PHARMACOLOGICAL PROPERTIES OF BUTAZOLIDIN AND OF ITS CALCIUM AND SODIUM SALTS

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Since the synthesis of butazolidin amongst a number of pyrazolidines by Stenzl *et al.* (1950) in the laboratories of J. R. Geigy S A., Basle in 1946, the product was used mostly in the form of the sodium salt in the pharmacological investigations carried out in common by Wilhelmi (1949), Domenjoz (1952) and Fleisch and Dolivo (1953). The sodium salt proved particularly suitable for parenteral administration owing to its good solubility in water.

Similar properties were shown also by other butazolidin salts with monovalent metals such as potassium and lithium, whereas those with bivalent metals such as strontium, magnesium and calcium are less soluble in water. Soon after the sodium salt of butazolidin became available, its calcium salt was synthesized and tested pharmacologically. It was imagined that in particular the intensive anti-inflammatory properties of butazolidin could be potentiated by the additional effect of calcium. However, as can be seen from our comparative tests of 8 butazolidin salts this hope was not altogether fulfilled (Wilhelmi, 1958a). On the other hand Adami (1956) had reported that butazolidin calcium showed greater analgesic and anti-inflammatory effect in the animal test but was less toxic than butazolidin. These findings, however, were confirmed by us only on oral administration and even then only partially, viz. with respect to toxicity in the rat and to only one out of the six inflammation tests used. As the abovementioned tests (Wilhelmi, 1958b) with parenteral administration show, retardation of the absorption of butazolidin due to calcium can shift the level of the product in the blood, which in turn might lead to certain changes in the intensity of effects in the animal test. In the new tests it was shown that even with oral administration of butazolidin and its calcium salt certain differences can be observed in the curves representing the course of plasma level of the drug.

The most important results of the tests with butazolidin sodium and butazolidin calcium will be summarized below. A number of illustrative supplements, particularly concerning butazolidin (free acid) and butazolidin calcium will be included. To assess the pharmacological properties of

pyrazoles it is necessary to consider the anti-inflammatory, antipyretic and analgesic effects together with the toxicity. For this purpose various methods were used, in particular several kinds of experimental inflammation. The concentrations of the drug in the blood and in the exudates were measured at various times and the results were correlated to the pharmacological effects.

METHODS

Formalin oedema in the paw of the rat was induced by the method described earlier by Wilhelmi (1952), where 0.1 ml of 0.75 per cent formaldehyde was injected into the dorsum pedis of the rat and the swelling measured 2 hours later by the immersion method. The reduction in swelling was calculated in per cent by comparison with untreated controls. In each test 20 to 40 or more male albino rats weighing 100 to 140 g were used.

Formalin peritonitis in the rat (Wilhelmi, 1958a) was induced by intraperitoneal injection of 1 ml of 1 per cent formalhehyde solution in male albino rats weighing 120 to 140 g. The preparations were injected subcutaneously immediately before the formaldehyde or administered by mouth 1 hour before. The rats were killed 9 hrs after the formaldehyde injection, or in special tests, 4, 8, 16 and 24 hrs after the formaldehyde. In this case each group consisted of an equal number of male and female animals. The ascites was measured and the number of nuclear cells and the protein content of the exudate determined. In the special tests mentioned above butazolidin concentration in the serum and the exudate was also determined (Herrmann, 1959).

Croton oil inflammation was produced in the ear of the mouse. This test was concerned with the influence of the drugs on the croton oil-induced permeability of the ear vessels of the mouse, and at the same time their effect on the normal permeability of the vessels (Wilhelmi, 1949). Trypan blue in doses of 200 mg/kg was injected intraperitoneally and the drugs given subcutaneously, 15 min before and 15 min after, or administered by mouth 45 min before and 15 min after. At the same time as the injections of trypan blue, the right ear of the animal was moistened with croton oil diluted with 2 parts of olive oil. The time of the first appearance of the dyestuff in the inflamed and the normal ear was determined.

Ultra-violet erythema in the guineapig was induced, as previously described (Wilhelmi, 1949, 1950), by irradiation of 4 small areas of skin on the back of each animal from which the hair had been removed. At most 2 hours after the ultra-violet radiation, the erythema had developed to full

intensity in the control animals. The drugs were administered in three (subcutaneous) or two (by mouth) separate doses in some cases before, and in some immediately after the irradiation. To assess the intensity of effect, the median effective dose (ED 50) was determined, i. e., the dose which completely or almost completely suppressed the formation of redness in half of the irradiated areas of skin.

