

The Role of Platelet-Activating Factor Antagonists in the Prophylaxis of Posttransplant Acute Tubular Necrosis

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Renal reperfusion injury can be defined as the renal damage suffered by a kidney because of reestablishment of blood flow after prolonged ischemia. Increased permeability for calcium ions, subsequent activation of endogenous phospholipases, and damage mediated by oxygen free radicals (1) are involved in this reperfusion injury. Inhibition of inflammatory mediators of endothelial origin, such as superoxide radicals and platelet-activating factor (PAF), is a possible way of protecting the injured endothelium following blood reperfusion (2).

Since its identification in the early 1970s, and along with its potent proaggregating properties of platelets, PAF (1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) has emerged as a mediator in acute, allergic, and inflammatory reactions (3). PAF is a glycerophospholipid elaborated by a variety of cell types including not only platelets, neutrophils, and endothelial cells (ECs), but also glomeruli and renal medullary cells (4-6). There is a general agreement that resting cells do not generate PAF, but on stimulation, formation of PAF takes place from a lipid found in membranes of many types of cells (7). The synthesis of PAF occurs by a sequential pathway involving deacylation in position 2 of 1-alkyl-2-acyl-*sn*-glycero-3-phosphocholine by phospholipase A₂, and subsequent acetylation in position 2 by acetyl-coenzyme A transferase (8). The inactivation of PAF also occurs in two

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steps. Intracellular and extracellular PAF is removed by PAF acetylhydrolase to form inactive lyso-PAF. Then, this metabolite is converted to 1-*O*-alkyl-2-acyl-glycerophosphocholine by an acetyltransferase. All enzymes required for PAF formation and metabolism are present in the kidney (4,9,10). Of interest is the finding that PAF has been characterized in human urine (11).

PAF can exert direct deleterious effects. Systemic administration of PAF into the renal artery induces hemodynamic changes in the anesthetized dog, with a dose-dependent reduction in renal blood flow, glomerular filtration rate, urine volume, and urinary sodium excretion, without changing both the systemic and the renal blood platelet counts (12). Further evidence that PAF's effect on the kidney was not mediated by thromboxane release from platelets was obtained after blocking prostaglandin and thromboxane synthesis with indomethacin. Under these conditions, the hemodynamic changes observed were even more intense (12). Thus, it was shown again that PAF's effects have to be considered synergistic with those resulting from prostaglandins, thromboxanes, and leukotrienes. Moreover, the time-dependent reduction in rat glomerular cross-sectional area induced by PAF has pointed to the possibility that the mediator decreases the glomerular filtration rate or the renal plasma flow by a direct effect on the glomeruli, on the basis of a possible modification of the filtration surface (13). In addition, PAF induces changes in cytoskeleton structures that control microvascular endothelial permeability (14). Moreover, it has been reported that PAF has a receptor biphasic effect on rabbit afferent arterioles, dilating them at low concentrations (mediated through endothelium derived relaxing factor) while constricting them at higher concentrations (mediated in part through cyclooxygenase products) (15).

PAF is also a chemotactic agent for neutrophils, inducing neutrophil aggregation and activation, and additionally, it potentiates the synthesis of leukotrienes and oxygen-derived free radicals from stimulated polymorphonuclear cells (PMNs) (16). Pretreatment with PAF reduces PMN adhesion to hydrogen peroxide activated cultured ECs, suggesting a specific desensitization process (17). Moreover, competitive PAF receptor antagonists block PMN adherence to cultured ECs in response to exogenous PAF in solution (18). Studies from cultured EC have demonstrated that hydrogen peroxide enhances the permeability of EC plasma membranes to Ca^{2+} . Increased intracellular Ca^{2+} can enhance PAF synthesis by the EC, by causing the generation of Ca^{2+} ionophores, such as lipid peroxides (17). PAF can also induce the release of tumor necrosis factor (TNF), a cytokine that also increases neutrophil superoxide production and adherence to ECs (19). Very low concentrations of PAF and TNF can "prime" neutrophils to respond in an enhanced manner to subsequent agonistic stimuli that would otherwise be ineffectual (19). The fact that PAF and various cytokines can induce not only the release of each other, but also their own generation in vivo indicates that self-generating positive feedback cycles may become established (19). In this respect, it is of interest that oxygen radicals may inhibit human plasma acetylhydrolase—the enzyme that catabolizes PAF—possibly causing the potentiation and the prolongation of PAF-induced inflammatory effects (20).

