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COMMUNICATIONS

Antiulcer and mineralocorticoid activities of carbenoxolone and desoxycorticosterone in rats

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Carbenoxolone is an antiulcer drug that, by strengthening the defensive mechanisms of gastric mucosa (Goodier et al 1967; Goodier 1968; Lipkin 1970; Gheorgiu et al 1973; Yeomans & St John 1974), represents a rational approach in peptic ulcer therapy, but its clinical usefulness may be limited by the occurrence of side effects related to its mineralocorticoid-like activity. It is still debated whether these mineralocorticoid properties are involved in the therapeutic activity, and it is unclear whether the drug acts topically or systemically. In fact, while the mineralocorticoid effects appear to be related to the plasma concentration of the drug (Baron et al 1978), an analogous relationship for the ulcer-healing action is not so convincing (Baron et al 1978; Reed & Davies 1978; Wright et al 1980).

In the present study, the relationship between the gastroprotective and the mineralocorticoid actions was examined by comparing the pharmacological effects of carbenoxolone with those of a standard mineralocorticoid agent, desoxycorticosterone acetate (doca). The effect of these two compounds on water and electrolyte balance was evaluated in adrenalectomized rats, whereas the antiulcer activity was assessed also in rats by comparing the compounds' protective action against stress-, ethanol- and acetylsalicylic acid (ASA)-induced gastric lesions. In these ulcer models, both drugs were tested intragastrically (i.g.) and intraduodenally (i.d.) establishing thereby whether a direct contact with the gastric mucosa is necessary for the production of the gastro-protective action.

Materials and methods

As in the following experiments fasted rats were used, the animals were placed in plastic cages with large wire mesh to avoid coprophagy and hydrolysis of carbenoxolone due to gastrointestinal microflora.

Mineralocorticoid activity

Male Sprague-Dawley rats (Charles River, Italy), 140–160 g, and fasted 24 h before the experiment were used. According to the method of Stafford et al (1955), four days after adrenalectomy the animals were given 5 ml of water i.g. and 1 h later 5 ml of 0·9% NaCl (saline) by the same route. After the urinary bladder had been emptied by suprapubic pressure, the animals were treated by gavage with the drugs and placed in pairs in metabolic cages. Urine was collected for 5 h, the volume recorded, and the Na^+ and K^+ concentration analysed by flame photometry. The $NA^+:K^+$ ratio was taken as the index of mineralocorticoid activity. Carbenoxolone and doca were administered i.g. at doses ranging from 3–100 mg kg^{-1} and 1–10 mg kg^{-1}, respectively.

Antiulcer activity

Stress-induced gastric lesions. Female Sprague-Dawley rats (210–230 g) fasted for 48 h were immobilized in suitable containers and exposed to 4 °C for 2 h (Senay & Levine 1967) after which the animals were killed and the stomachs removed and opened along the greater curvature. The length of each lesion in the glandular portion was measured in blind conditions using a stereomicroscope under a 10× magnification. The sum of the length (mm) of all lesions for each rat was taken as the lesion index. Carbenoxolone and doca were administered i.g. at the dose of 100 mg kg^{-1} 15 min before exposure to stress.

Ethanol-induced gastric lesions. Female Sprague-Dawley rats (180–200 g) fasted for 24 h received 1 ml of absolute ethanol as described by Robert et al (1979). One h later the animals were killed. The evaluation of the degree of gastric lesion was performed in blind conditions by use of an arbitrary scale from 0 (no lesions) to 5 (100% of lesioned glandular portion). The tested drugs were administered i.g. or i.d. (under light ether anaesthesia) 1 h before the injurious agent, at the dose of 100 mg kg^{-1}.

Acetylsalicylic acid-induced gastric lesions. I. Male Sprague-Dawley rats (180–200 g) fasted for 24 h were treated orally with 200 mg kg^{-1} of ASA. The animals were killed 5 h later. Each separate lesion was measured and scored in blind conditions following the schedule proposed by Carmichael et al (1978). The tested drugs were administered i.g. concomitantly with ASA at the dose of 100 mg kg^{-1}.

