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Influence of disodium etidronate on salicylic acid absorption in the rat

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Disodium etidronate (disodium ethane-1-hydroxy-1, 1-diphosphonate), or simply etidronate, is a diphosphonate that has been investigated extensively in man. The compound is effective in reducing ectopic calcification and excessive bone resorption when chronically administered, and it is presently used clinically in the treatment of Paget's disease.

Etidronate is highly ionized at physiologic pH, having pK_a values of 1·7, 3·1, 7·5 and 11·5 (Grabenstetter et al 1967). Its gastrointestinal absorption is low and erratic. Gural (1975) estimated the oral absorption of etidronate in man to average 2·3% of the administered dose. Similar findings have been reported (Recker et al 1973).

Etidronate binds calcium both in solution and on crystalline surfaces (Francis 1969; Grabenstetter et al 1971). Calcium is involved in the structural integrity of the gastrointestinal membrane barrier and the removal of calcium by chelators, such as edetic acid (EDTA), results in a dramatic increase in the permeability of the membrane to various substances (Windsor 1961; Schanker 1961; Tidball 1964; Cassidy & Tidball 1967; Poiger et al 1979).

The object of the present investigation was to determine if etidronate's chelation properties might influence the absorption of other drugs. The in situ technique reported by Doluisio et al (1969) was selected for the study, and salicylate was selected as a model drug since its in situ absorption has been reported.

Methods

Male, albino Sprague-Dawley rats, 190–310 g, were housed in wide mesh metal cages to minimize coprophagy and were fasted 14 to 16 h before surgery. Water was freely available. A modification of the anaesthesia technique reported by Youth et al (1973) was used. Each animal was given ketamine hydrochloride (Ketajeit, Bristol Labs, 50 mg ml^{-1}), 60 mg kg^{-1} i.m., followed within 10 min by sodium pentobarbitone (Nembutal sodium, Abbott Labs, 50 mg ml^{-1}) 16 mg kg^{-1} i.p. When additional anaesthetic was required during the experiment, 0·1 ml of ketamine was given i.m. initially. If more anaesthetic was required after 5 min, 4 to 8 drops of pentobarbitone were instilled on the intestinal tract in the peritoneal cavity.

After anaesthesia was achieved, a 35 cm jejunal segment just distal to the suspensory ligature that marks the end of the duodenum was cannulated, placed back in the abdominal cavity and rinsed with sodium chloride 300 mOsm kg^{-1} (saline) until the effluent was clear. The segment was filled with clean saline for 15 min, then the saline was expelled by air.

The rectal temperature was monitored throughout and kept as close as possible to normal by means of a rheostatically controlled heating pad and an overhead work-light.

All solutions introduced into the intestine were preheated to 37 °C, and adjusted to pH 6·4 with either sodium hydroxide or hydrochloric acid and if necessary, sodium chloride was added to bring the osmotic pressure to 300 mOsm kg^{-1}.

In one experiment, salicylic acid and various concentrations of etidronate (up to 0·08 M) were concomitantly administered. The range of etidronate concentrations was equivalent to the human therapeutic range determined by dividing usual dose by usual gastrointestinal fluid volume. In another series of experiments, the various etidronate solutions were introduced into the segment and allowed to remain for 0·5, 1, or 2 h. The etidronate solution was then expelled by air, the intestinal segment rinsed with 10 ml of saline, the salicylate solution instilled, and the absorption rate of salicylic acid determined. Saline was used as the control (for the 0·0 M etidronate concentration).

The initial concentration of salicylic acid was 2·0 mg ml^{-1}, and 0·1 ml samples were removed at preselected time intervals. The samples were diluted with 3·0 ml of 0·1 M NaOH, and the u.v. absorption was read against a saline blank at 296 nm. Absorbance values were converted to concentrations using a previously prepared standard curve. The absorption rate was calculated by logarithmic-linear least-squares analysis of drug concentrations remaining in lumen vs time.