Oxygen radicals are generated primarily via the xanthine-oxidase system in ischemic tissues and via the NADPH-oxidase system in immigrated neutrophils (21,22). In some clinical situations—such as cadaveric renal transplantation—a postischemic injury may occur when molecular oxygen is reintroduced to the graft immediately after blood reperfusion, leading to the production of large amounts of superoxide and secondarily derived cytotoxic species capable of causing massive tissular damage. Oxygen radicals generated during the reperfusion of ischemic organs can stimulate the synthesis of PAF. Along with

the lysis of ECs, oxidant species such as hydrogen peroxide have the capacity to induce the synthesis and accumulation of PAF by both bovine pulmonary artery and human umbilical vein ECs in a concentration-, time-, and calcium-dependent manner that is temporally dissociated from lytic cell injury (17). Thus, hydrogen peroxide can be considered as an agonist such as thrombin, histamine, or leukotrienes C₄ and D₄ that are capable of initiating the rapid EC-dependent PMN adhesion; in contrast to certain cytokines (TNF and interleukin-1), which induce the interaction of ECs and PMNs in a delayed manner (18,23). Following the stimulus of ECs by certain agonists, PAF is coexpressed in the surface of the cell with P-selectin, an integral membrane glycoprotein located in platelets and in Weibel-Palade bodies of endothelial cells (23,24). Upon activation of the endothelium, the Weibel-Palade bodies fuse with the plasma membrane, and P-selectin is rapidly translocated to the EC surface. Then, the tethering of particular subsets of leukocytes is initiated. Selectins mediate a degree of adhesion that is strong enough to induce rolling along the vessel wall but not capable of stopping leukocytes completely (25,26). This strong adhesion is obtained after a triggering step, in which integrins are activated in a way that EC-PMN adhesion can stop the rolling leukocyte. In addition to important trigger factors such as interleukin-8 and complement products, PAF is believed to stimulate PMNs, resulting in the EC-PMN adhesion by a leukocyte integrin-dependent mechanism, based on the binding of heterodimeric membrane glycoproteins called β_2 integrins (CD11_a-CD18 in the case of lymphocytes and CD11_b-CD18 in the case of neutrophils and monocytes) to counter receptors expressed on endothelium, namely, intercellular adhesion molecules 1 and 2 (ICAM-1 and ICAM-2). PAF associated with ECs activates PMNs by binding to a cell surface receptor, and promoting upregulation of CD11_a-CD18 and CD11_b-CD18 (25). CD11_b-CD18 is also the receptor for C3bi, one of the breakdown components of the third component of complement C3 (27). Moreover, interaction of β_2 integrins with biological surfaces induces the prolonged release of oxidant species in response to chemotactic ligands (28). The final step consists on transendothelial migration of PMNs, which takes place under the influence of promigratory factors, with further degranulation and release of proteinases into the extracellular sites. Tethering, triggering, strong adhesion, and motility are sequential steps in adhesion of leukocytes to endothelium that have inherent specificity since it requires that all elements are present in the correct combination for the particular responding leukocyte (25).

PAF AND PAF ANTAGONISTS IN EXPERIMENTAL RENAL ISCHEMIA-REPERFUSION

Over the years a great deal of evidence has accumulated supporting a role for PAF in experimental ischemia-reperfusion-induced damage in different organs and in kidneys (19). It has become apparent that, although there are a great number, PAF is one of the most important mediators involved in the inflammatory circuit generated in ischemic organs. Significant protection against such damage is afforded by a range of structurally unrelated PAF antagonists.

Moreover, numerous evidences are available for the implication of PMN in the development of injuries after an ischemic event in various organs (29-31). PMN are well known to play an important role in the transformation of an ischemic event into an inflammatory response. Recent studies (32,33), using hypoxic human umbilical vein endothelial cells incubated with PMN, have proved that hypoxia itself can activate endothelial cells, and this

activation can account for the PMN increased adherence. These studies also showed an induction of PAF synthesis, and an inhibitory effect of this response using PAF antagonists, and anti-ICAM-1, anti-CD18/CD11b, and anti-GMP-140 monoclonal antibodies. These observations are of interest since they confirm that PMN kinetics and margination in hypoxic damage are regulated by the same mediators as in inflammatory damage.

Numerous other studies, in the whole organ, have proved the role of PAF in ischemia-reperfusion-induced leukocyte adherence and activation in myocardial (29), hepatic (30), and intestinal (31) warm ischemia models. Also, other studies have proved the role of PAF and the protective effects of a PAF antagonist after cold ischemia in organ preservation (34,35).

Initial studies on PAF participation in acute renal failure were conducted by Pirotzki et al. (36). These authors injected intravenously to rats several doses of PAF that resulted in a fall of GFR associated with a reduction in urinary flow rate. Electron microscopy examination revealed the presence of platelets in the glomeruli, as well as loss of fixed anionic charges of the capillary wall (colloidal iron staining). These glomerular alterations and renal functional changes were prevented by BN 52021, a PAF receptor antagonist. Other authors, administering PAF to dogs, confirmed these observations in GFR and reported also a fall in renal plasma flow after PAF injection (37). They suggested that it could be due to arteriolar vasoconstriction and/or mesangial cell contraction. It has been also reported that glomeruli isolated from kidneys after a temporal ischemia of 60 min (38), and that isolated rat kidneys subjected to several stimulations (4) released increased amounts of PAF-acether.

