RESEARCH PAPER

SOME PHARMACOLOGICAL PROPERTIES OF 5-CHLORO-2,4-DISULPHAMYLTOLUENE "DISAMIDE" AN ORALLY ACTIVE DIURETIC AGENT

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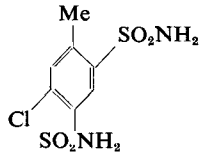
5-Chloro-2,4-disulphamyl toluene, "Disamide", is a non toxic orally active diuretic agent which has been compared with acetazolamide and chlorothiazide. Disamide at half the dose of chlorothiazide caused a greater diuresis and Na^+ excretion but had a slightly less Cl^- excretion. The carbonic anhydrase inhibitory activity was 0·4 times that of acetazolamide but unlike the latter Disamide still caused diuresis after prolonged administration. Disamide and chlorothiazide were found to have no effect on blood pressure or respiration. The anti-convulsant activity in mice is similar to acetazolamide. The new diuretic agent has no antibacterial, hypoglycaemic or analgesic properties.

5-CHLORO-2,4-disulphamyltoluene was synthetised in our Chemical Research Laboratories. It is a non-hygroscopic white crystalline powder with a molecular weight of 284·6. It is odourless and practically tasteless, melting at about 260°. It is insoluble in water and dilute hydrochloric acid, slightly soluble in ethanol and soluble in cold sodium hydroxide solution.

It has the structural formula (I).



(I)

METHODS

Acute Oral Toxicity

Male albino mice weighing approximately 20 g. each were fasted overnight. Three groups of 20 animals were given a suspension of Disamide by stomach tube in an aqueous suspending medium[1]. The volumes were adjusted to 0·5 ml./20 g. body weight. The mice were kept for 7 days.

A group of ten male albino rats was given a single large dose, by stomach tube, of Disamide suspended in 2·5 ml. of the aqueous suspending medium per 100 g. body weight, a second group of 10 rats was given the vehicle alone. They were carefully observed for 8 hours following administration and on the following day. The rats were killed on the seventh day.

Chronic Oral Toxicity

Sixty immature female albino rats weighing between 74 and 112 g. were divided into four groups of 15 animals with mean weights not differing by more than 3·4 g. Three groups were given 75, 150 and 300 mg./kg. respectively of Disamide in 1 ml. of aqueous suspending medium per 100 g. and the fourth group was given the vehicle alone. The animals were dosed daily by stomach tube 5 days a week for 13 weeks. Weekly weights were recorded and the doses adjusted accordingly. The rats were kept under identical conditions and given water and diet 41 *ad libitum*, except when the diuretic responses were being investigated. Haematological studies were made on the 300 mg./kg. and control groups at the end of the fourth and tenth weeks and on all groups at the end of the thirteenth week. Twenty-four hours after the last dose 5 rats from each group were killed and sections of lung, liver, kidney, spleen, stomach, heart, thyroid, pituitary, adrenal and ovary were prepared for histological examination. The remaining animals were kept under observation for a further 9 weeks.

Urine, Na^+, K^+ and Cl^- Excretion

Oral diuretic activity was investigated in mature male albino rats using a modification of Lipschitz, Hadidian and Kerpezar's[2] method. Groups of 8, 10 or 20 rats were deprived of food and water overnight. The following morning a 0·9 per cent saline load, 25 ml./kg., was given by stomach tube together with varying amounts of Disamide, chlorothiazide or acetazolamide suspended in 5 per cent gum acacia. The control animals were given the saline and 5 per cent gum acacia only. Each group was placed in wire meshed cages over a large funnel and the urine collected for 5 or 8 hours following administration. Urinary sodium and potassium were estimated with a flame photometer and the chloride content by King and Wooton's[3] method. At 4, 9 and 13 weeks the diuretic responses of the rats in the chronic toxicity experiment were determined. The usual daily dose was administered in a saline load of 25 ml./kg. and the urine collected. A similar procedure was followed for acetazolamide except that the diuretic responses were recorded initially, after the first dose, and at 3, 5, 8 and 13 weeks. The mean urinary excretion was expressed as ml. or m-equiv./kg. for 5 or 8 hours and the diuretic activities were expressed relative to the controls taken as unity.

