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Dose-dependent absorption of disodium etidronate

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The gastrointestinal absorption of disodium etidronate (as [^{14}C]disodium etidronate) was investigated in the rat proximal jejunum in-situ. Studies using various initial concentrations of the drug suggested that etidronate absorption occurred by passive diffusion at initial concentrations below 0·08 M. At initial concentrations above 0·08 M, the rate of absorption was significantly greater than would be expected if passive diffusion was the only mechanism responsible for absorption. Etidronate absorption is not mediated by the carrier mechanism responsible for phosphate ion absorption.

Disodium etidronate (disodium 1-hydroxyethylidenediphosphonate), EHDP, or simply etidronate, is a diphosphonate that has been shown to be effective in reducing ectopic calcification and excessive bone resorption when chronically administered. It is presently being used clinically in the treatment of Paget's disease. Etidronate is highly ionized at physiological pH, having pK_a values of 1·7, 3·1, 7·5 and 11·5 (Grabenstetter et al 1967). As might be expected, 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. Other studies have reported similar findings (Recker & Saville 1973). Michael et al (1972) found less than 10% of the dose administered by intragastric cannula was absorbed in the rat, rabbit and monkey, and slightly greater than 10% was absorbed in the dog. Wasserman et al (1973) reported that the absorption of etidronate from ileal segments of the chick, in-situ, was 13% of the administered dose. However, little is known about the mechanism by which etidronate is orally absorbed.

We have examined the oral absorption mechanism of etidronate in the rat, in-situ. Experiments were carried out to determine the site of maximal etidronate absorption and if etidronate is absorbed by a passive or active process. Additionally, it was determined if the phosphate ion transport system influenced etidronate absorption.

Methods

Site of maximum etidronate absorption. Male, albino, Sprague-Dawley rats, 190–310 g were housed in wide mesh metal cages to minimize coprophagy and were fasted 14–16 h before the experiment. Water was freely available. Animals were anaesthetized using a modification of the technique reported by Youth et al (1973) and Shrewsbury et al (1982). After anaesthesia had been achieved, an abdominal mid-line incision was made, and a segment of the gastrointestinal tract cannulated as follows: stomach — the pyloric sphincter and the oesophagus, duodenum — the pyloric sphincter and 4 cm distal to the suspensory ligature which identifies the end of the duodenum with the bile duct ligated, jejunum — 4 cm distal to the suspensory ligature and 15 cm distal to that incision. The segment was rinsed with saline (NaCl 300 mmol kg^{-1}, pH 6·4) until the effluent was clear. Saline was allowed to remain in the segment for 15 min and was then expelled by air. A 0·12 M [^{14}C]etidronate solution was then instilled into the segment, and allowed to remain for 3 h. After which the segment was rinsed with 3–4 ml of saline, the washings collected in a preweighed scintillation vial, and the amount of [^{14}C]etidronate determined by liquid scintillation spectrometry (Tricarb, Model 3320). The washed segment was excised and placed in a preweighed scintillation vial. 15 ml of Soluene 100 (Packard) was added, and the vial was placed in a 50 °C oven for 24 h. After cooling, the vial was weighed to determine the amount of solubilized tissue, and the amount of [^{14}C]etidronate determined by liquid scintillation spectrometry.

During the experiment, each animal was kept warm with the use of a rheostatically controlled heating pad and an overhead work-light. The rectal temperature was monitored (Telethermometer, model 47) throughout the experiment and maintained at normal.

All solutions introduced into the intestine were 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 mmol kg^{-1}. The solutions were preheated to 37 °C by immersion in a circulating water bath.

Mechanism of etidronate absorption. The jejunal segment of the gastrointestinal tract was used to study the mechanism of etidronate absorption. Various initial concentrations of [^{14}C]etidronate, reflective of human therapeutic concentrations, were instilled in the segment and treated according to the procedure described above. In addition, a 22 gauge catheter was inserted into the jugular vein after anaesthesia. Sterile saline was

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infused at a rate of 1 ml h^{-1} to increase the production of urine. Urine was collected when spontaneously excreted during the experiment and directly from the bladder by hypodermic syringe at the end of 3 h. The urine was pooled in a preweighed scintillation vial and the amount of [^{14}C]etidronate determined.

The jejunal segment was also used to determine if etidronate absorption is mediated by the same carrier mechanism responsible for phosphate ion transport. Etidronate absorption was compared using 0·06 M [^{14}C]etidronate alone or with 0·12 M monobasic sodium phosphate.

