ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Sept. 1976, p. 555-563
Vol. 10, No. 3
Copyright © 1976 American Society for Microbiology
Printed in U.S.A.

Can Mannitol Reduce Amphotericin B Nephrotoxicity?
Double-Blind Study and Description of a New Vascular Lesion in Kidneys

WARD E. BULLOCK,* ROBERT G. LUKE, CHARLES E. NUTTALL, AND DINYAR BHATHENA

Divisions of Infectious Disease and Nephrology, Departments of Medicine and Pathology, University of Kentucky College of Medicine, Lexington, Kentucky 40506*

Received for publication 16 March 1976

Eleven patients with systemic mycotic infections were treated with amphotericin B, 1 mg/kg, on alternate days. Five patients also received mannitol (M), 1 g/kg, in the amphotericin infusion, while six served as controls (C). Renal function studies prior to therapy were repeated at a total cumulative amphotericin B dosage of 25 mg/kg; renal biopsies were obtained from 10 patients. Inulin and creatinine clearances decreased in both the C and M groups, significantly so in the latter. Urinary concentrating ability of five patients (2C, 3M) decreased as did the capacity of three (1C, 2M) to acidify urine after an acid load. Neither the peak and valley levels of amphotericin B in serum nor the urinary excretion thereof differed between the C and M groups. Striking vacuolization of smooth muscle cells, previously unrecognized, was observed in the media of arterioles and arteries in all renal biopsies. Tubular calcification was present in both groups. In summary, M therapy (1 g/kg) did not protect against the nephrotoxicity of amphotericin B. A unique lesion of the renal vasculature secondary to amphotericin B is described.

Many of the side effects associated with amphotericin B therapy can be ameliorated by therapy every other day and by judicious cotherapy with such agents as heparin, corticosteroids, and antiemetics. Unfortunately, there has been little success in reducing the nephrotoxic effects that often restrict therapy. These include reduction in glomerular filtration rate (GFR) and renal blood flow (5, 24) and abnormalities of distal tubular function. The latter are manifested histologically by intratubular and interstitial calcium deposition (25) and functionally by impaired urinary concentrating ability (13), and by renal tubular acidosis with secondary potassium wasting (4, 5, 17).

Although individual susceptibility to the nephrotoxicity of amphotericin B appears to vary widely (18), the likelihood of at least transient renal impairment is high with the large dose (1.5 to 2.5 g) usually required to treat adequately many of the systemic mycotic infections (5). It has been suggested that alkali therapy might prevent some aspects of amphotericin B nephrotoxicity (17), and in an experimental rat model administration of bicarbonate during amphotericin therapy was shown to improve several measures of renal function (11). Unfortunately, similar studies in man are not available.

More recently, it has been reported that administration of an osmotic diuretic, mannitol, significantly reduces the nephrotoxicity of acute amphotericin B administration to dogs (12). Mannitol therapy appeared to prevent a rise in the blood urea nitrogen and serum creatinine (Cr) values as well as the degeneration of renal tubular epithelium that occurred when amphotericin B was administered alone. Clinically, mannitol has been employed extensively as cotherapy with amphotericin B, and protective effects on the kidney have been claimed in one small uncontrolled study (20). Because of widespread interest in the therapeutic potential of mannitol (3), we have conducted and here report the results of a double-blind controlled study designed to evaluate the capacity of this agent to diminish the nephrotoxicity of amphotericin B in man.

MATERIALS AND METHODS

Patient selection. Potential subjects for study were screened for suitability as participants by four criteria: (i) proof of active systemic mycotic infection; (ii) no prior amphotericin B therapy; (iii) normal renal function as judged by urinalysis and levels of blood urea nitrogen and serum creatinine; (iv) absence of diabetes mellitus and hypertension (diastolic pressure > 90 mm of Hg). Patients meeting these criteria were advised of the study verbally and in writing. Those giving informed consent were then entered into a treatment protocol approved by the

University of Kentucky Human Investigation and Studies Committee. Upon entry into the study, renal function of all patients was evaluated by measurement of inulin clearance (C_{In}), creatinine clearance (C_{Cr}), and urinary concentrating and acidifying ability. Serum electrolytes, calcium, phosphorus, and uric acid were measured, and urine cultures were performed. An intravenous pyelogram was normal in all patients.

