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Studies of Capreomycin Nephrotoxicity

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Nephrotoxicity induced by the administration of the tuberculostatic antibiotic capreomycin in laboratory animals has been described by Welles *et al.* (1966). In confirmatory studies in rats, it was found that the lesion induced by capreomycin was similar in histologic development to that caused by 5-hydroxytryptamine (5-HT) (Fiore-Donatti and Erspamer, 1957; MacDonald, 1959). In addition, capreomycin caused acute depression accompanied by cyanosis and edema in the extremities, which was also similar to the toxicity induced by 5-HT (Jasmin and Bois, 1960).

These findings suggested that the nephrotoxicity of capreomycin might be mediated by 5-HT, which is released by the administration of capreomycin. The present study was carried out to investigate this possibility.

METHODS

5-HT releasing activity of capreomycin in rats. Male rats of the Wistar strain, initially weighing 100–130 g, were used. The animals were kept under standard conditions with free access to rat chow and tap water. A nephrotoxic, nonlethal dose of 400 mg/kg of capreomycin as the disulfate was injected intraperitoneally. At 1, 4, 24 hours, 2, 4, and 7 days after the injection, the necks of the animals were dislocated without causing immediate death and rupture of cervical vessels. The peritoneal cavity was opened quickly and 1 ml of blood was collected from the aorta, using a previously heparinized syringe.

In another experiment, capreomycin was injected subcutaneously for 5 or 7 consecutive days. Twenty-four hours after the last injection, blood was collected by the method described above. The small intestine, including the duodenum, and an interscapular region of the skin, previously dehaired with an electric clipper, were excised. The intestinal contents were removed before homogenization of the tissue with a motor-driven glass homogenizer. The skin was minced with scissors and homogenized with emery in a mortar. 5-HT content in the whole blood and the tissues was determined fluorometrically according to the method of Bogdanski *et al.* (1956). This method was adequate to assay whole blood, since the lower limit of sensitivity is 0.1 μg/ml and each sample was shown to contain more than 0.4 μg/ml of 5-HT.

Effect of adrenergic blocking agents upon the development of capreomycin-induced renal damage. In an attempt to inhibit renal damage induced by the administration of capreomycin, adrenergic blocking agents were used: chlorpromazine, 10 mg/kg; hydralazine, 20 mg/kg; dibenamine, 15 mg/kg; LSD-25, 0.5 mg/kg; propanolol, 5 mg/kg; and pronethalol, 5 mg/kg. (In preliminary experiments it was shown that the

above doses given daily, intraperitoneally, for 5 days caused no signs of renal toxicity.) One half of the dose was injected intraperitoneally 1 hour prior to the subcutaneous injection of 200 mg/kg or 400 mg/kg of capreomycin. The subcutaneous route was chosen to avoid direct contact with the adrenergic blocking agents at the injection site and because rats could not tolerate intraperitoneal administration of 400 mg/kg. The remaining half dose of the adrenergic blocking agent was injected 7 hours after capreomycin administration since the maximum level of 5-HT in the blood continues from 1 to 24 hours after single intraperitoneal administration of capreomycin. This injection schedule was continued for 5 consecutive days. Twenty-four hours after the last injection, the animals were sacrificed by a blow on the head and the kidneys were removed. Histologic evaluation of the renal damage was performed on formalin-fixed, paraffin-embedded sections stained with hematoxylin and eosin. The damage was limited to the renal cortex, so grading was expressed according to the degree of the cortical necrosis. A relative scale from (–) to (++++) was adopted to indicate the renal damage: (–) no renal lesions, (±) a small number of tubules affected, (+) about 10% of the renal cortex necrotized, (++) renal damage involving more than 20% of the renal cortex, (+++) renal damage involving more than 40% of the renal cortex, and (++++) renal damage involving more than 70% of the renal cortex.

Effect of capreomycin on mast cells. In the experiment on mast cells, a nephrotoxic dose of 400 mg/kg of capreomycin was injected intraperitoneally. Animals were sacrificed 16 or 48 hours later, and a piece of ileum with its mesentery was carefully dissected from an area close to the cecum. Four pieces of the mesentery of each rat were removed and fixed in a mixture of formalin: 95% ethanol: distilled water: acetic acid (3:10:20:0.5), and stained with 0.025% thionin in carbonate buffer solution at pH 4, with stirring for 90 seconds. Stretched and dried mesenteric specimens on glass slides were defatted with acetone, cleared in xylene, and mounted with Canadian balsam. The number of normal and degranulated mast cells was counted in a population of 100 cells per piece of mesentery.

For study *in vitro* of the mesenteric mast cells, separated mesenteries were incubated in Krebs-Ringer phosphate buffer solution in a 10-ml glass container with various amounts of capreomycin for 1 hour at 37°. Slides were prepared as described previously.

