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Comparative Neurotoxicities of Amphotericin B and Its Mono-Methyl Ester Derivative in Rats

KENNETH R. REUHL,[1]* MANISHA VAPIWALA,[1] MARIA T. RYZLAK,[2] AND CARL P. SCHAFFNER[2]

Neurotoxicology Laboratories, Department of Pharmacology and Toxicology, College of Pharmacy,[1] and Waksman Institute,[2] Rutgers-The State University of New Jersey, Piscataway, New Jersey 08855-0759

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The intracisternal administration of amphotericin B (AmB) and its mono-methyl ester derivative (AME), via direct intraventricular injection (0.01 to 5 mg/ml, 6 μl) in adult female Wistar rats, revealed that AmB was significantly more toxic than AME, as measured by weight loss, lethargy, death, and central nervous system histopathology. Light and electron microscopy confirmed a greater neurotoxicity for AmB, manifested as edema and modest gliosis extending along and beyond the injection tract. Neuronal degeneration and myelin damage were present in AmB-treated (1 mg/ml) animals but were present only modestly in animals treated with AME at a fivefold greater concentration. Intravenous administration of AmB to adult female Wistar rats as five daily doses of 5 mg/kg of body weight resulted in significant weight loss and some deaths. Histopathologic examination of the brains, spinal cords, and sural nerves of surviving animals revealed neurotoxicity manifested by neuronal degeneration, gliosis, and myelin edema. In sharp contrast, similar treatment with AME at a 10-fold greater dose resulted in neither death nor significant neurotoxicity. The administration of five daily doses of a mixture of AME-AmB (9:1; wt/wt) at 50 mg/kg of body weight resulted in neurotoxicity. These results indicate that AmB exhibits significantly greater in vivo neurotoxicity than AME.

Parenteral amphotericin B (AmB) has been the most effective antibiotic for the treatment of serious systemic mycotic infections, conditions that result in high morbidities and mortalities. Its use, however, has been associated with serious toxic side effects, most notably, nephrotoxicity. Numerous attempts have been made to modify the molecule chemically in the hope of producing a less toxic and equally effective derivative (38). The AmB mono-methyl ester (AME) is a derivative that is reportedly highly soluble in water (31, 39) and that has excellent in vitro and in vivo antifungal activity (1, 2, 21, 22, 26).

With apparent reduced parenteral toxicity in experimental animals (24, 25), in particular, significantly reduced nephrotoxicity, clinical evaluation of AME for the treatment of deep-seated systemic fungal infections was initiated (15). During this initial trial nephrotoxicity was rare, even at doses five times greater than those usually used with AmB. Clinical efficacy was also encouraging, since several patients who were thought to be terminal survived their mycotic infections. As the trial progressed, several patients developed a progressive neurologic disorder (20). There was a clinical association between the administration of AME in some of the patients and the appearance of neuropsychiatric aberrations. Analysis of the clinical AME preparations by high-pressure liquid chromatography (HPLC) (20) revealed, in addition to AME and residual AmB, numerous components later described as overmethylated AmB derivatives (36). The many components in AME preparations were initially detected and separated by HPLC (20) and analyzed by fast atom bombardment (36). Autopsy studies of 14 patients treated with AME for focal, disseminated, and nervous system mycotic infections revealed diffuse white matter degeneration in the central nervous system (CNS) that was most marked in the cerebral hemispheres (8). This neurotoxicity was described as "diffuse leukoencephalopathy" since the lesions were limited to brain white matter, were widely distributed, and did not have discrete borders. Severe leukoencephalopathy included myelin sheath disintegration, an increased ratio of axons to myelin, macrophages filled with lipid debris, and numerous large fibrillary astrocytes (8). The neurotoxicity described after impure AME administration appeared to be related to prolonged treatment with large cumulative doses.

Considering the correlation between the manifestation of neurological dysfunction and the appearance of leukoencephalopathy, the exact role of AME, its impurities in clinical preparations, and the CNS mycotic infection itself became the subjects of several reports (16, 17, 40). Studies in normal dogs (7, 19), which were given AME preparations of 57 to 65% purity intravenously for 30 to 60 days, appeared to confirm the development of neurological dysfunction and leukoencephalopathy. Additional studies (30) in dogs with a related chemical derivative of AmB revealed behavioral abnormalities and/or white matter gliosis after prolonged intravenous treatment with high doses.

More recently, the comparison of the neuropathologies of high doses of AmB and pure AME by injection into the peripheral nerves of rats revealed significant neurotoxicity (12, 13). The effects of AmB were far more severe, as evidenced by a more extensive peripheral nervous system degeneration and significantly fewer remyelinating axons, indicating that pure AME is less neurotoxic than AmB. The greater neurotoxicity of AmB compared with that of pure AME was also confirmed in studies with cultured rat glial cells consisting of astrocytes and oligodendrocytes (34).

Since the neurotoxicity observed after the intravenous administration of impure AME to patients was manifest as diffuse leukoencephalopathy (8), the current studies were designed to determine the potential CNS toxicities of AmB and pure AME alone and in combination after administration to rats by the direct intraventricular and intravenous routes.

* Corresponding author.

MATERIALS AND METHODS

Animals. For the intraventricular administration studies, adult female Wistar rats (weight, 150 to 180 g; Taconic Farms, Kingston, N.Y.) were individually housed in polycarbonate cages with wood shavings as bedding. For the intravenous studies, adult female Wistar rats (weight, 175 to 200 g) were used. The animals were provided water and food (Rat Chow 5012, Purina Mills, Inc., Richmond, IN) ad libitum and were maintained on a 12 h-12 h light-dark cycle.