Investigational protocol. After completion of renal function studies, amphotericin B (hereafter designated as A), supplied and labeled under a Food and Drug Administration investigational new drug number by E. R. Squibb & Co., was administered intravenously in three increasing doses until the full dosage schedule of 1 mg/kg of body weight, given every other day, was attained. Full doses were given in 500 ml of 5% dextrose in water over a 4-h period. Assignment of patients to a control (C) group receiving A therapy alone or to a mannitol (M) treatment group was made by a physician and a pharmacist not associated with the daily care of study patients. M was added without labeling to the A solution of patients chosen to receive M cotherapy so that the contents of an infusion, other than A concentration, were unknown to both the attending physicians and patients during the course of therapy. In all cases, the dose of M administered with A was 1 g/kg of body weight. This dose is in the range used to treat acute renal failure (16) but below that producing toxic effects (9). Attending physicians prescribed medications from a preselected panel of drugs when necessary for relief of various side effects caused by A. These included: (i) heparin, 1,000 U or less added to each 500 ml of infusion to control phlebitis; (ii) 20 mg of hydrocortisone or its equivalent delivered as an intravenous bolus immediately prior to each A infusion; (iii) Proclorperazine, 10 mg, orally, 0.5 h before infusion of A and every 8 h to a total of three doses; (iv) aspirin, 600 mg, orally, 0.5 h prior to infusion and every 6 h as needed for reduction of fever (>102°F) induced by therapy; (v) $NaHCO_3$ for replacement of bicarbonate when serum CO_2 fell to 18 meq/liter; (vi) KCl, 1 g, orally three times daily, as needed for serum potassium levels less than 3.8 meq/liter.

Criteria for the removal of a patient from study were: (i) interruption of A therapy as the result of noncompliance, (ii) nephrotoxicity sufficient to elevate Cr to 3.5 mg/100 ml, or (iii) to decrease the C_{Cr} to 25% or less of the pretreatment control value. In fact, no patient was dropped from the study for these reasons.

In addition to clinical assessment of patients, mandatory clinical studies at weekly intervals included weighing, 24-h urine collection, complete blood count, urinalysis, and measurement of serum electrolytes, Cr, blood urea nitrogen, and erythrocyte sedimentation rate. At intervals of 2 weeks to 1 month, appropriate X-ray studies, fungal cultures, and fungal serologies were repeated. At a total cumulative A dosage of 25 mg/kg of body weight, all renal function studies were repeated. In addition, percutaneous renal biopsy was performed under fluoroscopic guidance in 10 patients, all of whom gave informed consent. Biopsy was not carried out in one patient (no. 8) because of severe kyphosis. After biopsy, the course of A was completed to a total mean dosage of 43 mg/kg of body weight in 10 patients. One patient failed to return after receiving 1.7 g.

Assay of A in blood and urine. After the third administration of A at full daily dosage (1 mg/kg of body weight), serum and urine specimen were collected for assay at weekly intervals from all patients. Blood for peak A levels was drawn within 30 min after completion of the infusion. For assay of valley levels, blood was drawn just prior to the next infusion 48 h later. Twenty-four-hour urine collections were started at the beginning of an infusion. During collection, urine was kept at 4°C, after which a 10-ml portion was removed and, along with serum, frozen at −60°C until assayed. The remaining urine was assayed for creatinine as a check on adequacy of collection. Serum and urinary concentrations of A were measured on coded specimens through the courtesy of the Squibb Institute of Medical Research, New Brunswick, N.J. Levels were determined by bioassay using the *Paecilomyces varioti* small-plate agar diffusion assay (21).

Renal function studies. All renal studies and renal biopsies were performed at least 24 h after an infusion of A on the "off" day of therapy. Inulin clearance studies were performed in the morning after a light breakfast. Two sequential hourly urine collections were taken during a modest water diuresis (100 ml/h), and the average value was used; a bladder catheter was employed for two patients. Inulin was measured by the method of Kulka (14). Serum and urinary creatinine were measured by an autoanalyzer (Technicon). Serum electrolytes, uric acid, calcium, and phosphorus were measured by the clinical laboratory. To measure urine concentrating ability, 5 U of pitressin tannate in oil (Parke, Davis & Co.) was given intramuscularly after voiding at 10 p.m. The osmolality of the next three voided urine specimens was measured by an advanced osmometer, and the highest value was selected. The "short" ammonium chloride loading test was performed by the method of Wrong and Davies (26); fresh urine was collected under mineral oil, and urinary pH was measured by a Beckman pH meter.

Histological methods. Light, electron, and fluorescence microscopy of renal biopsies was carried out by one of us (D.B.) without knowledge of which patients received M. For light microscopy studies, serial sections (1 to 2 μm thick) were made of formalin-fixed tissue and stained by hematoxylin and eosin, periodic acid Schiff, periodic acid silver methenamine-Masson trichrome, and von Kossa methods. For electron microscopy, 1-mm tissue cubes were fixed in 3.5% buffered glutaraldehyde, postfixed in 1% osmium tetroxide for 1 h, embedded in epon, and sectioned with subsequent double staining by lead citrate-uranyl acetate. For immunofluorescence studies, sections of snap-frozen tissue were stained by the direct technique with fluorescein-conjugated goat antisera to human immunoglobulins G, A, and M, βIC, and fibrin.

