PIPERAZINE ADIPATE: A NEW ANTHELMINTIC AGENT

PART II. TOXICOLOGICAL AND PHARMACOLOGICAL STUDIES

BY B. G. CROSS, A. DAVID and D. K. VALLANCE

From the Biological Laboratories, The British Drug Houses Ltd., Godalming, Surrey

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THE purpose of this paper is to report the results of some pharmacological and toxicological investigations of piperazine adipate[1].

Pure piperazine adipate was used unless otherwise stated. In the acute toxicity studies in mice, pure and technical piperazine adipate and technical piperazine hydrate were examined simultaneously. To determine the effect on the hæmopoietic system of rabbits 250 mg./kg. was administered subcutaneously, but due to its low solubility[1] it was necessary to use the formulation as described in the text.

RESULTS

(*a*) *Acute Oral Toxicity*

(*i*) *Mice*

Male albino mice (ex-Evans, Stockwell), weighing approximately 20 g. each, were starved overnight and given one of the following preparations: pure piperazine adipate, technical piperazine adipate or technical piperazine hydrate. The compounds were administered as suspensions in 5 per cent. mucilage of acacia by means of a metal catheter, the 4 doses varying from 3 to 10 g./kg. The volumes were adjusted to 1 ml./20 g. of body weight. 10 animals were used at each dose, and from the mortalities after 7 days the LD50 and limits of error (P = 0·95) were calculated according to the method of Litchfield and Wilcoxon[2]. The results are recorded in Table I. All mortalities occurred within the first 24 hours, but there were

TABLE I

THE ACUTE ORAL TOXICITIES OF THREE PIPERAZINE COMPOUNDS IN MALE ALBINO MICE

Compound	LD50 g./kg.	Limits of Error (P = 0·95) g./kg.
Piperazine Adipate (pure)	11·4	9·2–14·0
Piperazine Adipate (technical) ..	8·2	7·0– 9·6
Piperazine Hydrate (technical) ..	4·3	3·3– 5·6

no immediate deaths. These results in mice demonstrate that on a weight for weight basis both the pure and technical piperazine adipate are considerably less toxic than technical piperazine hydrate.

(*ii*) *Rats*

Female albino rats (B.D.H. strain) weighing approximately 200 g. each were starved overnight, and piperazine adipate (10·0, 6·7, 4·5 and 3·0 g./kg.) was administered as a suspension in 5 per cent. mucilage of acacia by

means of a rubber catheter. The volume was adjusted to 5 ml./100 g. of body weight. 5 animals were used at each dose level and an additional group received an equivalent volume of the acacia alone.

Within 1 hour all the animals, including the controls, developed diarrhœa or passed fæces which although formed were softer than normal. The former condition was more evident in the groups receiving 10·0 and 6·7 g./kg. and persisted in some of those animals for 48 hours. No other toxic effects were observed apart from lethargy, which was very marked at the higher dose levels. There was no immediate mortaility, but at 10 g./kg. all the rats died within 24 hours and one rat given 6·7 g./kg. died on the second day following administration. No further deaths occurred during the 7-day observation period. The LD50 as estimated by Karber's[3] formula was 7·9 g./kg.

(*b*) *Subacute Oral Toxicity*

The possibility of cumulative toxic effects occurring on prolonged administration was studied by the addition of piperazine adipate to the diet (ground rat diet 41 as supplied by Associated London Flour Millers, Ltd.) of immature female albino rats (B.D.H. strain) for a period of 8 weeks. The amount of food was sufficient to satisfy hunger, and the piperazine adipate content was such that each animal received approximately 300 mg./kg. daily. 14 rats received this diet and 15, comprising the controls, received a diet identical in all respects other than the omission of the piperazine adipate.

During the experimental 8 weeks period each treated rat received a total of approximately 17 g. of piperazine adipate per kg. of body weight without the development of any apparent toxic effects. The animals were weighed at weekly intervals and no significant difference between the experimental and control groups was observed. The mean initial weight of the piperazine-treated rats was 116 g. (range 104 g. to 132 g.) and at the termination of the experiment the mean weight was 165 g. (range 141 g. to 185 g.). The mean initial and final weights of the control group were 114 g. (range 99 g. to 126 g.) and 162 g. (range 129 g. to 190 g.) respectively. The growth curves for the two groups are shown in Figure 1.

Autopsy of the animals at the end of the experiment revealed no gross pathological changes. Histological examination of lung, heart, liver, spleen, kidney, brain, spinal cord, peripheral nerve, stomach, small and large intestine did not reveal any significant difference between control and experimental groups.

