Pentoxifylline in Amphotericin B Toxicity Rat Model

KISHOR M. WASAN,1,2 KIUMARS VADIEI,1 GABRIEL LOPEZ-BERESTEIN,1,2 REGINA R. VERANI,3 AND DAVID R. LUKE1,2†*

Department of Pharmaceutics, University of Houston;1 Clinical Immunobiology and Drug Carrier Section, The University of Texas M.D. Anderson Cancer Center,2 and Department of Pathology and Laboratory Medicine, The University of Texas Medical School at Houston,3 Houston, Texas 77030

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The mechanism of acute nephrotoxicity following the administration of amphotericin B (AmpB) remains unclear despite a number of studies describing hypermagnesuria, hyperkalaemia, and hemodynamic changes. The present experiments attempted to elucidate the mechanism by using a novel hemorheologic probe, pentoxifylline (PTX). Acute studies were performed with rats given single intravenous doses of AmpB (1 mg/kg of body weight) with or without intraperitoneal PTX (45 mg/kg). Renal function, assessed by insulin clearance (ClIN) and electrolyte handling, and morphology were compared with those of controls given sterile water and PTX. A significant decrease in ClIN was not observed in rats given AmpB and PTX or in the controls was found in rats given AmpB. Electrolyte handling was not different among groups. Whereas pronounced (3 and 4+) on a scale of mild to significant (1+ to 4+) vascular congestion was found in rats given AmpB, rats coadministered PTX had mild (1 and 2+) medullary and glomerular vascular congestion. In chronic studies, intravenous AmpB (1 mg/kg per day) or sterile water was coadministered with intraperitoneal PTX (45 mg/kg every 12 h) or saline for 10 days. Mean ClIN of rats coadministered AmpB and PTX was not significantly different from that of PTX control rats (1.61 ± 0.19 versus 1.31 ± 0.29 ml/min per g of kidney weight). A 46% decline in ClIN was found in rats treated with AmpB and saline (P < 0.05). Renal sodium and potassium excretions were increased in both AmpB-treated groups compared with controls. Coupled with histologic evidence of the acute studies, these data suggest that the benefit of PTX in the prevention of AmpB-induced nephrotoxicity is, in part, due to vascular decongestion.

Amphotericin B (AmpB) remains the most effective and widely used antibiotic for the treatment of systemic fungal disease in humans (11, 12). Its use is frequently limited by the development of nephrotoxicity manifested by renal vascular resistance with diminished glomerular filtration rate and renal blood flow (7–10, 17, 20). The inability to concentrate urine, as well as hyperkalaemia and hypermagnesuria, are prominent clinical features of AmpB-associated nephrotoxicity. To date, the mechanism of this toxicity is not well understood, but it may be related to the interaction of the drug with membrane-bound cholesterol to form aqueous channels; thus, increased permeability to small solutes and decreased electrical resistance may occur at the cellular level (1, 4, 6). The net effect is renal vasoconstriction and interstitial changes in electrolyte handling.

Pentoxifylline (PTX) is a unique hemorheologic agent useful in the treatment of intermittent claudication and other vascular diseases (16). We have previously shown beneficial effects of PTX in the treatment of nephrotoxicity following cyclosporine (5), glycerol, cisplatin, and mercuric chloride administration (21; unpublished data). Furthermore, complete restoration of glomerular filtration and renal blood flow has been associated with PTX use following ischemic events (13; D. R. Luke, K. L. Berens, and R. R. Verani, Renal Failure, in press). Morphologically, we have found significant reductions in vascular congestion in rat kidneys treated with PTX. The use of indomethacin does not interfere with the posts ischemic benefit of PTX, suggesting that the arachidonate pathway does not play a major role in its mechanism of action.

The present study investigated the influence of PTX coadministration on both the acute and chronic nephropathies associated with AmpB. More importantly, the role of vascular congestion is implicated in the pathogenesis of AmpB-associated nephrotoxicity.