In another experiment on mast cells *in vivo*, 400 mg/kg of capreomycin was administered intraperitoneally; 1 hour later the rats were sacrificed. Snout, paws, and scrotum, which were cyanotic and edematous, were separated and the subcutaneous mast cells of these organs were examined microscopically (Selye, 1965).

Species difference of capreomycin-induced renal damage. The species difference in renal damage induced by capreomycin was investigated using white male mice of DS strain (18–20 g), white male rats of Wistar strain (110–130 g), golden hamsters (90–100 g), white male guinea pigs (120–130 g), white male rabbits (1.8–2.0 kg), and male cats (2–4 kg). Daily doses of 200–2000 mg/kg of capreomycin were administered subcutaneously for 5 consecutive days. Animals were sacrificed 24 hours after the last injection, and histologic examinations of the kidneys were performed.

RESULTS

5-HT Releasing Activity of Capreomycin in Rats

From 1 hour to 24 hours after the single intraperitoneal administration of 400 mg/kg of capreomycin, the level of 5-HT was increased to about twice the normal value

Effect of Adrenergic Blocking Agents upon Development of Capreomycin-Induced Renal Damage

The adrenergic blocking agents, chlorpromazine, dibenamine, and LSD-25, showed some inhibitory effect on the development of capreomycin-induced renal damage, but the adrenergic β-blockers, propanolol and pronethalol, showed none. Hydralazine exacerbated the renal damage by capreomycin (Table 2).

Effect of Capreomycin on Mast Cells of Rats in Vivo and in Vitro

A prostrated and dyspneic state with cyanotic and edematous extremities was observed after a single intraperitoneal administration of 400 mg/kg of capreomycin in rats. Mast cells in these extremities and in the mesentery showed severe degranulation. Quantitative observations of mesenteric mast cell degranulation after 16 and 48 hours of drug administration are shown in Table 3.

In the study, *in vitro* capreomycin at a concentration of 125 μg/ml or more produced degranulation of mesenteric mast cells with a definite dose-response relationship. At a concentration of 500 μg/ml, the metachromatically stained granules seldom remained in the cytoplasm. Disruption of mast cells, which has been known to occur after treatment with histamine liberators (Bhattacharya and Lewis, 1956; Parratt and West, 1957a), was not evident. Degranulation of mast cells by capreomycin means that the metachromatic granules were discharged, but disintegration or disruption of the cytoplasm did not occur.

Species Differences of Capreomycin-Induced Renal Damage

Although nephrotoxicity was seen in rats at a daily dose of 200 mg/kg, only slight cortical necrosis was seen in mice at a dose of 1000 mg/kg. In the hamster, guinea pig, rabbit, and cat, the typical cortical necrosis of the kidneys seen in rats was not observed. As shown in Table 4, none of the animals of the species used in these studies tolerated 2000 mg/kg/day for 5 days, but most animals survived a dose of 1000 mg/kg/day. In the hamster and rabbit slight desquamation or karyorrhexis of the epithelium of the proximal tubules was the only lesion encountered.

DISCUSSION

The nephrotoxic property of capreomycin in laboratory animals has been described by Welles *et al.* (1966); and it has been confirmed that the main toxicologic problem is its renal toxicity. The present investigation was carried out to determine some mechanisms of capreomycin-induced renal damage with special consideration to its 5-HT releasing activity, which might be responsible.

It is known that not only 5-HT (Fiore-Donatti and Erspamer, 1957; MacDonald, 1959), but also its metabolic precursor, 5-hydroxytryptophan, and the 5-HT releaser reserpine are nephrotoxic (Garattini and Valzelli, 1965). The sequence of renal damage mediated by 5-HT is vasoconstriction (Greco *et al.*, 1956; Craig, 1966), ischemia, and necrosis (Waugh and Pearl, 1960), with the necrosis localized mainly in the proximal tubules of the cortex (MacDonald, 1959; Waugh and Pearl, 1960). The glomeruli are usually not involved. It is probable that if capreomycin-induced renal damage is due

TABLE 2

EFFECT OF ADRENERGIC BLOCKING AGENTS UPON DEVELOPMENT OF CAPREOMYCIN-INDUCED RENAL DAMAGE IN RATS

Capreomycin, s.c. (mg/kg/day)	Challenge: Adrenergic blocking agents	Challenge: Dose, i.p. (mg/kg/day)	Number of rats	Renal damage (cortical necrosis)				
200[a]	Saline	—	5	−	±	+	+	+
200	Chlorpromazine	10[b]	5	−	−	−	−	+
200	Hydralazine	20	5	++	++	++++	++++	++++
200	Dibenamine	15	5	−	−	−	−	−
400	—	—	5	++	+++	++++	++++	++++
400	Chlorpromazine	10	5	+	+	+	+	+
400	Hydralazine	20	5	++++	++++	++++	++++	++++
400	Dibenamine	15	5	+	+	+	++	+++
400	LSD-25	0.5	5	−	+	+	++	+++
400	Pronethalol	5	5	++	++	++	+++	++++
400	Propanolol	5	5	++	+++	+++	+++	++++
—	Saline	—	5	−	−	−	−	−
—	Chlorpromazine	10	5	−	−	−	−	−
—	Hydralazine	20	5	−	−	−	−	−
—	Dibenamine	15	5	−	−	−	−	−
—	Pronethalol	5	5	−	−	−	−	−
—	Propanolol	5	5	−	−	−	−	−