(*c*) *Actions on Smooth Muscle*

The actions of piperazine adipate were studied on the isolated duodenum of rabbit and segments of guinea-pig's ileum suspended in a 50-ml. bath of ærated Ringer's solution at 35° C. The compound was added as a 4 per cent. aqueous solution. In the rabbit's duodenum preparations, no effects on the normal rhythmic contractions were observed with amounts below 10 mg. Larger amounts, from 10 to 40 mg., added to the bath for 2 to 8 minutes, caused an increase in tone (Fig. 2). This effect, however,

was reversed on washing out. Similar responses were obtained with piperazine citrate at the same dose levels. In the guinea-pig's ileum preparations, the spasmogenic action of piperazine adipate was again observed, with amounts varying between 4 mg. and 8 mg. The addition of 6 mg. of piperazine adipate produced a contraction which was 70 per cent. of the response caused by the addition of 0·2 μg. of acetylcholine. The response to piperazine adipate was, however, counteracted by the addition of methantheline bromide, a known spasmolytic, 0·3 μg. causing a 75 per cent. reduction in the response to 6 mg. of piperazine adipate.

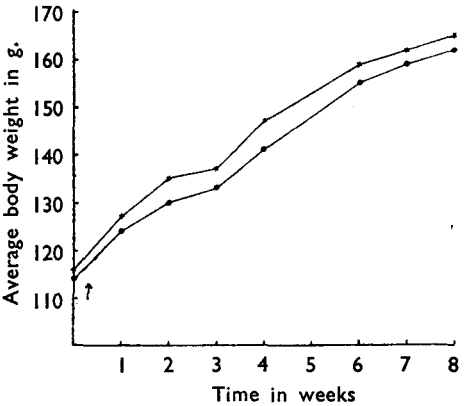

FIG. 1. Effect of piperazine adipate (300 mg./kg. per day) on the growth of immature female rats.
×——× Treated group.
●——● Control group.
↑ Indicates where treatment commenced.

The action of piperazine adipate on the intestine *in situ* was examined in 3 rabbits and 2 cats. The rabbits were anæsthetised with urethane (1 to 1·5 g./kg. intravenously) and the abdomens opened. The movements of a 3 cm. length of jejunum were recorded by attaching one end to a fixed rod and the other end to a small vertical lever whose movements were transmitted *via* a thread to a frontal writing lever. The abdominal cavity was

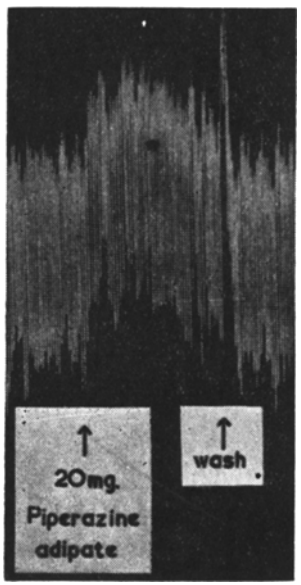

FIG. 2. Effect of piperazine adipate on the isolated rabbit duodenum (20 mg. in a 50-ml. bath for 4 minutes).

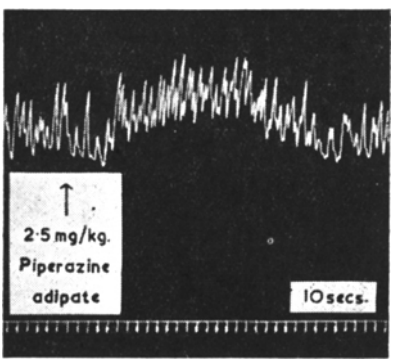

FIG. 3. Effect of intravenous injection of piperazine adipate (2·5 mg./kg.) on intact jejunum of a 3·8 kg. cat anæsthetised with chloralose.

filled with warmed Ringer's solution maintained at 37° C. Intravenous injection of varying amounts (3 to 15 mg./kg.) of piperazine adipate was generally followed by an increase in tone and a slight increase in the frequency of contractions. The cats were anæsthetised with ether followed by chloralose (50 to 60 mg./kg. intravenously). A water-filled balloon was placed in the jejunum through an abdominal incision, and the intestinal contractions were recorded by means of a water-air transmission system connected to a small piston recorder. Intravenous injections of piperazine adipate (1 to 2·5 mg./kg. in one animal and 5 to 20 mg./kg. in the other) were followed by an increase in tone, and occasionally by an increase in the frequency of contractions (Fig. 3).

