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BTS 39 542, A DIHYDROPHTHALAZIN-1-YLACETIC ACID WITH HIGH EFFICACY DIURETIC ACTIVITY

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1 BTS 39 542, a novel dihydrophthalazin-1-ylacetic acid, has high efficacy diuretic activity in mice, rats, rabbits and dogs. It is twice as potent as frusemide in mice and dogs, ten times as potent in rats and twenty times as potent in rabbits.

2 BTS 39 542, like frusemide, exerts its major effects in the loop of Henle and increases renal blood flow but does not affect glomerular filtration rate in dogs.

3 The ratio of the excretion of the major cations (sodium plus potassium) to that of the major anion (chloride) after either BTS 39 542 or frusemide varied with species. In rats and rabbits the ratio was approximately unity but in mice and dogs the ratio consistently exceeded unity.

4 A method for evaluating diuretics based on potency and relative potassium excretion is described.

Introduction

Orally active highly efficacious diuretics which cause marked excretion of water and electrolytes were first described in the 1960s. The most widely used agent, frusemide (Muschawek & Hajdu, 1964), is a sulphonamide and subsequently related compounds with similar activity such as bumetanide (Ostergaard, Magnussen, Nielsen, Eilertsen & Frey, 1972) and piretanide (Merkel, Bormann, Mania, Muschawek & Hropot, 1976) have been described. Non-sulphonamide high efficacy diuretics have also been described. Ethacrynic acid, a phenoxyacetic acid derivative (Bayer, Baer, Michaelson & Russo, 1965) was developed at the same time as frusemide and recently the activity of the pyrazolinone derivative, muzolimine (Moller, Horstmann, Meng & Loew, 1977) was reported. This paper reports the activity of one of a series of dihydrophthalazin-1-ylacetic acids described by Bristow, Macey, Nichol & Sim (1977). This compound 2-(1,2,3-benzothiadiazol-5-yl)-7-chloro-4-hydroxy-1,2-dihydrophthalazin-1-ylacetic acid (BTS 39 542, Figure 1) is structurally unrelated to any known high efficacy diuretic.

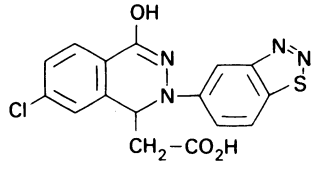

Figure 1 Structure of BTS 39 542.

Methods

Estimation of potency and potassium excretion

Diuretic potency is expressed as the dose required to cause excretion of 5 mmol/kg Na^+ and the effect on potassium excretion was assessed by estimating the potassium excretion at this level. Dose-response curves for both sodium and potassium excretion were established using linear regression analysis and the dose of diuretic causing 5 mmol/kg Na^+ excretion and the potassium excretion at this dose were calculated. The estimated potassium excretions obtained for the test compound and the standard were compared by Student's *t* test.

Diuretic studies in conscious rodents

Male Hough Porton mice (20 to 30 g) or female Boots Wistar rats (80 to 100 g) were fasted overnight with access to water in groups of 35 and 10 respectively. The next morning the animals were distributed randomly into groups of 5 and dosed orally with a suspension of the compound in 30 ml/kg 0.25% Cellosize in distilled water. Each group of animals was placed in a metabolism cage and urine collected for 3 h. The technique for mice was described in detail by Sim & Hopcroft (1976) and rat studies were similar but larger metabolism cages and collecting vessels were used.

For potency estimation studies in mice,

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BTS 39 542 and frusemide were given at dose levels of 3, 10, 30 and 100 mg/kg with 8 groups of 5 mice at each dose level. In rats, BTS 39 542 was given at doses of 0.3, 1, 3 and 10 mg/kg, frusemide at 10, 30 and 100 mg/kg in 5 groups of 5 rats at each dose level.

Diuretic studies in conscious rabbits

Female New Zealand white rabbits (2 to 3 kg) were fasted overnight with free access to water. Next day they were placed in plastic rabbit holders and their bladders catheterized with sterile nelaton catheters (8FG) (Portex Ltd). The bladder was drained and washed with 10 ml sterile distilled water. A plastic gag was placed in the mouth and a Jaques rubber catheter (12FG) (Warne Ltd) was inserted into the stomach. Compounds were administered via the catheter as suspensions in 10 ml/kg 0.25% cellosize in distilled water. Urine was allowed to flow freely into measuring cylinders and collected hourly for 3 h. The bladder was washed with 10 ml sterile distilled water at each collection and washings added to the urine.

