Amphotericin B Nephrotoxicity: The Adverse Consequences of Altered Membrane Properties

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ABSTRACT
Amphotericin B (AmB) has been in clinical use for more than 30 yr but has remained the most effective drug for treatment of serious fungal infections. Its use has increased in recent years, as the result of increases in aggressive intensive care support and increased numbers of immunocompromised patients. Nephrotoxic manifestations are common, and this is the major factor limiting the clinical use of the drug. A number of recent studies have contributed to a better understanding of the mechanism by which AmB exerts its nephrotoxic effect. AmB alters cell membrane permeability and probably as a consequence alters tubular and vascular smooth muscle cell function, leading to various tubular transport defects and vasoconstriction. Decreased RBF appears to play a major role in AmB-induced reduction GFR, and recurrent ischemia may be the basis of permanent structural nephrotoxic effects. Salt loading is the only measure proven by controlled prospective study to ameliorate AmB nephrotoxicity in humans. Liposomal AmB and the formulation of an emulsion of AmB in lipid may provide a protective effect based on altering the affinity of AmB for mammalian cell membranes, while preserving high efficacy against fungal cells. However, further studies are needed to evaluate the efficacy and safety of these new AmB formulations.

Key Words: Amphotericin B, vasoconstriction, renal toxicity, membrane permeability

Amphotericin B (AmB), a polyene antibiotic, remains the most effective drug in treating systemic fungal infections. Even with the emergence of the newer drugs (1-3), AmB remains in general the drug of choice in most severe infections (3-10). The use of AmB over the last three decades has increased enormously, especially during the last decade (11-13), an increase in part because of our better understanding of its side effects and administration guidelines, but more important, because of the increase in the incidence of systemic fungal infections and our abilities to diagnose these infections (13) (see Table 1).

AmB can produce a wide variety of acute and chronic side effects (see Table 2). A number of toxic effects are regularly observed during the infusion of the drug and, in general, appear to be highly dependent on the rate of infusion. A variety of other chronic toxic effects have been observed to develop after the drug has been administered for days or weeks, the most important of which is nephrotoxicity. Nephrotoxicity is quite common; it is the side effect that most often limits the clinical use of this drug (14,15). Our understanding of the mechanisms by which AmB exerts its nephrotoxic effects has grown over the last 30 yr. The purpose of this article is to review the current available data on AmB nephrotoxicity in general and to summarize some recent advances in understanding its effect on vascular smooth muscle.

EFFECT OF AMB ON MEMBRANE PERMEABILITY

It is widely accepted that polyene antibiotics exert their antifungal effect by altering the membrane permeability of the fungal cell, leading to the loss of intracellular elements that are essential for viability (16,17). Fungi, but not bacteria, possess ergosterol in their cell membranes, a sterol similar to cholesterol. This lipid has a particularly high affinity for polyene antibiotics (18). However, AmB also binds to cholesterol molecules found in mammalian cell membranes, albeit with some lower affinity. It has been shown that AmB forms intramembranous pores after binding to sterol molecules in artificial and biologic membranes. As a consequence of pore formation, membrane permeability to monovalent ions is greatly increased (16,19,20). It is this effect of AmB on membrane properties that may underlie all of its nephrotoxic effects (21). Although it is customary to divide the nephrotoxic effects of AmB into tubular effects on one hand and vascular effects on the other hand, both types of toxicity may arise from the direct effect of the drug on cellular membranes.

TUBULAR DEFECTS
Renal Tubular Acidosis

In 1962, Sanford et al. reported the existence of compensated metabolic acidosis in four of their six AmB-treated patients (22). Only one patient was tested with an acid load and demonstrated to have a diminished ability to acidify the urine. McCurdy et al.
TABLE 1. Factors contributing to the increased prevalence of fungal infections

<table>
<thead>
<tr>
<th>Factor</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased Numbers of Immunocompromised Patients</td>
<td></td>
</tr>
<tr>
<td>AIDS and longer surviving AIDS patients</td>
<td></td>
</tr>
<tr>
<td>More aggressive cancer chemotherapy</td>
<td></td>
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<tr>
<td>Increase in transplantation operations</td>
<td></td>
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<tr>
<td>More Aggressive Intensive Care Medicine in Adults and Premature Newborns</td>
<td></td>
</tr>
<tr>
<td>Increased Use of Prosthetic Devices and Long-Term Indwelling Intravenous Lines</td>
<td></td>
</tr>
<tr>
<td>Increased Broad-Spectrum Antibiotic Usage</td>
<td></td>
</tr>
<tr>
<td>Greater International Travel and Exposure to Foci of Endemic Mycoses</td>
<td></td>
</tr>
<tr>
<td>More Parenteral Nutrition and Increased Use of Lipid Suspensions</td>
<td></td>
</tr>
</tbody>
</table>

*Modified from Ref. 13.