Statistical methods. In most instances, the significance of differences between group data was determined by the Student t test (paired if appropriate). Serum levels and urinary excretion of A were analyzed by means of a split plot design (22). Urinary pH measurements were corrected to whole numbers for calculation of mean and standard error of the mean.

RESULTS

General clinical features. All 11 patients who volunteered for study were Caucasians and ranged in age from 19 to 75 years. The infecting organism in each patient is listed in Table 1. Fungus could not be isolated from a lung biopsy of one patient. However, the pathology and a complement-fixing antibody titer of 1:256 against yeast and mycelial-phase antigens of *Histoplasma capsulatum* were regarded as diagnostic of histoplasmosis. The M-treated (n = 6) and C groups (n = 5) differed in two respects (Table 1): (i) the average age of the M group (58 ± 4.7 years) was greater than that of the C (48.4 ± 8.8 years) ($P > 0.1$); (ii) one of six patients in the M group was female, whereas of five in the C group three were females.

The clinical response to A therapy was favorable among patients of both groups (Table 1). Five of six patients in the M group were considered to be clinically arrested at the termination of therapy and at followup checks ranging from 12 weeks to 2 years post-treatment. Likewise, infection was cured or arrested in four of five in the C group. Arrest was defined by four criteria: (i) clinical well being; (ii) failure to isolate the infecting fungus from sputum or other sites; (iii) stabilization or improvement in X-ray findings; and (iv) decrease of complement-fixing antibody titer to *H. capsulatum* in patients with histoplasmosis. Patient no. 2 relapsed 8 months post-treatment as judged by a marked rise in complement-fixing antibody titer and weight loss; sputum cultures did not give fungal growth. Side effects attributable to A infusion were witnessed with equal frequency in the M and C groups; these included nausea, chills, fever, and malaise.

Renal function. In the overall group of 11 patients studied prior to therapy, the C_{In} was 113 ± 9 ml/min per 1.73 m^2 (mean ± standard error of mean), the C_{Cr} was 86 ± 7 ml/min per 1.73 m^2, and the Cr was 0.9 ± 0.04 mg/100 ml. The mean urinary osmolality (U_{osm}) after pitressin was 659 ± 79 mosm/kg (excluding patient no. 4 with a normal inulin clearance but who failed to concentrate in association with a persistent urinary tract infection secondary to an indwelling catheter necessitated by her neurological state). The mean minimum urinary pH after NH_4Cl was 5.2 (patient no. 4 excluded).

At the "target" dose of 25 mg/kg of A, there was a considerable drop in the inulin and creatinine clearance of both the C and M groups (Fig. 1; Table 2). Of interest, two patients in the C group showed very little change in GFR during A treatment (Fig. 1, no. 2 and 4). The mean xenith level of Cr during A therapy in the C group was 1.8 ± 0.3 mg/100 ml and 1.5 ± 0.2 mg/100 ml in the M group ($P < 0.1$). Overall, older patients appeared to have a less marked drop in GFR. Thus, in patients over 60 years of age from both groups (n = 5), the mean drop in C_{Cr} was 10 ± 4 ml/min as compared to 55 ± 3 ml/min in patients less than 60 years of age (n = 6) of both groups ($P < 0.001$).

Five patients (2C, 3M) showed a drop in concentrating ability, and three (1C, 2M) showed a drop in minimum hydrogen ion concentration after ammonium chloride loading (Table 3; Fig. 2 and 3). Signs of sodium depletion were carefully sought, but not found, in any patient.

TABLE 1. *Clinical features of 11 patients*

Group	Patient no.	Age (yr)	Sex	Wt (kg)	Treatment	Pathogen[a]	Site	Total A dosage (g)	Result
Control	1	19	F	53	C	B	Disseminated	2.5	Arrested[b]
Control	2	61	M	50	C	H	Lung, cavitary	2.3	Relapse
Control	3	50	F	54	C	B	Lung	2.4	Arrested
Control	4	70	F	50	C	C	Meninges	2.4	Cure
Control	5	42	M	70	C	H	Lung, cavitary	2.6	Arrested
Mannitol	6	41	M	91	M	B	Disseminated	2.5	Arrested
Mannitol	7	58	M	59	M	H	Lung, cavitary	2.5	Arrested
Mannitol	8	75	M	43	M	B	Disseminated	2.0	Arrested
Mannitol	9	50	M	42	M	H	Lung, cavitary	1.7[c]	Improved
Mannitol	10	62	F	53	M	H ?	Lung	2.0	Arrested
Mannitol	11	62	M	42	M	H	Lung, cavitary	1.8	Arrested

[a] Abbreviations: B, *Blastomyces dermatididus*; H, *Histoplasma capsulatum*; C, *Cryptococcus neoformans*.
[b] See text for definition.
[c] Failed to complete therapy.