For potency estimation in rabbits, dose levels of 3, 10, 30 and 100 mg/kg BTS 39 542 and 10, 30, 100 and 300 mg/kg frusemide were used. At least 4 rabbits were used at each dose level.

Diuretic studies in conscious dogs

Beagle bitches (7.5 to 13 kg) were fasted overnight but allowed access to water. The next day they were placed in a quiet room where they stood supported by harnesses suspended from frames for the duration of the test. Their bladders were catheterized with sterile nelaton catheters (8FG) (Portex Ltd) fitted with short lengths of rubber tubing. The rubber tubing was closed with a clip and anchored to the harness to ensure retention of the catheter during the experiment. The bladders were drained and washed with 20 ml sterile distilled water. Compounds were given orally in gelatine capsules and urine collected hourly for 4 h. The bladder was rinsed with 20 ml sterile distilled water after each collection and the washings added to the urine.

For potency estimation studies, dose levels of 3, 10 and 30 mg/kg of each compound were given to 6 dogs in 6 × 6 cross over experiment. Tests were performed at weekly intervals.

Clearance studies in conscious dogs

Male Beagle dogs (17 to 18 kg) were placed in supporting harness and allowed to stand quietly for the duration of the experiment. The bladder and left and right cephalic veins of each dog were catheterized at the start of each experiment.

Creatinine and p-aminohippurate (PAH) clearance

The dogs were fasted overnight with access to water. On the next day loading doses of creatinine (50 mg/kg) and PAH (5 mg/kg) were each given intravenously in 1 ml/kg sterile water and an intravenous infusion of 75 mmol/l sodium chloride solution containing 1750 mg/l creatinine and 600 mg/l PAH was given at 1.7 ml/min throughout the test to maintain constant blood levels. Clearance studies were started after a 40 to 60 min equilibration period when urine flow had stabilized. Urine was collected for 10 min periods and a blood sample was taken at the mid-point of each period. After three control periods the diuretic was given intravenously and clearance measurements continued for 1 h. Creatinine and PAH concentrations in urine and plasma were measured by the techniques of Raabo & Walloe-Hansen (1972) and Brun (1951) respectively.

Table 1 The effects of BTS 39 542 and frusemide on electrolyte excretion

Species	n	*Compound*	*Oral dose* (mg/kg)	Na^+ (mmol/kg)	K^+ (mmol/kg)	Cl^- (mmol/kg)	$\frac{Na^+ + K^+}{Cl^-}$
Mouse	8*	BTS 39 542	3	4.25 ± 0.44	1.09 ± 0.07	4.53 ± 0.49	1.18
	8*	Frusemide	10	5.49 ± 0.30	1.59 ± 0.07	6.12 ± 0.31	1.16
	8*	Control	—	0.68 ± 0.13	0.55 ± 0.10	0.59 ± 0.13	2.08
Rat	5*	BTS 39 542	3	4.63 ± 0.23	1.21 ± 0.05	6.08 ± 0.33	0.96
	5*	Frusemide	30	4.02 ± 0.20	1.19 ± 0.04	5.37 ± 0.22	0.97
	5*	Control	—	0.27 ± 0.03	0.51 ± 0.07	0.54 ± 0.07	1.44
Rabbit	6**	BTS 39 542	10	5.67 ± 0.18	0.74 ± 0.06	6.33 ± 0.19	1.01
	4**	Frusemide	100	5.03 ± 0.35	0.68 ± 0.05	5.73 ± 0.47	1.00
	4**	Control	—	0.43 ± 0.04	0.35 ± 0.05	0.47 ± 0.06	1.66
Dog	6**	BTS 39 542	10	4.84 ± 0.57	1.14 ± 0.15	5.34 ± 0.56	1.12
	6**	Frusemide	30	4.95 ± 0.60	1.18 ± 0.10	5.61 ± 0.55	1.09
	6**	Control	—	0.52 ± 0.25	0.21 ± 0.05	0.42 ± 0.19	1.74

Values shown are means ± s.e.mean. *Number of groups of 5 animals. **Number of animals.

