

Research Papers

Dioxatrine, a potent and specific rumenal ulcer-preventing agent in rats

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When rats are starved with free access to a 20% glucose solution, extensive rumenal ulcers are found in almost all animals at the end of the third week. The formation of these ulcers may be inhibited by dissolving adequate amounts of two anti-acetylcholine agents in the glucose solution. A minimum of about 8 mg/kg of atropine sulphate daily is required for significant activity. Submaximal mydriatic effects are observed after oral administration of this dose. Dioxatrine, a tertiary amine of novel structure, inhibited ulcer formation at dose levels devoid of significant mydriatic activity. Dioxatrine prevents experimental ulcers in rats in one twentieth the dose of atropine.

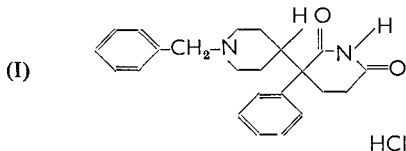
THE frequent occurrence of rumenal ulcers in starved rats was first reported by Büchner, Siebert & Molloy (1928) and was used to study the effects of drugs on ulcer formation by Grandjean (1948) by Visscher, Seay, Tazelaar, Veldkamp & Vanderbrook (1954) and by Zbinden, Pletscher & Studer (1959).

In an effort to develop an orally active drug, capable of preventing the formation of gastric ulcers at low doses without producing side-effects, we investigated many substances by a method in which rats are fasted for three weeks with free access to a 20% aqueous glucose solution containing the substance under investigation.

A few potent anti-acetylcholine drugs prevented rumenal ulcer formation in this test. All the other compounds investigated were either inactive or promoted ulcer formation, for example, reserpine.

Dioxatrine (I), an anti-acetylcholine tertiary amine of novel chemical structure recently synthesised in this laboratory, is of special interest in that it was the only compound which was capable of significantly decreasing the rate of rumenal ulcer formation without producing severe mydriasis, and of completely inhibiting the formation of ulcers in our experimental conditions.

Of the anti-ulcer compounds tested, dioxatrine was the most potent. We now present the experimental evidence of the activity of this drug compared with atropine.



± 1-Benzyl-4-(2,6-dioxo-3-phenyl)-3-piperidyl)-piperidine hydrochloride (Dioxatrine) $C_{23}H_{26}N_2O_2 \cdot HCl = 398.92$. Melting point: 300° (decomp). Solubility in H_2O : 11.7 mg/ml at 20° .

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Methods

THE RUMENAL ULCER TEST

Male Wistar rats of an inbred strain with an initial weight of 240 to 260 g were caged in groups of 5. Each cage (20 × 23 × 30 cm) had 12 × 12 mm mesh to prevent coprophagia. They were fasted for 21 days with free access to a 20% aqueous glucose solution in which a known amount (5, 10, 20 . . . mg/litre) of the substance under investigation was dissolved. At the end of the 21st day the animals were killed and the stomachs removed, split along the entire great curvature, rinsed with tap water and pinned on 10 × 10 cm cork plates under moderate stretching for inspection of the mucosa. A trained observer, with no knowledge of the regimen to which each mucosa had been subjected, then used the following arbitrary score system for expressing the degree of ulceration of the rumenal mucosa: score 1: no ulcers; score 2: one to ten small, or one to five big ulcers; score 3: extensive ulceration involving 25 to 75% of the total rumenal surface; score 4: widespread ulceration involving more than 75% of the surface. Two groups of five rats were used for each dose level. Ridit analysis is used for the statistical analysis of these data (Bross, 1958).

MYDRIATIC ACTIVITY

Inbred male Wistar rats with a weight of 200 to 250 g were used. Before and $\frac{1}{4}$, $\frac{1}{2}$, 1, 2, 4, 8, 32 and 56 hr after oral administration of the substance under investigation the pupil diameter was measured with a micrometer and expressed in 1/25 mm units.