Results

The percent of etidronate absorbed from the various segments of the gastrointestinal tract was calculated using the equation:

$$\text{Percent absorbed} = \frac{\text{dose} - \text{lumen} - \text{tissue}}{\text{dose}} \times 100$$

where 'dose' is the amount of [^{14}C]etidronate initially placed in the lumen of the gastrointestinal segment, 'lumen' is the amount of [^{14}C]etidronate remaining in the lumen fluid, and 'tissue' is the amount of [^{14}C]-etidronate in or bound to the wall of the gastrointestinal segment.

The average percent absorbed when 0·12 M [^{14}C]-etidronate was instilled in various gastrointestinal segments was 11·76% ± 15·52 (s.d.) from the stomach (n = 12), 32·46% ± 10·69 from the duodenum (n = 10), and 38·13% ± 10·87 from the jejunum (n = 12). Significantly more [^{14}C]etidronate was absorbed in either portion of the intestinal tract than from the stomach in the 3 h (*t*-test, $P < 0{\cdot}05$). There was no significant difference in the percent of [^{14}C]etidronate absorbed from the jejunum and duodenum.

The jejunal segment was exposed to various initial [^{14}C]etidronate concentrations for 3 h to investigate the mechanism responsible for [^{14}C]etidronate absorption. Table 1 summarizes the data. The amount of [^{14}C]-etidronate absorbed versus the initial instilled concentration is shown in Fig. 1. At low concentrations, the data fit a linear relation which passes through the origin;

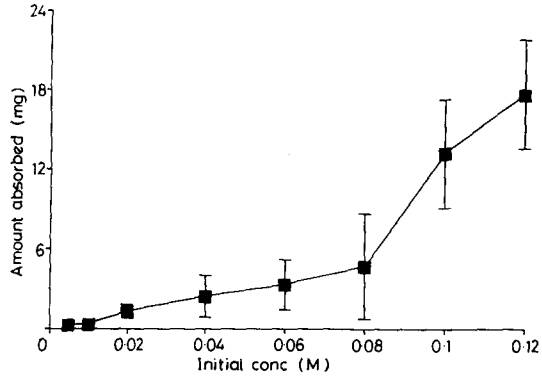

FIG. 1. Amount of [^{14}C]etidronate absorbed from rat jejunum, in-situ, versus initial concentration of [^{14}C]etidronate.

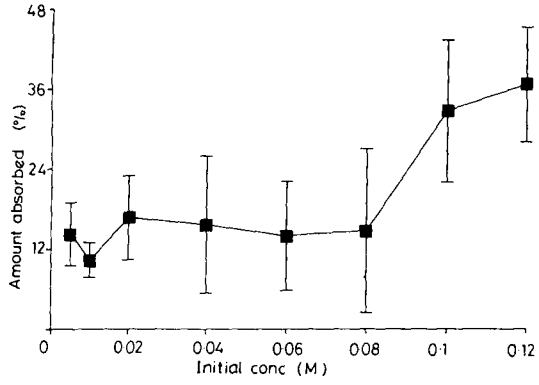

FIG. 2. Percent of [^{14}C]etidronate absorbed from rat jejunum, in-situ, versus initial concentrations of [^{14}C]etidronate.

however, the relation deviates markedly in a positive direction at initial concentrations above 0·08 M. The percent of etidronate absorbed as a function of the initial concentration is shown in Fig. 2. The plot indicates that the same percent of [^{14}C]etidronate is absorbed in 3 h at initial concentrations between 0·005 and 0·08 M. A marked positive deviation in the relation is again seen at initial concentrations above 0·08 M.

Table 1. Absorption of various concentrations of [^{14}C]etidronate from rat jejunum, in-situ.

Initial etidronate concn (M)	n	Absorbed (%)	Absorbed (mg)	Urine (%)	Jejunum (mg)
0·005	6	14·25 ± 4·73[a]	0·28 ± 0·09	2·24 ± 1·66	0·60 ± 0·11
0·01	5	10·34 ± 2·60	0·40 ± 0·11	2·27 ± 0·24	1·30 ± 0·22
0·02	9	16·66 ± 6·30	1·32 ± 0·51	4·56 ± 1·86	2·72 ± 0·35
0·04	4	15·57 ± 10·25	2·42 ± 1·56	3·58 ± 3·63	4·36 ± 1·08
0·06	10	13·83 ± 8·11	3·28 ± 1·86	3·60 ± 4·50	6·74 ± 1·25
0·06[b]	5	12·94 ± 3·31	3·06 ± 0·79	4·37 ± 1·16	8·91 ± 0·99
0·08	12	14·61 ± 12·22	4·66 ± 3·91	4·02 ± 3·30	9·00 ± 1·77
0·10	11	32·39 ± 10·53	12·98 ± 4·05	7·35 ± 4·08	12·13 ± 2·18
0·12	12	36·20 ± 8·53	17·43 ± 4·09	7·55 ± 6·14	13·49 ± 2·42