MATERIALS AND METHODS

Drugs. AmpB (Fungizone; E. R. Squibb & Sons, Princeton, N.J.) was reconstituted with sterile water; the reconstituted preparation remains stable for 7 days. PTX (Sigma Chemical Co., St. Louis, Mo.) was dissolved in sufficient saline for a resultant concentration of 45 mg/ml; since stability studies have not been performed, PTX solution was prepared fresh daily.

Animals. A total of 51 rats (male albino CD, 150 to 200 g; SASCO Breeders, Houston, Tex.) were housed in an animal facility with a 12-h light-dark cycle and controlled temperature and humidity. Powdered food (Purina rodent chow) and distilled water were unrestricted throughout the study. Rats were acclimated to individualized housing in a metabolism cage (Nalge/Sybron Corp., Rochester, N.Y.) for 2 days prior to study (K. Vadiei, K. L. Berens, and D. R. Luke, Lab. Anim. Sci., in press). Control animals were pair fed with drug-treated rats to avoid renal functional changes secondary to weight loss (Vadiei et al., in press). The experimental design was approved by the Animal Care Committee of the University of Houston. All procedures were in accordance with guidelines established by the Committee on the Care and Use of Laboratory Animals of the National Institutes of Health.

Acute studies. Rats (n = 27) were given intravenous (i.v.)
AmpB (1 mg/kg of body weight) or sterile water via the penile vein under light ether anesthesia. After 1 h, each rat was randomized to receive either a single intraperitoneal (i.p.) dose of PTX (45 mg/kg) or saline. Six rats received saline and sterile water (controls), six were given sterile water and PTX, nine received AmpB and saline, and six were given sterile water and AmpB. None of the rats received AmpB and PTX 1 h apart. Rats were placed in individualized metabolism cages for urine collection. Creatinine levels in serum were determined by using ion-selective electrodes (NOVA I+1 Autoanalyzer). Serum samples were analyzed for creatinine concentrations by a modified Jaffe reaction (Beckman Creatinine Analyzer II).

Data analysis. CL\(_{\text{IN}}\) was calculated by standard noncompartmental methods. The elimination rate constant (\(k_e\)) was iterated by nonlinear regression of the terminal counts per minute time points. The area under the serum concentration-time curve (AUC) was calculated by trapezoidal rule and extrapolated to infinity by the equation AUC = AUC\(_{\text{O-\tau}}\) + Ct\(_{\text{Ct}}\), where Ct is the counts per minute of \(^{3}H\) in the blood sample obtained at the last time point. CL\(_{\text{IN}}\) was estimated by the equation CL\(_{\text{IN}}\) = D/AUC, where D is the counts per minute of injected solution multiplied by the volume injected (0.25 ml). Sodium excretion rate was estimated as the product of urine sodium concentration and urinary flow rate. Excretion rate of potassium was calculated in a similar manner.

The degree of vascular congestion on histologic examination was graded in the two drug-treated groups of rats in the acute study on a scale of mild to significant (1+ to 4+). Electrolyte excretion rates and CL\(_{\text{IN}}\) were compared between groups by analysis of variance (PCANOVA; Human Systems Dynamics). Critical differences were assessed by Newman-Keuls post hoc tests. A difference was considered significant if the probability of chance explaining the results was reduced to less than 5% (\(P < 0.05\)). All data are expressed as mean \(\pm\) standard deviation.