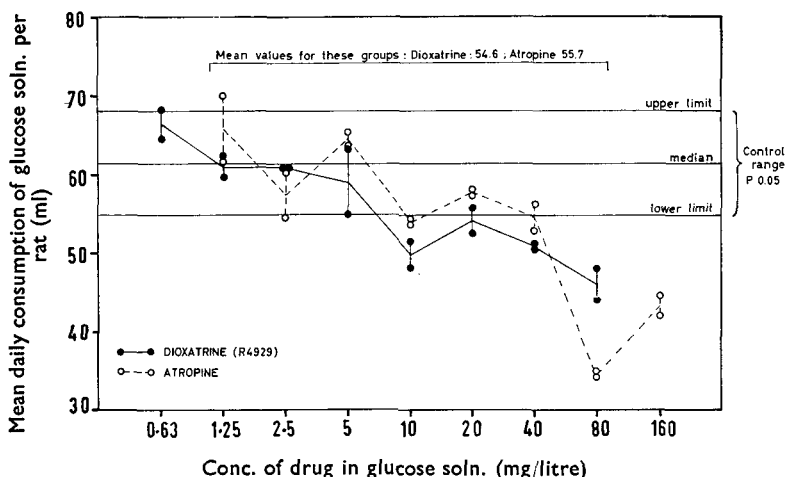


FIG. 1. The anti-acetylcholine drugs dioxatrine and atropine inhibit drinking in starved rats with free access to a 20 per cent aqueous glucose solution. Both substances are approximately equipotent in this respect. Each point represents a group of five rats.

Results and discussion

In a series of 140 control rats, fasted with free access to a 20% aqueous glucose solution, typical rumenal ulcers were found at the end of the third week in 139 animals (score 1 in one rat, score 2 in 5 rats, score 3 in 126 rats, score 4 in 8 rats). All these controls were still alive after 21 days, but their weight fell from 250 ± 10 g initially to 221 g after 7 days, 202 g after 14 days and 184 ± 10 g after a fast of 21 days. Drinking also gradually decreased from an average of 85 ml of glucose solution per rat on the first experimental day and an average of 92 ml per rat on the third day, to 68 ml on day 7, 55 ml on day 14 and 36 ml on day 20. The median control value for the entire 3 week period was 61.5 ± 6.5 ml of glucose solution per rat and per day.

Anti-acetylcholine drugs are known to block drinking by a central effect and eating by a peripheral effect (Stein, 1963). As shown in Fig. 1 dioxatrine and atropine were found to inhibit drinking of glucose solution in fasting rats. Significant inhibition was observed with both drugs in concentrations of 10 mg/litre of glucose solution or more. Both substances were approximately equipotent in this respect.

TABLE 1. FREQUENCY DISTRIBUTION OF RUMENAL ULCER SCORES IN GROUPS OF 10 RATS AFTER A FAST OF 21 DAYS WITH FREE ACCESS TO A 20 AQUEOUS GLUCOSE SOLUTION CONTAINING VARIOUS CONCENTRATIONS OF DIOXATRINE OR ATROPINE SULPHATE

Concentration in mg/litre	Dioxatrine-scores				Atropine-scores			
	1	2	3	4	1	2	3	4
0.63	0	1	9	0	—	—	—	—
1.25	0	3	7	0	1	1	8	0
2.5	2	4	4	0	0	2	6	0
5	2	3	5	0	0	3	8	1
10	2	5	3	0	0	2	7	1
20	8	1	1	0	0	1	9	0
40	9	1	0	0	4	2	3	1
80	10	0	0	0	0	6	3	0
160	—	—	—	—	4	3	1	0
Totals	33	18	29	0	9	20	45	3

No mortality, except with the two highest concentrations of atropine (1/10 and 2/10). Lowest active concentrations: 2.5 mg of dioxatrine per litre and 40 mg of atropine per litre ($P < 0.05$).

As shown in Fig. 2, the mean body weight at autopsy of rats treated with high doses of dioxatrine or atropine was significantly lower than expected from the control data. This effect is only partly due to reduced glucose consumption (Fig. 3). Eight groups of 5 treated rats, 4 with each drug, were found to have normal body weight values at autopsy in spite of a significantly reduced glucose intake, whereas only two groups, one with each drug, showed abnormally low body weights at autopsy after having consumed a normal amount of glucose solution.

The effects of both drugs on ulcer formation are shown in Table 1. Significant reduction (χ^2 test, $P \leq 0.05$) of ulcer formation was observed in six dioxatrine-treated groups of ten rats (2.5 to 80 mg/litre) and in three atropine-treated groups (40 to 160 mg/litre), the ratio of the lowest active concentrations being 1 to 16. With dioxatrine the stomachs of all

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rats treated with the 80 mg/litre concentration were free of ulcers and the concentration protecting half of the rats was about 15 mg of dioxatrine per litre. With atropine however only 4 out of ten rats were protected with the highest and toxic (2/10 mortality) concentration of 160 mg/litre.

