Retardation by Methylprednisolone of the Synergistic Toxicity of Endotoxin with Sparsomycin or Pactamycin[1] (34298)

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Enhanced toxicity in mice resulting from a synergistic interaction between bacterial endotoxin and the antitumor antibiotic sparsomycin or pactamycin was demonstrated previously by Karp and Bradley (1). Because this may have clinical significance, various agents have been tested for ability to prevent the enhancement. Inasmuch as steroids have been repeatedly referred to in the literature (2, 3) as effective protectants against the lethal action of bacterial endotoxins, this class of compounds was included in our screening procedure. Methylprednisolone (11β,17α,21-trihydroxy-6α-methyl-1,4-pregnadiene-3,20-dione) when used prophylactically at 100 mg/kg, retarded the toxic effect of endotoxin in combination with sparsomycin or pactamycin. The antihistamine chlorpheniramine maleate (Chlortrimeton) and the anti-inflammatory agent phenylbutazone were ineffective against the pactamycin–endotoxin toxicity.

Materials and Methods. Adult, male, Balb/sy, RFW, or New York State Research Lab mice (Swiss-albino) all weighing about 25 g were employed. Sparsomysin and pactamycin were dissolved in 0.15 *M* NaCl (pH 7.0) to final concentrations of 50 and 75 μg/ml, respectively. Methylprednisolone was suspended in 95% ethanol and diluted with 0.15 *M* NaCl (pH 7.0) to give a final solvent concentration of 63–71% ethanol and 5–12.5 mg of steroid/ml. These methylprednisolone suspensions were administered subcutaneously in 0.2 ml. Lower concentrations of steroid (0.6 and 1.0 mg/ml) were suspended directly in 0.15 *M* NaCl (pH 7.0). This resulted in a fine suspension which was administered subcutaneously in 0.5 ml. All methylprednisolone suspensions were prepared 4 or 5 days before the initial injection in each experiment. Controls consisted of mice receiving the methylprednisolone preparation only and solvent alone. The amount of steroid given was always equal to the highest concentration used in each experiment.

The bacterial endotoxin, Difco lipopolysaccharide W from ST0901 (Difco Laboratories, Detroit, Michigan), was suspended in 0.15 M NaCl (pH 7.0) to final concentrations of 50 and 100 μg/ml 3–4 days before the initiation of each experiment. Single injections of both sparsomycin and endotoxin, or pactamycin and endotoxin, administered intraperitoneally simultaneously in 1 ml each, constituted the challenge.

Chlorpheniramine maleate (Schering Corporation, Bloomfield, New Jersey) was diluted with 0.15 *M* NaCl (pH 7.0) to a concentration of 100 μg/ml immediately prior to initial intended use. Control mice consisted of those receiving only chlorpheniramine in the same amount and frequency of injection as each group of mice being tested. All chlorpheniramine injections were administered subcutaneously.

Phenylbutazone was suspended, homogenized, and diluted in 0.15 *M* NaCl adjusted to pH 8.0. The pH was adjusted with a Beckman Zeromatic pH meter using dilute NaOH. Homogenization was done manually with a tissue homogenizer. The phenylbutazone suspensions were prepared 3 days before initial use. All injections of phenylbutazone consisted of 3 mg delivered subcutaneously in

[1] This study was supported in part by a contract N00014-68-A-0510-0002 between the Office of Naval Research, Department of the Navy and the Medical College of Virginia Commonwealth University.

[2] Recipient of an A. D. Williams Fellowship.

a volume of 0.5 ml. Control groups received only phenylbutazone, the amount corresponding to the dose and frequency of injection of the test groups.

All solutions were kept frozen at —20° until thawed in a tepid water bath just before use. The time of challenge injections was termed zero time. Deaths were recorded every 24 hr for 72 hr. All injections were made between 9:00 a.m. and 12 noon. All levels of significance were determined by comparing the protected groups with the unprotected groups of the same experiment.

Results. Those animals receiving methylprednisolone with a toxic combination of bacterial endotoxin and sparsomycin demonstrated an increase in survival time during the first 24-hr period ($p < 0.001$) and 48-hr period ($p < 0.05$) when compared to the control group (Table I). The amount of steroid given ranged from 0.5 to 2.5 mg/mouse. The greatest protection was observed with those mice receiving 2.5 mg of methylprednisolone/mouse. The amount of protection after 24 hr was highly significant ($p < 0.001$) and was still significant after 48 hr ($p < 0.01$). Multiple injections did not appreciably affect the survival rate. These additional injections consisted of 2.5 mg administered either 11 hr before or 26 hr after challenge in addition to the 2.5 mg of methylprednisolone given 1 hr before zero time. A few controls died, with mortality increasing when two injections of the methylprednisolone preparation were administered to an animal within 10–27 hr of each other (Table I). When 63% ethanol (without steroid) was administered 1 hr prior to challenge, it resulted in more deaths at each observation period than in the challenge group (at 24 hr: 58 vs. 50%; at 48 hr: 75 vs. 67%; at 72 hr: 92 vs. 67%).