Results and discussion

The influence of increasing concentrations of etidronate on the absorption half-life of concomitantly administered salicylic acid from the in situ rat small intestine is shown in Table 1. At etidronate concentrations between 0·002 and 0·01 M, the absorption half-lives of salicylic acid tended to be less than the control, but only the 0·002 M was statistically different from control (unpaired t-test, $P < 0·05$). At etidronate concentrations between 0·02 and 0·08 M, the half-lives were not significantly different from the control but indicated a

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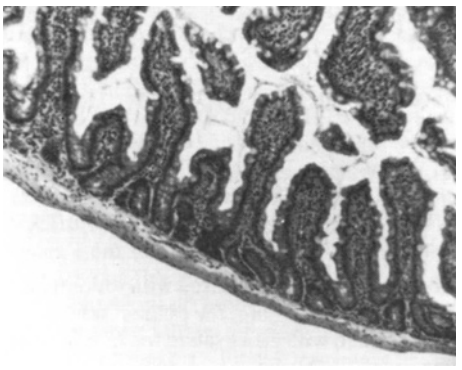

FIG. 1. A photomicrograph of the rat jejunum after a 0·5 h exposure to saline (200X).

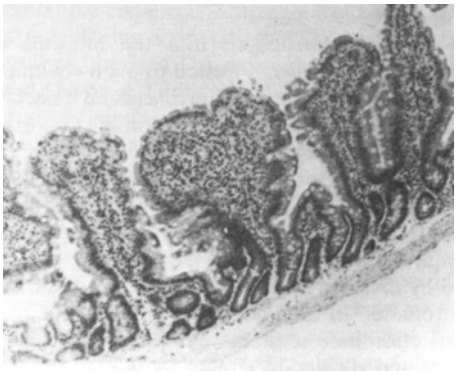

FIG. 2. A photomicrograph of the rat jejunum after a 0·5 h exposure to 0·004 M of etidronate (200X).

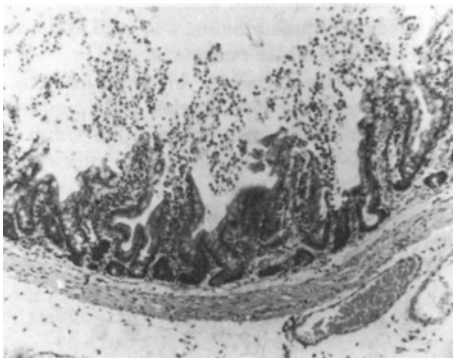

FIG. 3. A photomicrograph of the rat jejunum after a 1 h exposure to 0·08 M of etidronate (200X).

trend toward a greater absorption half-life with increasing concentrations of etidronate.

The results suggest that lower etidronate concentrations might alter the integrity of the intestinal membrane barrier, resulting in an enhancement of salicylic acid absorption. These results were similar to the effect of edetic acid on salicylic acid absorption where calcium depletion from the intestinal mucosa by chelation was the probable mechanism (Feldman et al 1969; Kunze et al 1972).

At higher concentrations of etidronate (0·02 to 0·08 M) the barrier appeared to become less permeable than in the control study. At even higher etidronate concentrations of 0·10 and 0·12 M, animal survival decreased dramatically. Forty-five to 50 min after the etidronate solution had been instilled into the jejunum, the rats became rapidly cyanotic and soon died even though rescue was attempted. Similar morbidity has been reported (Nadai et al 1972, 1975) using EDTA. Therefore, the influence of 0·10 and 0·12 M of etidronate on salicylic acid absorption could not be determined.

To further understand the dual influence of etidronate on the absorption of salicylic acid, segments were exposed to various concentrations of etidronate for various periods of time before instilling the salicylic acid solution. The results (Table 2) show a general trend toward greater absorption half-lives of salicylic acid with increasing concentrations of etidronate and exposure time.

At etidronate concentrations between 0·04 and 0·08 M, a distension of the intestinal segment could be observed with the naked eye. This was accompanied by the appearance of large amounts of a viscous secretion on the mucosal surface. Similar effects have been reported in rats (Berstock et al 1980) when the gastric mucosa was exposed to cytotoxic drugs. The degree of distension and the volume of the secretion increased with increasing concentrations of etidronate and with time of exposure to the chelator. At the same time, the rates of salicylic acid absorption decreased suggesting that etidronate causes changes in the structure of the gut membrane which would interfere with salicylic acid absorption. The histological condition of the gut wall was therefore investigated using the experimental conditions that produced both an increased and a decreased absorption of salicylic acid.

