NEPHROTOXICITY OF AMPHOTERICIN B

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The introduction of amphotericin B (AmB) in 1956 heralded a new era in the treatment of systemic mycoses. Many of these diseases had been fatal because of a lack of effective treatment. For example, the fatality rate of cryptococcal meningitis had been 100 percent; with AmB, the rate decreased to 15 percent (1). Unfortunately, along with the therapeutic successes of AmB, serious toxicities were observed, among which was nephrotoxicity. The nephrotoxicity of AmB has been the greatest limiting factor in its use (2, 3). Despite this problem, AmB is the predominant antifungal drug used today (3-6) and will probably continue to be so for a number of years. This study reviews the history of AmB with respect to nephrotoxicity, examines the theories explaining nephrotoxicity and reviews measures taken to ameliorate the nephrotoxicity. Some new formulations of AmB are described.

EARLY EXPERIENCE

Clinical observations. Amphotericin B is a natural product isolated from an actinomycete, Streptomyces nodosus, originally found in a soil sample from the Orinoco River region of Venezuela (1, 7, 8). In vitro studies revealed that AmB had a wide spectrum of antifungal activity (8-10). The drug initially was administered orally, but large single doses (as much as 8 g) of oral crystalline AmB (C-AmB) only occasionally resulted in measurable blood levels and therapeutic efficacy (8, 11). Intramuscular administration of C-AmB resulted in local pain and poor bioavailability (8). Intravenous administration of C-AmB produced therapeutic successes but there were problems because of its insolubility in water (10). To be given intravenously, the infusion bottle containing the C-AmB required agitation every ten minutes to keep the drug in suspension. This frequently resulted in erratic, and sometimes dangerous, concentrations of the drug in the plasma, even when the infusion volume rate was constant.

E.R. Squibb and Sons, Princeton, NJ, which had originally isolated AmB, marketed a 0.7

combination of sodium deoxycholate and AmB, which had been lyophilized and subsequently mixed with 5 percent dextrose solution, producing a “solubilized” form of AmB (10). Sodium deoxycholate is a detergent. Microscopic examination of a deoxycholate-AmB (D-AmB) mixture revealed that it was actually a colloidal solution (12). This form of AmB could be given intravenously with an appearance rate in the plasma that was steadily proportional to the infusion rate. Therapeutic successes were subsequently noted with D-AmB in the treatment of cryptococcosis, histoplasmosis, blastomycosis, coccidioidomycosis, moniliasis, and other fungal infections (8, 10, 11, 13).

In conjunction with these successes, it was apparent that administration of D-AmB resulted in acute toxic reactions in most patients (8, 10). These reactions included fever, nausea, vomiting, abdominal pain, phlebitis at the peripheral infusion site, and rarely, anaphylaxis, arrhythmias, and death (14). It was found that slow or intermittent infusion could reduce the frequency and severity of acute toxicity (10). Administration of nonsteroidal anti-inflammatory drugs (13), antihistamines (10), narcotics, and corticosteroids (15) before or with AmB seemed to obviate some of infusion-related symptoms.

Unfortunately, administration of D-AmB also resulted in an untoward effect that lasted beyond the duration of the infusion, namely, nephrotoxicity. "Nitrogen retention" was described as a side effect of AmB in 1958 (10, 11), occurring in more than 50 percent of those treated (16, 17). These patients were observed to have increased blood urea nitrogen (BUN) within two weeks after initiation of therapy (18). Further characterization of this change revealed that serum creatinine increased (19), the inulin clearance (a measure of glomerular filtration rate [GFR]) decreased (20, 21), the para-aminohippurate (PAH) clearance (an approximation of renal blood flow, [RBF]) decreased (18, 20, 22), the secretion of PAH and phenolsulfonphthalein (measures of tubular function) decreased (17, 22), and the urine specific gravity and concentrating ability decreased (17, 20, 22, 23). Compensated metabolic acidosis was also observed.
(18). The decrease in inulin clearance is proportionally greater than the decrease in PAH clearance, that is, the GFR decreases more than the RBF. Not infrequently, the decrrement in renal function progressed so that therapy had to be interrupted. When AmB was stopped, renal function usually improved over a period of months (19, 22), suggesting a functional deficit. However, defects in tubular function sometimes persisted and permanent renal damage from AmB was suspected (16, 21).

Renal tubular acidosis (RTA), hypomagnesemia, and hypokalemicemia also occurred with AmB therapy. A urine acidifying defect was noted to be prevalent (24-28). The site of the RTA was believed to be the distal tubule because of the inability of the affected kidney to excrete an acid load and the lack of accompanying glycosuria or aminoaciduria (proximal tubular defects). Hypokalemia occurred in some patients on AmB, requiring potassium supplementation and occasionally resulting in severe complications (29). Patients were noted to waste potassium in the urine (11, 29), and a tubular defect was implicated. Renal magnesium wasting also was observed (30), and occurred despite a decreased tubular load of magnesium, again suggesting a tubular resorption deficit.

Amphotericin-B nephrotoxicity in humans can be separated into: pretubular and tubular effects. Restated, the pretubular effects include a decrease in RBF and GFR and the tubular effects include loss of electrolytes in the urine (especially potassium and magnesium), distal tubular acidosis, a decrease in tubular secretion, and loss of urinary concentrating ability. These effects, which were described 20 or more years ago, still encompass the known effects of AmB on renal function.

Histopathologic factors. The initial clinical observations of AmB nephrotoxicity did not prove that permanent renal damage was occurring, but this effect was subsequently shown by histologic examination of biopsy and autopsy specimens. Hyaline casts and amorphous debris could be demonstrated in the urine at some time in almost all patients treated with AmB (18, 22, 31). Variably, the presence of white blood cells, proteinuria, tubular cells, and crystals also could be shown (18, 22). The abnormal urine sediment would resolve after completion of therapy. Renal tissue specimens obtained from biopsies or at autopsy of patients who received AmB revealed calcium deposits in the tubule lumen, tubule cells, and interstitium (18, 32-34). Nephrocalcinosis occurred in one patient who received as little as 1.56 g, but not in a second patient until 9.8 g was administered (18). Articular vacuolization with subintimal proliferation was noted in other biopsy studies (35, 36). Further characterization of the histopathologic factors using renal biopsies from patients before, during, and after AmB therapy confirmed the aforementioned findings and also demonstrated thickened basement membranes, tubular cell vacuolization, epithelial cell proliferation in the glomeruli, and glomerular hyalinization (16, 31). Such studies provided evidence that permanent renal injury could result from AmB administration. The renal histopathologic factors in dogs and rats were similar to those found in humans (34, 37, 38).