(*d*) *Blood Pressure Responses in Anæsthetised Cats*

Blood pressure changes were recorded in 5 cats anæsthetised with ether followed by chloralose (60 to 80 mg./kg. intravenously[5]). Carotid arterial blood pressures were recorded by a mercury manometer, and intravenous injections of piperazine adipate given *via* the femoral vein. The amount of piperazine adipate required to produce a definite response varied considerably in the different animals. With one exception, however, amounts below 50 mg./kg. had no effect on blood pressure. Doses between 50 and 200 mg./kg. produced an immediate depressor response which rarely exceeded 50 mm. of mercury. 1 cat was particularly sensitive, a hypotensive response being elicited following 7 mg./kg. Recovery was rapid in all cases, the blood pressure returning to normal within 1 to 3 minutes following administration. The effect of atropine sulphate was examined in 1 animal; 0·6 mg./kg. failed to modify the depressor effect of piperazine adipate (60 mg./kg.).

The above responses occurred following the rapid intravenous injection of piperazine adipate. In contrast, the slow intravenous infusion of 500 mg./kg. over a period of 30 minutes had no demonstrable effect on blood pressure. The animal had, however, previously given typical depressor responses to 100 and 200 mg./kg. injected rapidly. The subsequent rapid injection of 100 mg./kg. again produced the typical response to rapid injection.

(*e*) *Effects on Heart*

(*i*) *In situ*

The action of piperazine adipate on the hearts of 2 cats, artificially respired under chloralose anæsthesia was studied *in situ*. A Cushny myocardiograph attached to the left ventricle was used to record the responses. Amounts of 50 to 120 mg./kg. in one preparation and 7 to 28 mg./kg. in the other, injected rapidly *via* the femoral vein, reduced the amplitude of the heart, but had only a slight effect on the rate (Fig. 4). The carotid blood pressure showed a concomitant fall. Recovery from both effects was rapid.

(*ii*) *Isolated Rabbit's Heart*

The cardiac effects were also studied on the isolated heart of the rabbit, perfused *via* the aorta through the coronary vessels with oxygenated

Ringer-Locke solution at 38° C. (Langendorff preparation). Piperazine adipate was administered by direct injections into the cannula. Records were obtained of the amplitude of the contractions and the rate was counted. Varying amounts (1 to 4 mg.) of piperazine adipate reduced the amplitude but only slightly reduced the rate (Fig. 5). Complete recovery occurred within 5 minutes. Smaller doses had little or no effect.

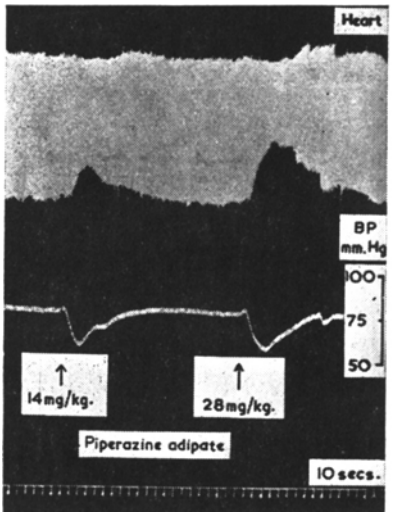

FIG. 4. Effect of intravenous injection of piperazine adipate (14 and 28 mg./kg.) on ventricular contractions and blood pressure in a 5·7 kg. cat anæsthetised with chloralose. Upper tracing: ventricular contractions; lower tracing: blood pressure.

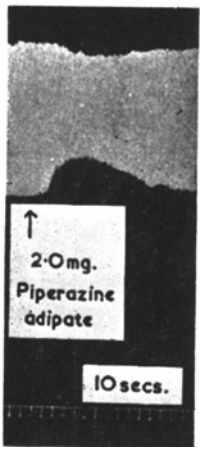

FIG. 5. Effect of piperazine adipate (2·0 mg.) on isolated perfused rabbit heart.

(f) Effects on Respiration

Respiratory effects were studied in 6 rabbits anæsthetised with urethane (1 to 1·5 g./kg.), using the method described by Gaddum[4]. The effects following the rapid intravenous injection of piperazine adipate were rather variable, but in general, doses varying from 2 to 16 mg./kg. produced slight stimulation of respiration, whereas larger doses had a depressant effect. The continued administration of piperazine adipate (4 per cent. solution) by fairly rapid intravenous injection resulted in death from respiratory failure in 2 rabbits. The doses required were 220 and 340 mg./kg. respectively.

(g) Effects on Hæmopoietic System

The effects of piperazine adipate on the hæmopoietic system were investigated on 8 female rabbits (B.D.H. stock) aged approximately 5 months. Due to solubility limitations the compound was given in the following preparation: piperazine adipate 10 g., phenol 0·2 g., sodium hydroxide solution B.P. 50 ml., distilled water 50 ml., and sufficient citric

acid B.P. to produce *p*H 7·4. Solutions so prepared were passed through a Seitz filter before use. A volume equivalent to 250 mg. of piperazine adipate per kg. of body weight was injected subcutaneously in 2 divided doses, 5 times a week for 5 weeks. A further 4 rabbits were given corresponding volumes of a formulation identical in all respects except for omission of the piperazine adipate.