Carbonic Anhydrase Inhibition

The carbonic anhydrase inhibitory activity was estimated by a method adapted from Roughton and Booth[4].

Anticonvulsant Properties

The anticonvulsant activities of Disamide and acetazolamide were determined against electroshock and leptazol-induced convulsions in unstarved male albino mice.

Varying doses of Disamide or acetazolamide in aqueous suspending medium, 0·5 ml./20 g., were given by stomach tube to groups of 20 mice.

Two hours later the mice were challenged either by a current of 15 mA applied for 0·2 seconds through ear electrodes or by an intravenous injection of 50 mg./kg. of leptazol. The absence of the hindleg tonic extensor component of the convulsion was regarded as evidence of protection. The ED50 values and confidence limits were calculated by the method of Litchfield and Wilcoxon[5].

Effect on Blood Sugar

This was investigated in three groups of four rabbits fasted overnight. Initial and hourly blood values for 5 hours following the administration of 50, 100 and 200 mg./kg. were estimated by Somogyi's[6] modification of Nelson's method.

Effect on Blood Pressure and Respiration

The carotid blood pressures of urethane anaesthetised male albino rats were recorded following the intraperitoneal injection of Disamide or chlorothiazide. The blood pressure and respiratory responses to Disamide given by stomach tube was investigated in three urethane-chloralosed cats.

Hypnotic Properties

The possible hypnotic properties of Disamide and chlorothiazide were investigated by observing their effects on sodium pentobarbitone treated mice. Chlorpromazine was used as the reference compound. Disamide and chlorothiazide were given in aqueous suspending medium and sodium pentobarbitone and chlorpromazine in water. The control mice were given the aqueous suspending medium only. All volumes were adjusted to 0·5 ml./20 g. body weight. Groups of 8 or 10 mice were given 25 or 50 mg./kg. of sodium pentobarbitone intraperitoneally 30 minutes after the administration by stomach tube of Disamide or chlorothiazide, 10, 50 or 250 mg./kg., chlorpromazine, 5 mg./kg. or aqueous suspending medium.

Analgesic Properties

The mouse tail pinch method of Bianchi and Franceschini[7] was used. Groups of ten mice fasted overnight were given 10, 40, 160 or 640 mg./kg. of Disamide and two other groups 40 and 80 mg./kg. of pethidine hydrochloride. The compounds were given by stomach tube in aqueous suspending medium, the volumes being adjusted to 0·5 ml./20 g. At 30, 60, 120 and 240 minutes the rubber covered bull dog clip was applied to the base of the tail for 30 seconds, and if no attempt was made to remove the clip a positive analgesic response was recorded.

Antibacterial Properties

The antibacterial activity was investigated using a double dilution tube assay against the following organisms which had been incubated for 24 hours in double strength Lemco broth. *Pseudomonas aeruginosa* (*Pseudomonas pyocyanea*) NCTC 8058, *Streptococcus faecalis* NCTC 775,

Proteus vulgaris NCTC 401, *Staphylococcus lactis* NCTC 189, *Escherichia coli* NCTC 86.

A solution of the disodium salt of Disamide was made by dissolving 0·71 g. in distilled water containing 0·165 g. NaOH and making up to a final volume of 100 ml. to give a 0·825 per cent clear solution. Serial dilutions were made and the 24 hours growth determined after incubation at 37°.

RESULTS

Acute Oral Toxicity

No deaths occurred in mice given 12 g./kg. In rats 9 g./kg. caused no deaths or neuromuscular disturbances, but there was an impression that the treated rats were slightly lethargic compared with the controls.