Ultra-violet inflammation on the skin of the rat (tested according to *Schikorr* (1932), modified by Wilhelmi (1952, 1960), was induced by ultra-violet radiation applied to 100 areas of skin in 13 albino rats weighing 150 to 200 g. The number of irradiated areas of skin showing no epithelial necrosis was determined. Slightly formed necrosis were counted as half free of necrosis. Signs of inflammation were looked for after 24 and 48 hrs, but the assessment was based predominantly on the finding after 24 hrs.

Granuloma pouch, a chronic proliferative form of inflammation, was induced by Selye's method (1953a, 1953b) in rats weighing 100 to 120 g by injecting 25 ml of air and 1 ml of 0·5 per cent croton oil in neat's foot oil subcutaneously in the back. The drugs were administered by mouth once a day for 14 days and the animals killed on the 15th day after the inducement of the granuloma, the diameter of the filled pouch measured, the weight of the granuloma and the amount of exudate and its protein content determined. By comparing these values with those of untreated control animals the degree of inhibitory effect of the drugs could be ascertained.

Yeast-fever in albino rats (Domenjoz, 1952) was induced by subcutaneous injection of yeast suspension. At the moment of a definite rise in temperature the drugs were administered by mouth or intraperitoneally. The course of the fever, as affected by the drugs, was followed for 2½ to 5 hrs.

In the analgesia test according to Woolfe and MacDonald (1944), where pain is induced by the application of heat to paws of albino mice the change brought about by drugs in reaction time was determined at various times and given as a percentage. Twenty to 60 animals per dose were tested, male and female albino mice weighing 14 to 18 g being used. The drugs were given orally.

To determine the toxicity, the median lethal dose was ascertained, after intravenous or oral administration of the drugs in mice and rats. These values were determined by interpolation, using the probability lattice. The animals were observed for a week after administration of the drugs.

The gastrotropic effect (Wilhelmi, 1958b) was investigated in fully grown female rats, particular attention being paid to the ulcerogenic effect of high doses of the drugs. The substances were administered in two doses at an interval of 14 hrs. Seven hrs after the second dose the animals were killed and dissected. The number of rats with ulcers and the intensity of the ulceration in each rat were determined. In the individual animal marked ulceration was designated by 3, moderate 2, and slight 1, very slight ½ and none 0. From the sum of the intensity figures of the individual rats divided by the number of animals used an index was calculated for each test group, which accordingly can be at most 3 (i. e. where the ulcerogenic effect is most marked).

RESULTS

For the interpretation of the results reproduced here in tabular form, it should be noted that the doses given parenterally for the most part relate to pure butazolidin. Thus the amounts of sodium and calcium injected with the pyrazolidine preparation have not been taken into account. In new unpublished tests, the effects of free butazolidin and butazolidin calcium given by mouth are compared. The doses represent on the one hand butazolidin pure substance and on the other hand butazolidin and calcium. In this case it seemed expedient to compare the preparations in this way, since both the calcium and the butazolidin have permeability-reducing properties so that in this case similar amounts of anti-inflammatory agent are administered.

TABLE I

Anti-inflammatory effect in formalin inflammation (rats)

		% inhibition compared with untreated controls		
	mg/kg	Butazolidin	Butazolidin sodium	Butazolidin calcium
Formalin	100 i. p.	...	41	39
Oedema	500 oral	34	...	33
Formalin	25 s. c.	...	33	18
Peritonitis	50 s. c.	...	40	27
	75 s. c.	...	53	33
	150 s. c.	...	44	43
	200 oral	66	...	32

When high doses of Butazolidin are administered, such as 500 mg/kg by mouth, some of the animals died during the 24 hr observation period.

TABLE II

Anti-inflammatory effect in ultra-violet inflammation

	Ultra-violet erythema guineapig ED 50 (mg/kg)	Ultra-violet inflammation rat mg/kg % areas free of necrosis	
Butazolidin	9 oral	300 oral	62
Butazolidin Na	7·5 oral 5.25 s. c.	300 s. c.	56
Butazolidin Ca	20 oral 6·75 s. c.	300 s. c.	44

The above-mentioned total amounts of the drugs were divided into 2 or 3 separate doses and administered to the guineapigs either by mouth 1 hr before and immediately after the u. v. irradiation or s. c. 45 and 5 min before and directly after. In the rat the drug was administered by mouth or s. c. 1 hr before and 6 and 22 hrs after the u. v. irradiation.