TABLE 2. Side effects of AmB

<table>
<thead>
<tr>
<th>Immediate Symptoms Observed During or Shortly After the Infusion</th>
<th>Common: fever, chills, anorexia, nausea, vomiting, headache</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less common: hypotension, flushing, vestibular disturbances</td>
<td></td>
</tr>
<tr>
<td>Rare: hyperkalemia, arrhythmias, anpea, convulsions, anaphylaxis</td>
<td></td>
</tr>
<tr>
<td>Delayed Symptoms Developing After Infusion</td>
<td></td>
</tr>
<tr>
<td>Local Symptoms, thrombophlebitis (common)</td>
<td></td>
</tr>
<tr>
<td>Nephrotoxic (common)</td>
<td></td>
</tr>
<tr>
<td>Tubular defects</td>
<td></td>
</tr>
<tr>
<td>Renal tubular acidosis</td>
<td></td>
</tr>
<tr>
<td>Renal concentrating defect</td>
<td></td>
</tr>
<tr>
<td>Hypokalemia and renal potassium losing</td>
<td></td>
</tr>
<tr>
<td>Other electrolyte disorders</td>
<td></td>
</tr>
<tr>
<td>Decreased GFR</td>
<td></td>
</tr>
<tr>
<td>Hematologic</td>
<td></td>
</tr>
<tr>
<td>Anemia (common)</td>
<td></td>
</tr>
<tr>
<td>Thrombocytopenia, eosinophilia, agranulocytosis (rare)</td>
<td></td>
</tr>
</tbody>
</table>

(23) measured acid excretion in seven patients who received AmB; five of them were not able to lower the urinary pH below 5.4 in response to acid loading. Titratable acid and total hydrogen ion excretion were diminished compared with values in normal controls; however, the percentage of hydrogen ion excreted as ammonium was not reduced. Subsequently, numerous reports have confirmed the defect in urine acidification induced by AmB in patients (24–26). The acidification defect appears to be dose related (23,24). For example, virtually all six patients studied by Patterson et al. who received >1 g of AmB had abnormal response to acid loading. Acidification defect precedes a significant fall in GFR (26) and is generally reversible within a few months of the end of therapy. None of 14 patients studied after 1 yr had abnormal response to acid loading (25). However, the incidence of clinically apparent acidosis with a moderate dose of AmB (<1 g) is quite low (<2%). Only 2 patients of 196 studied by Stamm et al. had a serum bicarbonate level below 20 mg/mL (27). Both the type of the acidification defect and the fact that it is often observed without a detectable reduction in GFR indicate that the acidosis cannot be simply attributed to renal insufficiency. Experimental studies have provided some insights into the mechanism of AmB-induced acidification defect. Steinmetz and Lawson have examined the effect of AmB on H⁺ ion secretion by isolated turtle bladder (28). They have demonstrated that although hydrogen ion secretion against a gradient was markedly reduced by AmB, H⁺ secretion remained normal if passive electrochemical forces across the epithelium were reduced. These results suggest that AmB-induced impaired acidification is caused by increased passive permeability of the luminal membrane and back diffusion of hydrogen ion rather than the failure of active transport. This is in agreement with the observation that in a rat model of AmB nephrotoxicity with renal tubular acidosis (29), alkali therapy prevented the defect in urinary acidification produced by AmB.

Renal Concentrating Defect

One of the most common effects of AmB therapy on the kidney is a loss of concentrating ability and polyuria. In one study, an abnormal concentrating ability was found in 46 of 47 patients (30). In more detailed studies in five patients, Bell et al. found that maximal concentrating ability after 24 hours of water deprivation was uniformly depressed; before treatment, maximum urinary osmolarity ranged from 628 to 1,064 mOsm/kg, but no patient exceeded 673 mOsm/kg after therapy (31). The defect occurs in patients who do not have azotemia (32); it is not responsive to vasopressin and is generally reversible (31). A number of explanations have been proposed. When AmB is discontinued, concentrating ability may correct whereas para-aminohippurate (PAH) clearance remains decreased (31), indicating a dissociation between the vasoconstriction and the concentrating defect. In acute infusion in rats, polyuria precedes the fall in inulin clearance (33). Therefore, it appears unlikely that the concentrating defect is merely a reflection of decrease in GFR or in the number of functioning nephrons, conditions known to affect urinary concentration (34,35).

