert detrimental effects above all on tubular epithelium. This can be attributed to the induction of iNOS resulting in high amounts of NO. In this situation, highly concentrated NO can exaggerate ischemia-induced damages due to its cytotoxic effecs, for example on tubular cells [27]. Selective blockade of iNOS with antisense oligodeoxynucleotides attenuated ischemic renal failure and improved tubular histology, compared to control animals [17]. Ischemia and consecutive reperfusion result in decreased NO-levels in the kidney without impaired NO-synthesis by the NO-generating enzyme NO-synthase (NOS) [12]. In a previous study, our group has shown that L-arginine-administration in slightly lower dosages could partly improve renal function after ischemia without CNI-treatment, whereas L-monomethyl-arginine, another unspecific NOS-inhibitor, had no effect [22]. For calcineurin inhibitors, an influence on intrarenal NO-synthesis was reported as well: CsA supplied with the drinking-water increases endothelial NOS-expression in the renal cortex of Wistar rats, most likely as counterregulation of the release of endothelin and other vasoconstrictors [4]. The group

of De Nicola observed in micropuncture studies that chronic CSA-treatment decreased single nephron GFR and plasma flow, due to disturbances in afferent arteriolar autoregulation, which was partially prevented by arginine-feeding [6]. In toxically impaired kidneys, NO-synthesis has shown to be well preserved, and the hemodynamic responses to NOS-activation by arginine infusion and NOS-inhibition are not impaired during CsA-administration [5]. L-arginine-administration for 10 days ameliorated CsA-induced decreases in GFR [1]. In human renal transplant recipients under TAC-based immunosuppression it was observed that infusion of aminoacid-solutions containing L-arginine has beneficial effects on graft function with amelioration of CNI-induced influences on GFR [19], specific effects on the NO-system have not been reported up to now.

The hypothesis of the second part of our study was that L-arginine administrated in abundance could, as well as in the previous study without CN-inhibition [22], reverse the effects of TAC on renal functional parameters in the situation of ARF. Administration of L-arginine together with TAC-infusion improved renal hemodynamics with significant increases in RBF and GFR and a decreased vascular resistance. Analysis of filtration fraction shows a decrease compared to control and TAC-groups pointing to stronger pre- than postglomerular vasodilation. These effects are in concordance with previous results (reviewed in [9]). It must be pointed out that a full recovery of renal hemodynamics could not be achieved by increasing the dosage of L-arginine and that only a temporary improvement during the drug infusion was observed. During the whole experimental period, mean arterial blood pressure remains unchanged. Urinary volume increased, showing the tubular responses which have been attributed to a rise of peritubular NO [11]. Because we have not measured intrarenal NO or its metabolites directly in the urine, we can not prove that the described changes are due to an increase in NO-production and not to other pathways of arginine-metabolism [26]. However, all effects of L-arginine reported here resemble the ones described for NO on the renal vasculature and could not be attributed to metabolites like agmatine or glutamate.

In a further set of experiments the effects to a blockade of the NO-system by administration of the competitive NOS-inhibitor L-NMMA were determined, in a dose sufficient to block the actions of an increase in NO caused by L-arginine-infusion in former experiments [22]. The rational of this was to exclude an excessive NO-production causing the deterioration of renal function in this model. L-NMMA-administration did neither during nor after cessation of the infusion, improve RBF and GFR, compared to control or TAC, although the systemic blood pressure was significantly enhanced, the latter pointing to a systemic blockade of NO-release. The decrease of filtration rate could be attributed to the known vasoconstricting effect on preglomerular arterioles as prediscribed by Schnackenberg et al. in dogs [20]. The diminished rise in RVR compared to TAC alone was not expected, the reason for NOS-inhibition not acting additively to TAC on RVR, but preventing the TAC-induced increase cannot be elucidated by our data or results from other groups, and has to be examined further.

In conclusion, systemic administration of tacrolimus in the state of a postischemic kidney causes a further deterioration of renal function due to an increase in vascular resistance. Systemic administration of L-arginine could reverse these changes in part, whereas full functional recovery could not be achieved. The mechanism most likely responsible for this is an increase in NO with its known vasodilating effects on preglomerular vessels. Tacrolimus-induced disturbance of renal perfusion seems not to be mediated through excessive NO-liberation, as was shown by an unspecific NOS-blockade.

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