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The effect of an aldosterone antagonist on the protective action of carbenoxolone on the gastric mucosal barrier

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The effect of an aldosterone antagonist on the protective action of carbenoxolone sodium on the gastric mucosal barrier has been studied in three dogs with Heidenhain pouches. The net fluxes of hydrogen ion and sodium ion were measured before, during, and after contact with a 10 mM bile acid solution at pH 2, in pouches which had not been treated with drugs, in pouches treated with carbenoxolone sodium and in pouches treated with both carbenoxolone sodium and the aldosterone antagonist, spironolactone

Hydrogen ion back diffusion from, and sodium ion gain by the untreated pouch was increased by 10 mM bile acid solution. Addition to the pouches of either carbenoxolone sodium alone or carbenoxolone sodium and spironolactone reduced the hydrogen ion back diffusion after exposure to the bile acid solution. The spironolactone did not change the protective effect of carbenoxolone on the gastric mucosal barrier. Carbenoxolone did not change the increased sodium ion diffusion caused by bile.

There are two main hypotheses for the aetiology of gastric ulcer (Rhodes, 1972). The first, states that an abnormality of the pyloric sphincter produces delayed gastric emptying and antral distension. This leads to increased production of the hormone gastrin, and the increased acid secretion that follows causes gastric ulcer. The second hypothesis, states that abnormality of the pyloric sphincter results in the reflux of bile from the duodenum into the stomach. The bile acids produce a gastritis, which may eventually lead to gastric ulceration. Neither of these hypotheses provide adequate explanation of the cause of gastric ulceration in all patients. There are several types of gastric ulcer, and each different type may have different aetiological factors.

Carbenoxolone sodium was the first drug that convincingly accelerated the rate of healing of chronic gastric ulcer (Doll, Hill & others, 1962). Its mode of action is uncertain. It has no direct effect on acid secretion, and it seems likely that it increases the defence mechanism of the stomach, either by stimulating or altering the physical characteristics of mucous secretion.

Its clinical use is limited by side effects due to salt and water retention and potassium loss (Davies, Rhodes & Calcraft 1974). Doll, Langman & Shawdon (1968), have shown that by combining carbenoxolone sodium with the aldosterone antagonist spironolactone the incidence of such side effects may be significantly reduced. Such a combination however, also reduces the rate of ulcer healing. The mechanism by which spironolactone administration blocks the healing effect of carbenoxolone is obscure. Aldosterone antagonists interfere with electrolyte transfer mechanisms in the gut as in the kidney (Elmslie, Mulholland & Shields, 1966), and it may be that ulcer healing is dependent upon the integrity of these mechanisms.

We have studied the effect of spironolactone on the protective action of carbenoxolone, using the gastric mucosa of canine Heidenhain pouches.

MATERIALS AND METHODS

Three dogs were prepared with Heidenhain pouches. The experiments, which were similar to Davenport's (Davenport, 1964, 1965, 1968), included three consecutive 30 min periods. In each period a solution was placed in the pouch and changes in volume and ionic (sodium and hydrogen ion) composition were determined. In the first and third periods a standard acid solution was used which contained 100 mM HCl, 15 mM NaCl and 78 mM mannitol (S.G. 1·007). In the second period, bile or control solutions were used. The difference between the net fluxes in the first and third periods was a measure of the effect of bile on the mucosal barrier. An increased loss of hydrogen ion from the solution, or gain of sodium ion indicated damage to the mucosal barrier.

The pouch was drained by a vitallium canula connected to a polypropylene Y tube. On one limb of the Y tube a three-way tap was connected by tubing to a vertical open-ended glass tube (converted burette). The three-way tap had a syringe attachment through which the solutions were introduced.

The standard acid solution (A) was at pH 1·2 with an osmolarity of 297 mOsm kg^{-1}. It contained 100 mM HCl, 15 mM NaCl and 78 mM mannitol.