TABLE III

Croton oil inflammation in the ear of the mouse

Retardation of appearance of trypan blue in the right (inflamed) and the left (normal) ear in min. Mean value from 20 animals

	mg/kg	right ear	left ear
Butazolidin	2× 75 s. c.	5.7 min	19.9 min
Sodium	2×100 oral	5.4 min	16.2 min
Butazolidin	2× 75 s. c.	6.5 min	19.2 min
Calcium	2×100 oral	4.6 min	17.9 min

TABLE IV.

Effect on the granuloma pouch in the rat

	mg/kg per day oral	Weight of granuloma per rat in g	Inhibition %	Amount of exudate per rat in ml	Inhibition %
Control	...	3.74±0.62	...	13.2±2.04	...
Butazolidin	100	2.62±0.51	30	7.3±1.34	45
Butazolidin Ca	100	2.83±024	24	6.0±1.08	55

The difference in effect between the two forms of butazolidin is significant with respect to neither the inhibition of the granuloma growth nor the reduction of exudation. During the tests with both butazolidin and its calcium salt the rats increased in weight similarly to the untreated control animals.

TABLE V

Antipyretic effect

	(Yeast fever, rats). Fall in temperature in °C		
	mg/kg	Total in 5 hr	Maximum
Butazolidin	100 oral	7.7	2.0
Butazolidin Na	100 oral	5.1	1.3
	100 i. p.	6.3	1.6
Butazolidin Ca	100 oral	4.5	1.2
	100 i. p.	6.3	1.7

The total fall in temperature in 5 hrs is a value obtained by planimetry of the temperature curve.

TABLE VI

Analgesic effect

(Hot-plate test, mouse)

	Average (maximal) increase in threshold over 1 hr in %		
	100 mg/kg	200 mg/kg	300 mg/kg
Butazolidin	20(21)	32(35)	38(40)
Butazolidin Na	11(13)	11(12)	22(29)
Butazolidin Ca	12(13)	19(22)	21(24)

The percentage increase in the threshold here denotes the prolongation of the individual reaction time.

Also in the Gross test (1947) butazolidin calcium and butazolidin sodium both showed moderate analgesic effect when injected intraperitoneally. The sodium salt was rather more effective than the calcium salt (Wilhelmi, 1958b). The analgesia test in the rabbit by electrical stimulation of the dental pulp (Gordonoff, 1958) showed for oral administration of butazolidin as a free acid and in the form of the calcium salt a slight analgesic effect. However, owing to the unfavourable absorption in the rabbit after oral administration, the results showed such a high degree of scatter that no dependence of effect on dose could be discerned.

TABLE VII

Acute toxicity

(LD 50 in mg/kg)

	Mouse		Rat	
	i. v.	oral	i. v.	oral
Butazolidin Na	94	935	113	855
Butazolidin Ca	104	600	104	1200
Butazolidin	...	905	...	468
Butazolidin Na +Ca gluconate	113	...	103	...

Calcium gluconate was injected subcutaneously in doses of 100 mg/kg 5 and 1 hrs before butazolidin. In oral administration all the doses mentioned were of pure butazolidin.

TABLE VIII

Ulcerogenic effect on the stomach of the rat

	mg/kg	No. of amimals with ulcers	Index (average)
Butazolidin	2 × 200 oral	10/10	2.2
Butazolidin Na	2 × 100 s. c.	19/20	1.63
	2 × 200 oral	10/10	1.55
Butazolidin Ca	2 × 1000 s. c.	9/10	1.6
	2 × 200 oral	20/20	1.48
Butazolidin Na +Ca gluconate	2 × 100 s. c. / 3 × 100 s. c.	10/10	1.4

Calcium gluconate was injected 38 and 34 hrs and directly before the second dose of butazolidin.