Hypokalemia is a common complication of AmB therapy (see below) and a well-established cause of a concentrating defect (36). An association between hypokalemia and the impairment of concentrating ability caused by AmB has been observed (23,24); furthermore, AmB, like hypokalemia (36), produces a vasopressin-resistant concentrating defect with normal diluting capacity (23,24). However, a significant impairment of maximum renal concentrating ability in patients (14) and in rats (29) has also been observed with normal serum potassium levels. This suggests
that other factors in addition to hypokalemia play a role in the pathogenesis of this defect.

The ability of the kidney to concentrate the urine is dependent on urea recycling and the maintenance of the urea concentration gradient in the medullary interstitium. Kavlock et al. have demonstrated a decrease in the fractional excretion of urea in neonatal rats treated with AmB (37). Subsequently, they have found that urea excretion in these AmB-treated rats was coupled to water excretion because both increased with the use of diuretics (38), whereas in control rats, urea excretion was not enhanced by diuresis. These observations suggest that the normally urea-impermeable membrane of the cortical collecting duct (39) may be altered by AmB. A failure to generate a high luminal urea concentration in the urea-permeable portion of the inner medullary collecting duct would lead to substantial reductions in medullary interstitial osmolality and a vasopressin-resistant concentrating defect. There is also direct evidence that AmB effects responses to antidiuretic hormone in the terminal portions of the collecting duct (40,41). In rat isolated inner medullary collecting ducts, perfused in vitro, baseline water and urea permeability was not altered by AmB, but the stimulatory effect of vasopressin was greatly reduced (41). This effect was reversible when AmB was removed.

It is of interest to notice that bicarbonate therapy has been observed to increase urine osmolality in rats treated with AmB (29). This suggests that an increase in intraluminal and/or intracellular pH may modify the ability of AmB to induce a renal concentrating defect. Diluting capacity has been reported to be unimpaired in patients treated with AmB; however, this is not well documented (23,24).

### Potassium Wasting

Hypokalemia during AmB therapy is quite common (12,14,22,26,30,42–45); levels as low as 0.8 mEq/L have been reported (22). It has been clearly documented that AmB induces renal potassium wasting (26,46–48) and can produce substantial potassium deficits (24,48). Potassium excretion increases acutely during the infusion period (23). AmB increases membrane permeability to potassium in red blood cells (49) and in isolated turtle bladder preparations (28); therefore, it is probable that AmB alters the permeability of the distal tubular cells, leading to an increase in the passive fluxes of potassium down its electrochemical gradient. Renin and aldosterone levels are not increased during AmB therapy (47,50).

The defect in urinary acidification, the potassium wasting, and the defect in maximal urinary concentrating ability observed in patients treated with AmB support the concept that AmB toxicity is directed at the distal tubule. This is in agreement with the pathologic findings of distal tubular degeneration and nephrocalcinosis (45,51,52). Furthermore, glucosuria, aminoaciduria, and phosphaturia, which reflect the function of the proximal tubule, are typically absent during AmB therapy (23,29).

### Other Electrolyte Disorders

**Hypomagnesemia.** Hypomagnesemia is another common feature of AmB therapy (24,53,54), and magnesium levels should be monitored closely in patients treated with AmB. The effect of AmB on urinary magnesium excretion is controversial. In two studies, AmB was found to increase the urinary excretion of magnesium (26,54), but in a recent study of 20 patients (55) and in an older study (24), urinary magnesium decreased significantly during AmB therapy despite mild hypomagnesemia. Therefore, it is unclear whether a renal defect is responsible for the AmB-induced hypomagnesemia. Alternatively, magnesium distribution in the body may be altered as a consequence of AmB's effect on membrane properties. It is important to realize that the therapy of hypokalemia associated with AmB can be quite resilient unless hypomagnesemia is corrected.

**Salt Handling.** Renal sodium wasting was reported in one patient by Butler et al. in 1964 (14). However, its presence has not been a consistent finding in humans (23,24) or in rats (29) treated chronically with AmB. We have observed, by micropuncture, that the acute infusion of AmB in rats induces significant diuresis and an increase in urinary chloride excretion but not in chloride delivery to the early superficial distal tubules (33). Chlorureteresis tended to subside shortly after the AmB infusion was terminated. These findings suggest that some impairment of collecting duct NaCl transport may exist during the period of drug infusion.

