PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 151, 329-332 (1976)

Endotoxin Toxicity in Rats Is Enhanced by Tilorone[1] (39203)

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Synergistic and lethal interactions between bacterial endotoxin and certain chemicals or drugs have been described. The latter include lead salts (1), alkylating agents (2), and inhibitors of protein, RNA, and DNA synthesis or metabolism (3-11). Tilorone is a relatively nontoxic drug which induces interferon (12, 13), depletes T-lymphocytes (14), and has antiviral (13, 15), anticancer (16), and immunosuppressive (17) properties. In view of the many potential clinical uses of tilorone in patients whose diseases might lead to development of endotoxemia, it is important to know whether tilorone and endotoxin interact synergistically. The present investigation reveals that tilorone enhances endotoxin lethality.

Methods. Male Lewis rats, 230-350 g, from Microbiological Associates, Inc., or bred in our laboratory, were kept in hanging cages and were fed Purina Laboratory Chow and tap water *ad libitum.*

Tilorone (2,7-bis(diethylaminoethoxy)-fluoren-9-one hydrochloride) was dissolved freshly in saline at 10 mg/ml and was administered without anesthetic by stomach tube at a dose of 100 mg/kg of body weight. *Salmonella enteritidis* lipopolysaccharide from Difco Laboratories was suspended in saline at 1 mg/ml; it was stored frozen, then defrosted and diluted with saline before use. The endotoxin was administered in a volume of 10 ml/kg through the penile vein with the aid of ether anesthesia.

Bilateral adrenalectomy was done through a middorsal incision under ether anesthesia, 4 days before endotoxin treatment. The incision was closed with sutures and clips, and saline was provided as sole fluid.

One day after endotoxin injection, surviving rats were anesthetized and sacrificed by exsanguination. Rats that died or were killed were subjected to gross and usually microscopic examination of kidneys, liver, adrenal, intestines, and lymphoid tissues.

Results. Tilorone is relatively nontoxic; the LD50 is 852 mg/kg for the oral route, and we have never observed deaths after a 100 mg/kg dose. Endotoxin is much more toxic (LD50 < 20 mg/kg (18)), but we had no fatalities after 2.0 or 0.5 mg/kg iv. However, 1 day after tilorone, as little as 0.01 mg/kg of endotoxin caused 45% mortality, and higher doses were almost uniformly lethal (Table I). Deaths were observed to occur 4 to 8 hr after endotoxin.

It is known that adrenalectomy increases susceptibility of rats to endotoxin (1, 19, 20). In our hands, adrenalectomy and tilorone pretreatments enhanced endotoxin toxicity to a similar degree (Table I). Therefore, it was conceivable that tilorone sensitized rats to endotoxin by damaging their adrenals or by antagonizing corticosteroid hormones. This possibility was excluded by studying the effects of endotoxin and tilorone in previously adrenalectomized rats.

TABLE I. TILORONE INCREASES ENDOTOXIN LETHALITY IN INTACT AND ADRENALECTOMIZED RATS.

Endotoxin (mg/kg)	Mortality[a] after endotoxin preceded by:			
	Neither	Tilorone[b]	Adrenex[c]	Both[b,c]
2.0	0/11	2/2		
0.5	0/2	4/4	2/2	
0.1		13/14	2/2	
0.01		5/11	8/9	4/4
0.001		0/9	0/7	8/8
0.0001				8/8
0.00001				0/7

[a] Numerator = number of rats that died within 24 hr after *S. enteritidis* endotoxin injection iv. Denominator = total number of rats.

[b] Tilorone 100 mg/kg by gastric tube 1 day before endotoxin.

[c] Adrenalectomy 4 days before endotoxin; saline by gastric tube 1 day before endotoxin.

[1] Supported by a grant from the Kroc Foundation.

Under these conditions, tilorone increased the toxicity of endotoxin approximately 100 times (Table I). Therefore, the effect of tilorone cannot be mediated by the adrenal gland.

A different endotoxin, *Escherichia coli* lipopolysaccharide 055:B5, was not lethal at a dose of 2 mg/kg to nine rats. When three rats were pretreated with tilorone, however, this dose of endotoxin also was lethal. Therefore, the tilorone effect was not restricted to the *S. enteritidis* product. An attempt to substitute rabbit anti-rat lymphocyte serum for tilorone did not succeed. This serum shares with tilorone the property of rapidly depleting T-lymphocytes (14). However, iv injection of 5 ml/kg of the serum did not sensitize rats to 0.5 mg/kg of *S. enteritidis* endotoxin injected 1 day later, although histologic examination of lymph nodes and spleen confirmed that the serum had depleted T-lymphocytes.

