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Protective effect of the PAF antagonist BN 52021 in an experimental renal warm ischemia model

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Abstract. Platelet activating factor is involved in warm ischemic damage. We studied the effect of the PAF receptor antagonist BN 52021 in an experimental model of 60 min of renal warm ischemia in which the left kidney was flushed with Euro-Collins solution and a right nephrectomy was performed. Eighty Wistar rats were divided into a sham-operated group, two control groups, and four study groups, according to the dosage and route of BN 52021 administration. BN 52021 was used in the flush solution at concentrations of 0.1 and 0.5 mg/ml, or intravenously prior to ischemia at 5 and 10 mg/kg body weight. Creatinine clearance per 100 g body weight, fractional sodium excretion, and conventional histology were studied. Rats that received BN 52021 intravenously showed a significantly higher creatinine clearance than controls. Intravenous BN 52021 produced a higher acceleration of renal function recovery at 10 mg/kg than at 5 mg/kg body weight. Conventional histology was better in animals that received BN 52021 at 10 mg/kg body weight than in controls. Addition of BN 52021 to Euro-Collins flushing solution showed no protective effect. We conclude that intravenous BN 52021 shows a renal protective effect against warm ischemia.

Key words: BN 52021, kidney transplantation, in rats – Kidney transplantation, in rats, BN 52021 – Kidney transplantation, ischemia – Ischemia, renal

Introduction

The glycerophospholipid platelet activating factor (PAF) is an inflammatory mediator elaborated by a variety of cell types including platelets, neutrophils, and endothelial cells [1]. It has been demonstrated that glomerular mesangial and medullary renal cells produce PAF [8] and that PAF production is increased after ischemia [5]. PAF is also a potent endogenous vasoactive factor capable of increasing vascular permeability [1]. In experimental animal models, intravenous administration of PAF produces a constellation of pathophysiological renal effects similar to those observed in ischemia and reperfusion injury. In the kidneys, it produces an acute reduction in renal plasma flow and a drop in glomerular filtration rate [9], which have been attributed to renal vasoconstriction and/or mesangial cell contraction [7, 8]. At high concentrations it induces a severe impairment of the renal function [9]. On the other hand, it has been reported that administration of the PAF antagonist BN 52021 minimizes cyclosporin A [10], gentamicin, and cisplatin [11] nephrotoxicity. Lopez-Farre et al. have recently reported that BN 52021 and alprazolam, two specific blockers of PAF receptor, significantly ameliorate renal function in rats with ischemic acute renal failure [6]. Moreover, they demonstrated an increase in PAF content in glomeruli from these rats and an increased release of PAF from the kidneys of these animals, thereby providing some evidence of the role of PAF in the physiopathology of experimental acute renal failure [6].

Euro-Collins (EC) solution has been widely used in clinical renal preservation. Recently, an acute tubular necrosis rate as high as 30% has been reported using EC solution, and some investigators have noted that this solution may lose its protective ability above 15 °C [4]. We have noted that EC solution does not seem to have preservation capacity during experimental renal warm ischemia [12]. To further study the protective effect of the PAF antagonist BN 52021 in ischemia-reperfusion injury, we attempted to modify the renal damage resulting from 1 h of warm ischemia on kidneys flushed with EC solution by using BN 52021 added to the flush solution or administered intravenously at different doses.

Materials and methods

Eighty male Wistar rats weighing 250–325 g were used. Animals were acclimated for 1 week prior to experimentation at the metabolic unit. Animals were fed standard rat chow and water ad libitum and placed in metabolic cages. Renal warm ischemia (RWI) and flush

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Table 1. Mean creatinine clearance per 100 g profile. Results are expressed as mean ± standard error of the mean (SEM). IS, Intrasolution; IV, intravenous. * $P < 0.05$ versus group 2; ** $P < 0.05$ versus groups 2–5; *** $P < 0.05$ versus groups 2, 3, and 6