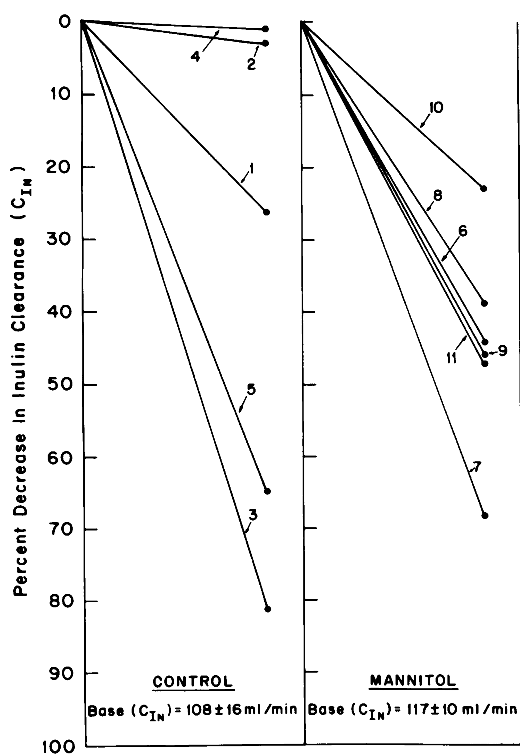

FIG. 1. *Change in GFR at a cumulative A dosage of 25 mg/kg. Data presented for patients no. 6 and no. 11 based on C_{Cr} values.*

All but one patient (no. 6) required supplemental potassium, and the nadir levels of serum potassium were not different beween the two groups (3.4 ± 0.2 meq/liter in C and 3.1 ± 0.2 meq/liter in M). Sodium bicarbonate therapy was not required in any patient; the mean nadir serum bicarbonate was 22.6 ± 0.5 meq/liter in the C group and 22.8 ± 0.5 meq/liter in the M group. The lowest serum bicarbonate observed was 19 meq/liter in patient no. 11. Urinary casts, mainly of the hyaline type, were seen frequently in four of five in the C group and in one of six in the M group. After completion of therapy, no casts were detected. Proteinuria was not detected by routine urinalysis in any patient during the study.

If the group of 11 patients is considered as a whole, a significant deterioration in GFR, urinary concentrating ability, and urinary acidification after an acid load was observed (Table 4).

Renal biopsies. At least 10 nonsclerosed glomeruli/biopsy were seen. By light microscopy, no specific glomerular lesion was found, and immunofluorescence studies were negative. Since 9 of the 10 patients were over 40 years of age, it is not surprising that arterial and arteriolar sclerotic changes were common. Tubular calcification was scored by an arbitrary scale on which 1+ signified minimal calcification (1 calcified focus/10 high-power fields). Extensive calcification (more than one focus/high-power

TABLE 2. *Changes in GFR during A therapy*

Patient no.	C_{In} (ml/min per 1.73 m²)[a]			C_{Cr} (ml/min per 1.73 m²)			Cr (mg/100 ml)		
	Base	Target	Δ	Base	Target	Δ	Base	Target	Δ
C group									
1	70	52	−18	76	24	−52	1.0	1.9	+0.9
2	120	117	−3	75	74	−1	1.1	1.0	−0.1
3	91	18	−73	96	36	−60	0.9	1.5	+0.6
4	95	94	−1	59	55	−4	0.8	0.9	+0.1
5	166	58	−108	128	68	−60	0.9	1.7	+0.8
	108 ± 16[b]	68 ± 17	−41 ± 21	87 ± 12	51 ± 10	−35 ± 14	0.9 ± 0.1	1.4 ± 0.2	0.5 ± 0.2
			NS[c]			$P < 0.10$[d]			$P < 0.10$
M group									
6	—	87	—	108	61	−47	1.1	1.7	+0.6
7	152	49	−103	107	60	−47	0.9	1.4	+0.5
8	125	76	−49	60	38	−22	0.8	1.1	+0.3
9	110	59	−51	96	36	−60	0.9	1.5	+0.6
10	93	71	−22	67	61	−6	0.7	0.8	+0.1
11	105	—	—	75	56	−19	1.0	1.0	0.0
	117 ± 10	68 ± 6	−56 ± 17 $P < 0.05$	86 ± 9	52 ± 5	−34 ± 9 $P < 0.02$	0.9 ± 0.1	1.3 ± 0.1	+0.3 ± 0.2 NS

[a] C_{In}, Inulin clearance.
[b] Mean ± standard error of mean.
[c] NS, Not significant.
[d] Probability (P) value for differences in GFR during A therapy as compared with pretreatment base-line values.