Antibiotics. AmB as Fungizone (deoxycholate formulation) was obtained from Bristol-Myers Squibb (New Brunswick, N.J.). AME was obtained from the New Jersey Department of Health, Trenton. The AME preparation analyzed by HPLC was estimated to be in excess of 93% purity and contained approximately 1.3% residual AmB. The antibiotics for intraventricular administration were dissolved in 5% aqueous glucose solution, filtered through a 0.45-μm-pore-size membrane filter, and diluted with sterile aqueous glucose to form treatment solutions with concentrations of 0.01, 0.1, 1.0, 2.5, and 5.0 mg/ml. For intravenous administration, solutions of 1.0 mg of AmB per ml, 10.0 mg of AME per ml, and 10.0 mg of a mixture of AmB-AME (1:9; wt/wt) per ml were prepared in reconstituted form in 5% aqueous glucose. For a control, 5% aqueous glucose was used. All solutions were sterile filtered prior to use.

Experimental treatment design. For intraventricular treatment, rats were anesthetized with sodium pentobarbital and were mounted on a Kopf stereotaxic frame. A small hole was drilled in the skull, and under stereotaxic guidance, 6 μl of one of the antibiotic test solutions was injected via a fine needle (30 gauge) into the lateral ventricle at a rate of approximately 1.5 μl/min. The needle was allowed to remain in place for 1 min following the end of the injection to minimize seepage. When the needle was removed, the wound was closed with wound clips and the animal was returned to its home cage for recovery.

To test the potential neurotoxicities of AmB, AME, and AmB-AME (1:9; wt/wt) after intravenous administration, 1 ml of the test compound solution was injected via the tail vein of rats anesthetized with sodium pentobarbital. In all, five consecutive daily doses of approximately 5 mg of AmB, 50 mg of AME, and 50 mg of AmB-AME (1:9; wt/wt) per kg of body weight were administered.

All animals from the intraventricular and intravenous treatment groups were observed twice daily for the next 5 days for signs of toxicity, including loss of appetite and weight, ataxia, irritability, and death.

Tissue preparation. Five days following intraventricular or the last intravenous treatment, the rats were anesthetized with sodium pentobarbital and perfused intracardially through the left ventricle with 300 ml of 0.9% aqueous saline; this was followed by perfusion with 500 ml of 3% glutaraldehyde–1% paraformaldehyde in 0.1 M phosphate buffer (pH 7.42). The tissues were then allowed to fix overnight at 4°C before dissection.

In the intraventricular study, the brains were removed and samples rostral and caudal to the injection tract were identified and carefully removed. Tissue samples of the perfusion-fixed rats of the intravenous study were taken from the sural nerve, the cerebellum, parietal cortex, caudo-putamen, and cervical spinal cord. After rinsing in phosphate buffer, the tissue samples were processed for histopathology. For electron microscopy, the samples were postfixed in osmium tetroxide, dehydrated in ascending concentrations of ethanol, cleared in acetone, and embedded in Epon-Araldite.

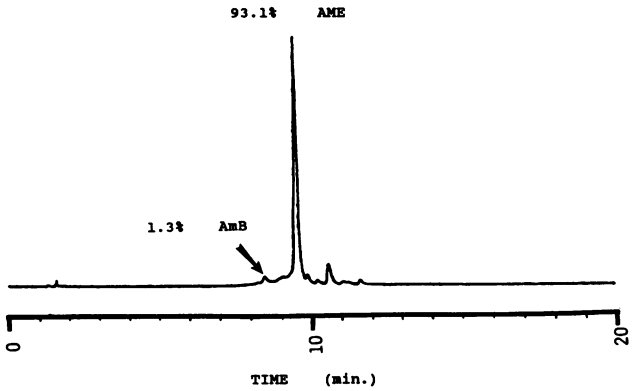

FIG. 1. HPLC analysis (Hewlett-Packard chromatograph model 1090 with diode detector) of AME-HCl (lot 5295-100A). The column was HP ODS Hypersil P/N 7991600-554 (5 μm by 10 mm by 4.6 mm [inner diameter]). The guard was Supelco Supelcoguard (LC-18, P/N 5-9064; cartridge, 5 μm by 2 cm). The mobile phase was H_2O-NaH_2PO_4-citric acid (0.05 equimolar, pH 3.7) at 80:20 (vol/vol) for 1 min, which was ramped to 30:70 (vol/vol) at 5%/min and held at 30:70 (vol/vol) for 9 min. The detector was set at 408 nm. AME-HCl in dimethylformamide at 0.1 mg/ml times a 0.01-ml injection volume was equal to 1.0 μg injected onto the column.

One-micrometer sections were stained with toluidine blue for screening. Thin sections (60 to 65 nm) were cut on a Reichert Ultracut E microtome, stained with uranyl acetate-lead citrate, and examined with a Zeiss 10CA electron microscope. Large coronal blocks of the remaining tissue were further fixed in 10% neutral buffered formalin, dehydrated in ascending concentrations of ethanol, and embedded in Paraplast. Six-micrometer sections were cut and stained with hematoxylin and eosine-phloxine or with a Holzer stain for astrocytes. Following initial examination, all slides and electron microscopic grids were coded and were evaluated blindly by a neuropathologist. The code was subsequently broken, and samples were reexamined by two reviewers.

RESULTS

The AME-HCl used in the present studies was 93.1% pure, as determined by HPLC analysis (Fig. 1). Approximately 1.3% residual AmB was present as a contaminant. The AME-HCl exhibited excellent solubility in water, and solutions could be sterilized by filtration.