II. The method employed was the same as that described in I, but the drugs (100 mg kg^{-1}) were administered i.d. and

* Correspondence.

the pylorus was ligated under ether anaesthesia to prevent intestinal reflux.

Drugs

The compounds and vehicles used were: carbenoxolone sodium salt (ISF, Milan) dissolved in distilled water, doca (Schering, Berlin) suspended in 0·5% Tween 80, and ASA (Merck, Darmstadt) suspended in 0·5% methocel.

Results

As shown in Fig. 1, carbenoxolone and doca produced a significant reduction in the urinary electrolyte excretion ratio of Na^+ and K^+, although a dose-effect relationship was not found. Since the minimal effective doses ranged between 1–3 and 3–10 mg kg^{-1} for doca and carbenoxolone, respectively, on this basis doca appeared to be, by oral route, at least three times more potent as a mineralocorticoid agent.

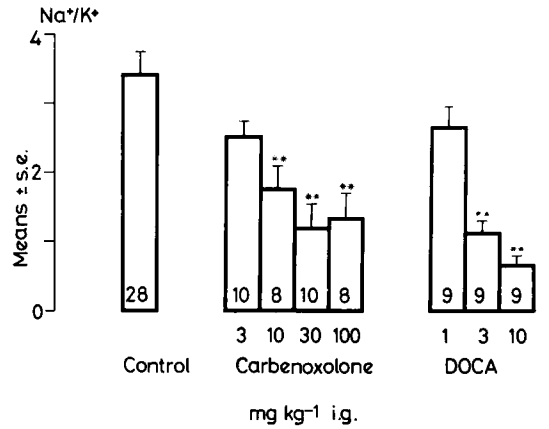

FIG. 1. Effects of intragastric administration of carbenoxolone and doca on urinary excretion ratio of Na^+ and K^+ in adrenalectomized rats. Each column represents the mean ± s.e. The numbers of rats are given in the columns. The data were analysed by Dunnett's test. ** $P < 0·01$ compared with the control group.

Intragastric administration of carbenoxolone at the dose of 100 mg kg^{-1} produced a considerable (−66·7%) and significant ($P < 0·01$) reduction in severity and incidence of gastric lesion produced by stress, whereas doca at the same dose level had no effect on gastric lesion formation (Table 1). Moreover, in the animals pretreated with doca 38% mortality was observed after stress exposure. The animals that died were excluded from the evaluation of gastric lesions.

Carbenoxolone, 100 mg kg^{-1}, produced a significant ($P < 0·01$) inhibition (−34·9% for i.g. and −44·1% for i.d. administration) of ethanol-induced gastric lesions irrespectively of whether it was given i.g. or i.d. (Table 2). Doca, at the same dose, failed to show any significant effect in this lesion model.

Unlike doca, the i.g. administration of carbenoxolone significantly ($P < 0·01$) inhibited (−72·4%) gastric lesion

Table 1. Effects of i.g. administration of carbenoxolone and doca on gastric lesions induced by stress in rats. The data were analysed by Dunnett's test.

Treatment (mg kg^{-1})	n	Lesion index: Mean ± s.e.	Lesion index: % Change	Incidence of lesions (%)
Control	49	4·36 ± 0·408		98·0
Carbenoxolone 100	20	1·45 ± 0·391	−66·7*	55·0
Doca 100†	21	4·45 ± 0·888	+2·1	95·2

* $P < 0·01$ compared with control group.
† 38% mortality was observed.
n = number of rats.

Table 2. Effects of i.g. and i.d. administration of carbenoxolone and doca on gastric lesions induced by ethanol in rats. The data were analysed by Dunnett's test.