Chronic Oral Toxicity

There was no significant difference, by Student's *t* test, between the weight increases of the treated and control groups. Red, white and differential counts and haemoglobin values of treated and controls were similar. Histological examination of lung, liver, kidney, spleen, stomach, heart, thyroid, pituitary, adrenal and ovary, by Professor T. Crawford, did not reveal any abnormality.

Urine, Na^+, K^+ and Cl^- Excretion

The diuretic responses in groups of twenty rats to oral administration of three doses of Disamide and chlorothiazide were investigated and the results recorded in Table I. The increased excretion of urine over the

TABLE I

THE MEAN ORAL DIURETIC RESPONSE AND RELATIVE ACTIVITIES OF DISAMIDE AND CHLOROTHIAZIDE, THE Na^+, K^+, AND Cl^- CONTENT AND IMBALANCE IN SALINE LOADED RATS

Compound	Dose mg./kg.	Urine excreted ml./kg./8 hrs.	Relative Activity	m-equiv./kg. 8 hrs. Na^+	K^+	Cl^-	Imbalance $Na^+ + K^+$ minus Cl^-
Disamide	7·5	9·6	2·4	2·0	1·0	1·9	1·1
	15	13·3	3·3	2·9	1·1	2·3	1·7
	30	19·0	4·7	4·1	1·3	3·2	2·2
Chlorothiazide ..	15	9·4	2·3	1·9	0·9	2·6	0·2
	30	12·2	3·0	2·6	1·0	3·3	0·3
	60	14·5	3·6	3·0	1·1	3·7	0·4
Controls	—	4·1	1·0	0·7	0·6	1·1	0·2

control value for both compounds was linear to log dose but not parallel, (Fig. 1). The doses required to give an increase of 6 ml./kg./8 hours are 8·3 mg./kg. for Disamide and 17·5 mg./kg. for chlorothiazide. The greater activity of Disamide was confirmed in a number of experiments which were combined to give mean responses for 600 rats; Table II records the results. Disamide 10 mg./kg. caused a slightly greater diuresis and Na^+ excretion than 20 mg./kg. of chlorothiazide, but the Cl^- excretion was

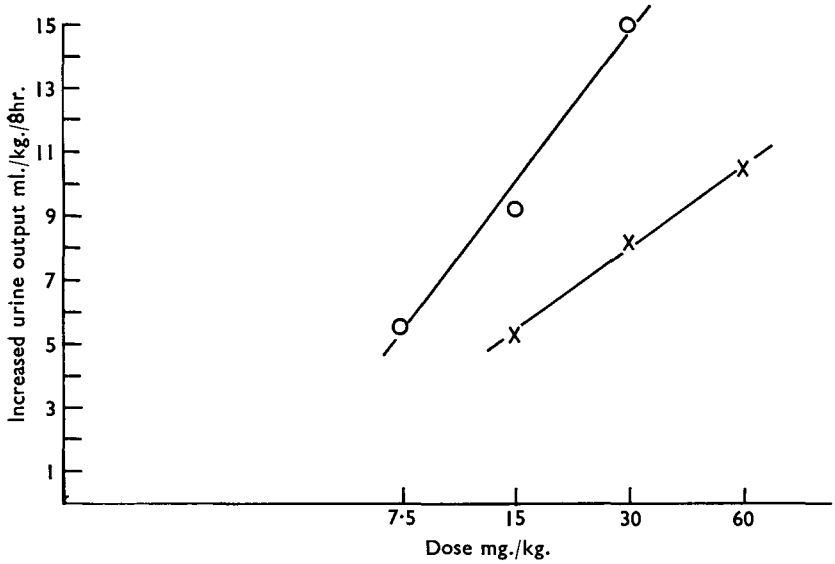


FIG. 1. The log dose/response for Disamide (O——O) and chlorothiazide (×——×), twenty rats per group.