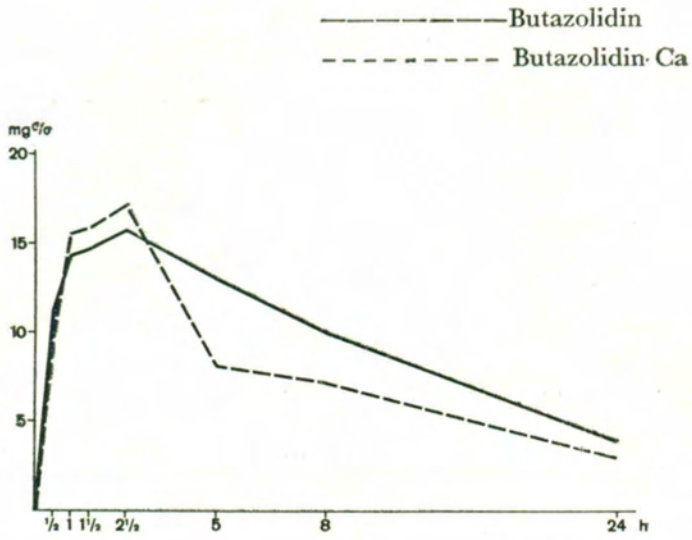

Fig. 1. Serum concentrations of butazolidin Na and butazolidin Ca after doses of 100 mg/kg s. c. in rats in each case

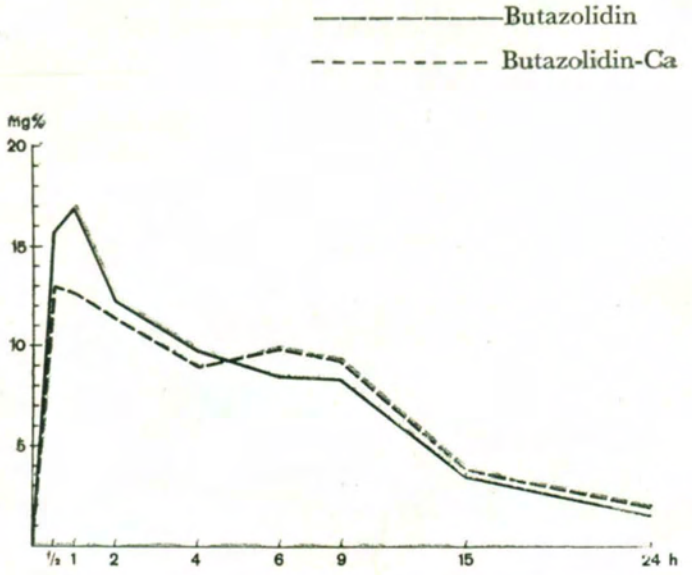

Fig. 2. Serum concentrations of butazolidin and butazolidin Ca after doses of 100 mg/kg by mouth in rats in each case

The absorption of butazolidin sodium and butazolidin calcium after subcutaneous injection shows small but characteristic differences in the blood level curves (Fig. 1). Unfortunately no corresponding curve for free butazolidin can be plotted, since it is only poorly soluble in water and cannot be injected.

In Fig. 2 the difference has been ascertained in the course of the serum level of the drug after oral administration between butazolidin and its calcium salt; the irregular absorption after this mode of administration should be noted.

After oral administration of 100 mg/kg of butazolidin in rats with formalin-induced peritoneal exudate, the drug rapidly reaches high concentrations in the ascites. The rise in concentration and with it also the onset of effect (inhibition of exudation) of the calcium salt proceeds rather more slowly than that of the free acid.

DISCUSSION

The tests show that on the whole the anti-inflammatory effect of butazolidin calcium is not essentially different from that of butazolidin sodium or free acid. In the various kinds of acute inflammation such as the formalin oedema, the croton oil inflammation in the mouse ear and partly also the formalin peritonitis in the rat, the 3 forms of the drug showed anti-inflammatory effects of similar intensity. In formalin peritonitis butazolidin calcium proved to be inferior to the other two preparations in the majority of doses used, as also in the ultra violet inflammation in the guineapig and to a certain extent also in that of the rat.

It seemed interesting to follow the behaviour of butazolidin calcium and the free acid also in chronic inflammation such as the granuloma pouch induced by Selye's method. As has been shown in earlier studies, antipyretics have no specific growth-inhibiting properties, so that the effect of butazolidin on the granuloma pouch is essentially an anti-inflammatory one. In this test the calcium salt was rather more potent than the free acid in reducing exudation but rather less so in inhibiting the formation of granulation tissue. As already mentioned, however, the difference in action between the two forms of the drug is not significant either in the formation of exudate or of granuloma, since the scatter in chronic forms of inflammation seems to be even greater than in acute forms.