Treatment (mg kg^{-1} route)	n	Lesion index: Mean ± s.e.	Lesion index: % Change	Incidence of lesions (%)
Control	47	2·47 ± 0·166		100·0
Carbenoxolone 100 i.g.	20	1·50 ± 0·256	−39·3*	80·0
100 i.d.	29	1·38 ± 0·201	−44·1*	75·9
Doca 100 i.g.	17	3·18 ± 0·287	+28·7	100·0
100 i.d.	13	1·92 ± 0·288	−22·3	92·0

* $P < 0·01$ compared with control group.
n = number of rats.

Table 3. Effects of i.g. and i.d. administration of carbenoxolone and doca on gastric lesions induced by ASA and ASA plus pyrolic ligation in rats. The data were analysed by Dunnett's test.

Lesion model	Treatment (mg kg^{-1} route)	n	Lesion index: Mean ± s.e.	Lesion index: % Change	Incidence of lesions (%)
ASA	Control	39	17·33 ± 1·720		100·0
	Carbenoxolone 100 i.g.	24	4·79 ± 1·189	−72·4*	45·8
	Doca 100 i.g.	16	17·19 ± 2·283	−0·8 −0·8	87·5
ASA + pyloric ligation	Control	24	24·50 ± 2·818		95·8
	Carbenoxolone 100 i.d.	24	8·63 ± 1·547	−64·8*	87·5
	Doca 100 i.d.	14	19·86 ± 2·612	−18·9	100·0

* $P < 0·01$ compared with control group.
n = number of rats.

formation induced by ASA (Table 3). In the second part of the experiment, ligature of the pylorus was performed, and the tested drugs were administered into the duodenum. Under these conditions carbenoxolone also exhibited a highly significant ($P < 0.01$) gastro-protective effect (−64·8%), whereas doca was found to be inactive (Table 3).

Discussion

Our results confirm that carbenoxolone is active against gastric ulcer formation induced by stress and ASA (Okabe et al 1976). In addition, they also demonstrate that the drug is active in preventing acute gastric lesions induced by ethanol in rats. This latter finding is indicative for a cytoprotective effect (Robert et al 1979) which might be mediated by a rise in the level of prostaglandins in the gastric mucosa, according to the recent evidence that carbenoxolone inhibits prostaglandins inactivating enzymes (Peskar et al 1976; Peskar 1980). In addition to its antiulcer action, carbenoxolone displays an appreciable mineralocorticoid-like activity, even if the drug is, by oral route and on a weight basis, at least three times less potent than doca.

Unlike carbenoxolone, doca tested at the same dose (which was far higher than the mineralocorticoid one) failed to show any significant protection of gastric mucosa in accordance with the results obtained by Robert et al (1970) on a forced exertion stress ulcer model in rats.

On the basis of these comparative results, a direct relationship between renal mineralocorticoid activity and antiulcer properties of carbenoxolone must be excluded, since doca would also be expected to inhibit gastric lesions. In a recent study, Dajani et al (1979) also concluded that the antiulcer action of carbenoxolone is not related to an aldosterone-like action. However, since the aldosterone antagonist spironolactone reduces the healing effects of carbenoxolone on human gastric ulcers (Doll et al 1968), the likelihood that the therapeutic action of carbenoxolone depends on the integrity of the electrolyte transport systems at the gastrointestinal level cannot be ruled out. Indeed, carbenoxolone has been shown to increase the active transport of sodium in the human rectal mucosa and spironolactone hinders this effect (Tomkins & Edmonds 1975), but the relevance of these findings to the therapeutic action at the level of gastric mucosa is unknown.

Another possible explanation for the negative interference of spironolactone is that advanced by Dajani et al (1979), who suggested that spironolactone may enhance metabolic degradation of carbenoxolone.

An additional interesting finding of our study is that carbenoxolone possesses an important gastric antiulcer action even when administered i.d. and, in ASA-induced lesions, even when intestinal reflux is prevented by ligation of the pylorus. Similar findings were obtained by Okabe et al (1976), who demonstrated that carbenoxolone inhibits gastric lesions induced by stress and ASA when administered intraperitoneally. These data clearly indicate that a direct contact of carbenoxolone with gastric mucosa is not necessary for its antiulcer action.

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