TABLE 3. *Changes in tubular function during A therapy*

Group	Pitressin test (U_{osm} [mosm/kg])			NH_4Cl load (urinary [H^+] $\times 10^{-7}$ eq [pH])		
	Base	Target	Δ	Base	Target	Δ
Controls (n = 5)	674 ± 110[a]	445 ± 50	−229 ± 80,[b] $P < 0.10$[c]	55 ± 20[a] (5.3)	41 ± 18 (5.4)	−14 ± 26, NS[d]
Mannitol (n = 6)	649 ± 118	516 ± 34	−132 ± 108, NS	64 ± 14 (5.2)	46 ± 16 (5.3)	−17 ± 17, NS

[a] Omits patient no. 4.
[b] Mean ± standard error of mean.
[c] Probability (P) value for differences in tubular function during A therapy as compared with pretreatment base-line values.
[d] NS, Not significant.

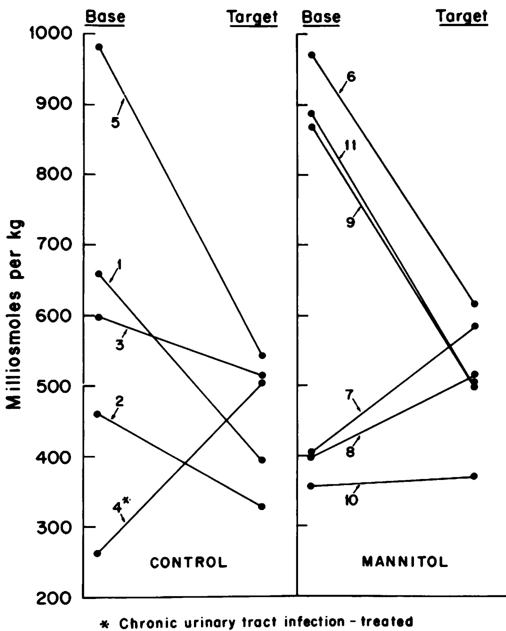

FIG. 2. *Change in urine concentration ability (pitressin test) at a cumulative A dosage of 25 mg/kg.*

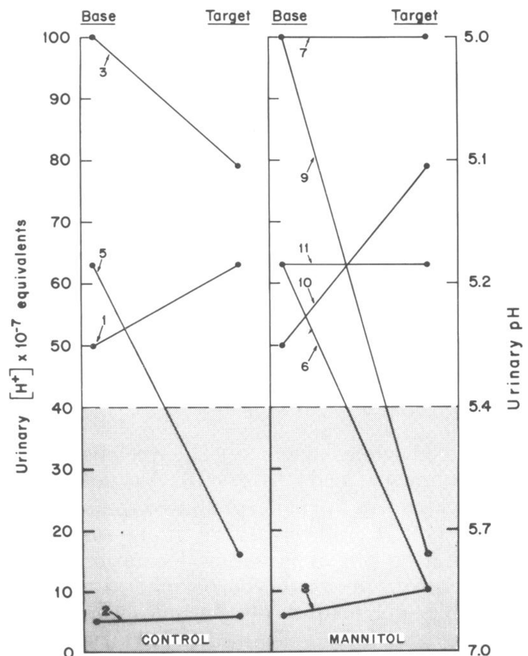

FIG. 3. *Change in urinary acidification ability at a cumulative A dosage of 25 mg/kg as determined by the short NH_4Cl loading test. Points falling within shaded area are below the normal range. Patient no. 4 excluded from figure.*

field) was scored as 3+ and intermediate calcification as 2+ (Table 5).

In varying degrees tubular calcification was observed in all but one patient (no. 4) and tended to be worse in the M group. There was, however, no correlation between tubular calcification and changes in glomerular and tubular function. There was no evidence of coagulation necrosis in the tubular lining cells, and there appeared to be no significant difference in the tubular cell cytoplasmic vacuolization between the M and C group.

A striking vacuolization in the media of small arterial and arteriolar profiles was seen in all biopsies (Fig. 4). The vacuolization was focal even in the most extensively affected biopsy specimens. Because of this, and the varying amounts of renal tissue sampled from case to case, qualitative assessment was necessarily arbitrary. Larger and frequently encountered lesions in small arteries and arterioles with relatively few uninvolved profiles were classed as 3+ (Table 5). Small vacuoles found in only an occasional vessel were classified as 1+ severity. Assessment of the severity of vacuolization was made "blindly" on two occasions by the same observer with good agreement.

There was no relationship between the degree of vacuolization and the arterial and arteriolar sclerotic changes. In ultramicroscopic studies of more severely involved biopsies, vacuoles were both more numerous as well as

TABLE 4. *Mean change of renal function in 11 patients during A therapy*

Renal function	C_{In} (ml/min per 1.73 m^2)[a]	C_{Cr} (ml/min per 1.73 m^2)	Cr (mg/100 ml)	Pitressin test (mosm/kg)	NH_4Cl load ($[H^+] \times 10^{-7}$ eq)
GFR	-48 ± 13.5[b] $P < 0.01$[c]	-35 ± 7.4 $P < 0.001$	0.4 ± 0.13 $P < 0.02$		
Tubular function				-171 ± 70 $P < 0.05$	-16 ± 6 $P < 0.05$

[a] C_{In}, Inulin clearance.
[b] Mean ± standard error of mean.
[c] Probability (P) values for differences in renal function during therapy as compared with pretreatment base-line values.