Fig. 1 is a photomicrograph of the intestinal segment treated for 0·5 h with saline to serve as a reference. The villi are obviously present and the mucosal surface is

Table 1. The influence of etidronate concentrations on the absorption half-life of concomitantly administered salicylic acid from the rat jejunum, in situ.

Concentration of etidronate (M)	n	Absorption half-life (min) (mean with s.d.)
0·0	4	6·87 (0·62)
0·002	4	5·11 (0·65)*
0·004	4	5·97 (1·19)
0·01	4	5·96 (1·86)
0·02	4	7·14 (1·73)
0·04	4	7·50 (0·86)
0·08	5	8·62 (1·51)

* $P < 0·05$, see text.

Table 2. The influence of etidronate concentrations and exposure times (0·5–2·0 h) on the absorption half-life of salicylic acid from the rat jejunum, in situ.

Concn of etidronate (M)	0·5 h		1·0 h		2·0 h	
	n	(mean with s.d.)	n	(mean with s.d.)	n	(mean with s.d.)
0·0	3	7·87 (0·64)	3	7·54 (1·19)	5	8·07 (0·81)
0·002	4	5·84 (1·28)		(a)		(a)
0·004	4	4·96 (0·87)*	4	6·64 (0·57)	4	7·75 (0·30)
0·01	6	6·91 (1·89)	6	5·78 (0·76)*	4	11·02 (1·13)*
0·02	10	9·34 (1·86)	8	9·34 (1·23)	7	11·60 (1·98)*
0·04	7	9·89 (1·20)*	6	10·65 (3·50)	11	15·20 (3·84)*
0·08	4	18·73 (4·88)*	4	29·58 (6·29)*	12	29·27 (9·60)*

* See footnote Table 1. [a] Not determined.

intact. Fig. 2 shows the mucosa after a 0·5 h exposure to 0·004 M of etidronate, a concentration that caused a significant increase in the absorption rate of salicylic acid. Oedema and infiltration with inflammatory cells are present in the villi, but the mucosal epithelium appears intact when viewed at a higher magnification. Fig. 3 shows an intestinal segment that was exposed to 0·08 M of etidronate for 1 h, the conditions where salicylic acid absorption was greatly reduced. The wall is grossly distended, the villi are partially or totally destroyed and only fragments of the epithelial lining remain. Many mitotic figures are present in the disrupted villous structures. Similar histological results have been reported for EDTA (Nadai et al 1972, 1975).

The indication is that at low concentrations of etidronate and relatively short exposure times, the attachments of the mucosal epithelial cells are loosened due to the removal of calcium from the mucosa and the oedema process. This would result in a decrease in the barrier properties of the membrane, especially if a significant amount of the drug is capable of diffusing between the cells via aqueous channels, as with salicylic acid (Kunze et al 1972). The inflammatory process may be the result of the loosening of mucosal cells or of direct irritation of etidronate to the intestinal wall.

With increasing concentrations of etidronate and increased exposure time, the intercellular binding becomes progressively weaker until the mucosal barrier is disrupted and the villi damaged. Such extensive destruction is due to etidronate's capacity to form polynuclear complexes with calcium (Grabenstetter et al 1971; Wiers 1971). Total absorbing surface area is reduced, leading to a decrease in the absorption rate of salicylic acid. The rate of diffusion would also be decreased and the diffusional path length increased by the appearance of the viscous secretion on the mucosal surface and the intestinal distension, respectively. Such phenomena would also decrease the rate of salicylic acid absorption.

It is not evident if the viscous secretion is the result of extensive cellular damage or etidronate irritating the intestinal wall. Nor is it clear if the distension results from the secretion distending the wall or loss in muscle tone due to calcium chelation.

However, it has been shown that therapeutic intestinal etidronate concentrations will influence the absorption of salicylic acid. An increased rate of salicylic acid absorption might be expected when low dosages of etidronate are given or when salicylic acid absorption is occurring in the lower segments of the intestinal tract. On the other hand, decreased rate of absorption might be seen when high dosages of etidronate are given or when salicylic acid is occurring in the first part of the intestinal tract.

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