Attentuation of Some Effects of Anaphylaxis by 1,18-Diamino-6, 13-diaza-9,10-dithiaoctadecane Tetrahydrochloride (WR 149,024)[1] (35486)

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S-[2-(5-Aminopentyl) aminoethyl]-phosphorothioic acid (WR 2823) has been shown to be an alpha-adrenergic blocking agent (1, 2). This compound prevents mortality from hemorrhagic shock in dogs (3) and other species (4); it also prevents mortality and some of the cardiovascular effects of endotoxin in dogs (5, 6).

We have speculated that the active form of WR 2823 is the symmetrical disulfide of the hydrolysis product of WR 2823 (2)—1, 18-diamino-6,13-diaza-9,10-dithiaoctadecane (WR 149,024). A small quantity of this compound was synthesized for biological testing. We have verified the alpha-adrenergic blocking properties of WR 149,024 (7).

An anaphylactic shock model in mice was developed to test for efficacy of the compound in that type of cardiovascular crisis. The results of these studies show that pretreatment of sensitized mice with WR 149,024 can reduce the severity of the effects of anaphylaxis.

Methods and Materials. WR 149,024 used in these experiments was synthesized by hydrolysis and oxidation of WR 2823 by Dr. D. L. Klayman, Department of Organic Chemistry, Division of Medicinal Chemistry, Walter Reed Army Institute of Research. This material occurred as a white, granular powder, very soluble in water. The chemical was dissolved in normal saline so that the volume of the injection was always 10 μl/g of body weight. All doses of WR 149,024 are expressed as the base.

Random bred male ICR mice from the Walter Reed Colony were sensitized by the intraperitoneal injection of a 7.6% solution of egg white in saline (10 μl/g) on days 1, 3, and 5. On day 8, the challenge dose of egg albumin (760 mg/kg, ip) was given. The egg albumin solution was made daily from material purchased from Fisher Scientific Co.

Sensitized animals were pretreated on day 8 with the chemical as indicated in Table I. Untreated control animals received 10 μl/g of normal saline as indicated.

The effects of the chemical on hematocrit values were studied by injecting sensitized animals with WR 149,024 or normal saline 1

TABLE I. Prevention of Mortality from Anaphylaxis in Mice by WR 149,024.

Dose of WR 149,024 (mg/kg, ip)	Time of injection before antigen (min)	Mortality	
		Treated	Untreated[a]
50	120	0/8[b]	8/10
	60	1/8[b]	6/10
	0	2/10	6/10
	+15	9/9[c]	6/9
30	60	3/10	10/10
17	60	5/10	
10	60	8/10[d]	

[a] "Untreated" mice received 10 μl/g normal saline instead of WR 149,024; χ^2 (treated vs untreated) = 6.50; χ^2 (homogeneity of untreated groups) = 0.34.

[b] Two deaths from drug in each group.

[c] χ^2 (homogeneity of treated groups) = 15.74; χ^2 (posttreatment cell only) = 11.35.

[d] χ^2 (treated vs untreated) = 10.94.

[1] A portion of this material was reported at the 1970 meetings of Fed. Amer. Soc. Exp. Biol. (Fed. Proc., Fed. Amer. Soc. Exp. Biol.) **29**, 420 Abstr. 1970).

TABLE II. Prevention of Hemoconcentration from Anaphylaxis in Mice by WR 149,024.

Treatment I	Treatment 2[a]	Hematocrit values[b]	Mortality
Saline	Egg white	67.1–76.3[c]	9/10
	Saline	45.3–48.1	0/10
WR 149,024[d]	Egg white	58.8–64.0	0/10
	Saline	40.8–43.0	1/10

[a] Treatment 2 was given 1 hr after treatment 1.

[b] Hematocrit samples taken 15 min after treatment 2.

[c] Hematocrit values expressed as 95% confidence interval for the mean ($n = 10$).

