

Comparison Between the Polymyxins and Gentamicin in Preventing Endotoxin-Induced Intravascular Coagulation and Leukopenia

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Three antimicrobial agents were evaluated as to their ability to neutralize the toxic effects of endotoxin in rabbits. These consisted of two cyclic polypeptides, polymyxin B sulfate and colymycin M (sodium colistimethate), and an aminoglycoside, gentamicin sulfate. Polymyxin B regularly prevented endotoxin-induced leukopenia, thrombocytopenia, and disseminated intravascular coagulation. Colymycin M had similar activity but was not as effective as polymyxin B. Gentamicin demonstrated no neutralizing ability in this study.

One of the many toxic effects of bacterial endotoxin in animals is activation of the coagulation mechanism with subsequent production of either localized or generalized thrombosis (5, 20, 22). Similarly, humans with gram-negative infections frequently manifest an activated coagulation mechanism which may progress to disseminated intravascular coagulation (DIC; 7, 8, 10). The antibiotic polymyxin B sulfate has been shown to neutralize endotoxin lethality in chick embryos and adrenalectomized mice (9, 14, 16) and to prevent endotoxin-induced leukopenia, thrombocytopenia, DIC, and renal cortical necrosis in rabbits (3, 6, 15). Polymyxin E (colymycin S, or colistin sulfate, and colymycin M, or sodium colistimethesulfonate) similarly reduces endotoxin lethality in chick embryos, but the colymycin M form is apparently less potent in this respect (16). This suggested to Rifkind that the free amino groups of the diaminobutyric acid found in polymyxin B and colymycin S were involved in the antiendotoxin ability of the antibiotics (16). A chemically dissimilar antimicrobial, gentamicin, which is used clinically, also has free amino groups and is effective against gram-negative organisms (2). The purpose of this investigation was to compare the endotoxin-neutralizing ability in rabbits between polymyxin B sulfate, sodium colistimethate, and gentamicin sulfate by using the generalized Shwartzman reaction as the model. The effect of these antimicrobial agents on endotoxin-induced leukopenia, thrombocytopenia, DIC, and renal cortical necrosis was evaluated.

MATERIALS AND METHODS

White rabbits (Californian strain, weighing 1.0 kg) of either sex were used. They were housed in an air-conditioned room and fed Purina rabbit chow and water ad lib.

Escherichia coli 0127:B8 endotoxin, Boivin type (Difco), was prepared fresh daily in pyrogen-free normal saline at a final concentration of 0.1 mg/ml.

Polymyxin B sulfate (Aerosporin, Burroughs-Wellcome and Co.) for injection contained 50 mg (500,000 units) of antibiotic per vial. Sodium colistimethate (colymycin M, Warner-Chilcott) contained 150 mg of colistin base per vial. Gentamicin sulfate (Garamycin, Schering Corp.) contained 40 mg per vial. All antimicrobial agents were made to a final concentration of 5 mg/ml with sterile pyrogen-free 5% glucose water.

The production of the generalized Shwartzman reaction was performed in the classical manner (5). All animals were given 0.1 mg of endotoxin per kg intravenously (preparative injection), and, 24 hr later, a second (provocative) endotoxin injection of 0.1 mg/kg intravenously. Simultaneous with the second endotoxin injection, the rabbits were given either normal saline, polymyxin B sulfate, sodium colistimethate, or gentamicin sulfate intravenously in the opposite ear vein. The timing of the antimicrobial injection is critical, as previously reported (6). Blood was obtained by cardiac puncture, utilizing a two-syringe technique, immediately before (0 hr) and again at 4 hr after the provocative injections. Those animals that survived the cardiac punctures were sacrificed 24 hr after the provocative injections, and the presence of bilateral renal cortical necrosis was noted. The blood samples were used to determine white blood cell counts (by routine hematological technique) and platelet counts by phase microscopy (1). Plasma was

TABLE 1. *Effect of polymyxins B and E and gentamicin on endotoxin-induced renal cortical necrosis^a*

Provocative agents (mg/kg)	Renal cortical necrosis (no. positive/total)
Endotoxin (0.1 mg) + saline	7/10
Endotoxin (0.1 mg) + polymyxin B (5.0 mg)	0/10
Endotoxin (0.1 mg) + colistimethate (5.0 mg)	0/14
Endotoxin (0.1 mg) + gentamicin (5.0 mg)	5/9

^a All animals were prepared with endotoxin and, 24 hr later, given endotoxin and saline, polymyxin B, colistimethate, or gentamicin as simultaneous injections.

obtained by anticoagulating 9 volumes of whole blood with 1 volume of 3.8% sodium citrate and centrifuging at 8,000 rev/min for 20 min at 4 C. The plasma samples were used to determine fibrinogen concentration (13) and levels of coagulation factors II (prothrombin; reference 12), V (18), and VIII (AHF; reference 19). The hematological data are expressed as mean values \pm standard error and are statistically analyzed for unpaired experiments.

RESULTS

Of the 10 rabbits treated in the classical manner, with two intravenous injections of endotoxin (0.1 mg/kg) 24 hr apart and no antimicrobial agent, 7 had bilateral renal cortical necrosis 24 hr after the second endotoxin dose. As shown in Table 1, similarly treated animals were given 5.0 mg of polymyxin B per kg, 5.0 mg of colistimethate per kg, or 5.0 mg of gentamicin per kg with the second endotoxin injection. None of the animals receiving polymyxin B or colistimethate developed the generalized Shwartzman reaction. Conversely, five of the nine rabbits given gentamicin with the endotoxin were noted to have renal cortical necrosis.