[a] Continued for 5 consecutive days.
[b] Continued for 5 consecutive days. Half dose was injected 1 hour before capreomycin injection and the rest 7 hours after capreomycin.

TABLE 3

EFFECT OF CAPREOMYCIN ON THE MESENTERIC MAST CELLS OF RATS

Treatment	Mast cell changes[a]	Rat No.:	At 16 hour 1	2	3	4	At 48 hour 1	2	3	4
In vivo										
Saline	Degranulation		5[b]	1	1	4	—	—	—	—
	Disruption		15	1	0	0	—	—	—	—
Capreomycin, 400 mg/kg, s.c.	Degranulation		40	38	29	45	21	34	20	25
	Disruption		0	0	0	0	4	2	0	5

		At 1 hour after incubation			
Rat No.:		1	2	3	4
In vitro					
Saline	Degranulation	0	0	4	6
	Disruption	2	3	4	0
Capreomycin					
500 μg/ml	Degranulation	83	90	95	98
	Disruption	0	0	1	0
250 μg/ml	Degranulation	62	75	93	95
	Disruption	2	0	0	0
125 μg/ml	Degranulation	6	10	15	27
	Disruption	0	1	5	5
62.5 μg/ml	Degranulation	0	3	2	6
	Disruption	1	2	1	0

[a] The number of normal, degranulated, and disrupted mast cells were counted in a population of 100 cells in each of 4 pieces of the mesentery per rat.
[b] Figures present the affected mast cells in percent.

TABLE 4

SPECIES DIFFERENCES OF THE CAPREOMYCIN-INDUCED RENAL DAMAGE

Species	Dose, s.c. (mg/kg/day)	Number of animals	Body weight		Kidney (cortical necrosis)	Other findings
			Start	Finish		
Rat	400[a]	5	120 g	105 g	3/5 (++++), 2/5 (+++)	—
	200	5	122 g	134 g	4/5 (+), 1/5 (±)	—
	Saline	5	118 g	134 g	5/5 (–)	—
Mouse	2000	10	20.0 g	—	—	10/10 Died on day 1
	1000	10	20.5 g	22.0 g	2/7 (+), 5/7 (–)	3/10 Died on day 1
	500	5	19.2 g	22.0 g	5/5 (–)	
	Saline	5	20.5 g	23.0 g	5/5 (–)	
Rabbit	2000	2	2.4 kg	—	—	2/2 Died on day 4
	1000	2	2.1 kg	2.2 kg	1/2 Slight desquamation of tubular epithelium, 1/2 (–)	—
	500	2	2.1 kg	2.2 kg	2/2 (–)	—
	250	2	2.2 kg	2.3 kg	2/2 (–)	—
	Saline	2	2.1 kg	2.2 kg	2/2 (–)	—
Hamster	2000	2	113 g	—	—	2/2 Died on day 1
	1000	2	128 g	113 g	1/2 Karyorrhexis in tubular epithelium, 1/2 (–)	—
	500	2	112 g	110 g	2/2 (–)	—
	250	2	118 g	115 g	2/2 (–)	—
	Saline	2	114 g	105 g	2/2 (–)	—
Cat	2000	1	2.8 kg	—	—	Vomiting, died on day 1
	1000	1	3.4 kg	3.2 kg	(–)	Vomiting
	750	1	3.1 kg	2.7 kg	(–)	Vomiting
	500	1	4.4 kg	4.0 kg	(–)	Vomiting
	250	1	3.1 kg	3.1 kg	(–)	—
	Saline	1	2.6 kg	2.6 kg	(–)	—
Guinea pig	2000	2	322 g	—	—	2/2 Died on day 2
	1000	2	335 g	315 g	2/2 (–)	—
	500	2	295 g	290 g	2/2 (–)	—
	250	2	323 g	348 g	2/2 (–)	—
	Saline	2	310 g	322 g	2/2 (–)	—

[a] Continued for 5 consecutive days.

to its 5-HT releasing activity, 5-HT antagonists and adrenergic blocking agents should be useful in inhibiting renal damage.