Dose dependency. Clinical evidence of permanent injury was demonstrated in patients who, while on AmB, either died from gradually worsening uremia (15) or required renal homotransplantation (33) for survival. These patients received large (greater than 14 g) cumulative doses of AmB. However, not all patients who received such large doses did so poorly. While there does seem to be a correlation between permanent renal injury and large cumulative doses of AmB, there is interpatient variability in the appearance and degree of AmB nephrotoxicity (39). One hundred patients were followed for at least 48 months and a persistent loss of renal function was seen in almost all patients receiving 5 g or more of AmB (15). About one-third of patients receiving 2 to 5 g had laboratory evidence of permanent renal damage. Other investigators (16) evaluated 45 patients for a minimum of three months after completion of AmB therapy and found that 44 percent of those who received more than 4 g of AmB had laboratory evidence of permanent renal injury, while only 17 percent of those who received less than 4 g had permanent injury. Data from others (39) revealed that, after one to 50 months of follow-up evaluation, five of seven patients receiving more than 2.5 g of AmB had a persistent loss of creatinine clearance and eight of 17 patients who received 0.5 to 2.0 g had a similar loss. It seems that permanent renal damage is more apt to occur with large doses of AmB. Permanent injury can occur with smaller doses, but is unpredictable (16).

That the use of AmB could result in serious and occasionally fatal nephrotoxicity that seemed dose dependent suggested that AmB therapy
PHARMACOKINETICS

Doses of Amb in the range of 0.5 to 1 mg per kg intravenously produce peak serum levels in the range of 1 to 3 μg per mL (40–42). Peak serum concentrations seem to plateau at doses above 50 mg (40). In serum, the drug is 95 percent bound to erythrocytes and lipoproteins (40, 43). The total volume of distribution in humans is about 3.2 to 4 L per kg (44, 45). The distribution follows a three compartment model (44): two quickly equilibrating compartments (the blood volume and the interstitium) and one slowly equilibrating compartment (the tissues). In autopsy studies in humans treated with Amb, the highest tissue concentrations of Amb were found in the liver (27 percent of total dose), followed by the spleen, lung, and kidney (46, 47). The muscle and the adipose tissue did not contain significant amounts of Amb. It has been suggested (46) that the reticulo-endothelial system in the liver, spleen, and lung removes the colloidal particles of Amb-deoxycholate from the circulation, accounting for the high Amb content of these tissues. The affinity of Amb for steroids may account for the slow elimination of Amb from the tissues.

In contrast to reports on the disposition of Amb noted in autopsy of humans, a study using radiolabeled Amb in nonhuman primates revealed that the highest concentration of the drug 24 hours after a single dose was in the kidneys, followed by (in decreasing order) the liver, spleen, adrenal glands, lungs, thyroid, heart, skeletal muscle, pancreas, brain, and bone (48). The elimination of Amb seems to be biphasic, with a 17 to 24 hour half-life and an 11 to 15 day half-life characterizing the initial and secondary phases, respectively (44, 45). Despite its long half-life, continued dosing of Amb does not result in progressively higher serum levels (40, 49). The drug is still detectable in serum and tissues up to one month after cessation of therapy (41, 46, 50). Amb does not seem to be metabolized, as shown by high-performance liquid chromatography analysis of human tissues (46). About 3 percent of an initial dose of Amb appears in the urine in 24 hours (42, 44). Forty percent of a single dose appeared in the urine of one patient in one week (42). However, absence of urinary excretion in another patient did not appreciably affect the elimination of Amb (49). In fact, the clearance of Amb from plasma may be higher in patients with renal failure compared with patients without renal failure (51). In a dog, 19 percent of a single dose of Amb was excreted in the stool in 11 days. In dogs with biliary diversion, the stool did not contain any Amb (52). Considering the high liver concentrations of Amb found in humans, it is reasonable to assume that biliary excretion is a significant route of elimination. However, urinary and biliary excretion do not totally account for the elimination of Amb. The pharmacokinetics in neonates is highly variable (53). All metabolic degradation routes currently are incompletely characterized (43).

The relevance of this information with respect to Amb nephrotoxicity is that the drug is concentrated in the kidney, among other organs. There is a small but significant urinary excretion, which means that the luminal side of the tubular cells is exposed to the drug. Because of the affinity of Amb for certain tissues, prolonged administration of Amb results in increasing tissue concentration (47), in the kidney and thus provides an opportunity for Amb to exert toxic effects directly on renal cells.

MECHANISM OF TOXICITY

The mechanism of Amb toxicity and specifically its nephrotoxicity, is incompletely understood. The following is a summary of the observations and hypotheses that surround the issue of Amb nephrotoxicity.

Administration to animals. Infusion of Amb (5 mg per kg over 30 minutes) into the renal artery of anesthetized dogs (54), results in an abrupt decrease in inulin clearance (by 90 percent), PAH clearance (by 88 percent), tubular secretion of PAH, and urine volume. Grossly, the infused kidneys blanch, soften, and decrease in size. The renal veins collapse. Pretreatment with adrenergic or ganglionic blocking agents does not affect these changes. Arteriography of the kidneys after Amb infusion shows no contrast in the cortex, an observation consistent with arteriolar constriction. All of these changes are reversible; the kidneys resume function after four to six hours. Pretreatment with Amb for six months did not produce tolerance to renal artery infusion of dog kidney. It is evident from this study that Amb is a renal vasoconstrictor.

Chronic administration of Amb to dogs resulted in dose-dependent azotemia and death when the daily dose was 0.5 mg per kg intravenously or greater (55, 56). Dosing every other day produced
increased survival and slower progression of azotemia. The intravenous administration of 5 mg per kg AmB as a bolus could result in ventricular fibrillation and death.

Administration of AmB (1 mg per kg over one hour) intravenously to anesthetized rats (57) resulted in 70, 40, and 25 percent decreases in inulin clearance, renal plasma flow (RPF), and glomerular capillary pressure, respectively, as well as a twofold increase in renal vascular resistance. There was a 55 percent decrease in the recovery of radiolabeled inulin that had been injected into the tubules. All of these changes were observed one to five hours after completion of AmB infusion. Inulin clearance and RPF returned to normal after 24 hours. It was concluded that AmB had two functional intrarenal effects: increased vascular resistance, and increased tubule permeability (demonstrated by the diffusion of labeled inulin out of the tubules). Because the decrease in inulin clearance was greater than the decrease in RPF, the site of the predominant vasoconstrictor effect of AmB was thought to be the afferent arteriole.

Effect on membranes. Investigation into the cellular toxicity of AmB and other polyene drugs revealed that they could lyse fungal cells and mammalian erythrocytes (58, 59). When added to the mucosal (but not serosal) side of a toad bladder preparation, AmB greatly increased membrane permeability to potassium, chloride, and thiourea, but less so to sodium (60). Permeability changes also were noted in mammalian kidney tubule cells (61, 62) and Candida albicans cells (63). AmB was found to be nontoxic to bacterial and other membrane systems not possessing sterol components (64). A sterol-containing membrane seemed a prerequisite for AmB to exert its increased permeability effect. The basis of the greater toxicity of AmB for fungal cells was its higher affinity for the fungal sterol, ergosterol, versus the mammalian sterol, cholesterol (65–69).