TABLE II

THE MEAN ORAL DIURETIC RESPONSE AND RELATIVE ACTIVITIES OF DISAMIDE AND CHLOROTHIAZIDE, THE Na^+, K^+ AND Cl^- CONTENT AND IMBALANCE IN SALINE LOADED RATS

Compound	Dose mg./kg.	No. of rats	Urine excreted ml./kg./5 hrs.	Relative Activity	m-eqiv./kg./5 hrs. Na^+	K^+	Cl^-	Imbalance $Na^+ + K^+$ minus Cl^-
Disamide	10	600	12·0	3·2	2·6	1·0	2·1	1·5
Chlorothiazide	20	600	10·3	2·8	2·2	0·8	2·9	0·1
Controls	—	600	3·7	1·0	0·7	0·5	1·0	0·2

TABLE III

THE MEAN ORAL DIURETIC RESPONSE AND RELATIVE ACTIVITIES OF DISAMIDE, CHLOROTHIAZIDE AND ACETAZOLAMIDE, THE Na^+, K^+ AND Cl^- CONTENT AND IMBALANCE IN SALINE LOADED RATS

Compound	Dose mg./kg.	Urine Excreted ml./kg./5 hrs.	Relative Activity	m-equiv./kg./5 hrs. Na^+	K^+	Cl^-	Imbalance $Na^+ + K^+$ minus Cl^-
Disamide	1	3·6	0·8	1·1	0·5	1·3	0·3
	3	6·0	1·4	1·5	0·8	1·5	0·8
	9	12·0	2·8	3·4	1·0	2·4	2·0
Chlorothiazide ..	9	7·1	1·7	1·7	0·8	2·4	0·1
	27	8·9	2·1	2·5	0·8	3·0	0·3
	81	12·4	2·9	3·7	1·2	4·2	0·7
Acetazolamide ..	1	6·6	1·6	1·6	0·5	1·1	1·0
	3	7·0	1·6	2·0	0·8	1·2	1·6
	9	13·3	3·1	3·1	1·0	1·5	2·6
Controls	—	4·3	1·0	0·9	0·7	1·5	0·1

slightly less. Tables I and II and Figure 2 show that the diuretic activities are accompanied by similar well marked increases in Na^+ and smaller but similar excretions in K^+. The Cl^- excretion for Disamide is lower and

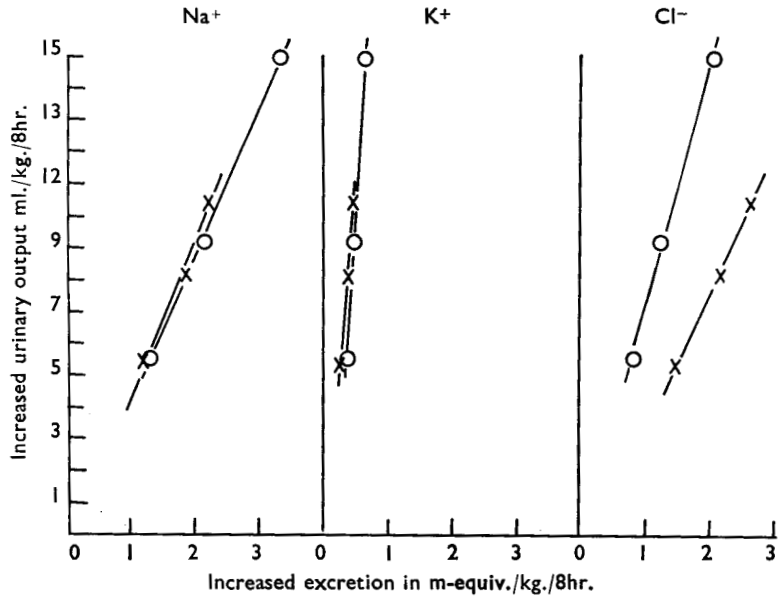


FIG. 2. The increase in Na^+, K^+ and Cl^- excretion with diuresis to Disamide (○——○) and chlorothiazide (×——×), twenty rats per group.