Examination of weekly blood samples showed no significant differences between the red, white and differential cell counts or hæmoglobin concentrations of the experimental and control animals. The treated animals did not gain weight to quite the same extent as the controls. These results are summarised in Table II. By the 14th day of the experiment both groups developed slight ulceration at injection sites, and, at autopsy small hæmorrhages were observed in the underlying tissues. No significant gross or microscopical changes were evident in the major internal organs.

TABLE II

THE MEAN BODY WEIGHTS, RED, WHITE AND DIFFERENTIAL BLOOD-CELL COUNTS AND HÆMOGLOBIN VALUES OF 8 FEMALE RABBITS INJECTED SUBCUTANEOUSLY WITH THE EQUIVALENT OF 250 mg./kg. OF PIPERAZINE ADIPATE 5 TIMES A WEEK FOR 5 WEEKS AND OF 4 CONTROL ANIMALS GIVEN THE VEHICLE ALONE

Group	Week	Weight, kg.	R.B.C. cells/ c.mm.	W.B.C. cells/ c.mm.	Hæmoglobin, per cent.	Lymphocytes, per cent.	Monocytes, per cent.	Neutrophils, per cent.	Eosinophils, per cent.	Basophils, per cent.
Experimental 8 animals	Initial	2·56	5,390,000	12,770	61	78·25	15·3	2·0	1·5	1·5
	1st week	2·67	5,255,000	10,625	60·25	78·6	18·0	2·0	0·25	1
	2nd ,,	2·66	5,522,000	10,870	60·6	74·2	21·5	2·0	1	1·5
	3rd ,,	2·66	5,430,000	10,900	60·0	75·5	21·5	1·5	1	0·25
	4th ,,	2·66	5,500,000	10,100	59·5	72·75	24·0	2·0	0·6	0·5
	5th ,,	2·68	5,329,000	10,900	60	74·5	21·3	2·0	2	0·25
Control 4 animals	Initial	2·52	5,420,000	12,300	61·7	79·0	16·0	3	0·75	1
	1st week	2·71	5,500,000	12,100	62·0	80·5	16·5	2	0·25	0·75
	2nd ,,	2·7	5,670,000	12,350	62·0	79·5	17·0	1·75	0·75	1
	3rd ,,	2·87	5,650,000	11,650	61·5	74·5	20·25	1·25	1·75	0·25
	4th ,,	2·84	6,000,000	11,500	62·0	75·0	18·25	2	1·25	1·0
	5th ,,	2·9	5,480,000	12,100	61·25	77·5	18·0	2·25	1·25	0·75

DISCUSSION

This investigation has shown that piperazine adipate is comparatively non-toxic, the oral LD50 of the pure compound in mice and rats being 11·4 and 7·9 g./kg. respectively. This provides an ample margin of safety as the maximum dose recommended for clinical use would not normally exceed 75 mg./kg. a day for 7 days. The only toxic effects observed in rats following single sublethal doses were lethargy and diarrhœa. The spasmogenic effect obtained on isolated intestinal muscle and intestinal muscle *in situ* might possibly be of significance in the latter connection, although the amounts required to produce such responses were undoubtedly very large.

It is evident that therapeutic oral doses in man are unlikely to cause either circulatory or respiratory disturbances. These effects were only observed following the rapid intravenous injections of extremely large doses.

Finally, the continued administration of 300 mg./kg. daily in the diet of immature rats for 8 weeks and the subcutaneous injection of 250 mg./kg.

daily (5 times a week) for 5 weeks in rabbits did not produce any pathological changes, apart from local necrosis at injection sites, probably attributable to the concentrated preparation used. In particular piperazine adipate did not have any deleterious action on the hæmopoietic system of rabbits.

SUMMARY

1. An investigation on animals of the toxicological and pharmacological properties of piperazine adipate, a new anthelmintic agent, is reported.

2. It is comparatively non-toxic orally, the LD50 of mice and rats being 11·4 and 7·9 g./kg. respectively.

3. It has a spasmogenic action on isolated intestinal muscle and intestinal muscle *in situ* when large doses are given.

4. Cardiac, circulatory and respiratory effects were slight, the rapid intravenous injection of large amounts being required to produce a response.

5. It has been safely administered over a prolonged period to rats and rabbits.

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