**Hyperkalemia.** Although hypokalemia is substantially more frequent, AmB has also been reported to cause hyperkalemia. In anephric patients, an acute rise in serum potassium during AmB infusion has been documented with rapid infusion (56). This effect may be the consequence of an immediate alteration of cell membrane K⁺ permeability and the efflux of potassium from cells. It has been recommended that if the rapid infusion of AmB is necessary in renal failure patients, it should be accompanied by simultaneous hemodialysis (56).

### Decreased GFR

Soon after its introduction to clinical use, it was recognized that AmB causes azotemia (14,15,31,32). The incidence of renal insufficiency during AmB therapy has varied widely (12,14,27). In one large study from 1964, 83% of 81 patients with a variety of fungal diseases were found to have an increase in serum creatinine of >1.5 mg/mL or in BUN of >20 mg/mL (14). More recently, in a prospective study of 194 patients with cryptococcal meningitis, 26% of patients were observed to have an increase in serum creatinine of more than 2.0 mg/mL (27). The lower incidence rates in the recent study probably reflect the effects of
lower doses and a more homogeneous patient population. Because AmB is widely used in septic patients and in patients receiving other nephrotoxic drugs, in clinical practice, renal insufficiency is very common, but the contribution of AmB to worsening renal function is often uncertain. Although it is generally believed that there is some synergism between nephrotoxic drugs, in a recent retrospective study of 50 patients, there was no association between AmB nephrotoxicity and concomitant aminoglycoside administration (12).

Azotemia secondary to AmB is usually largely reversible. The incidence of persistent damage has been shown to be dose dependent. Chronic renal failure was observed in 44% of patients receiving more than a total of 4 g of AmB, whereas only 17% of patients receiving less than 4 g had persistent azotemia (14). In another study, only 8% of patients who received less than 1 g had chronic renal insufficiency (27).

In most clinical studies, GFR has been assessed from either serum creatinine or creatinine clearance, which as is well known to nephrologists, provides a rather imprecise measure of GFR. It is possible that the frequency of GFR depression would be higher if more precise methods had been used to assess it. The feasibility of using lithium as a marker for GFR has never been assessed with AmB. In experimental animals, a reduction in GFR has been confirmed by the demonstration of a reduction in inulin clearance (33,57-59). However, concern has been raised that even inulin might not accurately reflect filtration rate because of leakage out of the damaged renal tubule. In one study, when inulin was microinjected into the tubules of AmB-treated rats (57), a substantial portion of the inulin was not recovered in the urine; however, we observed virtually 100% inulin recovery in rats infused acutely with AmB, indicating minimal if any effect of the drug on tubular inulin permeability (33).

**EFFECT ON RBF**

A reduction in PAH clearance concomitant with the reduction in GFR has been documented during AmB therapy in a number of clinical studies (15,22,24,31). There is some concern about the accuracy of PAH clearance in estimating RBF in this setting, because PAH extraction is incomplete and PAH uptake may be inhibited by AmB (60). However, an acute vasoconstrictive effect of AmB on renal circulation, measured directly by an electromagnetic flowmeter, has been well established in dogs (50,61,62) and rats (58,63) (Table 3). Severe vasoconstriction has also been documented angiographically in dogs during AmB infusion into the renal artery (64) and in vitro, on isolated vessels from the aorta, renal artery, and glomerular arteriole of the rabbit (33).

As indicated in Table 3, decreased RBF contributes significantly to the reduction in GFR during the acute infusion of AmB in dogs and rats. Similar reductions in RBF occur during chronic AmB administration in rats (58,59) and in humans (31). The role of decreased RBF as a major contributor to the decreased GFR is also suggested by the paucity of structural alterations and the lack of correlation between azotemia and the severity of morphologic changes (31,45,57).

**THE SEARCH FOR THE MEDIATOR OF AMB VASOCONSTRICTION**

The mechanism of AmB-induced renal vasoconstriction has been the focus of many studies during the last three decades. It was shown in early studies that ganglionic or adrenergic blockade does not prevent this vasoconstriction in dogs (64), nor does renal denervation in rats (58). Phentolamine, an α-adrenergic antagonist, was associated with a modest reduction in the force generated by AmB in isolated rings of rabbit aorta in vitro (33), indicating a possible minor contribution of norepinephrine released from nerve endings within the vessel wall to AmB-induced vasoconstriction. Potent vasodilators, such as hydralazine and nitroprusside, were also ineffective in preventing AmB-induced vasoconstriction (63,64). The blockade of angiotensin II with saralasin or a receptor blocker had no or minimal effect (58,61,63), consistent with the observation that renin levels are not increased during AmB therapy (47,50). More recently, Heyman et al. (65) have demonstrated no increase in endothelin plasma levels after the acute infusion of AmB in rats, despite a 43% reduction in RBF (measured by pulsed Doppler technique). They have also shown that endothelin release from cultured bovine aortic endothelial cells did not increase in the presence of AmB at a high concentration (10⁻⁵ M). However, they observed a significant increase in endothelin plasma levels in hypodemic, but not in euvoletic, rats treated chronically with AmB. These results suggest that