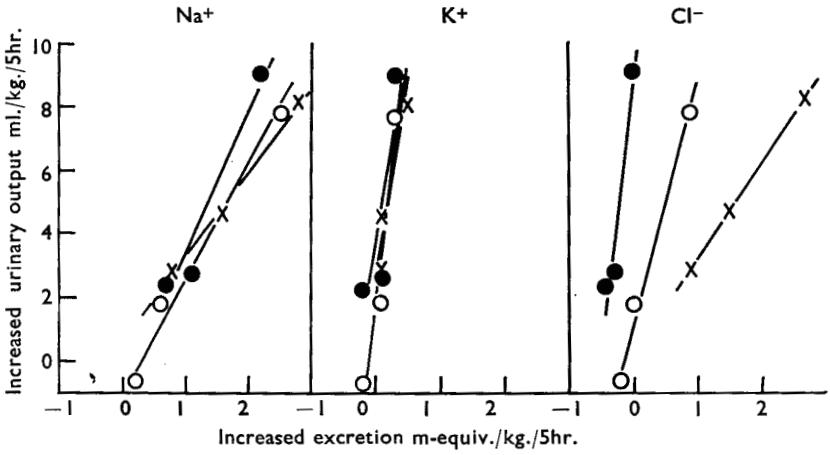


FIG. 3. The increase in Na^+, K^+ and Cl^- excretion with diuresis to Disamide (○——○), chlorothiazide (×——×) and acetazolamide (●——●), eight rats per group.

consequently the imbalance between Na^+ plus K^+ and Cl^- is higher. This can be explained by a greater inhibition of carbonic anhydrase by

Disamide resulting in a greater excretion of bicarbonate. As this enzyme-inhibiting activity of Disamide is lower than that of acetazolamide the diuretic activity of Disamide was directly compared with chlorothiazide and acetazolamide, Table III and Figure 3. This experiment again confirmed the greater diuretic activity of Disamide compared with chlorothiazide and showed that it has about the same activity as acetazolamide. Acetazolamide had no effect on Cl^- excretion and consequently the electrolyte imbalance with acetazolamide was considerably higher than Disamide.

The rapid development of tolerance to acetazolamide is due to its carbonic anhydrase inhibitory properties[8]. As Disamide has some carbonic anhydrase inhibitory activity the diuretic responses in the chronic toxicity experiment were investigated and compared with the responses to repeated daily doses of acetazolamide. Table IV records the results.

TABLE IV

THE MEAN INCREASED URINARY EXCRETION IN RATS AT INTERVALS FOLLOWING REPEATED ORAL ADMINISTRATION OF DISAMIDE AND ACETAZOLAMIDE

Time in weeks	Dose mg./kg.	Urine ml./kg./5 hrs.	
		Disamide	Acetazolamide
Initial	75	—	16·3
"	150	—	20·6
"	300	—	23·0
3	75	—	3·9
"	150	—	2·0
"	300	—	0·8
4	75	9·9	—
"	150	9·6	—
"	300	9·1	—
5	75	—	5·8
"	150	—	5·0
"	300	—	4·5
8	75	—	5·6
"	150	—	4·6
"	300	—	5·0
9	75	9·5	—
"	150	11·6	—
"	300	7·5	—
13	75	9·7	3·6
"	150	11·4	3·8
"	300	11·6	5·0

Acetazolamide showed a marked diminution in diuretic activity following 3 weeks daily treatment with 75, 150 and 300 mg./kg. and this persisted throughout the 13 weeks observation period. In contrast the diuretic activity of Disamide was considerably greater.

Carbonic Anhydrase Inhibition

The relative *in vitro* activities of Disamide and chlorothiazide taking acetazolamide as unity were: Disamide 0·40; chlorothiazide 0·009.

Anticonvulsant Properties

Disamide has similar anticonvulsant properties to acetazolamide against leptazol and electroshock induced convulsions. Table V records the results.

Effect on Blood Sugar

Disamide has no hypo- or hyperglycaemic effect on rabbits after the administration of 200 mg./kg. by stomach tube.