The clinical findings following direct intraventricular administration of a single dose of each of AME and AmB are given in Table 1. While both compounds demonstrated toxic effects, AmB was by far the more toxic compound, with an apparent toxic dose between 10- and 20-fold less than that of AME. After administration of the highest concentration of AmB, one-third of the animals died, and the others lost weight and were lethargic. Conversely, even the highest concentration of AME administered was associated with only a 14% weight loss and no deaths.

Histopathology confirmed the impression of substantially greater toxicity of AmB after intraventricular administration. By light microscopy (Fig. 2a), a modest reactive gliosis was observed along the needle tract in all animals. Concentrations of AmB of 1.0 mg/ml or greater were associated with the formation of substantial edema extending over 2.5 mm from the injection tract. Some degenerating neurons were noted, particularly in the AmB-treated animals (Fig. 2b), but

TABLE 1. Clinical signs following intraventricular treatment[a]

Drug (concn [mg/ml])	Wt loss (% of control)	Lethargy or irritability[b]	No. of deaths/total no. of rats tested
AmB			
0.01	3.0	0	0/4
0.1	3.3	±	0/4
1.0	−4.0	+	0/4
2.5	−7.9	+	1/5
5.0	−18.1	++	2/6
AME			
0.01	−0.8	0	0/3
0.1	4.1	0	0/3
1.0	6.4	0	0/3
2.5	2.5	0	0/3
5.0	3.9	0	0/3
50.0	−14.0	+	0/3

[a] Clinical signs and weight were observed twice daily for 5 days following intraventricular administration. Lethargy and irritability were determined by observation or brief handling.

[b] 0, no clinical signs; ±, marginal effects; + and ++, positive and severe clinical effects, respectively.

degenerating neurons were observed only in rats given the highest dose of AME (5.0 mg/ml). With AmB treatment, edema was prominent along the injection tract and in the periventricular white matter. Edema was both inter- and intramyelinic and was associated with the separation of fascicles of myelinated axons and loosening of myelin lamina. In contrast, neither AME nor the glucose control induced significant edema beyond a 1.0-mm rim surrounding the needle tract.

Neuronal degeneration was observed ultrastructurally in animals administered AmB at concentrations of 1.0 mg/ml and greater. The changes consisted of dilation of cisternal organelles (Fig. 3a) and clumping of the chromatin. Similar changes were observed in adjacent glial cells, suggesting that the effects were not cell specific. Astrocytic processes appeared to be increased in number. Similar but much less frequent changes were noted in animals given a high dose of AME (5.0 mg/ml) via the intraventricular route of administration (Fig. 3b). Only occasional degenerating cells were encountered in the glucose controls, and these were confined to the immediate vicinity of the needle track.

The daily intravenous administration of AmB at a dose of 5 mg/kg of body weight for 5 consecutive days resulted, after 5 additional days of no treatment, in modest signs of clinical intoxication, including weight loss and lethargy. Histologically, evidence of neural damage was noted in the peripheral nervous system (sural nerve) and in perivascular astrocytes and neurons within the CNS. Changes of peripheral nervous system myelin, consisting of mild loosening of myelin lamina, interstitial and Schwann cell edema, and lengthening of the node of Ranvier, were noted in four of six of the animals (Fig. 4). These changes resembled those observed following direct injection of AmB into the peripheral nerve, but they were significantly less severe (12, 13). CNS changes consisted of edema of perivascular astrocytes, myelin "ball" formation, some white matter edema, and rare degenerating neurons containing abundant lysosomes (Fig. 5a and b). Capillary pericytes appeared swollen and contained an increased number of lysosomes. Endothelial cells appeared unremarkable.

Animals treated intravenously with AME (50 mg/kg of body weight per day for 5 consecutive days) lost weight during the first 2 days of treatment, but the weights recovered and thereafter were similar to those of control animals. No other signs of toxicity were evident during treatment or 5 days after the cessation of treatment, when the animals were sacrificed. By light and electron microscopy, both peripheral nervous system and CNS tissues appeared to be essentially normal. A moderate decrease in myelin compaction and some perivascular edema were observed in the sural nerve samples of 33% (two of six) of the animals, but axons and most myelin sheaths otherwise appeared to be unremarkable. Mild perivascular edema and astrocyte swelling were noted in CNS tissues, but significant neuronal injury of any kind was extremely rare.

The results for animals treated with the combination of AmB and AME proved to be the most revealing. The intravenous administration of AmB-AME (1:9; wt/wt) at 50 mg/kg of body weight per day for 5 consecutive days resulted in neurotoxicity which was similar in nature but less severe than that seen in animals given AmB alone. Injury was noted in five of six animals. The most frequent changes observed were the loosening and edema of peripheral nervous system myelin and a modest degree of interstitial edema. Within the CNS, perivascular edema, astrocytic swelling, and occasional degenerating astrocytes and neurons (Fig. 6) were observed, but only to a limited extent. As with the peripheral nervous system changes, the damage to CNS structures resembled that seen in animals treated with AmB, but it was less severe and less frequent.

DISCUSSION

AmB is a member of the large group of antifungal microbial products designated as polyene macrolides. These are noted for their ability to bind specifically to sterol components of cellular membranes resulting in the disruption of cell permeability, loss of intracellular metabolites, and eventual cell death. Differences in the degree of host and fungal cellular membrane interaction determine the clinical utilities of these drugs. With the discovery of AME, it was immediately noted that, compared with AmB, its parenteral toxicity in experimental animals was significantly reduced (24, 25). Similar results were also obtained in studies that compared the in vitro toxicities of both drugs in mammalian cell cultures (10, 11).