**TABLE 3. Acute AmB effect on RBF and GFR**

<table>
<thead>
<tr>
<th>Ref. No.</th>
<th>% Δ RBF (by Magnetic Flowmeter)</th>
<th>% Δ GFR</th>
<th>Total Dose (mg/kg)</th>
<th>Rate of Infusion (mg/Kg per min)</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>62</td>
<td>50</td>
<td>23</td>
<td>0.5</td>
<td>0.025</td>
<td>Dogs</td>
</tr>
<tr>
<td>50</td>
<td>32</td>
<td>90</td>
<td>1</td>
<td>0.1</td>
<td>Dogs</td>
</tr>
<tr>
<td>58</td>
<td>44</td>
<td>76</td>
<td>1.2</td>
<td>0.02</td>
<td>Rats</td>
</tr>
<tr>
<td>66</td>
<td>62</td>
<td>89</td>
<td>2.0</td>
<td>0.1</td>
<td>Dogs</td>
</tr>
<tr>
<td>61</td>
<td>64</td>
<td>94</td>
<td>2.5</td>
<td>0.1</td>
<td>Dogs</td>
</tr>
</tbody>
</table>
endothelin does not mediate the acute vasoconstrictive effect of AmB but do not rule out the possibility that this peptide might have some role in the chronic effects of AmB. Low-dose dopamine or fenoldopam, a selective dopamine DA$_1$ agonist, had no effect on the AmB-induced vasoconstriction in dogs (61,66), although low-dose dopamine combined with saralasin had an attenuating effect (61). In a recent study in rats, the use of an adenosine receptor antagonist, DPSPX, did not prevent the AmB-induced reduction in RBF (67). Thus, a large number of manipulations are without a substantial blocking effect on AmB-induced vasoconstriction.

Certain manipulations have been observed to block or blunt AmB-induced vasoconstriction. These include salt loading (50,55,68) and pretreatment with furosemide (50) or the administration of aminophylline (62,63,69) or verapamil (58). These diverse manipulations share the common property of producing inhibition of the tubuloglomerular feedback (TGF) (70–73).

**TESTING THE ROLE OF TGF ACTIVATION**

It is known that an acute increase in flow or NaCl concentration in the distal tubule at the macula densa produces a reduction in filtration rate. This phenomenon, which has been called TGF, is probably mediated through the juxtaglomerular apparatus and is a physiologic protective mechanism to minimize unregulated salt and water loss (74,75). It has been speculated by a number of investigators that AmB increases renal vascular resistance by activating the TGF mechanism (50,62,63,66,69,76). Because AmB can alter ion fluxes in biologic membranes (19,20), it seemed possible that AmB may increase salt concentration or transport at the macula densa and thus produce TGF-dependent vasoconstriction. In support of this hypothesis, it was observed that the changes in the determinants of glomerular ultrafiltration caused by AmB administration were similar to those induced by the activation of the TGF mechanism (75,76). We performed a series of micropuncture studies to test this hypothesis directly (33). The acute infusion of AmB produced a reduction in single-nephron GFR; however, the blockade of the macula densa signal by proximal fluid collections did not prevent the reduction in filtration rate. After the acute infusion of AmB, distal chloride concentration, the presumed signal in TGF-mediated vasoconstriction, did not increase and we were unable to detect any measurable increase in TGF sensitivity. Our results do not provide support for a role of the TGF mechanism in the AmB-induced reduction of GFR.

**DIRECT VASOCONSTRICION AS A POSSIBLE CAUSE FOR AMB-INDUCED NEPHROTOXICITY**

Because TGF activation did not appear to be responsible for the vasoconstriction, we performed studies to examine whether the effect of AmB on RBF could be a direct vascular effect. For these experiments, we used *in vitro* preparations of isolated afferent arterioles, renal artery, and aorta from rabbit. We observed that AmB is a potent vasoconstrictor of preglomerular arterioles (Figure 1) and both renal and nonrenal arteries (Figure 2) (33). The contractile response of isolated vessels to AmB was significantly reduced by atrial natriuretic peptide and theophylline. The methylxanthines may alter the vascular response to AmB by inhibiting phosphodiesterase and increasing intracellular cyclic nucleotide levels. Because of the dose range used, an effect mediated by adenosine receptor blockade is less likely. These results provide possible alternative explanations, other than TGF blockade, for the beneficial effect previously demonstrated in intact animals of both salt loading and pretreatment with aminophylline.