An examination of AmB and sterol structure can aid understanding of this selective affinity. The structures of cholesterol, ergosterol, and AmB are illustrated in Figure 1. The sterols have a hydrophobic backbone, which resides within the lipid bilayer of the membrane, and a hy-
drophilic end (the hydroxyl group), which is at the membrane surface. Amphotericin B is a polyene macrolide (64, 70). A macrolide is a lactone ring (a carbon-backboned ring joined by an ester linkage) with numerous hydroxyl groups. Polyene refers to the seven double bonds in the ring. Amphotericin B has a hydrophobic side (the polyene chain), a hydrophilic side (the saturated chain with the hydroxyl groups), and a hydrophilic head (the carboxyl group at C-16). The attraction between AmB and sterols seems to be the result of hydrogen bonding between the hydrophilic groups of AmB and sterols and to van der Waals forces between the hydrophobic portions of the molecules (64–71).

The relative contributions of each of the binding sites to the overall attractive force between AmB and sterols may be the basis for selective affinity of AmB. In vitro studies have attempted to define these contributions, but the results are conflicting. One investigation, using a protein-free lipid and sterol vesicle as a model, concluded that hydrophilic interaction between the polar heads is predominant (71). Another in vitro study using a different model found that AmB had a 15-fold greater affinity for membranes containing ergosterol compared with cholesterol (72). This in vitro finding is closer to the situation observed in vivo. The molecular basis for the selective binding seen in vivo was believed to reside in the extra double bonds in the ergosterol backbone and in its alkyl chain (Fig. 1) (64, 73). This provided a flat molecular surface with which the flat polyene portion of AmB could interact. The better "fit" between ergosterol and AmB increases the efficiency of the hydrophobic attraction.

The binding of AmB and sterol conceivably could produce a membrane pore (Fig. 2). In this model, AmB molecules are situated in the membrane with their hydrophobic side facing outward toward the sterols and the hydrophilic side facing and lining the inside of the pore. This would allow flux of water, electrolytes (anions more than cations, monovalents more than divalents), and solutes smaller than sucrose through the membrane (64). The resulting permeability change might cause cell swelling, lysis, and death (74).

Another possible mechanism by which AmB may alter membrane permeability is by inhibition of the sodium and potassium pump. Others (75) showed that AmB (5 μM) can completely inhibit this pump in human erythrocytes, resulting in potassium leakage from the cell into the interstitium. Although altered membrane permeability is induced by AmB, it may not be the mechanism of cell death.

Other membrane studies examined changes in proton exchange induced by AmB. Investigators using the turtle bladder (76, 77) and rat distal tubule (78) found that in the presence of AmB, cells could not produce a net outward flux of protons. Amphotericin B did not have an effect on proton secretion or bicarbonate permeability,
but allowed the membranes to become “leaky” for protons. This formed a basis on which AmB-induced RTA could be explained. Renal tubular acidosis seems to be a back diffusion, not a secretion, problem. The resultant acidifying defect could worsen urinary potassium losses because potassium may be pulled into the tubular lumens as net proton secretion decreases and the proton and sodium exchange mechanism falters (28). That is, potassium ions (in place of protons) may be “exchanged” in the distal tubule for sodium ions. This provides a rationale for alkalization of the urine through sodium bicarbonate administration. Alkaline tubular fluid during AmB therapy would decrease the proton gradient through which proton back diffusion occurs, allowing for net proton secretion. Potassium substitution in the sodium and proton exchange then would be prevented and urinary potassium losses would be less.

Role of oxidation and eicosanoids. We have described the so-called sterol hypothesis (64) of AmB toxicity and lethality. According to this hypothesis, AmB uptake by a target cell is dependent on the presence of sterol in the cell membrane, and the toxic and ultimately lethal effect is the result of disruption of membrane integrity through pore formation. However, other evidence indicates that the sterol hypothesis probably does not entirely explain the lethal action of AmB on renal cells. Others (79) studied four polyene antibiotics with varying permeability-inducing potencies and did not find a relationship between the degree of potassium permeability and lethality. Strains of Candida albicans resistant to the lethal effects of AmB still have permeability changes induced in the cell membrane by AmB (80). These resistant strains have increased, decreased, or unchanged levels of membrane ergosterol compared with wild C. albicans (64).

Others (73) found that changing the culture medium could modify in vitro cell killing by AmB without affecting the permeability effect. It was also determined (81) that AmB-induced lysis of human erythrocytes was retarded in the presence of either low oxygen tension or added catalase and was accelerated with the addition of ascorbate. Malonyldialdehyde, an end product of lipid peroxidation, was produced during AmB-induced red blood cell lysis. Potassium permeability was unaffected by manipulation of oxygen, catalase, or ascorbate. The hypothesis generated (82) to explain these findings was that cellular killing by AmB was primarily a result of oxidative injury to the cell membrane and that the permeability changes secondary to pore formation may or may not contribute to lethality. This hypothesis was supported by the observation that AmB auto-oxidizes in solution to form free radicals (83). Further evidence for the oxidative hypothesis was the independence of the permeability changes from the catalase, ascorbate, and oxygen-dependent AmB-induced killing of C. albicans (82, 84). Resistant strains of C. albicans have also been shown to have a two- to fourfold increase in catalase levels (85).

In vivo studies with AmB supported the hypothesis of oxidative damage and also suggested a role for eicosanoids. Using the rat lung, others (86) showed that a 1 mg per kg intravenous bolus infusion of AmB in rats resulted in acute pulmonary injury with vascular extravasation of protein and fluid. Histologic examination revealed disruption of endothelial integrity. There also was a pulmonary vasoconstrictive effect that could be attenuated with papaverine. Oxidative metabolites were produced; antioxidants reduced the lung injury. Interestingly, inhibition of leukotriene production decreased both pulmonary vasoconstriction and injury induced by AmB, suggesting a role of eicosanoids in the AmB toxicity of rat lung.

Infusion of AmB (1 mg per kg) into awake sheep decreased lung compliance and increased pulmonary artery pressure and airflow resistance, that is, there was smooth-muscle constriction (87). Thromboxane B2, a stable metabolite of the potent vasoconstrictor thromboxane, was found to peak in the lymphatic effluent correlated in time with the pathophysiologic changes. Pretreatment with ibuprofen attenuated the effects of smooth-muscle constriction, suggesting that thromboxane was the mediator of these acute changes (88). While there have been no direct investigations into the role of eicosanoids in AmB nephrotoxicity have been done, it is conceivable that AmB could release eicosanoids in the kidney to cause hemodynamic changes. It has been shown that AmB stimulates prostaglandin E2 release from human mononuclear cells in vitro (89).

The action of AmB at the cellular level has not been completely elucidated (73). Permeability changes because of AmB are well characterized, but there seems to be a dissociation between these changes and lethality. Amphotericin-B-associated oxidative injury seems plausible. Prostaglandins and leukotrienes may have a previously
unemphasized but important role in AmB toxicity. Further work needs to be done.

Manipulation of nephrotoxicity in animals. Concurrent with in vitro attempts to determine the mechanism of AmB toxicity, in situ and in vivo experiments investigating the prevention of nephrotoxicity were performed. Others (90), using a perfused rat kidney model, showed that AmB produced injury to the medullary thick ascending limb and that this injury could be prevented with ouabain. Their view was that by increasing membrane permeability, AmB increased oxygen-dependent transport, that is, the cellular compensatory response to altered membrane permeability, beyond the capacity of the relatively hypoxic medulla to support it. This chain of events ultimately would result in cell death, although the exact mechanism was not specified. When AmB-increased transport activity was blocked by ouabain, a sodium and potassium pump toxin, cellular injury was prevented.

