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Renal Histopathology Associated with Different Degrees of Amphotericin B Toxicity in the Dog. (29878)

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It has been reported recently that administration of amphotericin B to patients with systemic fungal diseases is followed by the development of renal tubular lesions, characterized by focal necrosis and calcification(1-4). Such lesions have been observed in this laboratory in 7 consecutively treated patients and in 3 dogs which had received large doses of amphotericin B and the description of these findings has been reported(4).

The present report contains descriptions of the gross and microscopic renal abnormalities in dogs given various doses of amphotericin B for periods of a few days to several months. Abnormalities of renal function in some of the dogs have been described elsewhere(5-7).

Materials and methods. Sixteen adult male mongrel dogs in good general health were given amphotericin B (Fungizone for infusion), prepared and administered intravenously as described previously(8). Blood urea nitrogen (BUN) determinations were made using an autoanalyzer (Technicon Co., Chauncey, N. Y.).

Renal biopsies were performed under anesthesia with sodium pentobarbital, using the Vim-Silverman-Franklin needle (V. Mueller Co., Chicago, Ill.) or by excisional wedge biopsy. The needle biopsies were performed after exposure of the left kidney by a muscle splitting subcostal incision, or after previous subcutaneous explantation of the left kidney by the technique of Page(9). Entire kidneys were obtained for histologic observation by nephrectomy or at post-mortem examination.

Specimens for histologic observation were obtained from 15 dogs before and after administration of amphotericin B and from an additional dog after drug administration. The specimens obtained prior to amphotericin B were all needle or wedge biopsies except for a single nephrectomy specimen. The specimens obtained following amphotericin B were whole kidneys except 2 which were biopsy specimens. Renal specimens were also obtained from 9 other dogs which had not received amphotericin B.

Biopsy specimens were fixed in 10% buffered formalin or formol sublimate, and entire kidneys were fixed in 10% buffered formalin after being bisected in a frontal plane. The sections were embedded in paraffin, sectioned at 6 μ, and stained with hematoxylin and eosin, alizarin and von Kossa stains.

Observations were made by one of us

(P.T.W.) upon the histologic preparations without knowledge of the amount of amphotericin B given. Abnormalities were coded on a scale of increasing severity from 0 to 4, referred to as the renal histopathologic index (RHI). Areas of tubular necrosis and calcification, basophilic thickening of tubular basement membranes and alterations in glomeruli were the lesions evaluated in the scoring (Fig. 1-3).

Renal histopathologic indices of Grades 1 and 2 represented kidneys in which occasional foci of nephrocalcinosis were present. Grade 1 included those specimens which contained 1 to 4 foci of nephrocalcinosis per section. Grade 2 included specimens in which there were 5 to 10 per section. Grades 3 and 4 represented lesions with tubular necrosis and calcification, or an association of these changes with pronounced basophilic thickening of the tubular basement membranes or infraglomerular reflux of tubular cells into Bowman's capsule. In Grade 3, lesions were present in as many as one-third of the nephrons, and in Grade 4, more extensive involvement was present.

Focal chronic pyelonephritis present in 3 specimens was described but not coded in the scale of 0 to 4. When more than one specimen had been obtained before or after administration of amphotericin B, the mean value for RHI was used.

Results. The renal histopathologic indices, blood urea nitrogen values, and details of administration of amphotericin B are described in Table I. The animals are listed in 4

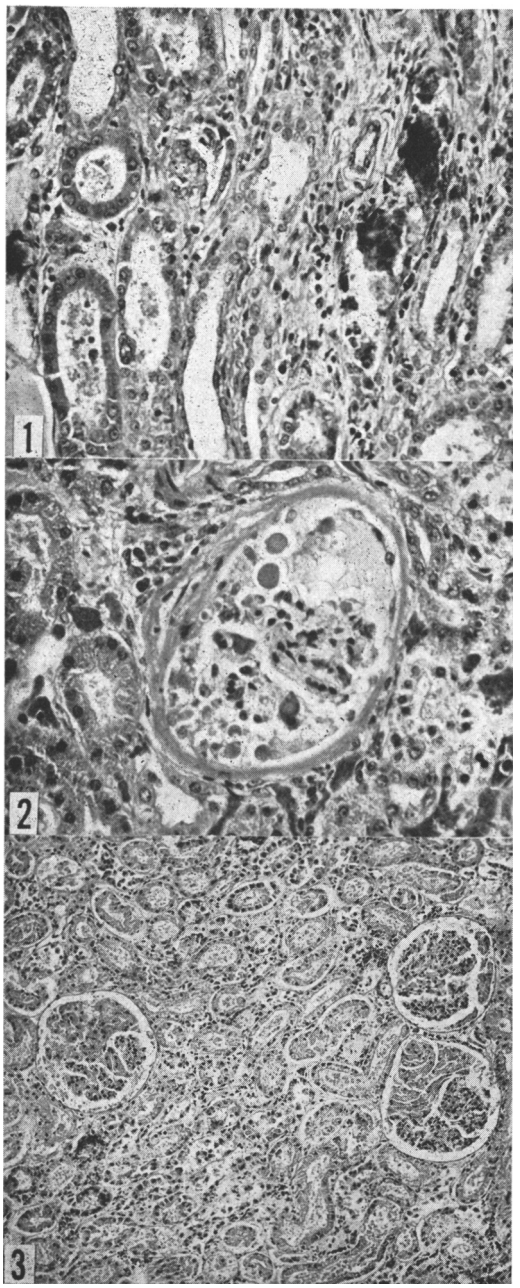
TABLE I. Renal Histopathology Associated with Different Degrees of Amphotericin B Toxicity.