[d] Dose of WR 149,024 was 50 mg/kg, ip.

hr before the challenge dose of egg white as indicated in Table II. Duplicate microhematocrit samples of 50 μl or less were taken from the retro-orbital sinus 15 min after the egg white or second simulated injection.

To study the effects of WR 149,024 on the cardiovascular effects of anaphylaxis, 12 sensitized mice were anesthetized with sodium pentobarbital and cannulated for recording blood pressure according to the method of Demaree *et al.* (8). The right carotid artery was cannulated using a 24-gauge needle shaft secured to the arterial wall with Collodion USP and attached to a pressure transducer. The left femoral vein was cannulated for egg-white injections by using a 27-gauge needle shaft. Electrocardiograms were recorded from needle electrodes placed under the skin of the extremities. The heart rate was recorded by using a cardiotach preamplifier actuated by the R wave of the ECG. Respiratory exhange was recorded by attaching a pneumotachograph screen to the tracheal cannula and to a differential gas pressure transducer. Two animals were prepared simultaneously and were assigned at random to untreated or treated groups. The treated group received WR 149,024 (40 mg/kg, ip), and the untreated group received normal saline (10 μl/g, ip). One hr later, a bolus injection of egg white was made into the femoral vein (100 mg/kg in 5 μl/g) in a 1-min injection. The animals were observed for 70 min; those surviving at that time were considered permanent survivors.

Results. The prevention of mortality from anaphylaxis by WR 149,024 is shown in Table I. When given 1 hr before the challenge dose of antigen, the effective dose range for WR 149,024 appears to be between 17 and 50 mg/kg. At 50 mg/kg given simultaneously with or up to 2 hr before the antigen, the drug was effective, but it was not effective when given 15 min after the antigen. Since 50 mg/kg is near the lethal range in mice, higher doses were not tested; pretreatment times greater than 2 hr were not tested.

The effects of the chemical on hematocrit values in mice are shown in Table II. When given 75 min before taking the hematocrit samples, WR 149,024 caused hemodilution in unchallenged mice and reversed about 40% of the hemoconcentration associated with anaphylaxis.

The blood pressure changes associated with the injection of antigen in sensitized mice are shown in Figs. 1 and 2. The cardiovascular changes observed in the untreated, anesthetized mouse were identical to those reported by McMaster and Kruse (9). There was an immediate pressor response followed quickly by a progressive hypotension and

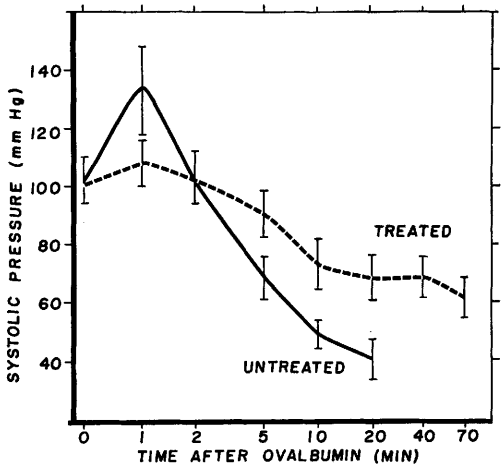

FIG. 1. Prevention by WR 149,024 of some of the effect of anaphylaxis on systolic blood pressure in anesthetized mice: (—) connects the means of observations made at logarithmic time intervals following the injection of antigen in untreated, sensitized mice; (vertical lines) represent ± 1 SEM ($n = 6$); (- - -) connects similar means for animals receiving WR 149,024 (40 mg/kg, ip) 1 hr before the antigen challenge ($n = 6$).

