Effect of Polymyxin B and Endotoxin on Renal Cortical Fibrinolysis[1] (37593)

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Although the role of the fibrinolytic system in the generalized Shwartzman reaction (gSr) has not been defined, several facts are clear. Glomerular fibrin deposition is promoted by inhibition (1–3) and prevented by activation (4–6) of the fibrinolytic system. In the rabbit, endotoxin fails to activate the circulating fibrinolytic system (7, 8) and inhibits renal cortical fibrinolytic activity (9). As polymyxin B has been shown to prevent the development of the gSr (10, 11), we have studied its effect on endotoxin-induced loss of renal cortical fibrinolytic activity in the rabbit. Further studies were carried out to delineate the effect of polymyxin B itself on cortical fibrinolytic activity.

Materials and Methods. Endotoxin. Endotoxin (lipopolysaccharide B from *E. coli* 026:B6) was obtained from Difco Laboratories, Detroit, MI. The preparatory and provocative doses ranged between 0.3 to 0.4 mg, given by maginal ear vein injection in 2.0 ml of isotonic saline.

Polymyxin B. Polymyxin B sulfate was obtained from Pfizer Laboratories, New York, NY. The contents of each vial (50 mg) was dissolved in 10 ml of sterile 5% glucose water. The inhibitory dose of polymyxin B (10 mg) was given by marginal ear vein injection in a volume of 2.0 ml.

Phosphate-buffered saline (PBS) consisted of 84 ml of 0.2 *M* Na_2HPO_4, 16 ml of 0.2 *M* $NaH_2PO_4 \cdot H_2O$ and 17 g NaCl, diluted to 2000 ml with distilled water (pH 7.4).

Animal studies. Eighty-seven 2.0 kg albino rabbits were studied in 4 groups.

Group 1 (Table I) contained 16 animals. Eight animals were given two injections of endotoxin and 8 received two injections of isotonic saline (2.0 ml) at 24 hr intervals. Immediately following the second injection, 4 endotoxin and 4 saline animals were given polymyxin B. All animals were sacrificed 6 hr following the last injection.

Group 2 (Table I) contained 8 animals given a single injection of endotoxin followed immediately by polymyxin B, 7 animals given endotoxin alone, 7 animals given polymyxin alone, and 7 animals given isotonic saline. All animals were sacrificed 2 hr following injection.

Group 3 (Table II) contained 15 animals given one injection of polymyxin B and 10 animals given isotonic saline. Three polymyxin B and 2 saline animals were sacrificed 1, 2, 3, 4, and 6 hr following injection.

Group 4 included 17 animals given polymyxin B. A single injection of endotoxin was given 2, 2.5 and 3 hr after polymyxin B to groups of 6, 6, and 5 rabbits. All animals were sacrificed 24 hr following the final injection.

Renal cortical tissue was obtained immediately after death and placed into buffered formalin and isopentane cooled in liquid nitrogen. The preparation of fibrin slides and the histological techniques for light microscopy were performed as previously described (12). Fibrin slides containing 2 renal cortical sections (4 μm in thickness) from each animal were fixed in 10% formalin after 40, 60, 80, and 100 min incubation. The slides were evaluated by light microscopy. The presence

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TABLE I. Glomerular Fibrin Deposition and Renal Cortical Fibrinolytic Activity in Rabbits Receiving Polymyxin B and Endotoxin.

Group	Protocol[a]	No. of animals	No. of animals with	
			Glomerular fibrin deposition	Cortical lysis
1	$E_1 \rightarrow E_2 + Px \rightarrow Sac.$	4	0	4
	$E_1 \rightarrow E_2 \rightarrow Sac.$	4	4	0
	$S_1 \rightarrow S_2 + Px \rightarrow Sac.$	4	0	3
	$S_1 \rightarrow S_2 \rightarrow Sac.$	4	0	4
2	$E_1 + Px \rightarrow Sac.$	8	0	0
	$E_1 \rightarrow Sac.$	7	0	0
	$Px \rightarrow Sac.$	7	0	0
	$S_1 \rightarrow Sac.$	7	0	7

[a] E_1 = preparatory injection of endotoxin; E_2 = provoking injection of endotoxin; Px = Polymyxin B; S = isotonic saline; Sac. = sacrificed. Group 1 animals were sacrificed 6 hr following the last injection. Group 2 animals were sacrificed 2 hr following injection.

of lytic activity was denoted by the appearance of a clear area within the fibrin film overlaying the tissue by 100 min incubation. Tissues for light microscopy were stained with hematoxylin–eosin and phosphotungstic acid–hematoxylin stains.

In vitro *studies.* To evaluate the direct effect of polymyxin B on renal cortical fibrinolytic activity, a frozen section from each of 3 normal rabbits, previously shown to have cortical lysis, was incubated at 37° for 1 hr in solutions of polymyxin B at a concentration of 10, 5, 2.5, 1.25, and 0.625 mg/ml in PBS; control sections were incubated in a similar manner in PBS alone. Following incubation, the slides were washed in PBS, dried and studied by the fibrin slide technique (incubation time of fibrin slides 3 hr).

Results. Group 1. All 4 rabbits given two injections of endotoxin developed the gSr, characterized by massive glomerular fibrin deposition on light microscopy (Table I). No histological abnormalities were detected in renal cortical tissue from rabbits given endotoxin plus polymyxin B, saline plus polymyxin B or saline alone. Renal cortical fibrinolytic activity was absent in tissue from the 4 rab-

TABLE II. Renal Cortical Fibrinolytic Activity in Animals Receiving One Injection of Polymyxin B or Isotonic Saline.