Group	n	BN 52021 Dosage	BN 52021 Route	Cr Cl/100 g Day 2	Cr Cl/100 g Day 7
1	6			0.49 ± 0.05	0.45 ± 0.02
2	13			0.08 ± 0.02	0.23 ± 0.03
3	14			0.11 ± 0.02	0.31 ± 0.03
4	8	0.1 mg/ml	IS	0.11 ± 0.03	0.37 ± 0.02*
5	12	0.5 mg/ml	IS	0.13 ± 0.03	0.38 ± 0.03*
6	14	5 mg/kg	IV	0.22 ± 0.03**	0.34 ± 0.03*
7	13	10 mg/kg	IV	0.28 ± 0.03**	0.43 ± 0.02***
P				0.0001	0.0001

Table 2. Mean fractional sodium excretion (FENa) and histological score. * $P < 0.05$ versus groups 2–4; ** $P < 0.05$ versus group 2; *** $P < 0.05$ versus groups 2 and 3

Group	n	FENa Day 2	FENa Day 7	Histological Score
1	6	0.2 ± 0.1	0.5 ± 0.1	2.8 ± 1.1
2	10	5.6 ± 1.5	2.8 ± 1.2	13.5 ± 1.6
3	13	2.7 ± 1.7	3.8 ± 3.3	11.2 ± 1.4
4	7	5.3 ± 3.0	0.7 ± 0.3	11.7 ± 1.4
5	12	2.0 ± 0.9	1.0 ± 0.4	13.0 ± 1.1
6	14	1.8 ± 1.2**	0.6 ± 0.2	11.2 ± 1.4
7	13	0.4 ± 0.1*	0.3 ± 0.1***	10.1 ± 1.2**
P		0.015	0.10	0.0008

solution perfusion were done as previously described [12]. Briefly, under intramuscular ketamine anesthesia (75 mg/kg body weight) laparotomy was performed and aorta, cava, and the left renal vessels were widely dissected. The aorta below renal arteries was cannulated with a 22-gauge needle, a small bulldog clamp placed over the aorta above the level of the left renal artery and, immediately, the flushing solution (at room temperature) perfused retrogradely into the aorta towards the kidney using a volume-controlled syringe pump (Harvard Apparatus). A total amount of 2 cc of flushing solution was perfused at a flow rate of 0.5 cc per minute. The left renal pedicle was occluded using a bulldog clamp, the abdominal cavity closed, and the animal placed in a warm cage for 60 min. Only kidneys that blanched completely were used in the study. After ischemia, the renal clamp was removed and contralateral nephrectomy was performed. BN 52021 was administered in two ways: added to the flush solution at 0.1 and 0.5 mg/ml, respectively, and intravenously 10 min before flush solution perfusion, at 5 and 10 mg/kg body weight, respectively.

Rats were divided into a sham-operated group, group 1 ($n = 6$), in which the surgical procedure was performed but no flushing and no ischemia were done, and two control groups, group 2 ($n = 13$), which received isotonic saline solution (ISS) flushing and RWI, and group 3 ($n = 14$), with EC flushing and RWI. Afterwards, a series of experiments using EC as flush solution were undertaken: group 4 ($n = 8$) received EC plus BN 52021, 0.1 mg/ml flushing and RWI; group 5 ($n = 12$) received EC plus BN 52021, 0.5 mg/ml flushing and RWI; group 6 ($n = 14$) received BN 52021, 5 mg/kg IV, EC flushing, and RWI; and group 7 ($n = 13$) received BN 52021, 10 mg/kg IV, EC flushing, and RWI.

Prior to surgery and on days 1, 2, 3 and 7 after surgery, weight and serum creatinine levels were determined. Also, prior to surgery and on days 2 and 7 after surgery, serum sodium levels were determined, 24-h urine was measured, and urine creatinine and sodium levels were determined. Blood was obtained from retro-ocular vessels by micropuncture. Serum and urine creatinine levels were measured using Kodak Ektachem DT slides (Kodak Ektachem DTSC Module, Kodak). Sodium levels were measured using a selective ion electrode system (Astra, Beckman). Creatinine clearance per 100 g body weight (Cr Cl/100 g) and fractional sodium excretion (FENa) were calculated with standard methods using all of these data.