Dog No.	Renal histopathologic index		BUN* (mg/100 ml)		Individual doses (mg/kg)	Total dose (mg/kg)	Duration of study† (days)
	After	Before	After	Before			
Group 1: Immediate severe toxicity and death							
U832	4	2	144	17	2.0	8.0	4
9107	4	1	200	16	1.0	11.0	12
T458	4	n.d.‡	276	28	.5–2.0	7.0	16
U885	4	0	240	23	.5	6.0	24
Group 2: Prolonged severe toxicity and death							
U491	4	2	195	13	.5	16.5	35
U580	4	0	288	15	1.0	50.0	103
9108	3	0	180	15	.5	45.5	188
T455	4	2	177	15	.5	48.0	190
U827	2	2	231	16	.25	46.75	191
U595	3	2	162	16	.25	28.0	235
Group 3: Moderate toxicity-general health excellent							
T489	3	0	36	23	.25	1.0	7
T466	2	0	102	23	.25–.5	4.25	132
T485	1	0	41	19	.25	3.75	34
T281	1	0	39	19	.25	1.0	14
ID91	1	3	38	18	.25	.75	28
T228	0	0	47	23	.25	2.75	21
Group 4: Controls							
U729		3		16			
U726		2		14			
K282		0		13			
G592		0		18			
T185		0		19			
T453T		0		20			
T330		0		23			
T189		0		24			
T347		0		24			

* Blood urea nitrogen.

† Time elapsed between first administration of amphotericin B and date when post-amphotericin B renal specimen was obtained.

‡ No pre-amphotericin B biopsy was performed. Died on 16th day after first dose of amphotericin B.



Photographs were prepared from sections stained with hematoxylin and eosin.

FIG. 1. Dog U491, lethal course of 33 doses of amphotericin B of 0.5 mg per kg every other day. Tubular degeneration and calcification. × 156. Renal histopathologic index grade 4.

FIG. 2. Dog T458, lethal course of 7 doses of amphotericin B of 0.5 to 2.0 mg per kg every other day. Compression and necrosis of capillary tuft, with proteinaceous material and basophilic droplets in Bowman's space and hyalinization of Bowman's parietal capsule. × 300. Renal histopathologic index grade 4.

FIG. 3. Dog U832, lethal course of 4 doses of amphotericin B of 2.0 mg per kg daily. Different stages of infraglomerular epithelial reflux into Bowman's space in 3 glomeruli. Tubular calcification is adjacent to one glomerulus. × 81. Renal histopathologic index grade 4.

groups according to their response to the drug. Slightly more than twice as many animals in the treated groups had pre-treatment renal histopathologic abnormalities than did untreated animals (47% *vs* 22%). However, results of blood urea nitrogen values before treatment are consistent with normal renal function in all groups. The data, therefore, are not further distinguished with respect to this finding.

Dogs in Group 1, in which toxic death occurred rapidly, received between 0.5 and 2.0 mg/kg of amphotericin B per dose. At death, blood urea nitrogen values were greatly elevated, ranging from 144 to 276 mg/100 ml. Tubular lesions in this group were considerably more extensive than in other groups and sometimes were associated with disruption of tubular basement membranes and interstitial deposition of calcium salts (Fig. 1). It was of interest that these lesions of maximum severity occurred after as few as 4 doses of drug.

The dogs in Group 2 developed severe chronic toxicity following administration of amphotericin B over prolonged periods. These dogs had blood urea nitrogen values which were similar to those in Group 1, ranging from 162 to 288 mg/100 ml. Despite the severe abnormalities in renal function of the dogs of Group 2, tubular lesions were less prominent. Tubular lesions were present, however, in all dogs. In contrast to Group 1, glomerular lesions and chronic focal pyelonephritis occurred with greater frequency in Group 2, so that the renal histopathologic indices were as high or nearly as high as those in Group 1 (Table II). Thickening of Bowman's parietal capsule, interstitial fibrosis, and hyalinization of glomeruli occurred only in dogs in Group 2. Moreover, Bowman's space contained proteinaceous material with basophilic spherules often compressing the capillary tufts (Fig. 2) in all dogs of

TABLE II. Histopathologic Abnormalities of Renal Glomeruli Following Amphotericin B.