TABLE V

THE NUMBER OF MICE PROTECTED AGAINST ELECTROSHOCK AND LEPTAZOL INDUCED CONVULSIONS TWO HOURS AFTER ORAL ADMINISTRATION OF DISAMIDE AND ACETAZOLAMIDE

Challenge	Dose mg./kg.	Number protected		ED 50 mg./kg. (95 per cent confidence limits)	
		Disamide	Acetazolamide	Disamide	Acetazolamide
Leptazol	75	7/20	5/20	174 (93–325)	143 (100–204)
	150	8/20	11/20		
	300	13/20	15/20		
Controls	—	0/20	0/20	—	—
Electro-Shock	5	—	0/20	38·5 (27·9–53·1)	29·2 (20·0–42·6)
	10	1/20	4/20		
	20	3/20	4/20		
	40	10/20	14/20		
	80	17/20	17/20		
	160	20/20	20/20		
Controls	—	0/20	0/20	—	—

Effect on Blood Pressure and Respiration

Disamide and chlorothiazide 500 mg./kg. intraperitoneally each had no effect on the carotid blood pressure of male rats anaesthetised with urethane.

The carotid blood pressure and respiration of three urethane-chloralosed cats was unaffected after the administration by stomach tube of 200, 400 and 1000 mg./kg. respectively of Disamide given in divided doses.

Hypnotic Properties

Disamide or chlorothiazide, 250 mg./kg., had no effect on mice given 25 mg. or 50 mg./kg. of sodium pentobarbitone intraperitoneally, whereas chlorpromazine 5 mg./kg. had a potentiating effect on the loss of righting reflex and sleeping time.

Analgesic Properties

Using the tail pinch technique in mice 640 mg./kg. of Disamide orally was inactive up to 4 hours after administration. Pethidine hydrochloride used as the standard gave satisfactory analgesic responses at 40 mg./kg.

Antibacterial Properties

Disamide has only slight antibacterial properties. There was some inhibition of bacterial growth at a dilution of 1 in 250, but at the other dilutions varying from 1 in 500 to 1 in 250,000 a profuse growth occurred in 24 hours incubation at 37° for all the organisms tested.

Discussion

Disamide is more than twice as active as chlorothiazide and has approximately the same activity as acetazolamide as a diuretic agent.

It has been shown that the diuretic activities of acetazolamide, Disamide and chlorothiazide are not parallel to their *in vitro* carbonic anhydrase inhibitory activities. Acetazolamide-like compounds exert their diuretic activity by inhibiting the carbonic anhydrase of the cells of the renal tubules thus preventing the re-absorption of bicarbonate from the glomerular filtrate causing an osmotic diuresis with a high urinary bicarbonate content[9]. Although chlorothiazide possesses some carbonic anhydrase inhibitory properties these play a minor role in its diuretic action which is characterised by a low bicarbonate and a high Cl^- excretion compared with acetazolamide; the Na^+ excreted is approximately equimolar to the Cl^-.[10,11]

In our experiments the Cl^- excretion after chlorothiazide administration is approximately equivalent to the combined Na^+ and K^+ output. This is not so with acetazolamide the Cl^- excretion being similar to the controls at all dose levels and the high electrolyte imbalance is due to the bicarbonate content. Disamide, like chlorothiazide, shows an increased Cl^- excretion with increased dosage and in the combined experiment the mean Cl^- excretion of 600 rats after 10 mg./kg. of Disamide is 2·1 times that of the controls. The electrolyte imbalance of Disamide is considerably less than acetazolamide and can be explained by its chloruretic and lower carbonic anhydrase inhibitory activities. Further evidence to support this dual action is seen in the diuretic responses following prolonged administration where it is reasonable to assume that if the diuretic activity of Disamide is due entirely to its enzyme-inhibiting properties a similar reduction in diuretic activity as observed with acetazolamide would have occurred.

The possible dual mechanism of Disamide distinguishes it from other known diuretics and may be of considerable clinical importance particularly in refractory cases.

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