On day 7 after surgery, rats were sacrificed under ketamine anesthesia and, after endovascular flushing with 4% paraformaldehyde, their kidneys were processed for conventional histology. Slides were reviewed blindly by two different pathologists and a semiquantitative analysis of each of the following histological parameters was graded on a scale from 0 to 3 +: tubular dilation, intratubular cell detachment, tubular vacuoles, cell necrosis, integrity of tubular brush border, interstitial edema and fibrosis, and interstitial cell infiltration. The histological score for each kidney was obtained from the sum of all of these parameters. Means ± standard error of the mean (SEM) are presented in the tables. Statistical analyses were done using an analysis of variance test. A posteriori individual comparisons were made using Fischer's test.

Results

Three rats from group 2 (ISS flushing) and one rat from groups 3 (EC flushing) and 4 (EC plus BN 52021, 0.1 mg/ml flushing) died of uremia. Weight and diuresis did not differ in any of the seven groups, either before surgery or during the experiment (data not shown). Tables 1 and 2 summarize the functional and morphological renal parameters during the experiment.

Intragroup Cr Cl/100 g comparisons showed the following results:

1. Mean Cr Cl/100 g did not differ between the control groups.
2. Groups 4 and 5, in which BN 52021 was added to EC solution at 0.1 and 0.5 mg/ml, were not different from controls, either on day 2 or on day 7 after surgery.
3. Only groups 6 and 7, in which BN 52021 was administered at 5 and 10 mg/kg body weight intravenously, showed significant amelioration of renal function on day 2 and on day 7 when compared to controls.
4. Group 7, in which BN 52021 was administered intravenously at 10 mg/kg body weight, showed a significant increase in Cr Cl/100 g on day 7 when compared to group 6, in which BN 52021 was administered intravenously at 5 mg/kg. Of note is that Cr Cl/100 g on day 7 in group 7 was not significantly different than Cr Cl/100 g in group 1 (sham-operated). On the contrary, the Cr Cl/100 g of the other groups was significantly lower than in group 1 on day 7.

Intragroup FENa comparisons showed that:

1. On day 2, FENa was significantly lower only in groups that received BN 52021 intravenously when compared to controls.
2. FENa in animals from the group receiving BN 52021, 10 mg/kg body weight intravenously (group 7) was similar to FENa in the sham-operated animals (group 1) and was below 1 (functional renal impairment range) in both groups.
3. On day 7, only the group in which BN 52021 was administered intravenously at a dose of 10 mg/kg body weight (group 7) showed a significant reduction in FENa when compared to controls.

Morphological studies showed a significantly lower mean score in group 7 (EC and BN 52021, 10 mg/kg IV) than in group 2 (ISS flushing). These differences were more pronounced with respect to tubular dilation, cellular necrosis, and edema (data not shown). There were no differences between the other groups.

Discussion

Our data show that the specific PAF receptor antagonist BN 52021 had a protective effect on a model of kidney flushing with EC solution and warm ischemia of 60 min. This effect was only observed when this agent was administered intravenously and it was more evident at higher doses. In contrast, when BN 52021 was added to the flushing solution, it did not seem to provide a significant protective effect since Cr Cl/100 g was not significantly different in comparison with controls.

Since BN 52021 has a half-life of 9–12 h [2], we administered only one dose of the drug prior to ischemia because we assumed that the drug would be effective for a long time after kidney reperfusion. Five milligrams per kilogram body weight is the most common dose of BN 52021 used in ischemic models [6], and in our study we used this and higher doses in order to evaluate a potential dose-dependent effect. The better results obtained with BN 52021 at 10 mg/kg body weight suggest that the drug might exert its protective effect in a dose-dependent manner, but further studies are needed to confirm this.

BN 52021 not only accelerated recovery of renal function but also decreased FENa. This result is in agreement with experiments performed by Lopez-Farre et al. [6] and suggests that renal impairment observed in rats that received BN 52021 was mainly functional, while renal impairment observed in control groups was due to acute tubular necrosis.