The exact role of AME in the progressive formation of CNS white matter degeneration or leukoencephalopathy among some patients during the first clinical trial was never clearly established. With considerable debate (8, 16, 17, 40), at best there appeared to be some agreement that the composition and total dose of AME, the duration of treatment, and the nature of the fungal infection were possible factors in the appearance of neuropsychiatric aberrations and leukoencephalopathy. The early lots of AME contained a variety of overmethylated ester derivatives, whereas later lots contained higher contents of AME and fewer overmethylated components. All clinical preparations, however, contained 2 to 8% AmB (18). Considering that AME, in all comparative toxicity studies, appeared to be less toxic than AmB, it was difficult to understand how AME could produce severe toxic CNS reactions in patients when the more toxic AmB did not. The first study (34) designed to compare the in vitro toxicities of AME and AmB on glial cells in culture clearly revealed that AmB is significantly more neurotoxic. Rat cortical glial cell cultures, consisting of astrocytes and oligodendrocytes, were 10-fold more susceptible to AmB than to AME. The myelin sheath in these cultures was

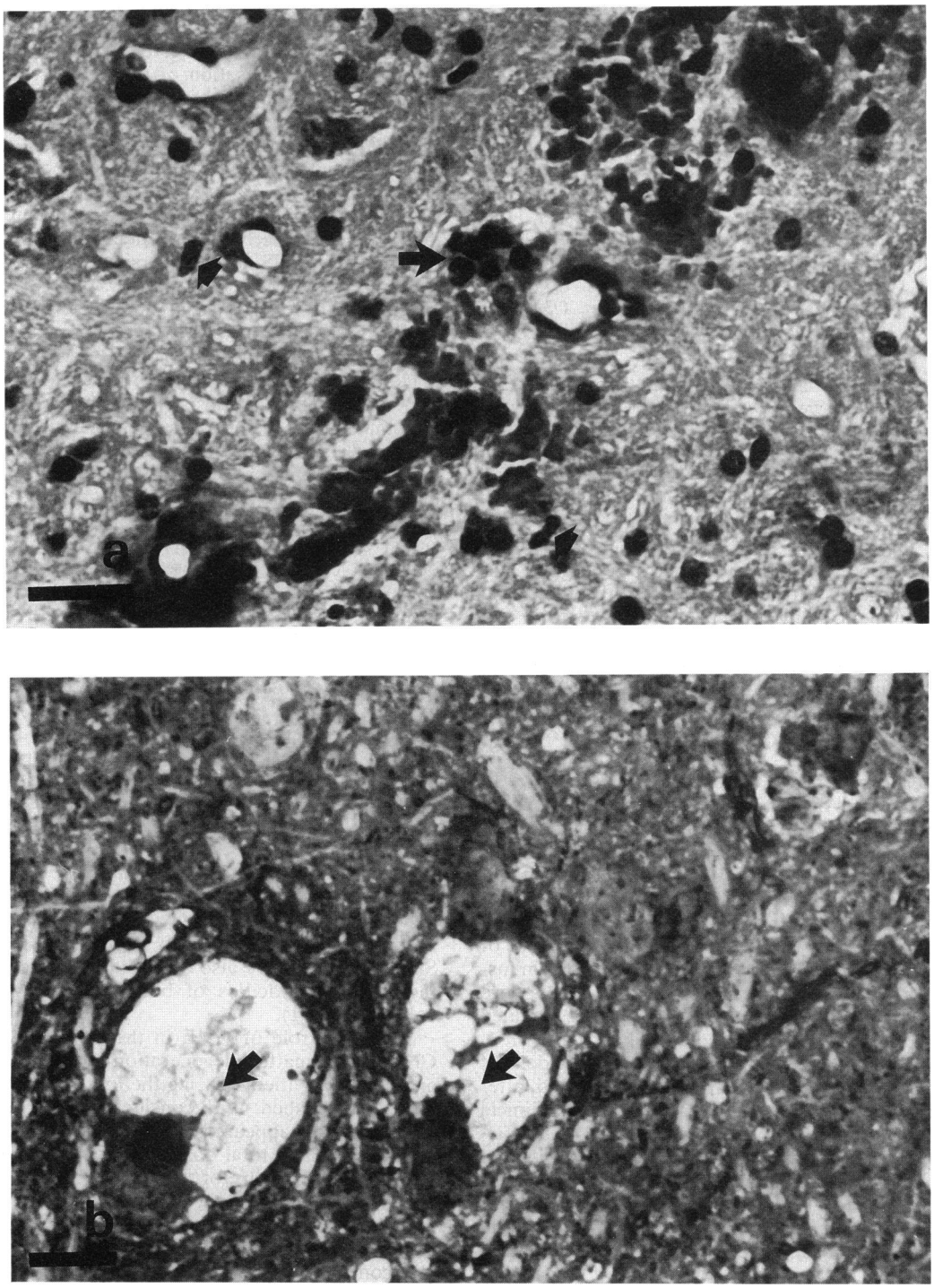

FIG. 2. (a) Light micrograph of needle track following administration of 2.5 mg of AME per ml into rat cerebral cortex. The needle path is marked by focal hemorrhage and an early proliferation of astrocytes (arrows). Some microglia are also visible (arrowheads). Mild edema extends less than 1 mm from the needle tract. (b) Light micrograph of rat cortex approximately 2.5 mm rostral of the needle tract following intraventricular treatment with 2.5 mg of AmB per ml. Two neurons (arrows) are undergoing degeneration. Mild edema is present in the cortical tissue. Bars, 25 μm.

disrupted by AmB, and its generation was inhibited. AME concentrations 10 times greater than the toxic concentration of AmB produced no such effect on the myelin sheath in cultured cells.