The bile solution (B) was prepared from a pool of bile collected from patients with T-tube drainage of the common bile duct; the bile acid concentration was measured by the steroid dehydrogenase method of Turnberg & Anthony-Mole (1969), and a 10 mM solution at pH 2 (titratable acidity 27 mM) was prepared with a sodium concentration of 100 mM. Aliquots of this were stored at −20° in air-tight containers.

The control solution (C) was also at pH 2 (titratable acidity 17 mM) and contained 100 mM NaCl. The osmolarity of solutions B and C was adjusted to approximately 340 mOsm kg^{-1} by adding mannitol.

Initially, the untreated pouches were tested with bile and control solutions in each dog on four occasions over four weeks; the interval between individual experiments was not less than two days. The pouches were then treated with 30 ml of a 0·33% solution of carbenoxolone sodium for 1 h daily over four weeks. After the first five preparation days, the experiments with bile and control solutions were repeated, again with four experiments in each dog. On each occasion, the pouches were treated with carbenoxolone sodium for 1 h and after an interval of 30 min the tests were made. The interval between experiments was not less than two days. A third series of experiments was then conducted in which the pouches were treated for 1 h with a 0·33% solution of carbenoxolone sodium, and for a second hour with a 0·083% solution of spironolactone. This procedure was repeated daily over four weeks. During this time each dog was also given 25 mg of spironolactone orally per day. After the first five preparation days, the experiments with bile and control solutions were repeated, again with four experiments in each dog. On each occasion, the dogs were given 25 mg of spironolactone orally, 2 h before the test; 30 min before each test the pouches were prepared with solution of carbenoxolone and spironolactone as described above. Again the interval between experiments was not less than two days. The experiments were carried out over three months.

The dogs were fasted for 18 h before each test. At the beginning of each experiment the pouch was washed clean of debris with solution A and then drained. The residual volume was assumed to be constant in the calculating of net fluxes. This residual volume was determined before the series of experiments by a phenol red dye dilution technique using the mean of 20 replicate estimations (George, 1968).

Residual volumes. The mean value (with two standard deviations) of the residual volume for each dog was (ml): dog 1, 1·40 (0·77); 2, 1·36 (1·18); 3, 0·92 (0·79).

The changes in hydrogen ion and sodium ion flux induced by bile cannot be explained by variation in the residual volume. We have used phenol red dye in similar experiments and have calculated the residual volume after each period. Minor changes in the residual volume were observed but these did not significantly affect the ionic flux. In preliminary work, carbenoxolone interfered with the dye dilution technique and the variable results may have been due to adsorption of the dye by increased amounts of mucus (unpublished observations).

40 ml of acid solution was then introduced into the pouch. After the solution had mixed with the residual volume, 10 ml was removed and the hydrogen and sodium ion concentrations were measured.

Thirty minutes after taking this specimen the pouch was emptied and the volume measured; the second specimen was also analysed for hydrogen and sodium ion concentrations. The same procedure was adopted for the second 30 min period, with bile or control solution in the pouch, and for the third period with the acid solution. On each occasion, decanted samples were analysed at the beginning and end of the period, and the volume change during each period calculated. During the experiment, the pressure within the pouch was kept constant by adjusting the height of the fluid column in the burette. Minor changes in the fluid column were unavoidable due to spontaneous contractions of the pouch.

Hydrogen ion concentration was determined by titrating with 0·01 N sodium hydroxide to pH 7. Sodium ion concentration was measured with a flame photometer. Measurements of hydrogen and sodium ion concentrations were made in duplicate. Net fluxes of hydrogen and sodium ions were calculated and expressed in μ mol per 30 min. Movement of ions from the mucosa to lumen was regarded as positive and vice versa. Changes in the net flux of hydrogen and sodium ions caused by bile and control solution were compared for the untreated pouches and both types of treated pouches, using the standard technique for analysis of variance, which extracted differences between dogs and interaction between dogs and treatment.