Salt loading may exert its protective effect by increasing atrial natriuretic peptide levels. In addition, it has been shown in rats that salt loading stimulates the endogenous production of the vasodilator nitric oxide (NO) (77,78). The protective effect of NO against AmB-induced vasoconstriction is suggested by our observation that *in vitro* endothelium-denuded vessels...
consistently generated more vasoconstriction than the intact vessels (Figure 2) (33). In a recent preliminary study, Bernardo et al. also suggested that increased NO production protects against the nephrotoxic effects of AmB (79). Although in this study, the administration of L-arginine (a substrate of NO synthesis) to rats treated acutely with AmB prevented a significant fall in GFR, there was no protective effect against the AmB-induced reduction in RBF (79). Therefore, it is possible that L-arginine supplementation protects against the AmB-induced fall in GFR by a nonvasoconstriction-dependent mechanism(s) (see below).

THE ROLE OF VOLTAGE-DEPENDENT CALCIUM CHANNEL ACTIVATION

Tolins and Raij demonstrated that verapamil inhibits completely the vasoconstrictive effect of acute AmB infusion in rats (58). They have also shown that cardizem, another calcium channel blocker, has a protective effect against the decrease in GFR and RBF during the chronic use of AmB in rats (59). We performed a series of in vitro studies to evaluate the role of voltage-activated calcium channels on the vasoconstrictive effects of AmB (33). Pretreatment with verapamil significantly blunted the vasoconstrictor effect of AmB on rings of rabbit aorta and the addition of verapamil to these rings during AmB-induced contraction significantly reduced the force generated in both intact and endothelium-denuded vessels. We also found that this vasoconstriction was dependent on the presence of extracellular calcium (33).

The abolition of constrictor effects of AmB in calcium-free solutions together with the inhibitory effect of the calcium channel blocker verapamil supports a dependence of AmB-induced vasoconstriction on calcium influx through voltage-dependent calcium channels. As indicated earlier, AmB incorporated into membranes generates channels that are preferentially permeable to univalent ions (16,19,20). Therefore, it is predictable that AmB causes an alteration in sodium influx, leading to cell depolarization. Depolarization may be followed by increased calcium influx through voltage-gated calcium channels (80). A depolarization effect of AmB has been demonstrated in skeletal muscle cell (81) and necturus gall bladder cells (82). We are not aware of a study where the effect of AmB on vascular smooth muscle cell membrane potential was evaluated directly; however, indirect evidence for a depolarizing effect of AmB is provided in our studies by the additional observation that low concentrations of ouabain significantly accelerated the onset of AmB actions (33). We suspect that the depolarizing effect of ouabain was additive to that of AmB in facilitating the entry of calcium (83). Furthermore, BAY K 8,644 a dihydropyridine agonist, did not cause vasoconstriction in untreated rings of rabbit aorta but produced constriction in the presence of subthreshold concentrations of AmB. It has been shown that the facilitation of calcium entry by dihydropyridine agonists can only be elicited if slight cell depolarization is present (84-86), an effect that may have been produced by exposure to AmB. Although there is some evidence that AmB at high concentrations can act as a calcium ionophore (87), one would not expect verapamil to block an ionophore effect. It therefore appears more likely that AmB alters calcium fluxes by changing membrane potential.

THE ROLE OF ARACHIDONIC ACID METABOLITES

There is also evidence that AmB activates the synthesis of arachidonic acid metabolites. The incubation of AmB with mouse macrophages or human mononuclear cells in vitro leads to a substantial increase in prostaglandin E2 production (88). In vivo, AmB administration to rats causes a marked increase in prostaglandin synthetase activity in the kidney (89). In a recent study, a specific thromboxane A2 receptor antagonist, SQ 29,548, was found to reverse AmB-induced renal vasoconstriction (90). The local release of potent vasoconstrictive metabolites of arachidonic acid, such as thromboxane A2, may mediate, in part, AmB's effect on smooth muscle tone. We speculate that AmB, by depolarizing vascular smooth muscle...
cells, activates voltage-dependent calcium channels; the resultant surge in intracellular calcium concentrations activates phospholipase A₂ (PLA₂), which is the primary enzyme regulating arachidonic acid release (91). In support of PLA₂ activation is the finding that a specific platelet-activating factor receptor antagonist blunted the renal vasoconstriction induced by AmB in rats (92). Platelet activating factor, an important lipid mediator, is also modulated by PLA₂ (91,93). Oxidative cell injury caused by AmB could also stimulate the release of arachidonic acid metabolites (90,94).