Osmolal clearance The dogs were deprived of food and water overnight and 2 h before being placed in harness were given an intramuscular injection of 6 u vasopressin BP. At the start of the test, a loading dose of 50 mg/kg creatinine was given intravenously and an intravenous infusion of 10 ml/min of sodium chloride solution, 150 mmol/l, containing 2.5 u/l vasopressin BP and 300 mg/l creatinine was begun. After a 50 min equilibration period, clearance studies were performed as described above. Urine and plasma osmolalities were determined cryoscopically by means of an Advanced Instruments osmometer.

Renal blood flow in the conscious dog

An electromagnetic flow-probe (Biotronix) was placed on the left renal artery of a Beagle dog (14 kg) under halothane anaesthesia using aseptic technique. The lead from the probe was brought subcutaneously to a button sutured on to the skin at the back of the neck. Three weeks after surgery experiments were started. The dog stood quietly supported by harness during the test and the probe was connected to an SE flowmeter. Mean and pulsatile blood flow were recorded on a 2 channel Devices recorder. Drugs were given intravenously via a catheter inserted into a cephalic vein at the start of each test.

Urine electrolyte determinations

Urine electrolyte concentrations were determined with an autoanalyser, sodium and potassium by flame photometry (Technicon method SE4-007 FH4) and chloride colourimetrically (Technicon method N-5b). Excretion was expressed as mmol/kg bodyweight.

Drugs and materials

The drugs used were BTS 39 542 (Boots Co. Ltd), frusemide BP (Hoechst Pharmaceuticals Ltd) and vasopressin BP (Parke Davies Ltd). Cellosize (hydroxyethylcellulose, QP 15,000) was obtained from Union Carbide Ltd, creatinine from Fisons Ltd and *p*-aminohippuric acid from B.D.H. Ltd.

Results

BTS 39 542, like frusemide, was a highly efficacious oral diuretic in all four species tested, causing comparable marked increases in urine volume and electrolyte excretion but having little effect on urinary pH. Effects on sodium excretion, the most important aspect of diuretic activity, illustrate the marked efficacy of both compounds. Control sodium excretion in all four species tested was approximately 0.3 to 0.7 mmol/kg (Table 1) and this was increased by both compounds to maximal values of 9 mmol/kg in mice, 6 mmol/kg in rats and rabbits and 5 mmol/kg in dogs.

The time courses of action of the two agents were approximately the same. In mice, rats and rabbits the peak effect occurred in the first hour and the duration was 3 h and in dogs the peak effect occurred in the second hour and the duration was 4 h. These differences may be due to the different methods of dosing, since the diuretic was given in a water load to the first 3 species.

BTS 39 542 was more potent than frusemide in all four species. The oral potency of BTS 39 542 was approximately twice that of frusemide in mice and dogs, ten times in rats and twenty times in rabbits (Table 2). In addition, in both rats and mice BTS 39 542 caused significantly less potassium excretion than frusemide (Table 2). In dogs and rabbits there were no differences in the kaliuretic effects of BTS 39 542 and frusemide but we observed that, at 5 mmol/kg sodium excretion, potassium excretion in rabbits was much lower than in the other three species.

Table 2 Calculated values for diuretic potency and relative potassium excretion after BTS 39 542 and frusemide

Species	*Compound*	*Oral dose (mg/kg) causing 5 mmol/kg Na^+ excretion*	*K^+ excretion (mmol/kg) at 5 mmol/kg Na^+ excretion*
Mouse	BTS 39 542	4.6	1.22***
	Frusemide	9.5	1.49
Rat	BTS 39 542	5.2	1.26***
	Frusemide	55.2	1.43
Rabbit	BTS 39 542	5.6	0.73
	Frusemide	97.3	0.69
Dog	BTS 39 542	13.8	1.19
	Frusemide	30.1	1.19

***$P<0.001$ for differences between potassium excretions at 5 mmol/kg Na^+ excretion after BTS 39 542 and frusemide.

There were species differences in the ratios of sodium plus potassium excretion to chloride excretion after either diuretic at all dose levels and sodium excretion levels. In rats and rabbits the ratio was approximately unity but in mice and dogs the ratio was consistently greater than unity. Representative examples at sodium excretion levels of 4 to 5 mmol/kg are shown in Table 1.

