Acute Renal Effects of Amphotericin B[1] (35549)

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Administration of the polyene antibiotic, Amphotericin B (AmB) for the treatment of mycotic diseases has been associated with abnormalities of renal function (1). Its chronic administration in man causes a reduction in glomerular filtration rate (GFR) and effective renal plasma flow (ERPF), renal tubular acidosis, natriuresis, and kaliuresis (2). In the dog, acute administration of AmB results in a similar decrease in GFR and ERPF apparently due to active renal arteriolar constriction (3). Studies on the toad and turtle bladder indicate that AmB increases the permeability of the membrane to water, urea and electrolytes (4, 5).

This study was designed to determine the acute effects of AmB on the renal tubule and on the intrarenal distribution of blood flow. These results confirm the previously demonstrated decrease in ERPF and GFR. In addition, there was an increase in the fractional excretion of sodium and potassium. Although free water clearance (C_{H_2O}) was decreased, the free water reabsorption curve (Tc_{H_2O}/C_{osm}) was unaffected, suggesting that the effect of AmB is predominantly at the distal tubule beyond the loop of Henle. The decrease in C_{H_2O} was probably due to a decreased distal tubular reabsorption of sodium and an increase in membrane permeability to water.

Materials and Methods. The experiments were performed on female mongrel dogs (12–20 kg) during hydropenia or water diuresis.

I. Water diuresis. Eleven dogs were included in this study. All food was withdrawn 16–18 hr before the experiment. Water was permitted *ad libitum*. The animals were anesthetized with sodium pentobarbital (30 mg/kg iv). An endotracheal tube was inserted and connected to a mechanical respirator. The femoral vessels were cannulated for blood sampling, fluid administration, and blood pressure recording. A Foley catheter was placed in the urinary bladder; and complete emptying of the bladder was assured by air washout. An oral water load amounting to 5% of the body weight was administered via a gastric tube, and supplemented by an equal amount of 2.5% dextrose in water given intravenously. Water diuresis was sustained by infusing 0.45% saline at 0.5 ml/kg/min. Inulin and *p*-aminohippurate solution were infused into the femoral vein to permit clearance measurements. After an equilibration period of 45–60 min or when water diuresis was achieved, 2–4 control urine samples were collected at 5–15-min intervals. Blood samples were collected at appropriate times. AmB (2 mg/kg) was then infused intravenously at a rate of 0.5 mg/min for approximately 30 min. Thereafter, urine was sampled every 10–15-min for approximately 1 hr.

II. Antidiuresis. There were 5 animals in this group. Thirty-six hr prior to study the animals were deprived of food and water. Sixteen to 18 hr before the experiment, 5 units of pitressin tannate in oil were given intramuscularly. After endotracheal intubation, vessel cannulation and initial blood and urine sampling, inulin and PAH were infused for clearance procedures. Ten percent mannitol was administered to establish a modest osmotic diuresis. Antidiuresis was maintained by a constant infusion of aqueous pitressin at 50 mU/kg/hr. When urine flow was stabilized, three 10-min urine collections were obtained and AmB solution was given as

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in group I. After administration of the drug was stopped, additional urine collections were obtained at 10–15-min intervals for 60 min.

III. Renal blood flow distribution. In three additional dogs undergoing water diuresis as in group I, the intrarenal blood flow distribution was determined using the washout of radioxenon (6). Studies were performed 30–90-min after the administration of AmB directly into the renal artery (0.5 mg/min for approx 30 min).

Inulin was determined by a modification of the method of Hubbard and Loomis (7) and PAH by the method of Smith *et al.* (8). Urine and plasma electrolytes were analyzed with a digital flame photometer. The xenon washout curve was analyzed as previously described (6, 9). Significance was tested by the matched paired *t* test.

Results. In all the experiments, the clearance of inulin and PAH fell after the administration of AmB. Blood pressure was unaffected by the drug.

I. Water diuresis. The results are summarized in Figs. 1 and 2. During the control periods in any given case no significant differences in sodium excretion were noted, so that there was no evidence for a sodium diuresis produced by the sustaining infusion. Immediately following the infusion of AmB there was a significant decrease in urine flow,

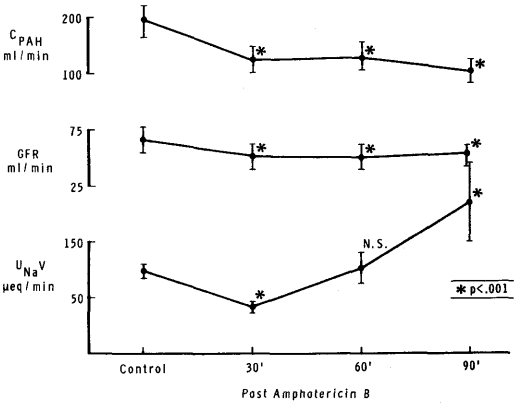

FIG. 1. Effect of amphotericin B on clearance of PAH (C_{PAH}), glomerular filtration rate (GFR) and rate of urinary sodium excretion ($U_{Na}V$) during water diuresis.