Smaller doses of tilorone and different time intervals between tilorone and *S. enteritidis* endotoxin reduced the mortality of the combination of agents. Administration of tilorone a few minutes or a few hours before, or 1 day after endotoxin was not fatal, but banded congestion of the small intestine (probably a sign of endotoxin toxicity) was observed at necropsy the next day (Table II). Even the incorporation of endotoxin in a water-in-oil emulsion did not void the obligatory temporal relationship to tilorone. Injection ip of 1 ml/kg of 0.1% endotoxin in an equal volume of Bayol F mineral oil–Arlacel A emulsifying agent (85:15) was lethal to all three rats pretreated with tilorone on the previous day, but was harmless

TABLE II. DOSAGE AND TIMING OF TILORONE FOR ENDOTOXIN SENSITIZATION.

Tilorone		
mg/kg	Time[a]	Mortality
0.4	D − 1	2/3
2.0	D − 1	1/3
10.0	D − 1	3/3
10.0	DO	0/14
10.0	D + 1	0/5

[a] Days before (minus) or after (plus) 2.0 mg/kg endotoxin.

TABLE III. SENSITIZATION TO ENDOTOXIN BY TILORONE IS SUBJECT TO TACHYPHYLAXIS.

Tilorone		
mg/kg	Time[a]	Mortality
10.0	D − 1	5/5
10.0	D − 4	0/5
10.0	D − 4 and −1	0/5

[a] Days before 0.1 mg/kg endotoxin.

to three rats that received tilorone on the same day.

Tilorone treatment 4 days beforehand did not sensitize to endotoxin, and it even interfered with the sensitizing ability of a second dose of tilorone given at the optimum time (1 day beforehand; Table III). This induced-hyporeactivity (tachyphylaxis) occurs with other tilorone effects also (13, 14, 21).

Histopathology. In several rats that were treated with tilorone and endotoxin, the renal glomeruli had fibrin thrombi, stained dark blue by phosphotungstic acid-hematoxylin, typical of the generalized Shwartzman reaction. Other instances were probably missed because tissues from many of the dead rats were badly autolyzed. Many livers had multiple foci of necrosis. Some adrenals had acute necrosis of medullary cells. Small intestine had focal congestion and hemorrhage. Some of these lesions were detected in rats that were sacrificed as well as in rats that had died.

Discussion. Most previous studies of endotoxin-drug synergy have been done in mice (3–10). However, there was no synergy between endotoxin and tilorone in that species (9; also personal communication from Merrell-National Laboratories), unlike our results in rats. The basis for this difference between mice and rats is unknown. Both species do react synergistically to endotoxin combined with some other agents, such as lead citrate (1, 10) or cyclophosphamide (2, 9). The magnitude of the endotoxin–tilorone interaction was not as great as the endotoxin–lead acetate synergy, but it was greater than most other reported interactions. It is obviously impossible to predict whether human species will react to tilorone like mice or rats with respect to endotoxin. Nevertheless, our results in rats

are important because clinicians can take appropriate prophylactic actions if they are forewarned of the possibility that tilorone might intensify the effects of endotoxemia in their patients.

We believe that endotoxin rather than tilorone was the lethal component of the combination, because of the intrinsically greater toxicity and speed of action of endotoxin, and because the pathological findings in kidney, liver, and intestines were similar to those elicited by endotoxin (22). Endotoxin was the lethal agent in some other combinations also (1, 3–11).

Drugs previously shown to act synergistically with endotoxin are known to impair DNA, RNA, or protein synthesis or metabolism, or to bind sulfhydryl groups. Presumably, one or more of these factors could affect the detoxification of endotoxin and thereby increase its toxicity. This suggests that sensitization to endotoxin could be related to the effects of tilorone on the reticuloendothelial system (23) or to its ability to inhibit DNA polymerase and RNA polymerase (24). An interrelationsip among tilorone's diverse effects is suggested by the fact that 1 day after administration is the time that interferon titers (13, 21), depletion of T-lymphocytes (14), and sensitization to endotoxin reach a peak. A role for the adrenal glands in sensitization to endotoxin has been excluded. This has been done previously only in the instance of the endotoxin-lead synergy (20).

The possibility that our tilorone preparation was contaminated with lead was excluded by a negative dithizone test for lead (kindly performed by Mr. Howard Lane), whereas a 10% contamination would be needed to reach a partly effective dose level (1). Even an indirect relation between tilorone and lead seems improbable because lead was effective if given at the same time as endotoxin but not if given 1 day before, which is quite the opposite of tilorone. Furthermore, l-cysteine hydrochloride, 100 mg/kg iv before tilorone, repeated before the injection of 0.1 mg/kg endotoxin, did not reduce mortality, although this sulfhydryl-containing compound is known to block the effects of lead acetate (25).

Summary. The relatively nontoxic drug, tilorone, greatly enhanced the susceptibility of rats to lethal effects of endotoxin. The magnitude of the synergy was similar to that produced by adrenalectomy, but the effect of tilorone was not mediated by the adrenal glands. The histopathologic effects of endotoxin plus tilorone resembled those produced by much larger doses of endotoxin alone, including instances of the generalized Shwartzman reaction.

We are indebted to the Merrell-National Laboratories, Cincinnati, Ohio, for the gift of tilorone.

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Received June 11, 1975. P. S. E. B. M. 1976, Vol. 151.