Group	No. of rabbits	Time sacrificed after injection of polymyxin B (hr)	No. of animals with cortical lysis[a]
3 Polymyxin B	3	1	3
	3	2	1
	3	3	0
	3	4	0
	3	6	2
3 Saline	2	1	2
	2	2	2
	2	3	2
	2	4	2
	2	6	2

[a] Renal tissue was normal by light microscopy.

bits developing the gSr and from 1 animal given saline and polymyxin.

Group 2. Cortical tissue from all group 2 animals was normal by light microscopy (Table I). Fibrinolytic activity was absent in cortex from all animals given one injection of endotoxin plus polymyxin and from those animals given a single injection of endotoxin or polymyxin B, but was present in all saline controls.

Group 3. Further studies were carried out to define the effect of polymyxin B on cortical fibrinolytic activity. Lytic activity was present in cortex from 3 animals sacrificed 1 hr following a single injection of polymyxin B (Table II). However, lytic activity was absent in tissue from 2 of 3 rabbits sacrificed 2 hr after injection, from all 6 animals sacrificed 3 and 4 hr after injection and from 1 of 3 animals sacrificed 6 hr following injection. Fibrinolytic activity was present in tissue from all saline controls. Cortical tissue from all group 3 animals was normal by light microscopy.

Group 4. No histologic abnormalities were detected in tissue from group 4 animals given a single injection of endotoxin 2, 2.5 and 3 hr following polymyxin B.

In vitro *studies.* Cortical fibrinolytic activity was absent in tissue from all animals incubated in polymyxin B at a concentration of 5 or 10 mg/ml (Table III). Lysis was absent in 2 of 3 tissue sections at 1.25 and 2.5 mg/ml, but was present in all tissue incubated at 0.625 mg/ml, and in all PBS controls.

TABLE III. Fibrinolytic Activity of Renal Cortical Tissue Sections from Normal Rabbits Following *in Vitro* Incubation in Polymyxin B.

No. of animals	Concn of polymyxin B (mg/ml)	No. of animals with cortical lysis
3	10	0
3	5	0
3	2.5	1
3	1.25	1
3	0.625	3
3	PBS[a]	3

[a] Phosphate-buffered saline without added polymyxin B.

Discussion. This study confirms the observation that polymyxin B will protect against the gSr (10, 11). Although the mechanism of endotoxin inhibition has not been defined, polymyxin B has been shown to have anticoagulant activity (13, 14). Corrigan and Bell's data (11) suggest that polymyxin must interact with endotoxin in the circulation before the latter becomes fixed to blood or tissues. In this and previous studies on the gSr (9), we have documented that cortical fibrinolytic activity is absent 6 hr following the second dose of endotoxin. This loss of lytic activity, as well as glomerular fibrin deposition, is prevented by polymyxin B.

Prior studies (9) have demonstrated disappearance of cortical lysis for periods up to 6 hr following a single injection of endotoxin. Of great interest was the observation that polymyxin B inhibits cortical fibrinolytic activity over a similar interval of time. The loss of lytic activity 2 hr after administration of both endotoxin and polymyxin B seems contrary to the expected neutralization of endotoxin by polymyxin B. As polymyxin B prevents the hematologic and tissue manifestations of endotoxin (11) and alters the ultrastructure of the endotoxin molecule (15), it is likely that loss of fibrinolytic activity in rabbits given both agents is secondary to polymyxin B. On the other hand, it is also true that polymyxin B is altered following interaction with endotoxin as demonstrated by inhibition of antibacterial activity (11, 16).

Although the mechanism of polymyxin B inhibition of renal cortical fibrinolytic activity has not been delineated in this report, it is clear that polymyxin inhibits lysis in a manner different from endotoxin. We have previously demonstrated that endotoxin in saline does not inhibit lysis *in vitro* (9). Polymyxin B, on the other hand, inhibits lysis of cortical tissue slices directly, in the absence of serum. The antibacterial activity of polymyxin B is felt to be the result of binding to phospholipids on cell membranes, resulting in membrane disruption and cell death (17). Polymyxin B has been shown to bind to kidney tissue (18, 19), and the nephrotoxicity of the drug is well known

(20). Thus polymyxin B may induce loss of renal cortical fibrinolytic activity *in vivo* by a direct effect on the kidney. The liberation of histamine from mast cells by polymyxin (21, 22) has been shown to separate vascular endothelial cells, exposing basement membrane and presumably increasing vascular permeability (23). This endothelial cell alteration may affect fibrinolytic activity.

We were unable to produce the gSr by giving endotoxin during the time period when renal cortical fibrinolytic activity was inhibited by polymyxin B. This observation stands in sharp contrast to the actions of endotoxin (9) and Thorotrast (24)—each of which prepare for the gSr and inhibit cortical fibrinolysis. That inhibition of cortical fibrinolysis did not prepare for the gSr is consistent with the concepts of McKay, Latour and Lopez (25), who believe that multiple factors including inhibition of fibrinolysis are involved in the production of the gSr.

Summary. When given with the second dose of endotoxin, polymyxin B prevented both glomerular fibrin deposition and loss of lysis. Of interest was the fact that polymyxin B inhibited cortical lysis over a time interval similar to that induced by endotoxin. *In vitro* studies revealed that polymyxin B inhibits cortical lysis in the absence of serum. Inhibition of lysis with polymyxin B did not prepare for the generalized Shwartzman reaction. These studies demonstrate that, although polymyxin B neutralizes the effect of endotoxin, it also inhibits renal cortical fibrinolytic activity by a mechanism different than that of endotoxin. That polymyxin B-induced loss of cortical lysis did not prepare for the Shwartzman reaction suggests that multiple factors are involved in the production of the generalized Shwartzman reaction.

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