The hematological data are presented in Table 2. As described above, all rabbits were given a preparatory injection of endotoxin; then 24 hr later and immediately before the second or provocative injection, whole blood was obtained and was designated "0 hr." Four hours later, another blood sample was taken and was designated "4 hr." As can be seen, those animals given only endotoxin developed significant leukopenia and thrombocytopenia during this time interval. In addition, we noted a 40% reduction in the activity of plasma coagulation factors II and V, a 55% reduction in factor VIII, and a 30% fall in the fibrinogen concentration. Those rabbits given polymyxin B sulfate (5.0 mg/kg) simultaneously

with the second endotoxin injection did not show a significant reduction in white blood cells, platelets, or coagulation factors. In fact, the platelets, factor V, factor VIII, and fibrinogen increased. In the colistimethate group, no significant change was noted in the platelet count or fibrinogen concentration. However, a significant leukopenia was observed, although not as severe as in the untreated group. Those animals given gentamicin sulfate (5.0 mg/kg) with the second injection of endotoxin showed leukopenia, thrombocytopenia, and reduced levels of factors II, V, and VIII and fibrinogen.

Polymyxin B sulfate was protective over a wide range of concentration. It was found that the smallest dose that provided complete endotoxin neutralization was 0.25 mg/kg given simultaneously with the second endotoxin injection. At a concentration of 0.1 mg/kg, the 4-hr mean values of five animals were: white blood cells, $2,557 \pm 320/\text{mm}^3$; platelets, $216,000 \pm 24,000/\text{mm}^3$; and fibrinogen, $421 \pm 35 \text{ mg}/100 \text{ ml}$. These results are similar to those obtained with colistimethate at a concentration of 5.0 mg/kg.

DISCUSSION

In the classical generalized Shwartzman reaction, the first endotoxin injection "prepares" the rabbit, probably by producing a functionally impaired reticuloendothelial system (5, 11, 21). The second or provocative injection, given 24 hr after the first, causes leukopenia, thrombocytopenia, and a precipitous and significant reduction in coagulation factors VIII, V, II, and fibrinogen which is fully developed within 4 hr. It is also during this 4-hr interval that DIC is produced and fibrin deposition appears in the kidneys (4, 5, 11, 21). Because this study was designed to evaluate the hematological changes, emphasis was placed on the 4-hr period following the second endotoxin injection.

As has been reported previously, rabbits given 5.0 mg of polymyxin B sulfate per kg simultaneously with the provocative dose of endotoxin failed to develop leukopenia, thrombocytopenia, DIC, and subsequent renal cortical necrosis (6). Similarly, renal cortical necrosis was not observed in those animals receiving 5.0 mg of colistimethate per kg. Unlike the polymyxin B group, these rabbits developed leukopenia, although reduction in the platelets and fibrinogen concentration did not occur. This suggests that DIC was not elicited and therefore fibrin deposition in the kidneys was prevented. The results would also suggest that the difference in the hematological data between polymyxin B and colistimethate was a function of concentration, because results

TABLE 2. Effect of polymyxins B and E and gentamicin on endotoxin-induced leukopenia, thrombocytopenia, and disseminated intravascular coagulation

Determination	Results at 0 hr	Results 4 hr after treatment with			
		Endotoxin + saline ^a	Endotoxin + polymyxin B	Endotoxin + colistimethate	Endotoxin + gentamicin ^c
White blood cells (per mm ³)	8,668 ± 568 ^a (32) ^b	2,075 ± 332 (22)	7,311 ± 807 (18)	4,208 ± 620 ^c (14)	1,872 ± 370 (14)
Platelets (× 10 ³ /mm ³)	140 ± 11 (34)	46 ± 5 (24)	293 ± 24 (18)	243 ± 29 (14)	78 ± 10 (14)
Factor II (%)	100 ± 6 (14)	59 ± 5 (21)	91 ± 8 (18)		51 ± 7 (5)
Factor V (%)	100 ± 6 (14)	60 ± 2 (21)	138 ± 13 (18)		36 ± 7 (5)
Factor VIII (%)	100 ± 10 (14)	45 ± 5 (20)	112 ± 15 (18)		27 ± 4 (5)
Fibrinogen (mg/100 ml)	324 ± 25 (14)	41 ± 26 (21)	394 ± 25 (18)	330 ± 25 (8)	251 ± 27 (12)

^a Mean ± standard error.

^b Number of animals.

^c Significant decrease from 0 hr ($P < 0.05$).

similar to the colistimethate data could be produced by reducing the polymyxin B dose 50-fold. Both polymyxins demonstrated endotoxin-neutralizing ability when used in concentrations presently recommended for clinical use; polymyxin B was clearly more effective than colistimethate.

Conversely, those animals given the aminoglycoside, gentamicin sulfate, reacted no differently from those given saline. Significant leukopenia, thrombocytopenia, and reduced levels of coagulation factors VIII, V, and II and fibrinogen (i.e., DIC), with the production of renal cortical necrosis, occurred in these rabbits.

Previous studies have demonstrated that the antimicrobial agents polymyxin B, colistin (colymycin S), and tyrocidine have endotoxin-neutralizing power (16). These cyclic cationic polypeptides have free amino groups which are thought to play an important role in the neutralization, because methylation of the free amino groups of colistin reduces the anti-endotoxin ability of this material. In addition, colistimethate is known to be less toxic and less active as an antibiotic than colymycin S (17). However, the presence of free amino groups is probably only part of the answer, because other cyclic cationic polypeptide antibiotics (capreomycin and viomycin) provide no protection; in addition, the polycationic proteins protamine and histone (16), and, as shown in this study, gentamicin, an aminoglycoside, also do not provide protection.

Thus, the data would suggest that, in addition to amino groups, a specific stereochemical configuration is likewise needed to permit endotoxin neutralization.

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