fractional excretion of sodium, and free water clearance. Ninety min after infusion of AmB, the clearance of PAH continued to fall while the GFR stabilized at a mean of 50–53 ml/min (82% of control). Urine flow recovered to about 70% of control and the clearance of free water to within 50%. The fractional excretion of sodium increased to more than 100% of control levels while that of potassium was inconsistently increased (5 of 11 dogs). Ninety min after the drug was given, the distal tubular load (C_{Na} +

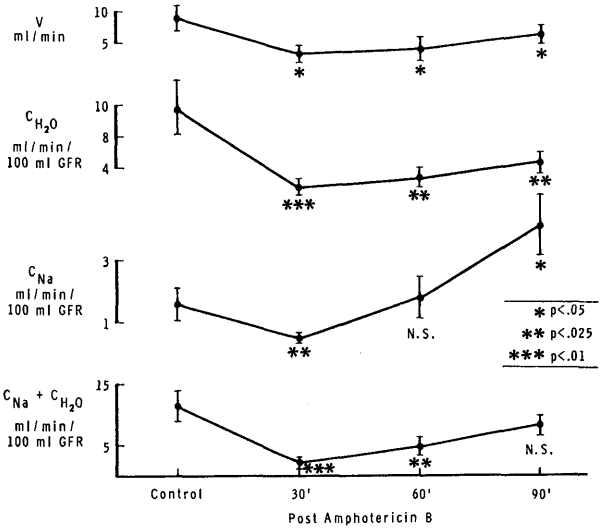

FIG. 2. Effect of amphotericin B on urine flow rate (V), free water clearance (C_{H_2O}), sodium clearance (C_{Na}) and distal tubular load ($C_{Na} + C_{H_2O}$).

C_{H_2O}/100 ml GFR) returned to control values and the absolute excretion of sodium ($U_{Na}V$) was significantly increased. The relationship between free water clearance/100 ml GFR and urine flow/100 ml GFR is shown in Fig. 3. While the correlation coefficient was 0.95 before the drug was administered (standard error of the estimate = ±3.06), the r value fell to 0.84 after AmB (standard error of the estimate = ±3.10). The change in the slope after AmB was significant ($p < .05$).

II. Antidiuresis. The drop in GFR and ERPF was comparable to that seen in studies during water diuresis. Tc_{H_2O}/C_{osm} was unaffected by the administration of AmB (Table I). Fractional sodium excretion sigsificantly increased after 90 min of AmB administration ($p < .025$). The changes in fractional potassium excretion were not significant ($p > .4$).

III. Xenon washout studies. Total renal blood flow decreased from 3.5 ± 0.1 to 0.9 ± 0.3 ml/g of kidney/min. Before AmB component I flow was 4.6 ± 0.3 ml/g/min and component II flow was 1.4 ± 0.1 ml/g/min. After the drug was given, components I and II could no longer be distinguished from each other. Regional flow distribution changes could not be correlated with the observed alterations in urinary electrolyte excretions.

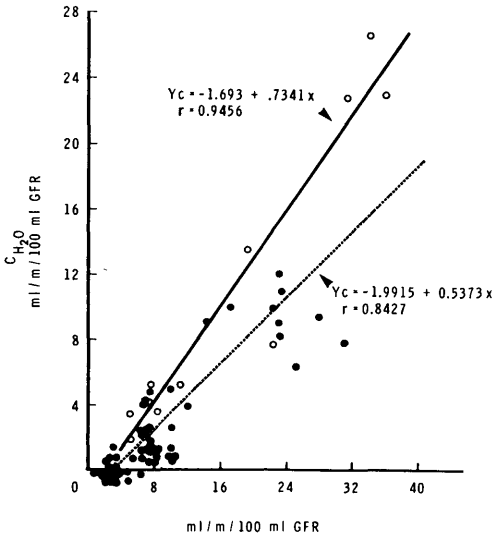

FIG. 3. Relationship between free water clearance (C_{H_2O}) and urine flow rate (abscissa) before and after administration of Amphotericin B. Open circles represent control periods and closed circles represent values obtained after Amphotericin B administration. The solid line represents the linear regression of the control points; the broken line represents the linear regression of the experimental points.

Discussion. Acute administration of AmB in the dog produces intense renal vasoconstriction (3). These studies confirm that AmB decreases the clearance of inulin and *p*-aminohippurate. Previous investigators have shown that the fall in the clearance of PAH is not due to depression of its transport mechanism (1). Moreover, the renal blood flow as measured by the washout of radioxenon was also reduced in these studies.