**RESULTS**

Acute studies. Mean creatinine levels in serum of rats given AmpB, independent of PTX therapy, were significantly greater than those of controls (Table 1). No differences in renal electrolyte handling were found between groups. PTX alone had no effect on CL\(_{\text{IN}}\) compared with effects observed in control rats (Fig. 1). AmpB treatment

**TABLE 1. Influence of PTX on AmpB-treated rats or sterile water controls**

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Serum creatinine (mg/dl)</th>
<th>Renal excretion (µg/min of):</th>
<th>Serum creatinine (mg/dl)</th>
<th>Renal excretion (µg/min of):</th>
<th>No. of rats with degree of kidney vascular congestion*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.5 ± 0.1</td>
<td>2.6 ± 0.4</td>
<td>3.9 ± 0.7</td>
<td>0.4 ± 0.1</td>
<td>2.6 ± 0.4</td>
</tr>
<tr>
<td>PTX only</td>
<td>0.4 ± 0.1</td>
<td>2.2 ± 0.2</td>
<td>3.8 ± 0.7</td>
<td>0.4 ± 0.1</td>
<td>6.3 ± 0.9</td>
</tr>
<tr>
<td>AmpB only</td>
<td>0.6 ± 0.1(c)</td>
<td>1.9 ± 0.7</td>
<td>3.2 ± 1.0</td>
<td>0.5 ± 0.0(c)</td>
<td>7.5 ± 2.1(c)</td>
</tr>
<tr>
<td>AmpB + PTX</td>
<td>0.6 ± 0.2(c)</td>
<td>2.7 ± 0.7</td>
<td>4.2 ± 1.0</td>
<td>0.3 ± 0.1</td>
<td>6.4 ± 1.0(c)</td>
</tr>
</tbody>
</table>

\(a\) Mean \(\pm\) standard deviation.

\(b\) Congestion ranged from none (0) to severe (4+).

\(c\) \(P < 0.05\) compared with control values.
was associated with a 51% decline in CL\textsubscript{IN} (P < 0.01); coadministration of PTX and AmpB resulted in 88% of the mean control CL\textsubscript{IN} (1.07 ± 0.12 versus 1.22 ± 0.35 ml/min per g of kidney weight; not significant).

Microscopic examination of kidneys showed a prominent degree of vascular congestion in the majority of the rats given AmpB and saline as compared with rats treated with AmpB and PTX (Fig. 2 and 3). All rats given AmpB and PTX had 1+ and 2+ vascular congestion in contrast to those given AmpB and saline, in which six of nine animals had 3+ to 4+ vascular congestion (Table 1). The vascular congestion was more significant in the inner medulla and inner stripe of the outer medulla but was also observed in cortical vessels and glomerular capillaries. The animals treated with AmpB and PTX had a larger number of discoid erythrocytes. There was no evidence of tubular necrosis or tubular degeneration in any of the animals.

**Chronic studies.** Rats were pair fed throughout the study period; hence, weight loss was not significantly different between groups. Similarly, kidney weights were not significantly different among treatment groups. Significant increases in creatinine levels in serum were observed in AmpB control rats compared with levels in rats given AmpB and PTX, sterile water and PTX, and sterile water and saline (Table 1). CL\textsubscript{IN} was markedly decreased in rats given AmpB and saline compared with CL\textsubscript{INs} in other groups (Fig. 1). Renal sodium excretion was significantly greater in all three treatment groups than in saline controls. However, increased potassium excretion was found in rats receiving AmpB with or without PTX.

**DISCUSSION**

The renal toxicity profiles following single and multiple doses of AmpB have been well characterized in both experimental animals and humans (5, 7–9, 13, 15–18). Consistent with drug-induced nephrotoxicities, decreased renal blood flow and significant increases in renal vascular resistance have been found in AmpB-treated animals. Coupled with findings of significant renal potassium and magnesium wasting, these data suggest both glomerular and tubular defects. Although an understanding of the mechanism of toxicity remains unclear, the renin angiotensin system and arachidonic pathway do not appear to play major roles (5, 16). Others (6, 15) have suggested renal artery constriction with subsequent ischemia and the generation of superoxide anions; hence, theophylline has shown improvement in function by blocking ischemia-related adenine effects (14).