A single injection of 2.5 mg of methylprednisolone/mouse, given 1 hr before challenge with pactamycin and endotoxin, resulted in significant protection for 72 hr ($p<0.01$). Methylprednisolone in a single dose of 300 μg of steroid/mouse given 0–2 hr before challenge failed to protect significantly against the toxic combination of pactamycin and bacterial endotoxin. Moreover, multiple doses of 300 μg of methylprednisolone given 2 hr before, with, and 2 hr after challenge with the toxic pair also failed to protect the mice (Table II). A few control mice died.

Chlorpheniramine was ineffective in protecting against the bacterial endotoxin–pactamycin synergy. Tested regimens included

TABLE I. Prolonged Survival of Mice Given Methylprednisolone and Challenged with Bacterial Endotoxin and Sparsomycin.

Treatment			Fraction of mice[a] dead after		
Endotoxin (μg)	Sparsomycin (μg)	Methylprednisolone (mg) (hr):	24	48	72
50	50	0	28/64	40/64	41/64
50	50	0.5–2.5; total	9/85	35/85	41/85
			$p<0.001$	$p<0.05$	NS
50	50	0.5–2.5; 1 hr before	9/75	26/66	32/66
			$p<0.001$	$p<0.05$	NS
50	50	0	22/42	29/42	30/42
50	50	2.5; 1 hr before	4/53	17/44	23/44
50	50	2.5; 1 hr before and 26 hr after	—	3/9	3/9
50	50	2.5; 11 and 1 hr before	0/10	6/10	6/10
50	50	2.5; total	4/63	26/63	32/63
			$p<0.001$	$p<0.01$	$p=0.05$
0	0	2.5; once	1/13	1/13	1/13
0	0	2.5; twice, 10–27 hr apart	1/10	2/10	2/10

[a] New York State Research Mice.

TABLE II. Effect of Methylprednisolone on Endotoxin–Pactamycin Toxicity.

Treatment			Fraction of mice dead after		
Endotoxin (μg)	Pactamycin (μg)	Methylprednisolone	(hr): 24	48	72
50	75	—	13/30	14/30	15/30[a]
50	75	300 μg; once	8/36	10/36	10/36[a]
—	—	300 μg	0/18	0/18	0/18[a]
50	75	—	5/12	9/12	9/12[a]
50	75	300 μg; 3 injections	4/12	9/12	9/12[a]
—	—	300 μg; 3 injections	0/6	0/6	0/6[a]
100	75	—	13/30	14/30	14/30[b]
100	75	2.5 mg; 1 hr before	5/41	5/41	5/41[b]
			$p < 0.01$	$p < 0.01$	$p < 0.01$
—	—	2.5 mg	1/12	1/12	1/12[b]

[a] Balb/sy mice.
[b] RFW mice.

multiple injections of 20 μg given five times (2 hr before, with, and 3, 6, and 9 hr after challenge); 10 μg four times (2 and 4 hr before, with, and 2 hr after challenge); and a single injection of 20 μg given 3 hr before or 1 hr before challenge. Phenylbutazone (3 mg) did not protect against the bacterial endotoxin–pactamycin synergy. Tested regimens included three multiple injections given 2 hr before, with, and 2 hr after challenge; and single injections of phenylbutazone given with the challenge.

Discussion. Methylprednisolone is a potent anti-inflammatory agent (2, 4), yet this attribute is not necessarily responsible for the observed decreased toxicity of the antitumor drug–endotoxin combination. The relative anti-endotoxin action of steroids does not necessarily correlate with their anti-inflammatory activities (2). The failure of this steroid to protect 72 hr after challenge with sparsomycin and endotoxin indicates that the clinical significance of this observation is limited. Methylprednisolone, however, may be a useful agent, given prophylactically, to reduce adverse side reactions during the pactamycin therapy.

The antihistamine chlorpheniramine did not reduce the pactamycin–endotoxin toxicity. This is the agreement with the general failure of antihistaminics to protect against endotoxin alone (5). Phenylbutazone was completely unsuccessful in alleviating the synergistic toxicity of the combination of bacterial endotoxin and pactamycin.

Summary. A large dose of methylprednisolone (100 mg/kg) given to mice 1 hr before challenge with sparsomycin and bacterial endotoxin significantly prolonged their survival time. The same dose of methylprednisolone given 1 hr before challenge with pactamycin and bacterial endotoxin protected mice against the synergistic toxicity. Phenylbutazone (120 mg/kg) and chlorpheniramine (4 mg/kg) failed to diminish the toxicity of the pactamycin–endotoxin synergy.

Sparsomycin, pactamycin, phenylbutazone, and 6α-methylprednisolone (Medrol) were generously supplied by Dr. G. S. Fonken, The Upjohn Company, Kalamazoo, Michigan.

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Received June 2, 1969. P.S.E.B.M., 1969, Vol. 132.