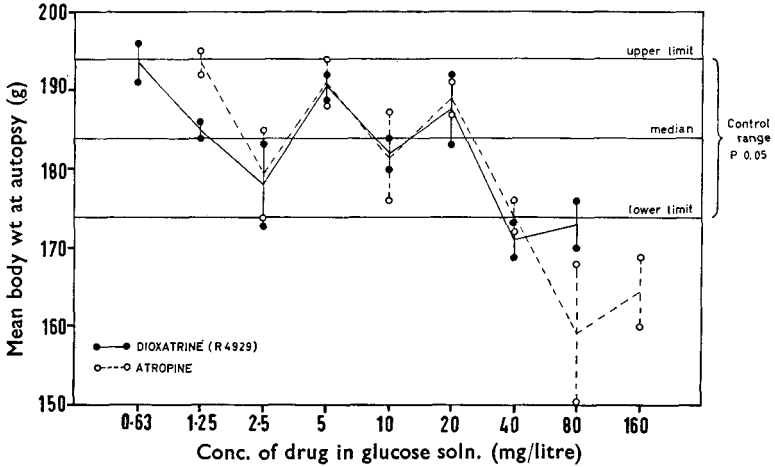


FIG. 2. The mean weight at autopsy of starved rats with free access to glucose solutions containing high concentrations of dioxatrine or atropine is significantly lower than the body weights of the control group. Each point represents a group of five rats.

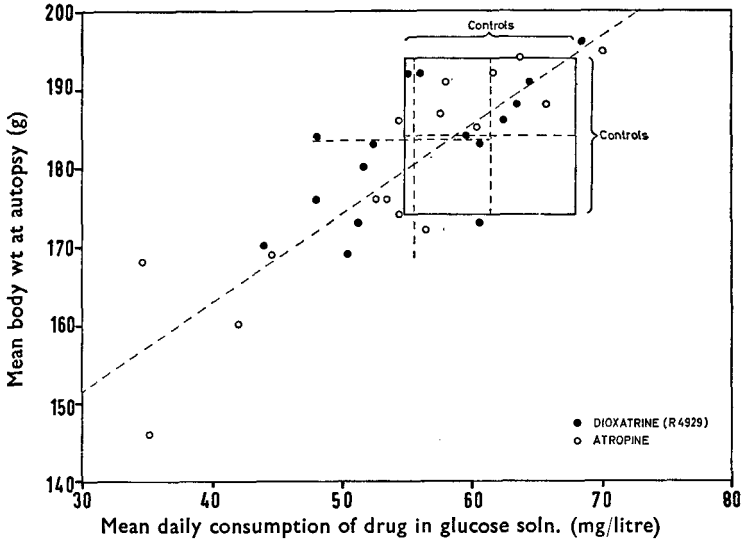


FIG. 3. Correlation of weight and glucose solution. Each point represents a group of five rats. As a whole, the weight of rats with low glucose consumption values is higher than would be expected from the control data. The square represents median values (broken lines) and confidence limits (solid lines) for the controls. Also represented are the median values for all treated rats (broken lines) as well as the calculated regression line for these animals (broken diagonal line).

Fig. 4 shows the correlation of the mean daily doses of dioxatrine and atropine absorbed by the treated animals throughout the three weeks and the degree of rumenal ulceration at autopsy, expressed in ridits. Ridit analysis (Bross, 1958) is a valuable statistical method for expressing the frequency distribution of measurements based on a nominal scale, e.g. the one to four score system used in this paper for measuring the degree of rumenal ulceration, in one meaningful symbol and for analysing its statistical significance. Using these dose-effect curves the lowest significantly active ulcer-preventing doses of dioxatrine and of atropine may be graphically estimated as respectively 0.1 and 2 mg/rat/day. Dioxatrine therefore may be said to be about 20 times more potent in this test than atropine sulphate.