BTS 39 542 and frusemide did not markedly affect creatinine clearance indicating that these agents do not affect glomerular filtration rate. However, both compounds increased PAH clearance during the first 20 min after dose indicating that they both increase renal blood flow. Figure 2 shows a typical response and this was confirmed in another dog with an indwelling flow probe (Figure 3).

In hydropenic dogs, free water re-absorption decreased with increasing diuretic-induced osmolal clearance after either BTS 39 542 or frusemide (Table 3). This indicates an action in the loop of Henle.

Discussion

In most studies of the diuretic action of compounds in conscious animals no more than two species have been examined simultaneously. The dog and the rat are the most commonly used species although there are studies in mouse (Sim & Hopcroft, 1976) and rabbit (Fukuchi, Katayama, Kakemi, Ueda & Koizumi, 1977). There is evidence that some diuretics, e.g. ethacrynic acid (Bayer *et al.*, 1965) quincarbate (van Dijk, Hartog & Boschman, 1976) are species specific and it seemed appropriate to study BTS 39 542 in a wide range of species.

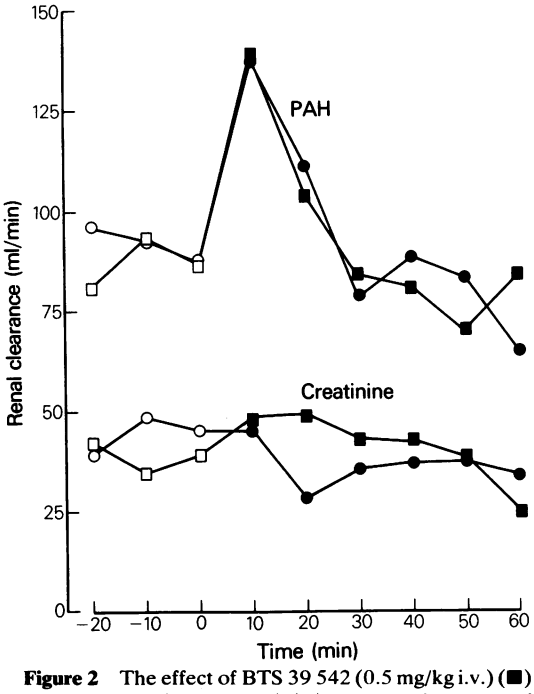

Figure 2 The effect of BTS 39 542 (0.5 mg/kg i.v.) (■) and frusemide (1 mg/kg i.v.) (●) on renal clearances of *p*-aminohippurate (PAH) and creatinine in the same conscious dog. Open symbols represent values before the diuretic and closed symbols those after the diuretic.

Our results show that BTS 39 542, a dihydrophthalazin-1-ylacetic acid structurally unrelated to other known diuretics, is an orally active diuretic in all four species tested and represents a novel chemical class for this type of activity. The high efficacy and similar time course of action suggest that BTS 39 542 has similar diuretic properties to frusemide. This was confirmed by osmolal clearance studies in dogs which indicated that both compounds exert their major effect in the loop of Henle. Moreover both compounds increased renal blood flow but did not affect glomerular filtration rate.

To facilitate quantitative comparison we devised a method of expressing diuretic potency by relating it to the dose causing an arbitrary sodium excretion value. For the purpose of the present study we chose a value of 5 mmol/kg sodium excretion, since, under our test conditions this level was near maximal for rats, rabbits and dogs and represented a marked diuretic effect in mice. We also estimated the potassium excretion at this sodium excretion level since marked kaliuresis is a disadvantage of diuretics such as frusemide and appears to be related to the level of sodium excretion. In practice we have found this technique extremely useful for quantifying activity during a diuretic screening programme since it can also be applied to moderate efficacy diuretics such as a bendrofluazide by setting appropriate sodium excretion levels.

BTS 39 542 was more potent than frusemide in all the species tested. It exhibited less interspecies variation in potency since it caused a marked diuretic response in each species after 5 mg/kg orally. In contrast, we found that the potency of frusemide varied considerably from species to species, being greater in mice than in the other three species. Similar interspecies variation in potency has been described for bumetanide which is highly potent in dogs but has much less activity in rats (Ostergaard *et al.*, 1972).