As far as the central effects are concerned, there is no great difference in antipyretic effect betweet the three forms of the drug, except that the free acid seems to be somewhat more active than the two salts. Since the central

analgesic effect of butazolidin is rather slight and can be manifested with certainty only in high doses, it is not easy to demonstrate differences in degree of effect. Again butazolidin in the form of the free acid administered by mouth was rather more effective in the hot plate test than either the sodium or calcium salt. The tests carried out in the rabbit by Gordonoff's method, owing to the degree of scatter in the results, show that the tested butazolidin preparations administered by mouth in any case have a certain analgesic effect, but that the intensity of effect of the individual preparations cannot be compared.

In determining the median lethal dose in the mouse and rat no significant difference can be demonstrated between butazolidin sodium and butazolidin calcium in intravenous injection. By administering separate high doses of calcium in addition to the butazolidin sodium the LD 50 can be very slightly increased in the mouse but not in the rat. Butazolidin calcium administered by mouth to rats shows a distinctly lower toxicity than the free acid and the sodium salt by mouth, while in the mouse butazolidin calcium seems to be rather more toxic than the other two forms of the drug. It is not known to what this difference in behaviour of butazolidin calcium in the two species of animal used can be attributed. The irregularities in the absorption generally observed after oral administration are to be held only partly responsible for these differences in action.

The differences in effect of the three forms of the drug on the stomach of the rat are less marked. It seems merely that butazolidin in the form of the free acid when administered by mouth has a somewhat more potent ulcerogenic effect than its two salts. In subcutaneous injection the two salts have equal ulcerogenic effect. By separate administration of high doses of calcium gluconate the ulcerogenic effect of butazolidin sodium (subcutaneously) is not reliably weakened.

The serum level curves after single doses of butazolidin (by mouth), butazolidin Ca (by mouth and subcutaneous) and butazolidin Na (subcutaneous) show certain deviations which may possibly account for some minor differences in effect of the various forms of the drug. However, such slight differences in effect would seem to have a certain significance only in the test animals used, were the serum level of butazolidin is known to show rapid rise and fall, so that changes even of short duration in the drug concentration could affect the pharmacological tests on the whole animal being carried out at the time. The fall in concentration in man however, is far slower. For instance, the half life of butazolidin after intravenous injection in man according to Burns *et al.* (1955) is about 2 or 3 days, but in the rat according to Herrmann (1959) about 3 hours.

Considered as a whole, apart from oral toxicity, no great differences in intensity of effect were manifested by butazolidin sodium, butazolidin calcium and free acid. Nevertheless, a certain calcium effect in parenteral administration of high doses of butazolidin calcium was feasible. However, butazolidin calcium cannot be injected in man since the calcium salt, unlike the salts with monovalent metal ions, has only a very limited solubility in water—little more than 1 per cent. It seems extremely doubtful that sufficient amounts of calcium are absorbed in oral administration of butazolidin calcium. In this case butazolidin is precipitated in the stomach as practically insoluble free acid. The calcium chloride formed at the same time is then converted into hardly soluble carbonic and fatty acid salts in the intestine.

SUMMARY

Butazolidin calcium in comparative tests showed sometimes a less potent anti-inflammatory effect (formalin peritonitis in the rat, ultra violet erythema in the guineapig, possibly also in the granuloma formation in granuloma pouch and in necrosing ultra violet inflammation in the rat), sometimes an equally potent effect (formalin oedema in the rat, croton oil inflammation in the mouse ear) and at the most with respect to inhibition of exudation in the granuloma pouch a somewhat more potent anti-inflammatory effect than butazolidin and/or its sodium salt.

Yeast-fever in the rat was brought down approximately equally by the two salts of butazolidin tested, while the free acid seemed to be somewhat more active.

In the hot plate test in the mouse butazolidin calcium and butazolidin sodium administered by mouth had a less potent analgesic effect than the free acid.

In parenteral administration butazolidin calcium and butazolidin sodium behaved similarly with respect to acute toxicity in mouse and rat (intravenous) and to ulcerogenic effect in the rat stomach (subcutaneous). When administered by mouth butazolidin calcium showed less toxicity in the rat, none in the mouse and—as the sodium salt—less ulcerogenic effect (rat) than butazolidin in the form of free acid.

Alterations in the course of concentrations of butazolidin in the serum of the rat after oral or subcutaneous administration of butazolidin calcium and sodium or the free acid of the drug might only partly explain certain differences in effect between the individual drugs in the acute animal test.

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