TABLE 5. *Histological changes in renal biopsy specimens*

Group	Patient no.	Tubular calcification	Medial vacuolization	Arteriosclerotic changes
Control	1	2+	1+	0
Control	2	2+	1+	2+
Control	3	1+	2+	3+
Control	4	0	2+	3+
Control	5	2+	1+	2+
Mannitol	6	3+	3+	2+
Mannitol	7	3+	2+	ND[a]
Mannitol	9	2+	2+	2+
Mannitol	10	2+	3+	2+
Mannitol	11	3+	3+	2+

[a] ND, Not determined. Larger arteries were not sampled.

larger, sometimes displacing the nucleus of the smooth muscle cells (Fig. 4B). Vacuoles appeared to evolve by fusion of dilated smooth and rough endoplasmic reticulum; in one instance, invaginating pinocytic vesicle formations suggested continuity with the peripheral plasma membrane of a medial cell. Vacuoles were more striking in arterioles than in the arteries, and occasionally they were seen in afferent arterioles. In general, changes were more marked in the M group (Table 5). To our knowledge, these changes have not been previously described as a consequence of A therapy.

Serum levels and urinary excretion of A. The peak and valley levels and urinary excretion of A were remarkably uniform for each patient during the course of therapy. Mean values for the C and M group are listed in Table 6 where it can be seen that there was no significant difference between the peak and valley levels of A measured in either group. Furthermore, there was no significant difference in the urinary excretion of A between the C and the M group, although excretion was slightly higher in the latter group.

DISCUSSION

Under conditions of this study, M in a dosage of 1 g/kg of body weight did not prevent the functional and histological manifestations of A nephrotoxicity. There was a significant and at least as great a fall in inulin and creatinine clearance within the M-treated group as in controls. Likewise, there was at least as much alteration of tubular function in the group treated with M as in the C group. Further, a newly recognized abnormality in the media of small arteries and arterioles appeared more severe in the M group.

A produces renal impairment by two substantially independent mechanisms. Firstly, an acute reduction in renal blood flow and GFR has been observed in man (2, 4, 5, 24), dogs (6), and rats (11). Infusion of the drug into the renal artery of a dog is associated with radiologically demonstrable intense vasoconstriction of small intrarenal blood vessels and cortical ischemia (6). Secondly, specific impairment of distal nephron functions, such as impaired concentrating ability (13), and distal renal tubular acidosis (4, 17), has been attributed to effects of the drug on tubular cell membranes; A interacts with membrane-bound sterols (1) to increase passive permeability to sodium, potassium, hydrogen ion, water, and low-molecular-weight solutes such as urea (1, 15, 23). In toad bladder studies, A increases membrane permeability only on the mucosal side of the membrane (15). Such effects on tubular cell membranes could explain potassium wasting and the inability to maintain a hydrogen ion and an osmotic gradient in the distal nephron.

Mannitol has both renal vasodilatory and "tubular flushing" effects (16) which might have been expected to influence favorably both of the nephrotoxic mechanisms associated with A therapy. In rats during renal hypoperfusion induced by aortic clamping (19), and after 2 h of complete obstruction of the renal artery (8), M can maintain glomerular filtration and urinary flow when infusion of saline cannot. There is also evidence in dogs of the ability of M to reduce renal vascular resistance in similar circumstances (7, 10). M increases medullary blood flow and reduces medullary hyperosmolality (9) and thus may diminish the levels of A