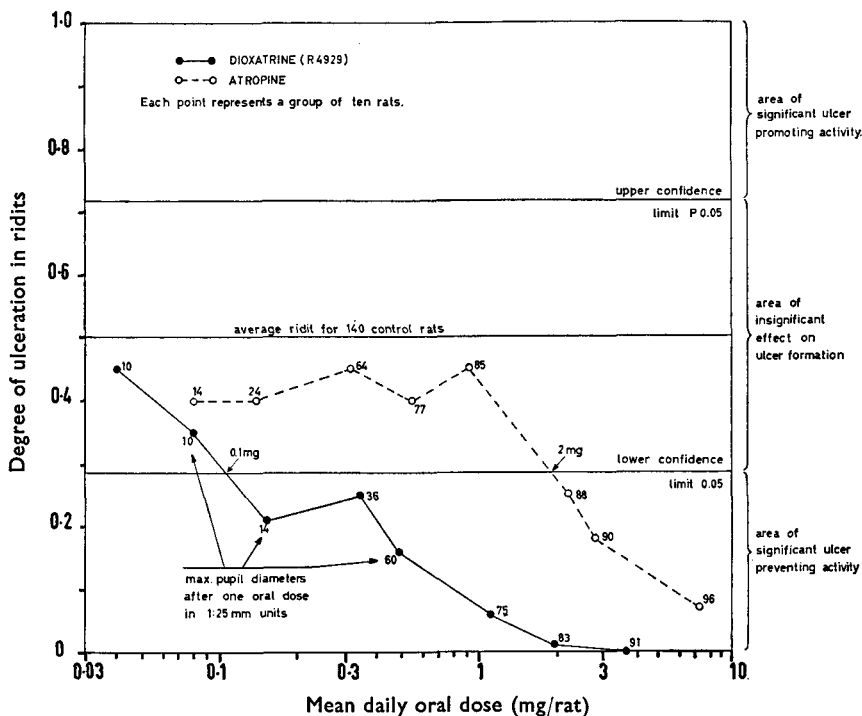


FIG. 4. Correlation of dose, degree of rumenal ulceration (in ridits) and maximal mydriatic effect. The lowest active ulcer preventing dose of dioxatrine produces almost no mydriasis and is about 20 times less than the lowest ulcer-preventing dose of atropine, which produces a pronounced mydriatic effect.

As an oral mydriatic drug, on the other hand, dioxatrine is only about half as potent (Fig. 5) as atropine, but is much slower and longer acting. With dioxatrine, mydriatic peak effects were observed about 4 hr after both oral and subcutaneous administration, as against about 3 to 4 hr with atropine.

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TABLE 2. SUMMARY OF EXPERIMENTAL DATA

	Dioxatrine	Atropine	Dioxatrine Atropine
Lowest active ulcer preventing dose level in mg./kg. orally (A)	0.4	8	1:20
Lowest active mydriatic dose level in mg./kg. orally (B) ..	0.6	0.3	2:1
Lowest active drinking inhibition dose level in mg./kg. orally (C)	2	2	1:1
Lowest active body weight lowering dose level in mg./kg. orally (D)	8	8	1:1
Relative values { A	1	1	1:1
{ B/A	3/2	3/80	40:1
{ C/A	5	1/4	20:1
{ D/A	20	1	20:1

Dioxatrine is furthermore a much more specific or selective anti-ulcer agent than atropine, i.e. at dose levels producing an equivalent degree of ulcer prevention, the mydriatic effects of atropine are much more pronounced than the mydriatic effects of dioxatrine.

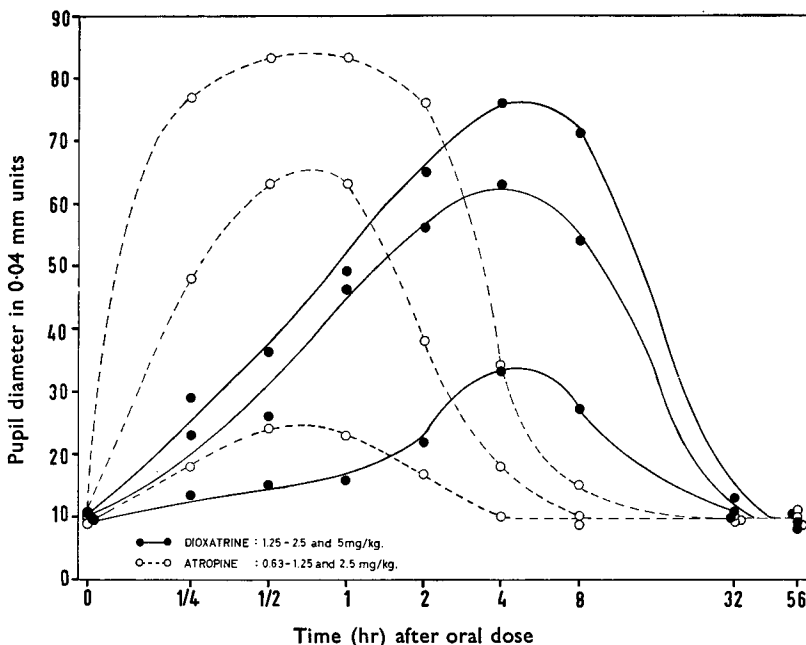


FIG. 5. As an oral mydriatic drug dioxatrine is about half as potent as atropine. Dioxatrine however has a slower onset and a much longer duration of action than atropine. Each curve represents a group of ten rats.

The lowest active oral anti-ulcer dose of dioxatrine, i.e. 0.1 mg per rat or about 0.4 mg/kg, is virtually devoid of mydriatic activity, whereas the equivalent oral anti-ulcer dose of atropine, i.e. 2 mg/rat or about 8 mg/kg produces submaximal mydriasis (Figs 4 and 5).

The most important experimental data, discussed above, is briefly summarised in Table 2.

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