In other in vivo studies that compared the neurotoxicities of AmB and AME, the direct injection of these drugs into peripheral nerves of rats also revealed that AmB is significantly more neurotoxic than AME (12, 13). With unusually

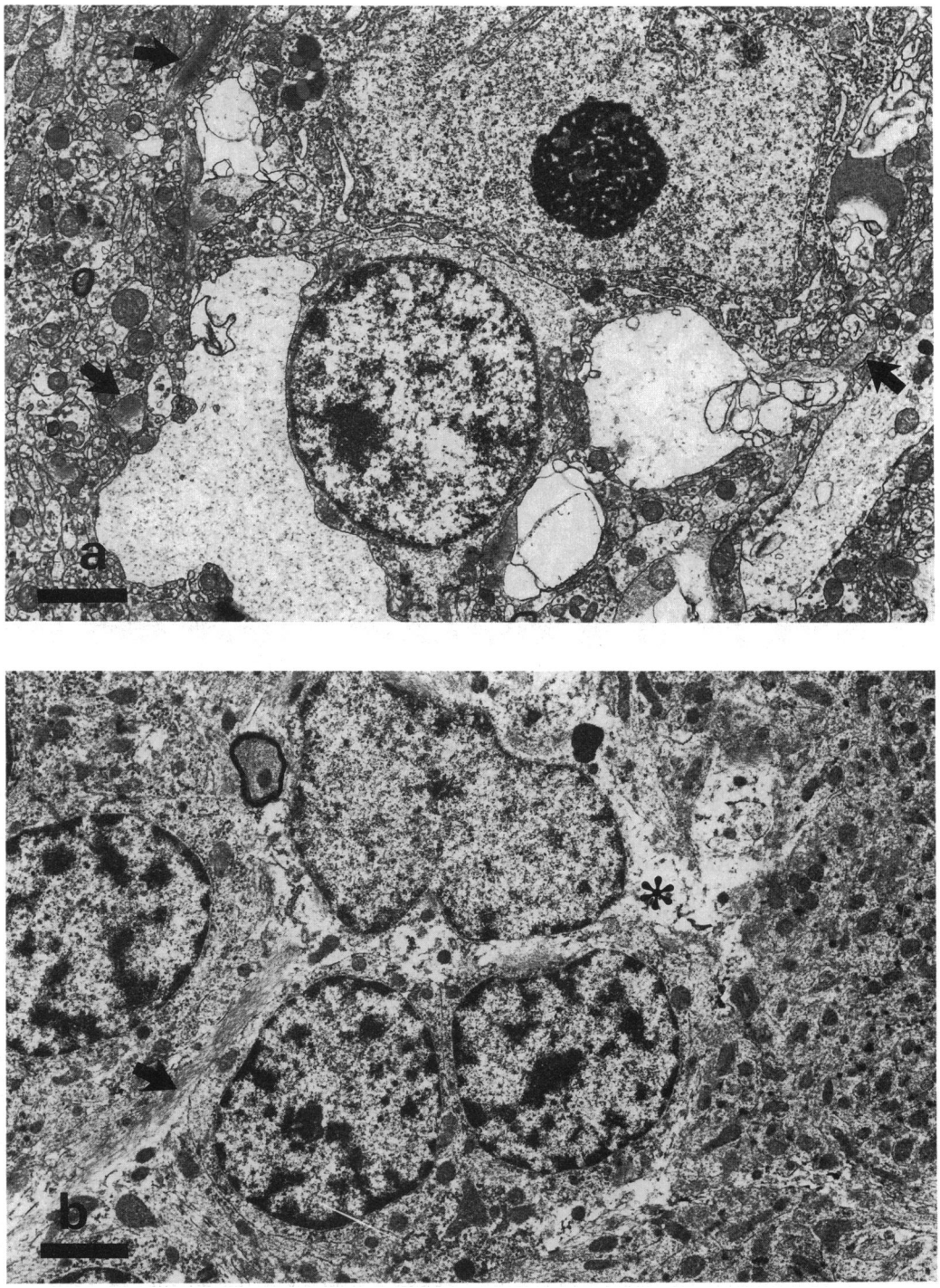

FIG. 3. Electron micrograph of the cortex 3 mm rostral to the needle tract following intraventricular administration of 1.0 mg of AmB per ml. Swelling of the cell process(es) and mild increases in the astrocytic processes containing glial filaments (arrows) are present. (b) Electron micrograph of the cortex 2 mm rostral to the injection tract following intraventricular administration of 5.0 mg of AME per kg of body weight. Mild edema (*) is noted in one small cell. Bars, 5 μm.

high concentrations (1 to 10 mg/ml) of both drugs, neurotoxicity was manifest by axonal degeneration, segmental demyelination, and myelin phagocytosis by macrophages; this was followed by eventual remyelination and axonal sprouting. Administration of AmB resulted in more pronounced axonal degeneration, significantly less remyelination of axons, and fewer regenerating sprouts than were obtained after administration of AME. At highly elevated concentrations, which are quite unlikely to be achieved in clinical practice, AME could also produce peripheral nerve alterations which