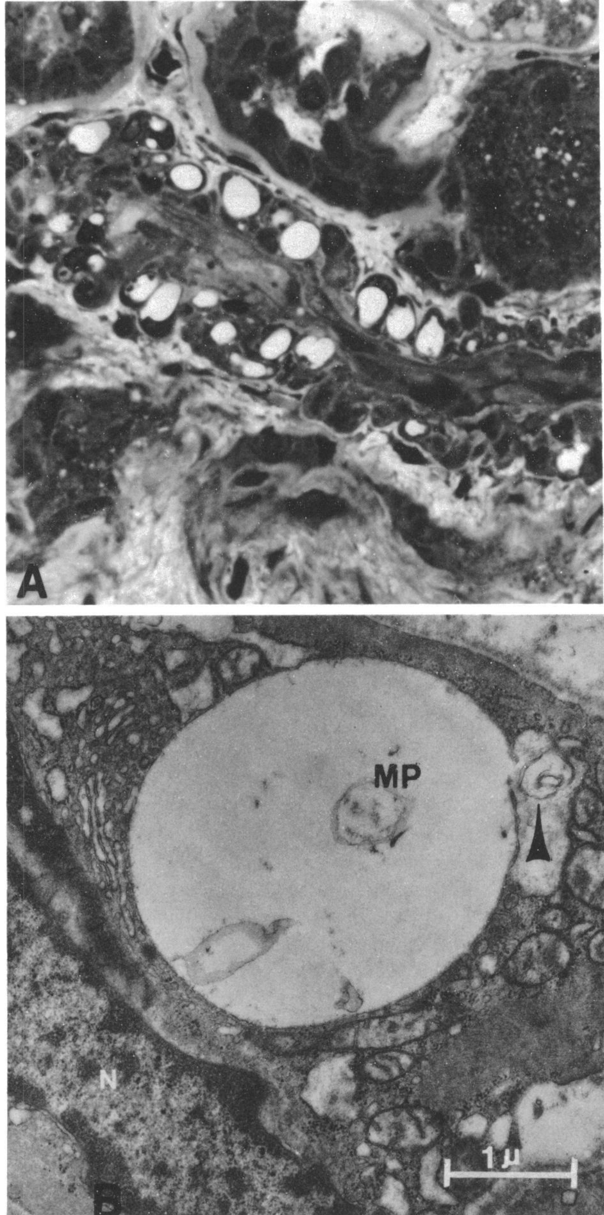

FIG. 4. *(A) Histological section of renal biopsy showing several vacuoles within the media of an arteriole. Epon-embedded, 1-μm section, using toluidine blue stain. ×160. (B) Electron micrograph of smooth muscle cell in the media of a renal arteriole. A large vacuole is situated centrally within the cell, near the nucleus (N). Membranes of the dilated rough endoplasmic reticulum (arrow) tend to break and form irregular membrane profiles (MP) suspended in the vacuolar lumen. ×9,500.*

in the vasa recta and, possibly, in the medullary interstitium itself. The osmotic diuresis induced by M infusion might be expected both to dilute A concentrations in the distal tubular lumina and to decrease luminal membrane contact time, especially if M also increased the GFR.

Despite the attractiveness of these hypothetically protective mechanisms and the previously reported protective effect in dogs (12), this controlled study has failed to confirm a beneficial effect of M. It is, of course, possible that a larger dose might have been protective, but the dose used (1 g/kg) was approximately twice that

TABLE 6. *Mean peak and valley serum levels and urinary excretion of A*

Group	No. of patients	No. of samples	Serum levels		Urinary excretion (mg/24 h)
			Peak (μg/ml)	Valley (μg/ml)	
Control	5	53	1.47 ± 0.05	0.36 ± 0.01	2.06 ± 0.27
Mannitol	6	58	1.60 ± 0.05[a]	0.42 ± 0.03[a]	2.20 ± 0.21[a]

[a] Differences from control values are not statistically significant.

used in the dog study (12) and in a report on the use of M in four patients (20). Further increase in M dosage might be hazardous for two reasons. Retention of M in the presence of a reduced GFR would tend to expand the extracellular fluid and cause hyponatremia; alternatively, osmotic diuresis may cause obligatory losses of sodium, water, and potassium which may be especially hazardous in patients receiving A. In addition, the histological and electron microscopic studies of renal biopsies from our patients suggest that a medial vacuolar lesion of arterioles and arteries may be more marked in patients receiving both A and M.

The vacuoles within renal vasculature appear to be made up of periodic acid Schiff stain-negative electron-lucent material which contains protoplasmic sap and some of the cytoplasmic organelles in varying stages of degeneration (Bhathena, D., W. E. Bullock, C. E. Nuttall, and R. G. Luke, submitted for publication). Continuity of vacuoles with the external cell membrane was suggested on electron microscopic study of at least one biopsy. It seems likely that these vacuoles are related to the increase in solute permeability of plasma membranes produced by A or to the intense renal vasoconstriction associated with administration of this drug or a combination of these effects. Whereas changes in membrane permeability appear to result from interactions of A with membrane-bound sterols to form pores (1), there is as yet no satisfactory explanation for the mechanism of vasoconstriction induced by A.

ACKNOWLEDGMENTS

We express appreciation to house staff members of the Department of Medicine at the University of Kentucky Medical Center for their outstanding care of the study patients and assistance with details of the treatment protocol. We wish also to thank the following: Peggy Herndon for assistance in preparation of infusion solutions, Marguerite Larsen for transport of specimens to the Squibb Laboratories for assay, Michael Kashgarian and Bernard Panner for helpful suggestions, and Catherine Fine for excellent editorial assistance.

This project was supported in part by a grant from E. R. Squibb & Sons, New York, N.Y. D.B. was supported in part by Public Health Service training grant AM 05701 from the National Institute of Arthritis, Metabolism, and Digestive Diseases.