[a] Mean ± s.d. [b] 0·12 M monobasic sodium phosphate.

The data in Table 1 indicate that the urinary recovery of absorbed [^{14}C]etidronate was approximately 25%, and reflected the sudden increase in [^{14}C]etidronate absorption above 0·08 M. Similar urinary recovery results have been reported (Michael et al 1972), although the authors offered no explanation for the marked deviation at higher etidronate concentration.

Table 1 also shows an apparent linear increase in the amount of etidronate in or bound to the jejunal tissue with increasing initial concentrations. There is no positive deviation at initial concentrations above 0·08 M as found in the other data of Table 1. The phosphate ion from monobasic sodium phosphate had no effect on [^{14}C]etidronate absorption as the four parameters were not statistically different from 0·06 M [^{14}C]etidronate alone ($P > 0·05$).

Discussion

The anatomical site of maximum [^{14}C]etidronate absorption in-situ is the segment of jejunum just distal to the suspensory ligature. It was expected that more [^{14}C]etidronate would be absorbed from the intestine than the stomach because of the difference in surface area between the two tissues. The results also show that [^{14}C]etidronate absorption is essentially the same in the segments of the intestinal tract studied.

However, the percent of etidronate absorbed from the intestinal segments is greater than reported in earlier investigations. In several animal species (Michael et al 1972), approximately 10% of the administered etidronate dose was absorbed. In chick ileal segments (Wasserman et al 1973), 13% was absorbed. Both of these studies used etidronate concentrations of 0·008–0·02 M. Table 1 shows that approximately 13% of the instilled dose was absorbed in this range of etidronate concentrations, in good agreement with the earlier studies. However, 0·12 M [^{14}C]etidronate is in the range where a marked deviation was observed, and the enhanced etidronate absorption would explain the higher results found in this part of the investigation.

The mechanism of [^{14}C]etidronate absorption does not appear to be an active transport process. Such a process would produce a plot of the amount of etidronate absorbed versus etidronate concentration which was initially linear with a positive slope passing through the origin, and would eventually reach a plateau indicating some maximum amount absorbed. Also, etidronate does not appear to be absorbed by a passive diffusion process since such a plot would have a linear relation with a positive slope passing through the origin that would not reach a plateau with increasing etidronate concentrations (see Fig. 1). Plots of percent of etidronate absorbed versus etidronate concentration would be constant for passive transport processes, or declining for active transport processes (see Fig. 2). The linear increase in the amount of drug in or bound to the jejunal tissue at initial concentrations about 0·08 M implies that tissue binding or uptake is not a significant limiting phenomenon in etidronate absorption.

It has been reported that inorganic phosphate is actively transported in the proximal jejunum (Harrison & Harrison 1961). Because of the similarity in structure between inorganic phosphate and etidronate, the experiment was done to determine if inorganic phosphate influenced the absorption of etidronate. The results show that etidronate is not absorbed by the process responsible for the absorption of phosphate ion.

Etidronate binds calcium both in solution and on crystalline surfaces (Francis 1969; Grabenstetter & Cilley 1971). A recent report has shown that 0·08 M etidronate in the in-situ rat jejunum caused a time dependent histological disruption in the mucosal surface (Shrewsbury et al 1982). It was proposed that etidronate removed mucosal calcium via chelation which lead to the structural alterations. It would be reasonable to assume that etidronate concentrations of 0·10 and 0·12 M would cause similar or more dramatic alterations due to the longer exposure time and higher concentration.

It may be concluded that etidronate is not actively transported in the in-situ jejunal segment. It does appear to be absorbed by passive diffusion at concentrations of 0·08 M or less. At concentrations above 0·08 M structural alterations in the mucosal membrane probably occur which evidently allow more etidronate to be absorbed than would be predicted in a passive diffusion process. Thus etidronate has a complex absorption mechanism which is dose dependent.

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