Because AmB had been shown to act as a renal vasoconstrictor, the role of dopamine and angiotensin II in AmB nephrotoxicity was evaluated (91). It was found that either dopamine or saralasin, a vasopressin antagonist, partially antagonized AmB renal vasoconstriction, and when combined, the two agents normalized RBF and increased urine flow in response to AmB.

The protective effects of the dopamine and saralasin combination were not as complete regarding renal vascular resistance, which increased 196 percent with AmB alone compared with 41 percent with AmB and dopamine and saralasin, or regarding glomerular filtration rate (GFR), which showed 94 and 21 percent decreases in GFR without and with the protective drugs. Dopamine and saralasin together did not have any effect on the AmB-induced increase in systemic and pulmonary vascular resistance. Fenoldopam, a dopamine agonist with renal vasodilating properties (92), prevented the AmB-induced decrease in creatinine clearance in the dog (93). There was no effect on urinary loss of sodium. The fenoldopam dogs that were pretreated did exhibit reduced tubular necrosis compared with dogs receiving only AmB. It seems that drugs that improve RBF can ameliorate the nephrotoxicity of AmB, suggesting that nephrotoxicity, at least in part, is secondary to vasoconstriction.

Further exploration of the hypothesis that AmB nephrotoxicity was secondary to its vasoconstrictive effects was done (94). It was postulated that AmB might activate tubuloglomerular feedback (TGF). Animals that were sodium deficient demonstrated a shutdown of GFR in response to TGF stimulation. The investigators observed that dogs depleted of sodium had an exaggerated renal hemodynamic response to an intravenous bolus of AmB, but that dogs loaded with sodium did not respond to the same AmB infusion. They also noted a 64 percent decrease in the renal arteriovenous difference in renin, that is, renal renin secretion, which argued against a causative role for renin in AmB vasoconstriction. These observations are consistent with the hypothesis that AmB nephrotoxicity is the result of TGF activation.

Sodium loading in the rat does not prevent the acute renal hemodynamic change induced by AmB (95). However, sodium loading in the rat is somewhat efficacious in preventing chronic nephrotoxicity. In one study, sodium loading prevented changes in serum creatinine, GFR, and RBF, which occurred in sodium-depleted rats over 21 days of AmB administration; however, tubular function (concentrating ability, potassium conservation) deteriorated in rats that were sodium loaded and depleted (96). Another study in rats revealed that sodium loading initially prevented loss of creatinine clearance, but by the end of three weeks of AmB administration, clearance had decreased 43 percent (97). In this study, rats were sodium loaded with the aid of mineralcorticoid treatment; it is known that cortisone and AmB given together in the rat display synergism in producing nephrotoxicity (98). Thus, the mineralcorticoid in this study may have been a confounding factor. Interestingly, AmB accumulates in the kidneys of rats that are sodium depleted to a greater extent (21 times the serum concentration) than in the kidneys of rats that are sodium repleted (eight times the serum concentration) (97). Sodium loading prevents accumulation of AmB in the rat kidney so that stimulation of TGF may not be the predominant mechanism of AmB nephrotoxicity in this species.

In the dog, dependence of the acute renal vasoconstrictive effects of AmB on the level of sodium repletion suggested (94) that AmB was producing vasoconstriction through TGF. Because adenosine was the proposed mediator of TGF (99), the next logical step was to see if antagonism of adenosine reduced AmB-induced vasoconstriction. Others (100, 101) found in anesthetized, instrumented dogs that an intrarenal infusion of aminophylline, an adenosine antago-
nist, at a concentration sufficient to antagonize the hemodynamic effects of an intrarenal infusion of adenosine, also blocked the acute decrease in RBF and GFR produced by an intravenous infusion of AmB. In the rat (101), aminophylline prevented the AmB-induced decrease in RBF, but did not prevent the decrease in GFR. A species difference is apparent. Chronic theophylline administration in the rat ameliorated nephrotoxicity, as measured by inulin clearance, after seven days of AmB (102). Unfortunately, theophylline had no effect on tubular losses of sodium and potassium in this setting. These observations imply two separate sites of AmB action, pretubular and tubular, the former, involving vasoconstriction and loss of GFR, may be blocked by theophylline.

As described previously, sodium loading or administration of aminophylline could attenuate the acute vasoconstrictive response in the dog and chronic nephrotoxicity in the rat of AmB. The action of nonspecific vasodilators so far has not been shown to counter these AmB effects (101). Others (103) found that verapamil, a calcium channel blocker, could prevent the acute AmB-induced decrease in RBF in rats and partially antagonize the decrease in GFR. They suggested there were two mechanisms for the decrease in GFR after AmB administration, a calcium independent mechanism and a calcium dependent one. In preventing chronic (ten day) AmB nephrotoxicity in the rat, diltiazem was effective (104), but nifedipine was not (105). Although diltiazem did not completely eliminate AmB-induced loss of GFR, the differences in GFR in the rats treated with diltiazem and AmB were not statistically different compared with rats in a control group that were drug-free. It may be concluded that, in the rat, vasoconstriction, which can be antagonized with calcium channel blockers, is a significant, but not the only, mechanism of AmB nephrotoxicity.

Recently, more evidence has implicated vasoconstriction and decreased perfusion as the cause of chronic AmB toxicity. Others (106) showed that pentoxifylline (PTX), a hemorheologically active methylxanthine useful in claudication and cyclosporine-induced renal failure (107), was able to prevent both the acute and chronic (after ten days) decreased inulin clearance produced by AmB in the rat. On histologic examination of the kidneys, the only significant finding was that the rats treated with PTX had decreased vascular congestion compared with rats in a control group. It could be implied that ischemic congestion secondary to vasoconstriction results in a loss of nephron mass. However, the urinary loss of sodium and potassium resulting from AmB administration was unchanged by PTX. It seemed evident that PTX could improve RBF and attenuate chronic AmB nephrotoxicity in rats, but tubular handling of electrolytes was unaffected. Using a rat candidiasis model, others (108) observed that combination therapy with PTX and AmB decreased both nephrotoxicity and antifungal efficacy compared with monotherapy with AmB; however, AmB combined with HWA-138, a PTX analog, decreased nephrotoxicity but enhanced antifungal efficacy. In murine candidiasis, HWA-138 alone was found to have an antifungal effect and HWA-138 and AmB together acted synergistically (108, 109).