The present experiments suggest a further explanation: the role of vascular congestion resulting from ischemic blood flow. In the absence of oxygen, erythrocytes cannot maintain homeostasis. Swelling and loss of deformability of cells occur in the microvasculature, impairing further oxygen delivery. This vicious cycle continues until the appearance of tissue necrosis (15). Multinucleated cells, such as neutrophils and polymorphonuclear cells, are attracted to the site of ischemia because of various chemotactic factors, including tumor necrosis factor, superoxide anions, and interleukin-1, released by circulating macrophages and monocytes. Indeed, leukostasis has been associated with AmpB use (2). Furthermore, congestion at the site of ischemia may involve platelet activation and thrombogenesis. PTX effects on vascular congestion were confirmed by our histologic studies. Significant vascular congestion was found in six of nine rats given AmpB and water. In contrast, mild vascular congestion was found in rats administered AmpB and PTX. The increased deformability of the erythrocytes was also observed in animals given AmpB and PTX. This is a known effect of PTX on the fluidity properties of erythrocytes (3).

PTX was used in the present experiments to test the role of vascular congestion in AmpB-associated nephrotoxicity. Similar to other methylxanthines, such as theophylline and caffeine, PTX affects adenosine receptors in renal vascular tissue. By preservation of ATP during ischemia, the loss of homeostatic properties of the erythrocyte is prevented, disrupting the vicious cycle of vascular congestion. Furthermore, PTX prevents migration and aggregability of neutrophils, ultimately increasing blood flow in the ischemic state.
Interestingly, despite the association of leukostasis with AmpB administration (2), prominent accumulation of neutrophils in the medulla was not observed in the present study. Previously, we have demonstrated benefits from the addition of PTX following in vivo renal artery occlusion in the autoperfused rat kidney model (Luke et al., in press) as well as in vitro in the isolated rat kidney perfused with cell-free buffer (unpublished data). Addition of a nonspecific cyclo-oxygenase inhibitor, indomethacin, did not prevent the beneficial effects of PTX. Hence, the effects of PTX are not limited to cellular components or prostaglandin synthesis but likely include modulation of adenine nucleotide metabolism. Further studies of the biochemical mechanism of protection of PTX are warranted.

As in the case of other drug-induced toxicities, the addition of PTX preserved glomerular function, as assessed by maintenance of CLIN, in both acute and chronic studies. The increase in glomerular filtration rates in rats given sterile water and PTX is evidence of the modest diuretic effect found in most methylxanthines. It could be argued that the dose of PTX (90 mg/kg per day) greatly exceeded the dose used clinically. We chose this dose to be consistent with other studies (5, 21), demonstrating its potential benefit in drug-induced renal diseases. However, recent experiments demonstrate similar amelioration of ischemic damage with a 1-mg/kg i.v. dose followed by a continuous infusion (Luke et al., in press). Others have demonstrated effects on neutrophils at concentrations lower than those clinically observed following a 400-mg dose (19); hence, a reduction in the dose of PTX may result in a similar benefit in the AmpB toxicity model. To date, a dose-response curve has not been studied. With PTX as a hemorheologic probe, the present data support the theory of vascular congestion as the primary factor for AmpB-associated toxicity. However, changes in renal electrolyte handling also occurred (in particular, increased potassium wasting). Although the absolute difference was modest in these experiments, hyperkalaemia has been a significant problem in the clinical setting. The study was designed to treat drug-induced nephrotoxicity with PTX administered after the introduction of AmpB. It is possible that ischemic damage to the medullary thick ascending limb had occurred prior to the onset of activity of PTX. Pretreatment with PTX may offer increased protection from medullary damage.

In summary, the coadministration of PTX and AmpB resulted in preservation of glomerular function, most likely mediated by vascular decongestion. Since the use of AmpB is associated with high morbidity with dose-limiting toxicity to the kidney, the clinical implications of the present experiments are profound. However, further studies characterizing the effects of PTX on the antifungal activity of AmpB in the infected-animal model are warranted.

LITERATURE CITED