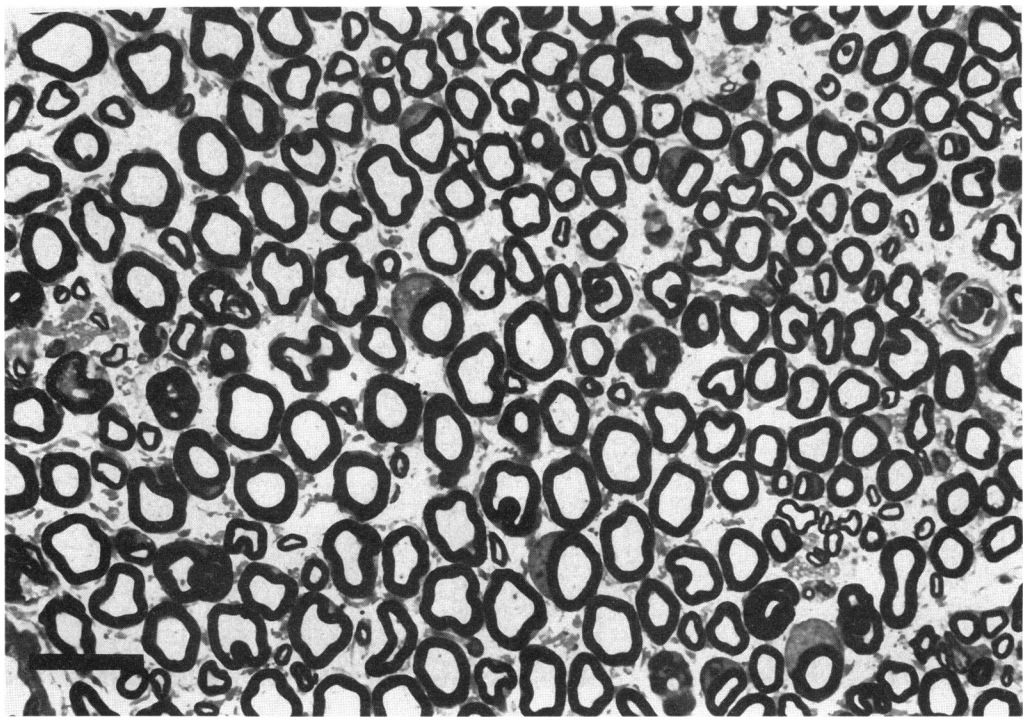

FIG. 4. Light micrograph of a 1-μm plastic section of sural nerve of rat receiving 5 mg of AmB per kg of body weight for 5 days. There is mild interfiber edema and some swelling of Schwann cells. In this field, the myelin is relatively normal, while the loss of myelin compaction was noted in other fields. Bar, 10 μm.

were, however, not as severe as those obtained after administration of AmB.

In the studies described here, the intraventricular administration of high levels of AmB and AME to rat brain resulted in neurotoxicity somewhat similar to that experienced in the intraperipheral nerve studies. Again, AmB produced much greater neurotoxicity manifested by edema and modest gliosis along and beyond the injection needle tract. Axonal degeneration associated with demyelination was observed in AmB (1 mg/ml)-treated rats, whereas AME produced only minor toxicity at a fourfold greater concentration.

The direct administration of AmB and AME to the peripheral nerves and the brain cannot be compared with their parenteral administration to patients, in which the pharmacokinetic characteristics of each drug and the nature of the fungal disease would control the ultimate levels of the drugs in tissue and blood. The results of the intravenous studies described here show that AmB can indeed produce neurotoxicity at near-lethal dosage levels (5 mg/kg of body weight), whereas AME does not. At dose levels that are 10 times greater (50 mg/kg), AME produced only minor neurotoxic effects. However, the neurotoxicity increased significantly when the AmB content of the AME preparation was increased to 10%.

Two studies comparing the neurotoxicities of AME and AmB have been carried out in dogs. The first study (19) used 14 mongrel dogs that were given 30 intravenous injections of AME (10 mg/kg of body weight), AmB (0.75 mg/kg of body weight), or 5% glucose. While AmB produced severe loss of body weight and nephrotoxicity, AME and 5% glucose both resulted in astrogliosis and pallor of the myelin in some dogs. The AME preparation contained 7% AmB. Thirty alternate-day injections of AME or 5% glucose produced greater neurotoxicity than that obtained with 30 injections given every day.

The second study (7) involved five mongrel dogs, three of which received approximately 6 to 7 g of AME as 26 to 31 intravenous injections over a period of 11 to 12 weeks. Two dogs were given 5% glucose. Cerebral white matter abnormalities were seen in the dogs only following AME treatment. Leukoencephalopathy was associated with diffuse myelin loss, oligodendrocyte depletion, accumulation of macrophages, fibrillary astrogliosis, and swelling or fragmentation of axons. No abnormalities were seen after glucose treatment. However, no dogs were treated with AmB alone. The neurotoxicity observed in that study (7) resembled that observed in our studies after AmB treatment.

Our results indicate that the AmB content of AME preparations may play an important role in the potential neurotoxicity of AME. The AME preparation used in the current studies contained 1.3% residual AmB and no overmethylated components. The AmB contaminant may very well have contributed to the more minor neurotoxicity observed in the present studies with intravenous AME at 50 mg/kg of body weight since the mixture of AmB-AME (1:9; wt/wt) produced significantly greater neurotoxicity at that dosage level.

It is interesting that all AME preparations used in the first clinical trial contained AmB (2 to 8%), as determined by HPLC (18). The report (8) of the presence of leukoencephalopathy in patients treated with high doses of AME (7 to 22 g) over periods from 6 to 22 weeks underscores the fact that extremely high doses of impure preparations were administered. Hence, considerable quantities of AmB were given simultaneously as a contaminant in the AME preparations.