According to the histological damage study, the protective effect with BN 52021 was only achieved when administered intravenously at 10 mg/kg but not at lower doses or when the drug was added to the flush solution. Though this histological benefit appears to be small in comparison to the bigger difference with the sham-treated group, we think that these data are of interest since previous studies failed to demonstrate histological protection when using BN 52021 [6]. However, these histological studies were carried out earlier in the postischemic period than was done in our study.

The protective effect of BN 52021 is derived from its PAF receptor antagonism and possibly from several intermediary mechanisms as well. First of all, PAF has a direct vasoconstrictory renal effect and it increases vascular permeability. PAF is also a mediator of the effects of endothelin on renal function and glomerular and mesangial cell contraction [7]. BN 52021 blocks the effect on the kidney of the high rate of endothelin production during ischemia or hypoxia [7]. PAF exerts a major action on neutrophil aggregation and activation [3]. It has been shown that PAF both primes and amplifies the release of superoxide anion and hydrogenion peroxides from resident neutrophils, macrophages, and endothelial cells [3]. Oxygen-free radicals appear to play a prominent role in mediating damage associated with renal ischemia and reperfusion.

In sum, the PAF antagonist BN 52021 shows a renal protective effect against warm ischemia and reperfusion injury, thereby substantiating the role of PAF in ischemic injury. Moreover, the use of PAF antagonists may introduce a novel approach to the treatment of ischemia reperfusion damage, which is currently recognized as a potential process in many mechanisms of disease.

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References

1. Braquet P, Touqui L, Shen TY, Vargaftig BB (1987) Perspectives in platelet activating factor research. Pharmacol Rev 39: 97–145
2. Conte JV, Katz NM, Wallace RB, Foegh ML (1991) Long-term lung preservation with the PAF antagonist BN 52021. Transplantation 51: 1152–1156
3. Droy-Lefaix MT, Drouet Y, Geraud G, Hosford D, Braquet P (1991) Superoxide dismutase (SOD) and the PAF antagonist (BN 52021) reduce small intestinal damage induced by ischemia-reperfusion. Free Radic Res Commun 12: 725–735
4. Hoelscher M, Klaess G, Kallerhoff M, Bretschneider HJ (1984) Is Euro collins solution losing its protective ability above 15 °C? Transplant Proc 16: 166–171
5. Lopez Farre A, Torralbo M, Lopez Novoa JM (1988) Glomeruli from ischemic rat kidneys produce increased amounts of platelet activating factor. Biochem Biophys Res Commun 152: 129–135
6. Lopez Farre A, Bernabeu F, Gomez Garre D, Ramon y Cajal S, Braquet P, Lopez Novoa JM (1990) Platelet activating factor antagonist treatment protects against postischemic acute renal failure in rats. J Pharmacol Exp Ther 253: 328–333
7. Lopez Farre A, Gomez Garre D, Bernabeu F, Montañes I, Millas I, Lopez Novoa J (1991) Renal effects and mesangial cell contraction induced by endothelin are mediated by PAF. Kidney Int 39: 624–630
8. Pirotzky E, Bidault J, Burtin C, Gubler MC, Benveniste J (1984) Release of platelet activating factor, slow reacting substance and vasoactive amines from isolated rat kidneys. Kidney Int 25: 404–411
9. Santos JC, Sanz E, Caramelo C, Hernando L, Lopez Novoa JM (1985) Effect of PAF-acether on renal function in dogs. Kidney Int 28: 587
10. Santos OFP, Boim MA, Bregman R, Draibe SA, Barros EJC, Pirotzky E, Schor N, Braquet P (1989) Effect of platelet activating factor antagonist on cyclosporine nephrotoxicity: glomerular hemodynamics evaluation. Transplantation 47: 592–595
11. Santos OFP, Boim MA, Barros EJG, Schor N (1991) Role of platelet activating factor in gentamicin and cisplatin nephrotoxicity. Kidney Int 40: 742–747
12. Torras J, Bordalba JR, Seron D, Carrera M, Castelao AM, Poveda R, Alsina J, Griño JM (1992) An experimental comparison between isotonic saline solution, Eurocollins and a flush solution with mannitol in the prevention of renal damage due to warm ischemia. Transplant Proc 24: 54–55