The mechanism by which PTX and its analogs reduce AmB nephrotoxicity is unclear. The mechanism probably does not involve hemorheologic modulation by itself. Pentoxifylline attenuates the decrement in inulin clearance induced by endotoxin infusion in rats, but vascular congestion is not a histologic component of this model (110). Pentoxifylline probably acts in this instance by modulating the inflammatory reaction, especially that involved with tumor necrosis factor (TNF). Tumor necrosis factor has multiple effects; intrarenal infusion into dogs causes marked diuresis and in rats results in acute tubular necrosis (111). The renal effect in humans is unknown. Tumor necrosis factor is produced mainly by inflammatory cells, but also in vascular smooth muscle. Amphotericin B has been demonstrated to induce TNF production in murine macrophages (112) and in humans (113). It is possible that AmB may stimulate intrarenal release of TNF, which might contribute to nephrotoxicity. This is a supposition; there are no directly supporting data. Pentoxifylline has been shown to block macrophage TNF release and both TNF and AmB activation of neutrophils (114, 115). Pentoxifylline also blocks numerous other inflammatory cell functions (116). However, inflammatory infiltrate is not a part of the histopathology of AmB nephrotoxicity, which creates doubt on a role of AmB-induced release of leukocyte TNF in the genesis of nephrotoxicity; moreover, TNF from vascular sources could participate. It would be reasonable to investigate the relationship, if any, between TNF, PTX and AmB.

Another possible mechanism through which
PTX may attenuate AmB nephrotoxicity involves prostaglandin release. Others (117) showed that micromolar concentrations of PTX stimulated release of prostacyclin, a vasodilator, from human vascular tissue and caused dose-dependent relaxation. This action possibly could counter AmB stimulated eicosanoid release, another putative mechanism.

The evidence from studies with animals indicates that sodium loading may prevent the acute and chronic decrease in GFR produced by AmB. Angiotensin II does not seem to have a direct role in acute toxicity. Aminophylline antagonizes acute loss of GFR. Calcium channel blockade may prevent the acute and chronic loss of GFR because of AmB. Dopamine agonism and blood viscosity manipulation seem to attenuate chronic toxicity without improving tubular dysfunction. Unfortunately, animal studies to date do not provide a unifying hypothesis to explain the decrease in GFR, tubular dysfunction, and loss of nephron mass that occur acutely and chronically with AmB administration.

Role of intrarenal regulatory mechanisms. Investigations independent of a focus on AmB nephrotoxicity have explored the mechanism of intrarenal regulation of GFR. Using micropuncture technologies, it was observed (118-120) that increased delivery of sodium chloride to the distal tubule resulted in a decrease in single nephron GFR. This phenomenon could be reproduced in the in situ kidney by infusing hypertonic saline solution into the renal artery (121, 122). The increased NaCl load to the distal tubules resulted in a decrease in GFR. In animal models, the filtration fraction also decreased, suggesting predominant afferent arteriolar constriction or a decrease in filtration coefficient. The dependence of GFR on distal luminal NaCl delivery is called TGF. It is a negative feedback system that decreases GFR when an increased flow of filtrate reaches the distal tubule. Theoretically, TGF would be important in acute renal failure (123) in which there is a loss of proximal resorption, with a resultant high flow of filtrate to the distal tubule. Tubuloglomerular feedback would be activated, GFR would decrease, and uncontrolled loss of water and electrolytes into the urine would be prevented.

The signal that initiates TGF seems to be an increased concentration of NaCl at the macula densa (124-126). The macula densa is intimately associated with the juxtaglomerular apparatus. In theory, upon initiation of TGF, the juxtaglomerular apparatus releases a paracrine factor to mediate the decrease in GFR. The identification of this factor is not certain, but adenosine and angiotensin in combination have been implicated (99). Experiments with micropuncture (127, 128) and in situ kidney preparations (129, 130) have been used to demonstrate that all the hemodynamic changes that occur in TGF can be mimicked with infusion of adenosine. However, angiotensin must be present for the adenosine reaction to occur. With in situ experimental preparations, both the adenosine (127, 129) and the TGF responses are exacerbated by a state of sodium depletion (high level of angiotensin), but these effects do not occur in an animal loaded with sodium (low level of angiotensin). Administration of angiotensin will restore TGF responsiveness in a rat loaded with sodium (131). The afferent arteriole is the likely site of the TGF effector mechanism (132, 133), that is, the site at which the putative paracrine factor stimulates a response.

The evidence that supports a physiologic role for TGF and adenosine in the kidney, and ultimately a role in AmB nephrotoxicity, is the response of the kidney to an ischemic insult. Ischemia induced by clamping the renal artery of an in situ animal kidney produces postclamping renal vasoconstriction (134) with a decrease in GFR and filtration fraction; in other words, a TGF response. Postischemic vasoconstriction in the kidney is in contrast to the "reactive hyperemia" that occurs in other tissues after an ischemic insult. Infusion of the venous effluent from a skeletal muscle bed that has experienced an acute increase in metabolic rate causes vasodilation of the vasculature of resting skeletal muscle, but vasoconstricts the vasculature of the kidney (135). This implies the presence of an agent released by a tissue under stress that produces vasoconstriction in the kidney and vasodilation in muscle.

Most evidence indicates that adenosine is the factor released by a tissue bed under stress, which serves to autoregulate the flow of blood to that tissue (136). Adenosine is released by the kidney (137, 138), skeletal muscle (139, 140), and heart muscle (139, 141) after periods of ischemia or increased metabolic rate. Infusion of adenosine at a physiologic concentration into an organ reproduces the vasoconstriction and vasodilation seen in the kidney (139, 142) and skeletal muscle, respectively. Two adenosine receptor subtypes have been identified, A1 and A2. Acti-
vation of each receptor results in vasoconstriction and vasodilation, respectively (143–145). The kidney is believed to possess predominately A1 receptors (145, 146). As mentioned previously, the posts ischemic renal vasoconstriction that probably is mediated by adenosine (with a permissive role of angiotensin) prevents uncontrolled loss of glomerular filtrate when tubular reabsorption fails.

In animal models, AmB produces an acute renal hemodynamic response that is identical to that produced by an arterial infusion of hypertonic saline solution or clamping ischemia (models of TGF) or infusion of adenosine (production of TGF by the putative mediator). As with TGF and the adenosine response, the AmB response of the kidney is exacerbated or absent in association with sodium depletion or sodium loading, respectively. The AmB response is antagonized by aminophylline (142) at concentrations that antagonize TGF and the adenosine response. Using these observations, it has been hypothesized (94) that AmB stimulates TGF presumably by increasing the permeability of the distal tubule to NaCl, which then triggers the juxtaglomerular apparatus to release adenosine. The subsequent TGF-mediated renal vasoconstriction was thought to be a mechanism of AmB nephrotoxicity.

A micropuncture study was performed in rats receiving AmB and showed, at the nephron level, that the action of AmB was similar to a TGF response (147). In addition, a decrease in filtration coefficient (Kf) was noted and it was also thought that there was mesangial cell contraction. Contraction of the mesangium, which can decrease Kf, and subsequently lower the GFR (148), had not previously been emphasized in explaining TGF.

In the in situ rat kidney, it was reported that an adenosine antagonist did not prevent acute renal vasoconstriction produced by AmB although TGF was inhibited (149). If adenosine mediates acute vasoconstriction because of AmB, then an adenosine antagonist should prevent AmB-induced vasoconstriction. Therefore, the study results are contrary to the AmB-TGF hypothesis. The previous observation that aminophylline antagonized AmB-induced vasoconstriction also is not in agreement with this newer data. However, this objection was explained by the fact that aminophylline has other effects aside from antagonizing adenosine, such as modulation of intracellular calcium.