**NONVASOCONSTRICTION-DEPENDENT EFFECTS ON GFR**

Several studies have revealed more prominent reductions in GFR than in RBF during the acute infusion of AmB (Table 3). Furthermore, a dissociation between the reduction in GFR and the fall of RBF was accomplished by pharmacologic manipulations (58,93), so other mechanisms may contribute to the reduction in GFR. AmB may decrease the glomerular ultrafiltration coefficient acutely (76), and/or the recurrent vasoconstriction and ischemia may have chronic structural effects on the number and surface area of filtering glomeruli, although most studies suggest only modest anatomical changes. It has been shown recently that chronic AmB administration is associated with an increase in urinary tubular cell excretion (95). This tubulotoxic effect was improved with fosfomycin, a lysosomal membrane stabilizer. It is also possible that AmB, by altering membrane permeability to ions, increases the amount of oxygen consumed in active electrolyte transport. This coupled with limited oxygen supply, owing to vasoconstriction, may lead to significant cellular injury, particularly at the level of the medullary thick ascending limb of Henle (96,97). Pentoxifylline, a hemorheologic agent, was found to ameliorate the fall in GFR induced by AmB in rats (98,99), although this agent appears to be quite nonspecific (100–102).

**PROTECTIVE MEASURES AGAINST AMB NEPHROTOXICITY IN HUMANS**

As indicated earlier, there are a number of animal studies showing a beneficial effect of certain maneuvers and drugs in preventing AmB toxicity, although very few of these manipulations have received careful clinical testing. In spite of the large number of patients treated with AmB, prospective randomized studies are difficult to conduct because the majority of patients are exposed to a number of nephotoxins and other potential renal insults.

Several retrospective or prospective noncontrolled studies have indicated a potential salutary effect of salt loading in AmB-induced nephrotoxicity (103–105). Recently, in a prospective, randomized, placebo-controlled study involving 20 patients, Llanos et al. have demonstrated a protective effect of salt loading (1 L of 0.9% NaCl) against reductions in renal function by AmB (55). Compared with the salt-treated group, creatinine clearance decreased significantly more in the control group. Although none of the patients had an increase in serum creatinine of >2 mg/mL, 4 of 10 patients in the control group and none in the salt-treated group had a doubling in their serum creatinine. Interestingly, AmB-induced hypokalemia was significantly worse in the saline-treated group.

In dogs, mannitol has been found to have a protective effect against the AmB-induced reduction in GFR (106). In humans, a case report (107) and a noncontrolled study in four transplant patients (108) also suggested a potential benefit from mannitol. However, in a small, prospective, double-blind, controlled study involving 11 patients, there was no protective effect of mannitol on the reduction of GFR measured by inulin and creatinine clearances (109). On the basis of this study, mannitol has not been used routinely in patients receiving AmB.

In dogs, furosemide also attenuates the reduction in RBF and GFR produced by AmB (50). To our knowledge, there is no human study to address directly the protective effect of furosemide. However, in a case-controlled study (110), diuretics administered during the course of AmB up to 3 days before the onset of nephrotoxicity conferred a 12.5-fold increase in the risk of nephrotoxicity, and there was a statistically significant linear correlation between total furosemide dose and nephrotoxicity. These findings suggest that in most clinical settings, protection with furosemide is unlikely to be achieved, perhaps because adverse effects of hypovolemia outweigh any direct protective effect.

The possibility, suggested by the animal studies reviewed above, that bicarbonate, aminophylline, and/or calcium channel blockers might be protective awaits examination by prospective clinical studies. All three types of manipulation would appear to have manageable toxicities and potential for clinical application. In a prospective, randomized, double-blind study, ibuprofen was found to ameliorate certain AmB-induced systemic effects, particularly fever and chills (88). However, in this study, kidney function was not assessed. In spite of evidence for a kidney-protective effect of cyclooxygenase inhibition in animal studies, this particular manipulation is obviously not a promising avenue in patients, in view of the propensity of nonsteroidal anti-inflammatory agents to worsen nephrotoxic injury (111). However, the selective inhibition of thromboxane formation or action would appear a more promising avenue for future study. It is important to bear in mind that any maneuver demonstrating protection against AmB-induced nephrotoxicity must concomitantly provide evidence of an unaltered fungicidal effect.