The increased sodium excretion and decreased free water clearance induced by AmB may be due to one or more of the following mechanisms. (i) The filtered load may be redistributed from the inner cortical nephrons to the superficial nephrons where the reabsorption of sodium and clearance of free water may be less (10, 11); (ii) AmB may alter membrane permeability allowing back diffusion of water from the tubular lumen into the interstitium; (iii) AmB may decrease the distal tubular transport of sodium. In several states of sodium diuresis, investigators have shown that the superficial nephron filtration was increased (10, 11), while the converse occurred in sodium retaining conditions. This mechanism was probably not operative in our study since the xenon studies indicated reduced flow to both compartments. Berliner and Davidson (12) have previously demonstrated that during water diuresis, a marked fall in GFR is accompanied by a rise in urine osmolality. The urine became hypertonic when the reduction in GFR was in the range of 55 to 30% of control. The rise in urine osmolality, which was independent of vasopressin, appeared due to a decreased solute load to the distal tubule; however, the final urine contained minimal amounts of potassium, chloride, and sodium. The decrease in GFR in this report was only 25% and, while the GFR remained depressed, the fractional excretion of sodium and potassium increased. The decreased free water clearance noted 30 min after beginning infusion of the drug may be explained by the

TABLE I. Effects of Amphotericin B on Urine Flow Rate (V), Sodium Clearance (C_{Na}), Potassium Clearance (C_K), Negative Water Clearance (Tc_{H_2O}), Osmolar Clearance (C_{osm}), and Glomerular Filtration Rate (GFR).[a]

Dog no.	Time (min)	V (ml/min)	(ml/min/100 ml GFR)				GFR (ml/min)
			C_{Na}	C_K	Tc_{H_2O}	C_{osm}	
D 2	C	0.25	0.24	16.8	1.4	1.7	91
	30	0.17	0.04	13.7	0.7	0.9	70
	60	0.24	0.05	26.7	1.5	2.0	47
	90	0.30	0.41	34.0	1.6	1.8	60
D 17	C	0.44	0.03	28.3	0.4	2.4	25
	30	0.44	0.04	20.3	0.4	2.3	25
	60	0.31	0.13	10.5	0.4	1.7	24
	90	0.30	0.24	13.0	0.3	2.2	16
D 18	C	0.80	0.04	11.3	5.3	6.1	70
	30	0.69	0.07	10.4	5.5	6.5	65
	60	0.70	0.20	8.9	6.9	8.1	58
	90	0.73	0.24	7.4	6.2	7.4	62
D 19	C	0.39	0.03	9.3	0.8	1.4	74
	30	0.38	0.05	18.5	2.4	4.3	28
	60	0.43	0.05	14.1	1.3	2.2	45
	90	0.66	0.15	12.1	0.7	1.6	67
D 27	C	0.24	0.02	14.8	1.1	1.5	74
	30	0.18	0.01	10.1	1.7	2.1	43
	60	0.20	0.02	12.4	1.4	2.0	35
	90	0.35	0.03	13.2	1.2	1.8	49

[a] The results at 30, 60, and 90 min were compared with control values using the matched pair t test. The decrease in GFR ($p<.05$) in all time periods and the increase in C_{Na} ($p<.025$) at 90 min are the only significant changes induced by Amphotericin B.

acute drop in GFR. At 90 min, however, when the distal tubular load ($C_{Na} + C_{H_2O}$/100 ml GFR) was not significantly different from control, urine flow (absolute and per 100 ml GFR) was still significantly lower. Therefore, the decrease in C_{H_2O} may not be due solely to the increase in C_{Na} but also to increased back diffusion of water due to AmB induced increase in tubular membrane permeability. Previous studies have demonstrated that AmB increased membrane permeability to water in the toad and turtle bladder (4, 5). In our studies, the increased permeability, if present, might be explained on this basis or by an effect of the infusion leading to increased secretion of ADH. The chronic administration of AmB in man produces renal tubular acidosis and renal wasting of sodium and potassium, suggesting that the drug has a selective toxicity to the distal tubule. Such an action would lead to an increase in C_{Na} with an equivalent decrease in C_{H_2O}. Although the magnitude of change in these parameters was not identical, this would seem to be in part an explanation for the results observed. Since Tc_{H_2O} was unaffected by AmB, the effect of the drug may be attributed to an action on the distal tubule beyond the loop of Henle.

It is interesting to note that while AmB-induced potassium wasting is well described in the literature only two cases of renal sodium wasting have been reported (13, 14). However, the frequency of either abnormality may be difficult to assess unless patients are specifically and carefully studied. Moreover, sodium retaining mechanisms (*e.g.*, reduced GFR and secondary hyperaldosteronism) may magnify the drug-induced kaliuresis while tending to minimize sodium loss.

Summary. The acute effects of intravenously administered AmB were studied

in 19 dogs during a water diuresis and antidiuresis. The decrease in C_{H_2O} cannot entirely be due to the decrease in GFR. Tc_{H_2O}/C_{osm} was unaltered, suggesting that the drug acts on the late distal tubule. The acute tubular effects may be due to alterations in tubular reabsorption of sodium and membrane permeability to water.

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