LITERATURE CITED

1. **Andrioli, T. D.** 1973. On the anatomy of amphotericin B-cholesterol pores in lipid bilayer membranes. Kidney Int. 4:337-345.
2. **Bell, N. H., V. T. Andriole, S. H. Sabesin, and J. P. Utz.** 1962. On the nephrotoxicity of amphotericin B in man. Am. J. Med. 33:64-69.
3. **Bennett, J. E.** 1974. Chemotherapy of systemic mycoses. N. Engl. J. Med. 290:30-32, 320-323.
4. **Burgess, J. L., and R. Birchall.** 1972. Nephrotoxicity of amphotericin B with emphasis on changes in tubular function. Am. J. Med. 53:77-84.
5. **Butler, W. T., J. E. Bennett, D. W. Alling, P. T. Wertlake, J. P. Utz, and G. J. Hill II.** 1964. Nephrotoxicity of amphotericin B: early and late effects in 81 patients. Ann. Intern. Med. 61:175-187.
6. **Butler, W. T., G. J. Hill, II, C. F. Szwed, and V. Knight.** 1964. Amphotericin B renal toxicity in the dog. J. Pharmacol. Exp. Ther. 143:47-56.
7. **Camishion, R. C., R. W. Solit, J. Iida, and W. F. Ballinger, II.** 1966. The effect of mannitol on renal vascular resistance. Surgery 59:1037-1042.
8. **Flores, J., D. R. DiBona, C. H. Beck, and A. Leaf.** 1972. The role of cell swelling in ischemic renal damage and the protective effect on hypertonic solute. J. Clin. Invest. 51:118-126.
9. **Gennari, F. G., and J. P. Kassirer.** 1974. Osmotic diuresis. N. Engl. J. Med. 291:714-720.
10. **Goldberg, A. H., and L. S. Lilienfield.** 1965. Effects of hypertonic mannitol on renal vascular resistance. Proc. Soc. Exp. Biol. Med. 119:635-642.
11. **Gouge, T. H., and V. T. Andriole.** 1971. An experimental model of amphotericin B nephrotoxicity with renal tubular acidosis. J. Lab. Clin. Med. 78:713-724.
12. **Hellebusch, A. A., F. Salama, and E. Eadie.** 1972. The use of mannitol to reduce the nephrotoxicity of amphotericin B. Surg. Gynecol. Obstet. 134:241-243.
13. **Holeman, C. W., and H. Einstein.** 1963. The toxic effects of amphotericin B in man. Calif. Med. 99:90.
14. **Kulka, R. G.** 1956. Colorimetric estimation of ketopentoses and ketohexoses. Biochem. J. 63:542.
15. **Lichtenstein, N. S., and A. Leaf.** 1965. Effect of amphotericin B on the permeability of the toad bladder. J. Clin. Invest. 44:1328-1342.
16. **Luke, R. G., and A. C. Kennedy.** 1967. Prevention and early management of acute renal failure. Postgrad. Med. J. 43:280-289.
17. **McCurdy, D. K., M. Frederic, and J. R. Elkington.** 1968. Renal tubular acidosis due to amphotericin B. N. Engl. J. Med. 278:124-131.
18. **Miller, R. P., and J. H. Bates.** 1969. Amphotericin B toxicity. Ann. Intern. Med. 71:1089-1095.
19. **Morris, C. R., E. A. Alexander, F. J. Burns, and N. G. Levinsky.** 1972. Restoration and maintenance of glomerular filtration by mannitol during hypoperfusion of the kidney. J. Clin. Invest. 51:1555-1564.
20. **Olivero, J. J., J. Lozano-Mendez, E. M. Ghafary, G. E. Knoyan, and W. N. Suki.** 1975. Mitigation of amphotericin B nephrotoxicity by mannitol. Br. Med. J. I:550-551.

21. **Shadomy, S., J. A. McCoy, and S. I. Schwartz.** 1969. Bioassay for hamycin and amphotericin B in serum and other biological fluids. Appl. Microbiol. **17**:497-503.
22. **Snedecor, G. W., and W. G. Cochran.** 1967. Statistical methods. Iowa University Press, Ames.
23. **Steinmetz, P. R., and L. R. Lawson.** 1970. Defect in urinary acidification induced *in vitro* by amphotericin B. J. Clin. Invest. **49**:596-601.
24. **Utz, J. P., J. E. Bennett, M. K. Brandriss, W. T. Butler, and G. J. Hill II.** 1964. NIH staff conference: amphotericin B toxicity. Ann. Intern. Med. **61**:340-343.
25. **Wertlake, P. T., W. T. butler, G. J. Hill II, and J. P. Utz.** 1963. Nephrotoxic tubular damage and calcium deposition following amphotericin B therapy. Am. J. Pathol. **43**:449-457.
26. **Wrong, O., and H. E. F. Davies.** 1959. The excretion of acid in renal disease. Q. J. Med. **28**:259-313.