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Dose-dependent effect of calcium and magnesium etidronate on salicylic acid absorption in the rat

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Disodium etidronate affected salicylic acid absorption from the rat small intestine, in-situ, when instilled into a jejunal segment for different exposure times before the salicylic acid absorption was measured. At low etidronate concentrations and short exposure times, the salicylic acid absorption rate was significantly increased compared with saline controls. At high etidronate concentrations and longer exposure times, the absorption rate was reduced. Etidronate precomplexed with calcium or magnesium ions at low concentrations still enhanced salicylic acid absorption but at high concentrations absorption of salicylic acid was close to saline controls. Intestinal mucosa exposed to high etidronate concentrations showed a progressive structural destruction but with the complexes, there was no visible alteration. It is proposed that a solubilized etidronate complex, formed either in-situ or administered as such, is responsible for enhancing salicylic acid absorption. This effect is hidden at high etidronate concentrations because of the deterioration of the mucosal surface and at high complex concentrations because these decrease the absorbing surface area and increase the viscosity of the lumen contents.

Disodium etidronate (etidronate, disodium 1-hydroxyethylidenediphosphonate) is used in reducing ectopic calcification and excessive bone resorption. It affects the absorption rate of salicylic acid from the rat small intestine, in-situ (Shrewsbury et al 1982). At low concentrations and with short exposures, etidronate causes an increase, while at higher concentrations and with longer exposure it causes a decrease in absorption. It also causes histological changes in the mucosa at all concentrations and exposures studied.

Etidronate binds calcium both in solution and on crystalline surfaces (Francis 1969; Grabenstetter & Cilley 1971) forming polynuclear complexes (Grabenstetter & Cilley 1971; Wiers 1971) so it has been proposed that calcium depletion from the intestinal membrane is responsible for the structural changes. A similar mechanism has been suggested for the increase in membrane permeability to various substances caused by edetic acid (Windsor & Cronheim 1961; Schanker & Johnson 1961; Tidball 1964; Cassidy & Tidball 1967; Poiger & Schlatter 1979).

We have examined the proposed mechanism by which etidronate causes histological changes in the intestinal mucosa.

Methods

Male,albino, Sprague-Dawley rats, 190–310 g, housed in wide mesh metal cages with free access to water, were fasted 14–16 h before surgery for which a modification

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of the anaesthesia technique reported by Youth et al (1973) was used (Shrewsbury et al 1982). A 35 cm jejunal segment distal to the end of the duodenum was cannulated, rinsed with saline (sodium chloride in water, 300 mOsm kg^{-1}, pH 6·4) until the effluent was clear, filled with fresh saline for 15 min, and then was expelled by air.

All solutions were preheated to 37 °C, adjusted to pH 6·4, and sodium chloride was used to bring the osmotic pressure to 300 mOsm kg^{-1} (Osmometer, Model 5120, Wescor, Inc., Logan, Utah).

Pretreatment solutions (saline (control), etidronate, calcium or magnesium etidronate) remained in the segment for 0·5, 1·0 or 2·0 h, and were displaced by 10 ml of saline followed by air before salicylic acid solution (2·0 mg ml^{-1}) was instilled.

Calcium and magnesium etidronates were prepared by mixing equimolar concentrations of calcium ion (as $CaCl_2.H_2O$) and magnesium ion (as $MgCl_2.6H_2O$) with disodium etidronate. The pH of the resulting solution was raised to 12·0 with 10% tetramethylammonium hydroxide. The suspension was filtered and washed twice with distilled water. The solid was dried over calcium carbonate in a dessicator. The 0·08 M magnesium etidronate preparation had an intrinsic osmotic pressure of 342 mOsm kg^{-1}, but was not expected to produce a significant osmotic effect (Kojima et al 1972).

Rectal temperature was monitored throughout (Telethermometer, Model 47, Yellow Springs Instruments Co., Yellow Springs, Ohio) and maintained at normal.

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

Concn etidronate (M)	Exposure time 0·5 h n	(Mean ± s.d.)	1·0 h n	(Mean ± s.d.)	2·0 h n	(Mean ± s.d.)
0·0 (saline)	3	7·87 ± 0·64[a]	3	7·54 ± 1·19	5	8·07 ± 0·81
0·002	4	5·84 ± 1 28		ND		ND
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*

[a] Half-lives in min^{-1}.
ND Not determined.
*$P < 0·05$, see text.

Table 2. The influence of etidronate (EHDP) complexes and exposure times on the absorption half-life of salicylic acid from the rat jejunum, in-situ.