Plante et al. reported a study on the recovery of renal function in the anesthetized rat subjected to 30-min occlusion on the left renal artery followed by reperfusion in a model of two kidneys—one ischemic (39). Pretreatment with an intravenous bolus of a PAF receptor antagonist markedly improved the recovery of glomerular filtration rate after acute ischemic renal injury. Rats treated with the PAF antagonist also exhibited an improved ability to excrete fluid and Na^+ . Other authors obtained similar results using an experimental model of acute renal failure induced in rats by clamping the renal artery for 60 min and contralateral nephrectomy (40). Results from this study showed a marked oliguria, decrease in renal plasma flow and GFR in rats subjected to ischemia, and a significant recovery of these parameters in rats pretreated either with BN 52021 or with alprazolam. Also, higher renal arteriovenous difference was seen in ischemic kidneys compared to nonischemic controls or ischemic kidneys pretreated with both PAF receptor antagonists (40), suggesting a role for PAF in the induction of renal ischemic injury.

Gentamicin and cisplatin are known nephrotoxic agents generally producing tubular cell injury. Nevertheless, renal dysfunction produced by both drugs, in association with minimal morphological alterations, suggest that hemodynamic factors may play a major role in their induced nephrotoxicity. Strong similarities between the effects of PAF, gentamicin, and cisplatin in renal microcirculation have been described (41). Moreover, simultaneous treatment with these drugs and a PAF antagonist ameliorated gentamicin nephrotoxicity and completely blunted cisplatin-induced alterations in glomerular hemodynamics, suggesting that PAF plays a role in nephrotoxicity induced by both drugs. On the other hand, PAF antagonists minimize cyclosporine nephrotoxicity by an amelioration of the vasoconstriction induced by the drug (42).

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, we have evaluated the protective effect of BN 52021 (43). Rats that received BN 52021 intra-

venously showed a significantly higher creatinine clearance than ischemic controls. Intravenous BN 52021 administration produced a more accelerated renal function recovery at 10 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 ischemic controls. Addition of BN 52021 to Euro-Collins flushing solution showed no protective effect. From these results we concluded that intravenous BN 52021 shows a renal protective effect against warm ischemia.

We have recently studied the role of PMN and PAF on the renal injury induced by cold ischemia in an isolated perfused rat kidney model (44). Our data showed that PMN contributed to renal cold ischemia-reperfusion injury, and that treatment with the PAF receptor antagonist BN 52021 attenuated this injury, suggesting that it is mediated by PAF. These conclusions were supported by some observations: First, addition of PMN during reperfusion induced hemodynamic alterations and worsened functional injury of previously hypothermically ischemic kidneys, whereas reperfusion with PMN did not cause functional nor hemodynamic injury in nonischemic kidneys. Second, the addition of the PAF receptor antagonist during reperfusion produced an amelioration of these hemodynamic and functional injuries induced by cold ischemia and PMN in a concentration-dependent manner.

Thus, PAF antagonists, as well as specific antibodies against selectins or integrins, could be considered new therapeutic tools in preventing the postischemic reperfusion injury in cadaveric organ transplantation.

PAF ANTAGONIST IN CLINICAL RENAL TRANSPLANTATION

Our group has recently reported a clinical study, the first one in this field, showing the efficacy of a platelet-activating factor antagonist, BN 52021, in the prevention of organ ischemic-reperfusion injury in clinical transplantation (45).

In this double-blind pilot study, 29 first cadaveric kidney transplanted patients were evaluated and randomly allocated to two groups, one receiving BN 52021 and the other placebo. Kidneys were from 20 donors, 10 in each group. In the BN 52021 group, the drug (i) was administered to the donors, 240 mg intravenously, just before kidney harvesting; (ii) was added to the University of Wisconsin preservation solution; or (iii) was administered to the recipients, 160 mg intravenously, before renal allograft blood reperfusion, and 12 h later 80 mg intravenously followed by 80 mg every 8 h for 4 days. Patients received placebo according to the same schedule. Patients receiving BN 52021 had significantly lower posttransplant renal failure, higher median diuresis, and faster decrease in creatinine values after transplantation than patients receiving placebo. Both groups of patients showed similar percentage of acute rejection episodes and actuarial graft survival in the third month after surgery.

In summary, our studies suggest that PAF antagonists may constitute valuable drugs in the prevention of acute renal failure in the clinical transplant setting. Larger multicenter trials are in progress to corroborate these promising initial results.

ACKNOWLEDGMENTS

This work was supported in part by a grant from FISS (94/1296); by a grant from "Fundació August Pi i Sunyer," Ciutat Sanitària i Universitària de Bellvitge; and by a grant from LASA Laboratories.

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