**THE ROLE OF RAPID AMB INFUSION RATE**

On the basis of practicality and convenience, there is a growing interest in increasing the rate of AmB
infusion from the current customary infusion time of 4 to 6 h. Although several recent studies suggest that it may be possible to reduce infusion time without markedly increasing general toxic symptoms such as fever and chills, none of these studies have critically addressed the effect of infusion time on renal function (112–117). There is some anecdotal evidence that rapid infusion may occasionally be associated with the development of malignant hypertension (118,119). In pilot studies, performed as a prelude to the micropuncture series discussed above, we observed that in the rat, the acute reduction in RBF with AmB infusion was much more marked with rapid infusion (P.B. Sawaya, unpublished observations).

There is some support from the clinical literature for the inference that the nephrotoxicity may be greater with rapid infusion. In one study (116), creatinine clearance, calculated by the Cockcroft and Gault formula, decreased in 6 of 11 patients in the rapid infusion group (45 min) and 2 of 9 patients in the slow infusion group (4 h). In a second study (117), 3 patients receiving AmB infusion over 4 h were compared with 22 patients receiving rapid infusion. In the rapid infusion group, the mean creatinine values doubled during AmB therapy, and 14 of 22 patients had an increase in serum creatinine of >0.5 mg/mL. Although the incidence of nephrotoxicity in this group was relatively high, in the small control group of three patients receiving the slow infusion, all had an increase in serum creatinine of >0.5 mg/mL. Clearly, these studies do not resolve whether or not rapid infusion is associated with increased renal risk. In our opinion, future evaluations of rapid AmB infusion rate should critically investigate its effect on renal function with accurate clearance techniques. In addition, studies carefully assessing RBF by isotopic or clearance methods (118,119) would be of interest because it is possible that the occasional development of malignant hypertension reflects a particularly intense renal vasoconstrictive response.

THE ROLE OF LIPOSOMAL AMB

Liposomes are small vesicles consisting of a phospholipid bilayer (120). Because of its lipophilic properties, AmB can be incorporated efficiently into liposomes. Liposomal-AmB (L-AmB) can be infused intravenously and is currently being evaluated as a therapeutic alternative. L-AmB is preferentially taken up by the reticuloendothelial cells and macrophages; therefore, drug delivery to sites of fungal infection may be enhanced. Because of changes in the interactions of the drug with mammalian cells, L-AmB appears to be less toxic and to enhance the therapeutic index of AmB by 20-fold or more (121–123). There is evidence for its effectiveness in the treatment of experimental fungal infections (124,125) and in clinical trials (126–128).

L-AmB is currently available only as a compassionate investigational drug in patients for whom conventional AmB therapy has been unsuccessful or for whom severe reactions to free AmB have been suffered. Initial reports suggest that L-AmB side effects are considerably less pronounced than those of free AmB and nephrotoxicity is probably less common (126,128). Serum creatinine levels increased in only 11 of 126 patients studied by Meunier et al. (128). It is possible that "new" side effects might emerge with the use of L-AmB. Levine et al. have described the occurrence of pulmonary edema with increasing pulmonary and systemic vascular resistances and decreasing cardiac index in a patient receiving L-AmB (129). Future prospective, randomized, controlled studies evaluating conventional AmB and L-AmB are needed to definitively evaluate the efficacy and safety of this new therapeutic modality. Similarly, further studies are needed to evaluate recent reports suggesting that other formulations of an emulsion of AmB in lipid reduce its side effects (130–133), including its renal toxicity (132,133).

SUMMARY

AmB has remained the "gold standard" therapy for serious fungal infections, and its use has been increasing during the last decade. Recently, several experimental studies have appeared in the literature that have helped us better understand the mechanism by which AmB exerts its nephrotoxic effect. By changing tubular cell permeability, AmB may lead to potassium wasting, decreased medullary tonicity, and diminished acidification capacity. By changing vascular smooth muscle cell permeability, AmB may lead to cell depolarization with the resultant opening of voltage-dependent calcium channels and muscle contraction. Increased intracellular calcium concentration may activate arachidonic acid metabolism and lead to the accumulation of vasoactive substances with a net vasoconstrictive effect. Renal vasoconstriction appears to play a major role in AmB-induced reduction in GFR, and recurrent ischemia may lead to structural and tubular damage and permanent nephrotoxic effects. Several protective measures have been shown to be effective in experimental studies. However, salt loading is the only measure proved by controlled prospective studies to ameliorate AmB nephrotoxicity in humans. The use of aminophylline, calcium channel blockers, and bicarbonate awaits future prospective studies. L-AmB is a promising new agent that may provide a unique protective effect on the basis of altering the affinity of AmB for mammalian cell membranes while preserving high efficacy against fungal cells.

REFERENCES