Concn of etidronate or complex (M)	Exposure time 0·5 h n	(Mean ± s.d.)	1·0 h n	(Mean ± s.d.)	2·0 h n	(Mean ± s.d.)
0·0 (saline)	3	7·87 ± 0·64[a]	3	7·54 ± 1·19	5	8·07 ± 0·81
0·004 EHDP	4	4·96 ± 0·87*	4	6·64 ± 0·57	4	7·75 ± 0·30
0·004 CaEHDP	15	5·65 ± 0·95*	6	6·57 ± 0·65	4	8·36 ± 2·17
0·004 MgEHDP	6	5·62 ± 1·05*	4	6·53 ± 0·89	3	6·58 ± 1·41
0·08 EHDP	4	18·73 ± 4·88*	4	29·58 ± 6·29*	12	29·27 ± 9·60*
0·08 CaEHDP	3	6·82 ± 0·31	3	11·45 ± 0·45*	4	12·50 ± 0·07*
0·08 MgEHDP	6	5·65 ± 0·79*	3	6·88 ± 1·79	3	10·75 ± 1·75*

See Table 1 for explanations.

Results

There was a tendency for salicylic acid absorption to be enhanced at low etidronate concentrations and short exposures (Table 1). The 0·5 h exposure with 0·004 M etidronate significantly enhanced salicylic acid absorption ($P < 0·05$, unpaired t-test). At 0·08 M, etidronate decreased the absorption of salicylic acid at all exposures. These concentrations were therefore selected for further study.

Pre-complexing 0·004 M etidronate with either calcium or magnesium also results in the etidronate-induced enhancement of salicylic acid absorption after a 0·5 h exposure (Table 2). The similarity in the salicylic acid absorption rates at all exposures suggests that pre-complexation did not alter the activity of etidronate. However, 0·08 M calcium or magnesium etidronate appeared to reverse the etidronate activity producing half-lives similar to saline control values. Salicylic acid absorption, though similar, was still slightly reduced with 1 and/or 2 h exposures to the complexes.

Discussion

Etidronate had a dual influence on the absorption rate of salicylic acid. At low concentrations and short exposure times, salicylic acid absorption was enhanced. Similar results have been reported with edetic acid (Feldman & Gibaldi 1969; Kunze et al 1972). At high concentrations and longer exposure times, salicylic acid absorption was dramatically decreased. At etidronate concentrations between 0·04 and 0·08 M, a distention of the intestinal segment could be seen and was accompanied by large amounts of a viscous secretion on the mucosal surface. Similar effects have been reported when the gastric mucosa was exposed to cytotoxic drugs (Berstock et al 1980). The degree of distention and the volume of the secretion increased with increasing concentrations of etidronate and 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 morphological condition of the gut wall was therefore investigated by scanning electron microscopy (SEM) using conditions that produced both an increased and a decreased absorption of salicylic acid.

There were no changes in the mucosal surface in segments exposed to saline or exposure to 0·004 M etidronate. However, there was a time dependent destruction of the mucosal surface with 0·08 M etidronate. The appearance of disjointed cells (confirmed by

energy dispersive X-ray analysis) and secretory material on the villi became progressively more evident with longer exposure times. These changes could account for the effect of high concentrations of etidronate on salicylic acid absorption. Etidronate's capacity to remove divalent ions from the mucosal surface could progressively weaken the intercellular binding of the epithelial lining leading to the observed deterioration of the villi structure. This would result in a decreased absorbing surface area, an increased diffusional path length, as well as an increased viscosity of the lumen contents.

Pre-complexed etidronate affected salicylic acid absorption in a manner similar to free etidronate at low concentrations, but had an opposite effect at high concentrations. Segments pretreated with 0·08 M calcium or magnesium etidronate for 1·0 and 2·0 h showed the mucosal surface to have no structural difference from the saline exposed mucosa.

It is documented (Borle 1975; Curtis et al 1980) that calcium efflux occurs when tissues are exposed to solutions low in calcium. At low etidronate concentrations, calcium efflux would produce some calcium etidronate complex in the lumen fluid: we suggest that this moiety is responsible for the enhanced salicylic acid absorption. At high etidronate concentrations, efflux still occurs and the calcium etidronate effect is still present, but the effect is overshadowed by the physical deterioration of the mucosal surface caused by the large concentration of uncomplexed etidronate.

The pre-complexed preparation of etidronate were either suspensions of large particle size (calcium) or colloidal dispersions (magnesium) of gel-like consistency. At low etidronate complex concentrations, the enhanced salicylic acid absorption is still seen due to the presence of the presumed active moiety, the etidronate complex. Solubility considerations would indicate that the same amount of complex is dissolved in the lumen fluid as when etidronate was administered alone and the complex formed in-vivo. The experimental results would seem to verify this.

At high etidronate complex concentrations, the decreasing absorption of salicylic acid with increasing exposure time may be due to physical phenomena since physiological damage was not observed. It is presumed that the portion of the etidronate complex dissolved is responsible for enhanced salicylic acid absorption, but that this effect is overshadowed. With calcium etidronate, the mucosal surface was visibly covered with small calcium etidronate particles which could easily be rinsed away when the segments were prepared for SEM examination. These particles would reduce the effective area available for absorption. This would seem reasonable since there was a smaller increase in the salicylic acid absorption half-life when the segment was pretreated with the magnesium complex which was colloidal and which remained dispersed. With magnesium etidronate, the increased salicylic acid absorption half-life may be due to an increased viscosity in the lumen fluid. The results indicate that such an effect may require at least 2 h to become significant.

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