RESULTS

In period I there was a net loss of hydrogen ion from the pouch (201 ± 133) and a net gain of sodium ion (209 ± 61) into the pouch. Fluxes of hydrogen ion out of the pouch and sodium ion into the pouch were also seen in period III, but their magnitude was dependent on the treatment of the pouch during period II. When control solution was placed in the pouch in period II, there were no significant changes in period III. When bile was placed in the pouch during period II, the fluxes of both hydrogen ion and sodium ion were significantly increased during period III (see Table 1). This confirms the results of Davenport. The increase in hydrogen ion flux caused by bile during period II was abolished by treatment with carbenoxolone alone or by carbenoxolone and spironolactone. The results are summarized in Table 1.

Table 1. *The change in ionic flux after placing control solution or bile during period II in the unprepared pouch and in the pouch prepared with carbenoxolone alone, and carbenoxolone plus spironolactone.* The values are the mean (with two standard deviations) of twelve experiments, four in each of three dogs.

		No treatment	Carbenoxolone	Carben. and spir.
H^+	Control	+28 (75)	+10 (81)	+14 (62)
	Bile	−43 (42)	+27 (64)	+52 (95)
	F1:18	15·1		
	P	<0·01	N.S.	N.S.
Na^+	Control	−18 (55)	−7 (77)	+2 (51)
	Bile	+103 (64)	+78 (87)	+70 (97)
	F1:18	58·7	13·9	9·6
	P	<0·001	<0·01	<0·01

DISCUSSION

Our experiments confirm that bile alters the permeability of the mucosal barrier in Heidenhain pouches to sodium and hydrogen ions. They also show that preparation of the pouch with carbenoxolone prevents the increased flux of hydrogen ion which usually follows exposure to bile. Spironolactone did not affect this protective action.

These results suggest that the mechanisms by which spironolactone reduces ulcer healing are not related to its topical action on the gastric mucosa.

Certain aspects of our results are difficult to explain. It can be seen that carbenoxolone preparation of the Heidenhain pouch prevents the increased hydrogen ion flux which normally follows bile damage. However, the increased sodium ion flux induced by bile remains unaffected. (Any interpretation of the mechanism responsible for these results must be speculative, as the nature of the gastric mucosal barrier is not fully understood.) If carbenoxolone increases mucus production (Dean, 1968), it is possible that mucus may buffer the hydrogen ion and prevent it from penetrating the mucosa, while sodium ion still leaks from the mucosa after exposure to bile.

This result is in contrast to the effect of amylopectin sulphate, which reduces both the increased flux of hydrogen ion and sodium ion induced by bile (Calcraft, Rhodes & others, 1974). The reason for the difference in the actions of amylopectin sulphate and carbenoxolone sodium is not known.

REFERENCES

CALCAFT, B. J., RHODES, J., CROSS, S., HOLE, D. & AUBREY, A. (1974). *Am. J. dig. Dis.*, **19**, 243–252.

DAVENPORT, H. W. (1964). *Gastroenterology*, **46**, 245–253.

DAVENPORT, H. W. (1965). *Ibid.*, **49**, 189–196.

DAVENPORT, H. W. (1968). *Ibid.*, **54**, 175–181.

DAVIES, G. J., RHODES, J. & CALCRAFT, B. J. (1974). *Br. med. J.*, **3**, 400–402.

DEAN, A. C. B. (1968). *A symposium on carbenoxolone sodium*, pp. 33–39. Editors: Robson, J. M. & Sullivan, F. M. London: Butterworths.

DOLL, R., HILL, I. D., HUTTON, C. & UNDERWOOD, D. J. (1962). *Lancet*, **2**, 793–796.

DOLL, R., LANGMAN, M. J. S. & SHAWDON, H. H. (1968). *Gut*, **9**, 42–45.

ELMSLIE, R. G., MULHOLLAND, A. T. & SHIELDS, R. (1966). *Ibid.*, **7**, 697–699.

GEORGE, J. D. (1968). *Ibid.*, **9**, 237–242.

RHODES, J. (1972). *Gastroenterology*, **63**, 1, 171–182.

TURNBERG, L. A. & ANTHONY-MOLE, A. (1969). *Clin. chim. Acta*, **24**, 253–259.