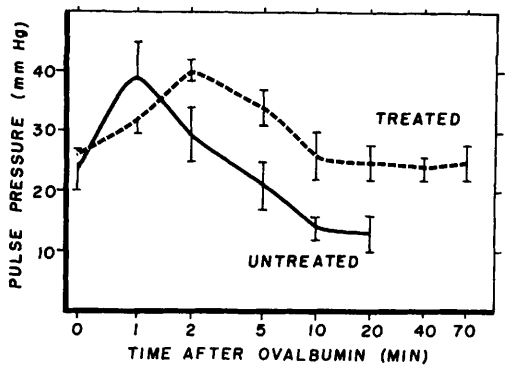

FIG. 2. Prevention of WR 149,024 of the loss of pulse pressure during anaphylaxis in anesthetized mice. For explanation see Fig. 1.

severe loss of pulse pressure. The heart rate increased from 420 to 550 beats/min within 2 min after the intravenous injection of the antigen. The heart rate gradually fell until death occurred in about 30 min after the antigen injection. WR 149,024 prevented most of the immediate pressor effect and much of the delayed depressor effect. The most striking effect of WR 149,024 was seen in the maintenance of the pulse pressure. The effect of WR 149,024 on heart rate was not remarkable. WR 149,024 appeared not to modify the base line cardiovascular parameters. Injection of egg white in nonsensitized mice caused no measurable cardiovascular changes and, of course, no mortality. All six of the untreated mice died in these experiments between 20 and 40 min after the antigen injection. Respiratory exchange usually stopped except for irregular gasps about 4 min before complete cardiovascular collapse. Thus, it appeared that death was due to respiratory failure secondary to severe, irreversible hypotension. All six mice treated with WR 149,024 were alive at 70 min after injection of the antigen.

Discussion. Bergman and Munoz (10) speculated that the signs of anaphylactic shock in mice are secondary to the cardiovascular crisis arising from a loss of vascular fluid. We have confirmed the cardiovascular crisis by direct measurement of blood pressure; the severe, progressive loss of arterial pulse pressure is consistent with this proposed mechanism.

WR 149,024 prevented some of the mortality, reduced the hemoconcentration, and moderated the cardiovascular changes produced by anaphylaxis in the mouse. The most striking effect on the cardiovascular responses to the antigen was related to the prevention of the loss of pulse pressure. These experiments provide no firm basis upon which to explain the mechanism by which WR 149,024 affects the hemodynamics of anaphylaxis in the mouse. Nevertheless, assuming that anaphylaxis and the drug act on the plasma volume only and not on red cell mass to modify the hematocrit, differences in the calculated plasma volumes reveal the correlation shown in Table III. The very close agreement in the two calculations of plasma volume differences between treated and untreated animals suggests that WR 149,-024 exerts its effect on hematocrit directly. If this is the case, WR 149,024 antagonizes hemoconcentration due to anaphylaxis by mobilizing extravascular fluids or by preventing the egress of vascular fluids by a mechanism unrelated to anaphylaxis. These experiments did not rule out some coincidental effects of the drug to antagonize the anaphylactic response by other mechanisms. Experiments are being planned to test the assumptions upon which the calculated plasma volumes are based and to examine the effects of the drug to block the release or actions of histamine and serotonin in the mouse.

WR 149,024 is an alpha-adrenergic blocking agent. The extent to which this antiadrenergic effect of WR 149,024 contributes to the survival mechanism remains speculative. In concurrent experiments, phenoxybenza-

TABLE III. The Effect of Anaphylaxis and WR 149,024 on Calculated Plasma Volume in Arbitrary Red Cell Volume Units.[a]

	Treated (T)	Untreated (U)	Difference (T − U)
Shocked	0.61	0.39	0.22
Control	1.41	1.16	0.25

[a] Calculated plasma volume (PV) from hematocrit (H), assuming no change in red cell volume (RV), is derived: by definition, $H = RV/(RV + PV)$; so transposing, $PV = [(1 - H)\,RV]/H$.

mine (5 mg/kg), a classical alpha-adrenergic blocking agent, prevented mortality from anaphylaxis in unanesthetized mice. As yet, however, we have not been able to demonstrate an effect of phenoxybenzamine to prevent the cardiovascular changes from antigen injection in anesthetized mice. Also we have not been able to rule out an antihistamine or antiserotonin action of WR 149,024 to prevent some of the effects of anaphylaxis.

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