Jasmin and Bois (1960) reported the inhibitory effect of adrenergic blocking agents on 5-HT-induced renal damage. The present data showed that some adrenergic blocking agents modified the renal damage caused by capreomycin. Chlorpromazine, dibenamine, and LSD-25 possessed some inhibitory effect, but at the dose level employed, hardly any inhibition was seen to result from the administration of the β-blockers propanolol and pronethalol. An unexpected finding was that hydralazine exacerbated the damage. This phenomenon was also observed in damage induced by polymyxin B, kanamycin, cephaloridine, and uranyl nitrate (Muraoka, 1967, unpublished). Studies on the mechanism of action of hydralazine on enhancement of the renal damage are now being undertaken.

Capreomycin caused degranulation of mast cells in rats, which is one sign of 5-HT release (Bhattacharya and Lewis, 1956; Parratt and West, 1957a). It is a well-known fact that mast cells of rats contain biologically active substances such as histamine, 5-HT, and heparin in high concentrations. This high concentration, particularly of 5-HT, is specific in rats and plays an important role in anaphylactic response (Benditt *et al.*, 1963).

In histamine liberation from mast cells, disruption of the cell is common, and at that time small amounts of 5-HT are released (Parratt and West, 1957b). In 5-HT release by reserpine, the cells do not show a severe change, such as disruption, which has been known to occur in histamine liberation, degranulation and swelling of the cytoplasm are generally seen (Bhattacharya, 1956; Parratt and West, 1957a).

The effect of capreomycin on mast cells *in vitro* was not disruption but degranulation. Since mast cells are thought to be one of the targets of capreomycin, and since they are an important source of 5-HT, the specific nephrotoxicity of capreomycin in rats may be due to the 5-HT which is released in the blood and acts through a constriction of the afferent vascular bed of glomeruli causing ischemia. Subsequently, cortical necrosis is seen at a dose that does not alter the general circulation; however, there still exists the possibility that capreomycin acts directly on the kidney or acts in some way other than the release of 5-HT.

If the greater part of the 5-HT released is of mast cell origin, the species in which 5-HT is not concentrated in the cell may be less sensitive to capreomycin nephrotoxicity. In the cat, rabbit, guinea pig, and hamster, 5-HT is not concentrated in mast cells (Parratt and West, 1957a; West, 1959). The mouse is similar to the rat in that 5-HT is concentrated in the mast cells but to a lesser degree (Parratt and West, 1957a). In the cat, rabbit, guinea pig, and hamster, hardly any necrotic changes are seen in the kidney except for slight desquamation or karyorrhexis of the epithelium of the proximal tubules in the rabbit and hamster at a daily dose of 1000 mg/kg.

These data suggest that capreomycin nephrotoxicity can be interpreted relative to its 5-HT releasing activity and one of the most readily released supplies of 5-HT is the mast cell. In the intestine, where 5-HT is localized mainly in the enterochromaffin cells of the mucosa (Erspamer and Asero, 1952; Barter and Pearse, 1955), the 5-HT contents increased slightly. In rats the increased blood level of 5-HT corresponded to a decreased level in the skin, which is rich in mast cells. On the other hand, when antibiotics such as streptomycin and chlortetracycline (Stacey and Sullivan, 1957) are

Saline 2 310 g 322 g 2/2 (−)

* Continued for 5 consecutive days.

administered, the increase of 5-HT in the intestine is related to other mechanisms (Sullivan, 1960).

One purpose of toxicity studies in laboratory animals is to predict the side effects of drugs and to minimize the risk when they are given to man. From the data presented, it is possible to speculate on the severity of capreomycin nephrotoxicity. In man, under normal conditions, 5-HT is not concentrated in mast cells as in rats (Ende and Cherniss, 1958; Parratt and West, 1957a; Sjoerdsma *et al.*, 1957). This is true also under abnormal conditions that accompany the proliferation of mast cells, such as urticaria pigmentosa and mastocytosis (West, 1957b; Sjoerdsma *et al.*, 1957). Therefore, it can be expected that capreomycin nephrotoxicity in man will be no more severe than that caused by other widely used nephrotoxic antibiotics.

SUMMARY

Studies of capreomycin nephrotoxicity in laboratory animals were performed, and it was confirmed that the rat was much more sensitive to capreomycin than the mouse, rabbit, hamster, cat, or guinea pig.

Adrenergic blocking agents such as chlorpromazine, dibenamine and LSD-25 showed some inhibitory effect on capreomycin nephrotoxicity, but propanolol and pronethalol showed practically none. Hydralazine exacerbated the damage.

The 5-HT releasing activity and mast cell degranulating property of capreomycin in the rat were demonstrated *in vivo* and *in vitro*.

Capreomycin-induced nephrotoxicity in the rat may be due, at least in part, to its releasing of 5-HT. Further, the difference in toxicity among the animals of the different species used was discussed in relation to the variation of 5-HT concentration in mast cells.

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