Others (150) determined in vitro that AmB in micromolar amounts has a direct vasoconstrictive effect on the aorta and renal artery of a rabbit, which is antagonized by calcium channel blockers, by an absence of calcium in the medium or by aminophylline. With micropuncture experiments, they demonstrated that interruption of the TGF loop did not prevent the AmB-induced decrease in single nephron GFR. In dissected, isolated glomeruli, the authors found that micromolar concentrations of AmB stimulated different arteriolar constriction. They concluded that AmB did not act indirectly through TGF as a vasoconstrictor but more directly, possibly by augmenting calcium channel activity. This hypothesis is consistent with the systemic vasoconstriction action of AmB previously observed in the dog (91). Furthermore, it was reported that 10^-8 M AmB can stimulate contraction of cultured mesangial cells (unpublished data). In vivo, mesangial contraction would decrease GFR by decreasing Kf. With these results, the role of TGF in AmB nephrotoxicity currently is being deemphasized and the role of direct vasoconstriction is being more actively investigated.

There is another mechanism through which AmB may generate renal vasoconstriction. Epidermal growth factor (EGF), a polypeptide produced in the kidney (151, 152) among many other tissues, has been shown to decrease GFR, RBF, and Kf when infused into the kidney of a rat (158). Similar changes occur when AmB is infused. Epidermal growth factor also stimulates contraction in cultured mesangial cells, as does AmB. Conceivably, AmB may stimulate EGF release. The changes in renal hemodynamics after AmB administration might be secondary to EGF effects. No studies have been done to examine the role of EGF in AmB nephrotoxicity. It should be noted that EGF is produced in the rat only in the thick ascending limb of the loop of Henle and the distal convoluted tubule (151, 152), making a direct route to the glomerulus seem difficult. The fact that AmB can stimulate mesangial cell contraction in vitro without an apparent source of EGF to act as a mediator also argues against a role of EGF in AmB nephrotoxicity. The role of EGF in the kidney is still being defined. Although there seems initially to be evidence to the contrary, the role of EGF as a mediator for AmB nephrotoxicity should be investigated.

Summary of experimental work. The mechanism of AmB nephrotoxicity has not been elucidated. The participation of adenosine, TGF, and renal vasoconstriction have been investigated, but the
exact role of each factor has yet to be defined. A correlation between the membrane effects of AmB and its physiologic effects has yet to be made. There are pharmacologic manipulations (aminophylline, sodium chloride) that can attenuate acute, and possibly chronic, AmB nephrotoxicity in animals.

**CURRENT STATUS IN HUMANS**

The problem of AmB nephrotoxicity. Amphotericin B use has been increasing. The amount dispensed by the hospital pharmacy at Duke University, Durham, NC, increased tenfold between 1978 and 1984 (154). Furthermore, the indications for use of AmB are broadening. Originally, AmB was administered primarily to patients with deep-seated mycoses whose premorbid state of health was relatively unremarkable (10). Currently, there is an increasing number of patients with conditions that predispose them to fungal infections, such as iatrogenic, viral, or malignancy related immunosuppression, total parenteral nutrition, bacterial sepsis, and broad-spectrum antibiotic therapy. Many patients currently have some degree of multiple organ failure, especially renal failure, before AmB therapy leading to a markedly increased mortality risk if fungal infection occurs (155).

The current role of AmB in contributing to renal failure is not clear. Forty-seven surgical patients who were treated with AmB for Candida infections were reviewed and it was concluded that AmB was not a significant factor in the occurrence of renal failure (155). In another review of surgical patients with Candida infections (156), only one of 55 patients had AmB stopped because of having renal failure; the patient whose AmB treatment was discontinued subsequently died as a result of Candida sepsis. Others (157) reviewed 272 patients undergoing bone marrow transplantation and found that 53 percent doubled their serum creatinine level and 24 percent required dialysis during the course of the transplant. The mortality rate was 84 percent in the dialysis group, 37 percent in the doubled serum creatinine not needing dialysis group, and 17 percent in the group that maintained acceptable renal function. Of the factors associated with acute renal failure in these patients, AmB administration had the highest relative risk (RR=7.7) followed by weight gain (RR=4.8), hyperbilirubinemia (RR=3.3) and an elevated baseline serum creatinine level (RR=3.0). Although AmB therapy was associated with significant morbidity and mortality rates in these patients undergoing bone marrow transplantation, in most instances AmB was first administered when the patient was already in poor condition, presumably from sepsis. In this clinical context it is difficult to determine if worsening renal function is secondary to the sepsis, the drug used to treat the sepsis, or both. Moreover, some patients experience an improvement in creatinine clearance while on AmB if the sepsis is controlled (155). Therefore, it is difficult to quantitate the contribution of AmB to renal failure in patients with multiple organ failure.

Part of the reason that AmB nephrotoxicity is not as salient as it used to be is that, in most instances, the total dose administered is smaller (2, 155, 156, 158, 159) compared with doses used 30 years ago. Candida infections, especially, seem to respond to a dose of 6 to 8 mg per kg (less than 1 g) in patients who are nonimmunosuppressed (155). A nonconsecutive group of 50 AmB-treated patients were reviewed and the mean AmB dose was 582 mg (2); nephrotoxicity occurred in 60 percent with the average maximal increase in serum creatinine being 1.55 mg per dL (range of 0.2 to 5.2 mg per dL). The authors did not find a correlation between the total dose of AmB and the degree of nephrotoxicity. One-hundred forty of 179 consecutive patients treated with AmB who had a median AmB dose of 306 mg (range of 1 to 2,200 mg) were reviewed (54); 15 percent had nephrotoxicity (undefined). Others (158) reviewed 65 surgical patients who had positive blood cultures for fungus; in the 25 patients who received AmB, the average dose was 166 mg. Thirty-five patients who had renal dysfunction, defined as a 100 percent increase over the baseline serum creatinine level while on AmB, were compared with 60 patients who received AmB without having nephrotoxicity and it was determined that the risk factors for AmB nephrotoxicity were a higher total dose (3.7 relative risk for each 50 mg of AmB), concomitant use of diuretics, and abnormal baseline renal function (159). Nephrotoxicity seems to be particularly severe in infants with low birth weight (53). While it is impossible to determine the precise role of AmB in the occurrence of nephrotoxicity in these retrospective studies, it is probable that AmB had a role in the occurrence of renal dysfunction in some patients who received less than one gram of the drug.

Others (160) performed a randomized, prospective blinded study of low dose AmB in 182 patients undergoing bone marrow transplan-
tion. Patients were given either 0.1 mg per kg per day of AmB or placebo as antifungal prophylaxis. Treatment generally lasted no more than 30 days; the total cumulative dose was less than 0.5 g. There was no significant difference in nephrotoxicity or other systemic toxicity between the AmB and placebo groups. The former had significantly increased survival rates that could not be attributed to the prevention of fungal infections. It is reasonable to conclude that low dose AmB in this population is not associated with clinical nephrotoxicity.