The pharmacokinetics of AmB and AME are quite differ-

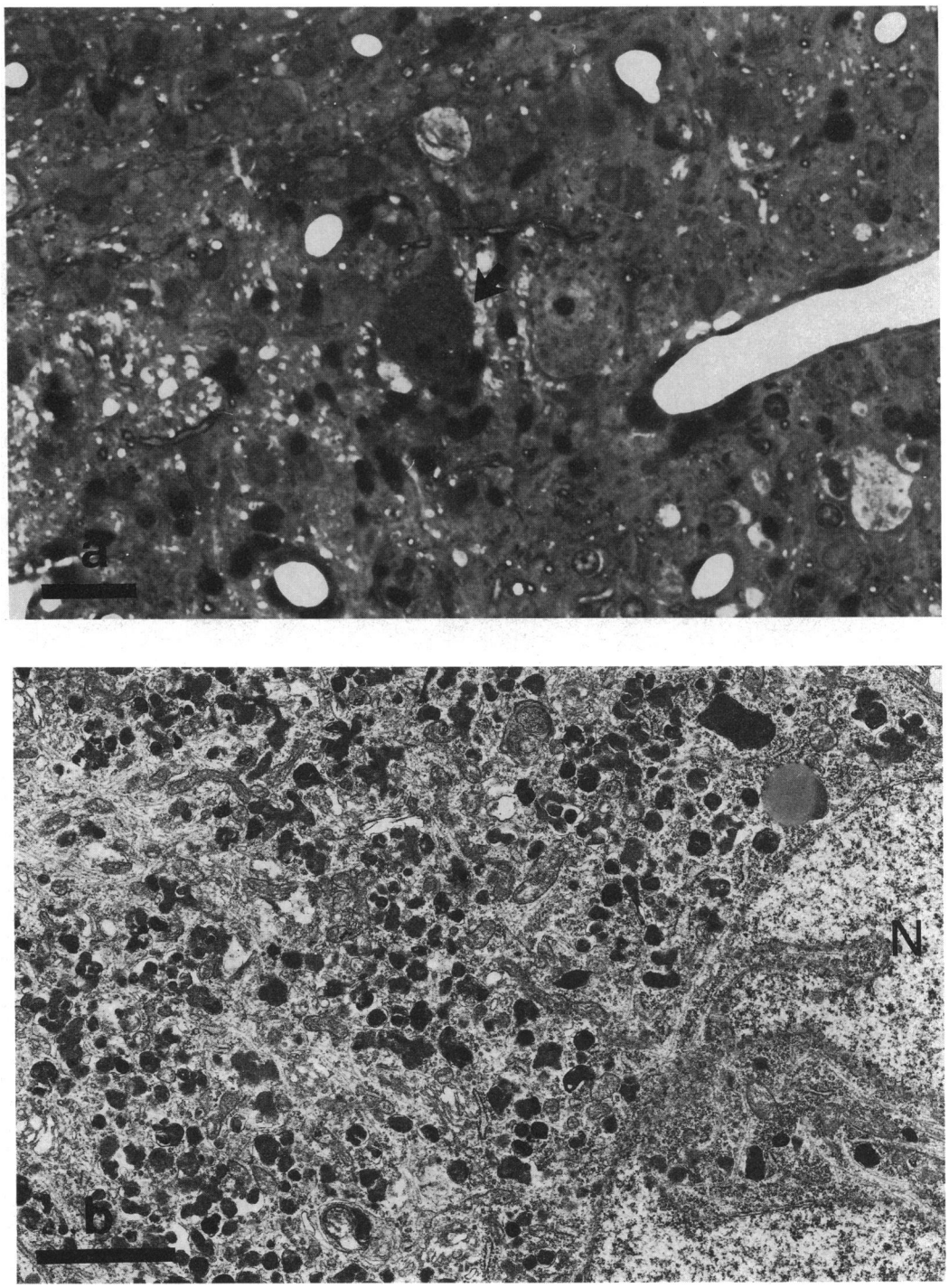

FIG. 5. (a) Light micrograph of a 1-μm plastic section of rat cortex following administration of AmB. A neuron (arrow) is undergoing degeneration and is surrounded by glial cells. Edema is present in the glial processes surrounding the neurons. Bar, 25 μm. (b) Electron micrograph of a degenerating neuron in a rat that received 2.5 mg of AmB per kg of body weight. The neuronal cytoplasm is filled with lysosomes (dark bodies). The neuronal nucleus (N) appears to be relatively normal. Bar, 5 μm.

ent and are significant factors in considering the neurotoxicities of these drugs. Studies with radiolabeled AME in mice (32) indicated that there is no detectable deesterification of the drug to AmB after intravenous or intraperitoneal administration. Additional studies with AME and AmB in the nonhuman primate (23) revealed that, after intravenous administration, the decline in drug concentrations in serum was consistent with a three-compartment, open pharmacokinetic model. The overall rates of elimination from the central compartment (blood-vascular) were about four times