BTS 39 542 caused a significantly lower potassium excretion than frusemide in rodents, indicating that it may have a slightly different effect from frusemide on the sodium potassium exchange mechanisms in these species. However, the absence of this difference in other species tested suggests that it is only of minor importance.

In our studies we also observed that after either

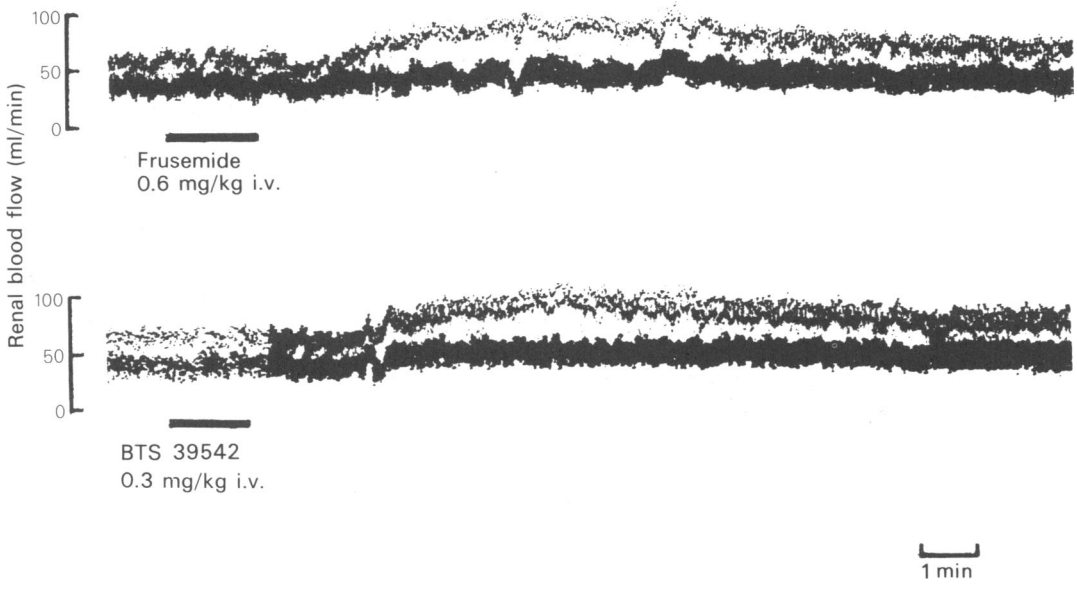

Figure 3 The effect of BTS 39 542 and frusemide on pulsatile renal blood flow in the same conscious dog.

frusemide or BTS 39 542 the ratio of the excretion of the major cations (sodium plus potassium) to that of the major anion (chloride) varied with species. We could distinguish two groups, rats and rabbits where the amounts excreted were approximately equal and mice and dogs where sodium plus potassium excretion was consistently greater than chloride. Chloride is actively transported in the loop of Henle with cations following and high efficacy diuretics inhibit active chloride transport (Burg & Stoner, 1976). The difference between cation and chloride excretion in mice and dogs must be balanced by excretion of another anion. This indicates that in these two species both BTS 39 542 and frusemide have some inhibitory effect at another site in the nephron.

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Table 3 Effects of BTS 39 542 and frusemide on free water reabsorption (T^cH_2O) and osmolal clearance (Cosm) in conscious hydropenic dogs

Time period	*T^cH_2O* (ml/min)	*Cosm* (ml/min)	*T^cH_2O* (ml/min)	*Cosm* (ml/min)
−20 min to −10 min	4.6	7.8	4.4	7.9
−10 min to 0	5.2	9.7	3.9	6.8
	BTS 39 542 1 mg/kg (i.v.) + 1 mg kg^{-1} h^{-1}		*Frusemide 2 mg/kg (i.v.) + 2 mg kg^{-1} h^{-1}*	
0 to 10 min	3.0	26.5	2.6	31.5
10 min to 20 min	−0.1	34.5	0.2	28.1
20 min to 30 min	0	35.0	0.1	35.4
30 min to 40 min	−0.1	30.1	0.7	32.0
40 min to 50 min	0.5	30.0	0.2	29.5
50 min to 60 min	0.6	26.5	0.9	29.3

Results are the means from two dogs 16 to 17 kg and are corrected to a standard glomerular filtration rate of 100 ml/min.

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