Prevention of AmB toxicity in humans. Mannitol.—Some investigators in the 1960s showed that mannitol, given in a timely manner, could reverse anuria and tubular necrosis in animal models of acute renal failure (ARF) (161-163). In an isolated perfused dog lung and kidney preparation, mannitol infusion increased RBF and decreased renal vascular resistance without decreasing the hematocrit level, an indirect measure of viscosity (164). This suggested that mannitol could increase RBF by an effect independent of its osmotic properties. Based on this information, mannitol was evaluated as a prophylactic agent in AmB nephrotoxicity. In a study of dogs receiving AmB for five days (165), a group given mannitol with AmB had smaller increases in BUN and serum creatine and minimal abnormal renal histopathology compared with a control group.

Mannitol was thought to be nephroprotective in patient reports of patients receiving AmB (166, 167). There also was an association of decreased ARF with mannitol infusion in patients prone to ARF in an uncontrolled study (162). A randomized double-blind study using mannitol (1 mg per kg) in the AmB infusion was performed in 11 humans (168). A protective effect was not demonstrated and a detrimental effect of mannitol on renal function when used with AmB was suggested. Mannitol is now not recommended for the prevention of AmB nephrotoxicity.

Sodium loading.—In the early clinical experience with AmB, it was noted that nephrotoxicity seemed worse in association with dehydration or sodium depletion. As reviewed previously, sodium repletion in animal models prevents AmB nephrotoxicity, with mitigation of TGF invoked as the mechanism. Others (169) reported five patients who had deterioration of renal function while on AmB who then were given a 150 to 300 mEq of sodium supplement per day and showed improved renal function. Subsequently, there have been three prospective, but uncontrolled, studies (170-172) and one retrospective study (171) indicating that sodium supplementation in the range of 85 to 170 mEq per day may decrease the incidence of AmB nephrotoxicity. Sodium was given as salt in the diet, as saline solution, or as the sodium salt of ticarcillin. However, because of the study designs, definite conclusions are difficult to formulate.

Fortunately, a prospective, randomized, double blind study of AmB therapy and saline solution supplementation in Peruvian patients who had mucocutaneous leishmaniasis, but who were otherwise healthy, was performed (173). One group of ten patients received 1 L of 0.9 percent saline solution (150 mEq of sodium) intravenously over one hour immediately before AmB; a second group of ten patients received 1 L of 5 percent dextrose in water in a similar manner. After ten weeks of AmB (dose approximately 1.5 g), the creatinine clearance of the group receiving saline solution was unchanged, while clearance in the group receiving dextrose had decreased 32 percent. None of the patients had AmB therapy interrupted because of nephrotoxicity. Interestingly, the potassium requirement in the group receiving saline solution was 70 mEq per day higher than in the control group receiving dextrose. All patients lost the ability to acidify urine, but the patients receiving saline solution lost this ability quicker.

It seems apparent that administration of saline solution mitigates the loss of GFR resulting from AmB, but at the sacrifice of worsened tubular dysfunction, that is, renal tubular acidosis and urinary potassium loss. Nevertheless, it can be recommended that in patients who can excrete a sodium load, 1 L of normal saline solution should be administered before a dose of AmB to help prevent nephrotoxicity.

Pentoxifylline.—One preliminary study has been reported on the use of pentoxifylline in humans with AmB-associated nephrotoxicity. The investigators (174) administered 400 mg orally of pentoxifylline, three times a day, to five patients undergoing bone marrow transplantation after they had at least a doubling of serum creatinine while on AmB and cyclosporine A (CsA). Compared with a group of ten patients in a control group, the group receiving pentoxifylline had a return to 150 percent of the baseline value of serum creatinine sooner (1.0 versus 5.8 days), allowing an earlier resumption of full dose AmB and CsA. More formal studies with pentoxifylline are in progress. Some bone marrow transplant
centers are now using pentoxyfilline routinely in conjunction with AmB.

Amiloride.—A randomized prospective study was performed in 20 patients treated with AmB, ten of whom received amiloride, a potassium-
sparring diuretic (175). The patients receiving amiloride required less potassium supplementation, had higher serum potassium levels, and less
urinary potassium loss than the patients in the control group. Three patients in each group had a minor elevation of serum creatinine. Amiloride
is thought to block sodium resorption in the distal tubule (176), with a resultant loss of sodium, calcium, and bicarbonate in the urine. Potas-
sium is spared secondary to interruption of sodium and potassium exchange. Although this pharmacologic manipulation is efficacious in reducing AmB-associated hypokalemia, amiloride may induce sodium depletion, a condition that predisposes to AmB-induced loss of GFR. Therefore, it is not clear whether or not amiloride should be recommended in conjunction with AmB treatment.

Sodium bicarbonate.—Others (26, 27) identified distal RTA in patients receiving AmB, that is, inability to excrete an acid load, associated with mild compensated metabolic acidosis and hypokalemia. Sodium bicarbonate has been found to be nephroprotective in rats receiving AmB (177). Although there have been no controlled studies in humans, the administration of NaHCO₃ to patients receiving AmB makes empiric sense because it provides a sodium load to maintain GFR and provides alkali to attenuate RTA and potassium loss. Whether NaHCO₃ should be used in place of saline solution (a proven nephroprotective agent) is debatable because it is unclear whether it is the sodium or chloride, or both, which is nephroprotective. It has been suggested that chloride is the signal that the macula densa detects (125). Nevertheless, a combination of NaHCO₃ and saline solution as prophylaxis seems appropriate. The bicarbonate deficit may be calculated and used as a dose guide (178). Alternatively, 50 mEq of NaHCO₃ and 1 L of saline solution per day can be used as a starting point.

Administration of AmB.—A variety of administration techniques have been described for AmB; most of them were created with amelior-
ration of acute toxicity (fever, chills, nausea) in mind. No single administration technique has been shown to produce less nephrotoxicity in humans than others. Some investigators (13) sug-
gest that dosing every other day results in less nephrotoxicity, but the data are anecdotal. Considering the half-life of AmB, every other day
dosing should be as efficacious as daily dosing, but there are no data in humans to support such a regimen. As far as duration of adminis-
tration is concerned, there has been one study in dogs (179) that compared AmB (1 mg per kg, six doses) given as an intravenous bolus in a minimal volume every other day to AmB (same dose) given intravenously over five hours in 1 L of D₅W every other day. Dogs receiving the bolus infusion had a lower inulin clearance and more abnormal histopathologies compared with dogs receiving the slow infusion. What part the additional fluid volume administered to the latter group of dogs have in the outcome, is unknown. Practically speaking, AmB is not given as a bolus to humans, so the clinical applicability of this animal study is moot.