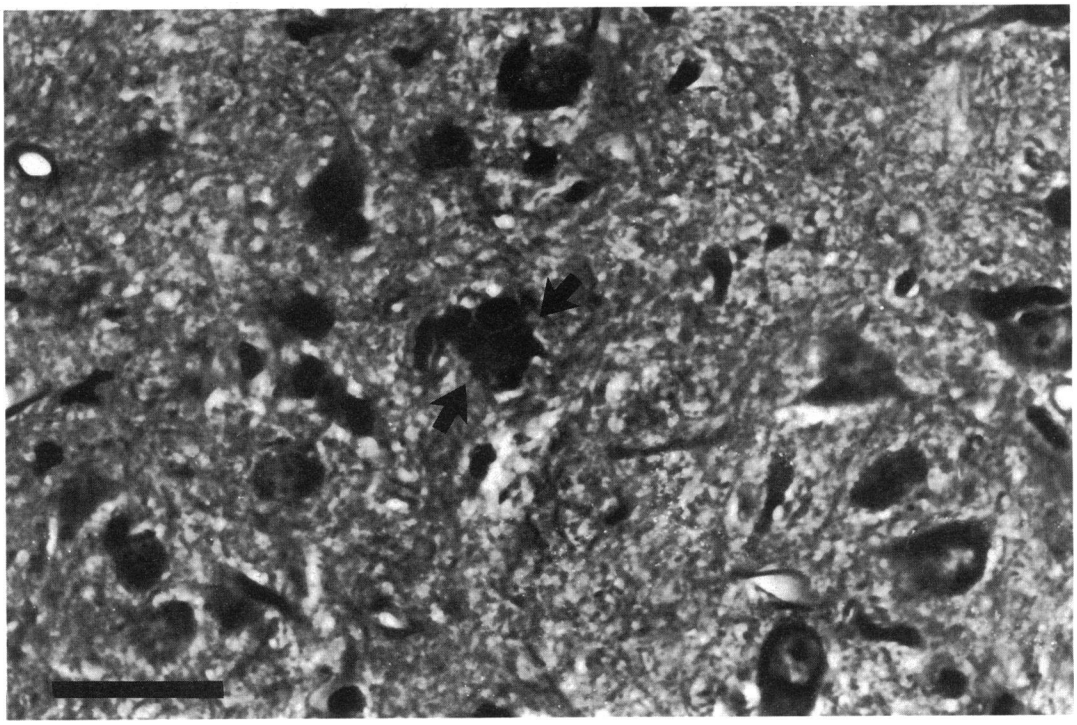

FIG. 6. Light micrograph of cerebral cortex from a rat that received a mixture of AME-AmB. Rare degenerating cells were observed (arrows). The frequency and severity of CNS injury were greater with the mixture than were observed with AME alone. Bar, 10 μm.

greater for AME than for AmB. Doses of AmB and AME of 1 and 5 mg/kg of body weight, respectively, were kinetically comparable. The biological half-lives of AME and AmB were 97 and 275 h, respectively, and the half-lives in serum were 2.5 and 17 h, respectively. Doubly radiolabeled AME and AmB (as the deoxycholate complex) were also studied in the nonhuman primate (27). While the AmB deoxycholate complex dissociated rapidly after intravenous administration, AME was metabolically stable. AME was more rapidly excreted in the urine than the more nephrotoxic AmB was. The passage of both drugs through the blood-brain barrier appeared to be similarly meager.

It is clear from the results of different pharmacologic studies that AME is more rapidly excreted from the body than AmB is. Nevertheless, extended therapy with AME at doses of 5 mg/kg of body weight on alternate days (18) might lead to the accumulation of AME and even greater accumulation of any contaminant AmB. The observed leukoencephalopathy (8) in those patients who received impure AME in large total doses over an extended period may have been due to the accumulation of AmB as well as AME.

Parental AmB therapy may result in multiple adverse reactions including neurotoxicity (37). Frequent among many neurological reactions are transient convulsions, drowsiness, headaches, and loss of hearing and vision. More serious neuropsychiatric disorders have been associated with myelin degeneration of peripheral nerves after intravenous therapy (14). Toxic delirium and electroencephalographic abnormalities have been seen after intravenous and, particularly, intrathecal treatments (42, 43). A more serious neurotoxic reaction after intrathecal AmB therapy is drug-induced myelopathy (3). Symptoms suggesting spinal cord dysfunction often occur after AmB intrathecal therapy, but these are attributed to arachnoiditis at or near the site of injection. In those studies, continued intrathecal therapy led to severe flaccid paraparesis. At autopsy, pathologic examination revealed a focal area of necrosis in one-half of the spinal cord, which was consistent with the clinical findings. More recently, cerebral and cerebellar leukoencephalopathy were seen in the brain of a patient who developed akinetic mutism after receiving 5.67 g of AmB as prophylaxis following total-body irradiation (6). These findings were similar to those reported after the administration of impure AME (8).

It was unfortunate that the first clinical trial with AME involved the use of several different impure preparations in which, at best, the AME content was 67% and the AmB content varied but was present at up to 8% or more. As seen in the present study, contamination of AME with AmB significantly increases neurotoxicity. Inadequate preclinical toxicology also contributed to the lack of understanding of potential neurotoxicity. At the same time, the absence of significant nephrotoxicity with AME was coupled with the experience of treating severe fungal diseases, encouraging the use of high doses over extended periods of time. Even with the higher rates of excretion of AME compared with those of AmB, alternate-day doses of 5 mg/kg of body weight could have led to the accumulation of AME.

It is interesting that current antifungal therapeutic research involves noncovalently bonded AmB preparations such as lipid complexes (liposomes) and colloidal preparations (29, 33, 41). These preparations generally exhibit reduced in vitro antifungal activity (35) and inferior in vivo chemotherapeutic activity (4, 5) in experimental animals. Similar to parenteral AME treatment, parenteral treatment with these AmB-lipid complexes also results in greatly reduced nephrotoxicity, although there may be damage to the kidneys when multiple high doses are given (28). The pharmacokinetic properties of these preparations appear to

be different from those of AME and AmB as the deoxycholate complex (9). Since it is believed that liposomes may act as reservoirs for free AmB, serious consideration should be given to the question of potential neurotoxicity. Currently, high doses are thought to be necessary for effective antifungal chemotherapy with liposomal AmB preparations.

In conclusion, the present studies with rats indicate that AME is a less neurotoxic drug than the AmB as the deoxycholate that is widely used in clinical therapy and indicate that additional chronic toxicological studies comparing pure AME with AmB should be conducted in other mammalian species, including nonhuman primates.

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