		Glomerular lesions*			
		Thickening Bowman's parietal capsule	Basophilic spherules in Bowman's capsule	Infraglomerular epithelial reflux	Hyalinization
Group 1	U832		+	+	
	9107				
	T458		+		
	U885				
" 2	U491		+	+	
	U580		+	+	+
	9108	+	+		
	T455	+	+		
	U827	+	+		
	U595		+	+	

* + = abnormality present.

Group 2 but in only 2 of the 4 dogs of Group 1. Likewise, infraglomerular epithelial reflux (Fig. 3) was seen in only one dog of Group 1, but in 3 dogs of Group 2. Another change seen only in Group 2 was extensive basophilic thickening of tubular basement membranes.

Dogs in Group 3 received 3- to 5-day courses of 0.25 to 0.5 mg/kg which were repeated at 1-2-month intervals. Periods of transient azotemia were related to dosage of drug administered, and were partially reversible. Histopathologic changes seen in this group were slight when compared to those of Groups 1 and 2.

Thus renal histopathologic changes were greatest in the dogs which developed very high blood urea nitrogen values following either high individual doses or high total doses of amphotericin B.

Gross abnormalities observed at postmortem examination in Groups 1 and 2 were emaciation and dehydration in all 10 dogs, the appearance of hemorrhagic gastritis or enteritis in 4, and recent hematemesis or melena in 3. The gross anatomic diagnoses of gastritis and enteritis were not confirmed histologically; only autolysis and vascular congestion were found histologically in these dogs. Congestion or edema was present in tissue sections from the spleens of 7 dogs, from the lungs of 6, and from the livers of 5. Focal myocardial inflammation or necrosis was observed in sections from 4 dogs. In addition, congestion of the adrenal medulla was observed in sections from 2 dogs, and a small submucosal gastric hematoma was present in sections from one dog.

Discussion. Extensive depositions of heavily staining calcium salts were present in convoluted tubules near the cortico-medullary junction of kidneys following large doses of amphotericin B. In untreated dogs, however, scattered deposits of calcium salts were predominant in the collecting tubules and failed to stain intensely in the alizarin preparation. Other changes noted in biopsy specimens of untreated dogs included pyelonephritis and minimal hydronephrosis. These abnormalities seen in specimens from untreated dogs have all been described by other investigators(10-11).

The lesions most commonly seen in the glomeruli of human patients treated with amphotericin B are fibrosis, hyalinization, thickening of the basement membranes and hypercellularity(12). The lesions observed in the glomeruli of 9 dogs of the present study had not been described previously. The finding of infraglomerular tubular reflux is especially interesting, and the difference between the human and canine studies cannot be explained fully. The dogs were allowed to develop greater renal damage than commonly occurs during treatment in man, and the glomerular lesions may represent a more profound alteration due to increased toxicity. The cause of infraglomerular reflux is not known, although anoxia was suggested as a stimulus for this change by Waugh(13) who

produced the abnormality by serotonin injection in rats. During acute and chronic amphotericin B toxicity in dogs(5) a decrease in renal blood flow of considerable magnitude has been observed which could cause renal hypoxia. In general, the severity of histopathologic changes in most dogs was related to the dose of drug received. This finding was of interest in view of the recent demonstration in man of abnormalities of renal function related to dosage of drug(14).

These experiments confirm previous studies by other investigators of hematemesis and melena associated with lethal doses of amphotericin B in dogs(15). The source of this bleeding, however, remains unclear. The necropsy findings of hepatic damage (5 dogs) cannot be interpreted completely. The changes observed are consistent with significant agonal hypotension, although blood pressures were not recorded in the last hours of life. Specific toxicity to the liver was not observed in random tests of liver function performed in some dogs, or in pathologic observations. The pulmonary edema and congestion of the spleen observed in some dogs may have been terminal events associated with severe uremia rather than due to specific action of amphotericin B. Myocardial abnormalities are somewhat more difficult to evaluate; cardiac lesions occurred less frequently and were less severe than the lesions in the kidneys, liver and lungs. However, alterations in cardiac rate and rhythm have been reported(16) following administration of amphotericin B to man, and recent studies in dogs indicate that the drug produces cardiac toxicity, perhaps mediated through acute electrolyte imbalance(17).

Summary. Renal specimens were obtained by biopsy or at necropsy examination from 16 dogs which had received amphotericin B. These specimens were compared with pretreatment specimens from 15 of these dogs, and with specimens from 9 other normal dogs. The severity of the histologic changes of tubular necrosis, tubular calcification, basophilic thickening of tubular basement membranes and infraglomerular epithelial reflux, was graded on a scale of zero to 4. Dogs which died of acute toxicity exhibited grade 4 renal histopathologic abnormalities which were predominantly tubular. Dogs which developed severe chronic toxicity developed a greater proportion of glomerular changes than seen in the acute group. The renal histopathologic changes were most extensive in dogs which received either large individual doses or large total doses. Dogs which developed transient azotemia following short courses of low doses of drug developed only slight histopathologic abnormalities.

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