Drug interactions.—AmB may interact with other drugs with resultant modulation of nephrotoxicity. In a retrospective study of patients un-
dergoing bone marrow transplantation, it was reported that the combination of AmB and CsA had an additive effect in producing nephrotoxicity (180). However, others (157) could not arrive at such a conclusion in their review of 272 pa-
tients undergoing bone marrow transplants. Amphotericin B in combination with parenteral pentamidine resulted in acute reversible renal failure in four patients with acquired immunodeficiency syndrome (181). The combination of flucytosine and AmB was more effective and less nephrotoxic than AmB alone at a higher dose (182), probably because the total AmB dose is less with the combination regimen. Another study (183) reported four patients who were believed to demonstrate synergistic nephrotoxicity with a combination of AmB and gentamicin. There have not been subsequent confirming studies. Serum aminogly-
coside concentrations in children receiving AmB were prospectively evaluated and 12 of 17 patients had impaired aminoglycoside clearance when AmB was added to the regimen (184); seven of these children did not have a concomitant increase in serum creatinine. Other than this mostly retrospective data, there is limited information regarding drug interactions with AmB. Caution is advised when adding other nephrotoxic drugs to AmB in a treatment regimen.

Other formulations of AmB. Liposomal AmB (L-
AmB).—In 1981, it was determined that L-AmB was less toxic to host cells than free AmB, yielding
a higher therapeutic ratio in the treatment of experimental fungal infections (185). Subsequently, numerous investigations have been performed to find a better carrier and delivery system for AmB than sodium deoxycorticosterone. Liposomes are vesicles composed of a bilayer lipid membrane that incorporates the AmB. Amphotericin B contained in liposomes was found to be efficacious in the treatment of murine candidiasis (186-189), histoplasmosis (190), and cryptococcosis (191). In comparison with conventional AmB, there was little, if any, toxicity with L-AmB and approximately equal potency. The use of liposomes permitted administration of a tenfold larger dose of AmB than that previously tolerated, resulting in increased survival of mice infected with fungus treated with L-AmB compared with similar mice treated with free or deoxycorticosterone-AmB.

The basis for the improved efficacy of L-AmB has not been completely investigated. The results of in vitro studies demonstrated that L-AmB was toxic to fungal cells, but not to mammalian cells (192, 193). Liposomal AmB did not induce increased membrane permeability or cell lysis as did D-AmB. In the in situ rabbit kidney, L-AmB did not produce any acute renal hemodynamic effects (194). Liposomal AmB is nontoxic to kidney cells in culture (195, 196). It is believed that the increased selectivity of L-AmB for fungal cells is based upon altered distribution of AmB or altered interaction of liposome-incorporated AmB with its objective. The latter explanation currently is receiving more emphasis (12) because the distribution of AmB and L-AmB are not very different (197) and because the toxicity of L-AmB is dependent on the lipid or sterol content of the liposome (198-200). The ideal liposome traits, that is, diameter, lipid type, relative amounts of lipid and sterol content, have not been identified (200-202). Also, it seems that there are other solubilizing agents, such as cholesterol sulfate (203), Intralipid®, Clinitec Nutrition, Deerfield, IL (201, 202), and sucrose esters (204-206) that can increase the therapeutic ratio of AmB. Investigations to identify a better carrier will continue.

With success of L-AmB in animal models, early clinical trials in humans were performed. Using standardized liposomes in a 14:1 combination with AmB, 46 patients with fungal infections who had failed AmB therapy were treated (207). Twenty-four complete responses and 22 treatment failures were reported. Thirty-two patients had mild fever and chills with L-AmB and 14 were noted to be hypokalemic or hypomagnesemic. The dose of L-AmB was 5 mg per kg per day; total doses were in the range of 75 mg per kg. There was no renal or neurologic toxicity noted in 18 patients for whom long-term follow-up information was available. Four patients who had acute renal insufficiency from AmB did not have worsening of renal function while on L-AmB. Liposomal AmB was given to 11 patients with carcinoma with suspected or proven fungal infections (208); there were three cures, two improvements, three failures, and three patients who were nonevaluable. Five patients had nausea and vomiting, seven had fever and chills, and two had ventricular extrasystoles secondary to hypokalemia. Azotemia was not observed, but all patients required potassium supplementation. Results of these studies suggest that L-AmB has less effect on GFR than AmB, but still adversely affects tubular function.

Liposomal AmB is now available as a commercial preparation, Ambisome, Vestar, Inc., San Dimas, CA (209). Ambisome cured 58 percent of 64 proven fungal infections among 126 patients who received the drug in Europe (210). Of the 71 patients who had a normal serum creatinine pretherapy, 11 had an increased serum creatinine after therapy. Fifty patients initially had a high serum creatinine; 17 of these normalized their serum creatinine during Ambisome therapy (211). Twenty-four patients had hypokalemia. The mean dose was 2.6 g (range, 50 mg to 16.8 g). Elevation of the serum concentration of hepatic enzymes was frequently noted, but the significance of this observation in this patient population is not known. It is evident that L-AmB has the potential to effectively treat serious fungal infections with less toxicity than AmB. Whether L-AmB will supplant AmB in the treatment of systemic mycoses remains to be seen.

Amphotericin methyl ester (AME).—Other formulations of AmB have been devised by altering the configuration of the AmB molecule at the C-16 position, the site of the carboxyl group (Fig 1). As described in the effects on membrane section of this report, this is the portion of the AmB molecule that binds to the hydrophilic head of cell membrane sterols. Blocking this binding site by esterification might decrease the affinity of the macroline for sterol, but it also could increase the relative selectivity for ergosterol over cholesterol (212). Without the polar head interaction, the hydrophobic interaction between AmB and sterol would then predominate; this inter-
action is stronger with ergosterol than with cholesterol.

Amphotericin methyl ester is AmB with an esterified carboxyl group at the C-16 position. In initial in vitro and in vivo studies, AME was found to have one-eighth or less the nephrotoxic potential of AmB in animals (213, 214) with only a slight diminution of antifungal potency (215–217). In accord with the aforementioned theoretical reasoning, AME displayed increased selective toxicity for fungal membranes compared with AmB. However, early clinical studies in humans with systemic mycotic infections revealed that severe leukoencephalopathy was associated with high total doses (greater than 5 g) of AME (218). This neurologic lesion was not noted in the control group. The exact cause of the AME-associated leukoencephalopathy is controversial (219, 220). Subsequent studies of AME in dogs failed to reproduce the neuropathic changes after a total dose of 0.3 g (221), but did reproduce them after 7 g (222). In the wake of this controversy, AME has all but disappeared from clinical practice.

Amphotericin B, a polyene macrolide antifungal agent, was noted to be nephrotoxic in humans soon after its introduction in the middle 1950s. Toxicity was manifested by increased serum creatinine and urea, decreased inulin clearance, urinal concentrating ability, and tubular secretion capacity, and the presence of renal tubular acidosis and hypokalemia. Histopathologic changes that are evident in the kidney after administration of variable amounts of AmB are a sign of permanent damage. Administration of AmB to animals resulted in renal vasoconstriction with a decrease in GFR. The mechanisms by which AmB causes cellular toxicity are alteration of membrane permeability and oxidative injury. The mechanism by which AmB causes renal vasoconstriction has been postulated to occur either by activation of TGF or direct vasoconstriction. Sodium loading attenuates AmB renal toxicity in humans and animals and theophylline and calcium channel blockers can decrease toxicity in animals. Mannitol is probably ineffective in preventing AmB nephrotoxicity. A new formulation of AmB, liposomal AmB, has an increased therapeutic ratio compared with conventional AmB.

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