J. Pharm. Pharmacol. 1989, 41: 339–341
Communicated August 9, 1988

COMMUNICATIONS

3-Amino-1-hydroxypropylidene-1,1-diphosphonate (APD): a novel enhancer of rectal cefoxitin absorption in rats

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Abstract—The promoting action of the calcium chelating compound EDTA on intestinal drug absorption is supposed to be based on Ca^{2+} depletion, inducing widening of tight junctions. The aim of the present study was to evaluate the effects of the calcium-binding agent 3-amino-1-hydroxypropylidene-1,1-diphosphonate disodium salt (APD) on rectal cefoxitin absorption in rats. The extent of rectal cefoxitin absorption was enhanced by 0·5 to 6% w/v of APD, on rectal infusion as well as on bolus delivery, the latter regimen tending to result in lower bioavailabilities. A maximal cefoxitin bioavailability of 85 ± 10% was achieved by infusion with 4% w/v of APD, compared with 14 ± 12% without APD.

The enhancing effects of calcium-binding agents, in particular disodium EDTA, on intestinal drug absorption have been reported in various studies. In concentrations of 1 to 7% disodium EDTA proved to enhance colonic absorption of e.g. cefoxitin (Nishihata et al 1985), inulin (Suzuka et al 1987) and fosfomycin (Ishizawa et al 1987) in rats. Its mechanism of action was suggested to be based on calcium complexation, inducing paracellular absorption enhancement (Cassidy & Tidball 1967). However, the promoting effect of EDTA on intestinal drug absorption appears to be accompanied by a damaging effect on mucosal integrity. Concentrations of 0·8 to 1% induced a reversible loss of rectal epithelial cells (Nakanishi et al 1983) and severe damage of small intestinal epithelium in rats (Nadai et al 1972). Furthermore, jejunal blood loss was observed in dogs (Tidball & Lipman 1962). These observations indicate that other compounds with a similar mechanism of action as EDTA but with a more benign effect on mucosal integrity are required.

Because of its ability to bind calcium and magnesium in hard water, 3-amino-1-hydroxypropylidene-1,1-diphosphonate (APD) has been used as an additive to detergents, to prevent "bathtube rings" (Francis & Centner 1978). Currently, this agent is under extensive investigation as a potential drug for the treatment of Paget's disease of bone and malignant hypercalcaemia (Mautalen et al 1984; Harinck et al 1987; Van Holten-Verzantvoort et al 1987). In these studies APD is used orally in a dose range of 300 to 600 mg daily. The oral bioavailability of APD has been estimated at 0·2% (Reitsma et al 1983).

Considering its calcium binding properties, APD was hypothesized to be a potential paracellular absorption promoting agent. The aim of the present study was to investigate the effects of co-administration of APD on rectal absorption of the poorly absorbed model drug cefoxitin in rats. As rate of delivery proved to affect the absorption promoting action of medium chain glycerides (Van Hoogdalem et al 1988a), and salicylate (Van Hoogdalem et al 1988b), the effects of APD were evaluated using two delivery rates.

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Materials and methods

Chemicals. 3-Amino-1-hydroxypropylidene-1,1-diphosphonate disodium salt (APD) was obtained as a gift of Dr P. Vermeij (University Hospital, Leiden, The Netherlands). Cefoxitin sodium (Mefoxin) was a gift from Merck, Sharp & Dohme (Haarlem, The Netherlands). Cefazolin sodium (Kefzol) was purchased from Eli Lilly & Co. (Nieuwegein, The Netherlands). All reagents used were analytical grade.

Animals. Male Wistar rats of laboratory breed, 170–200 g, were used. Food was withdrawn 16 h before the experiments, whereas water was freely accessible throughout the experiment. Each rat participated once.

Drug solutions. For i.v. administration a solution of cefoxitin sodium (15 mg mL^{-1}), made isotonic by addition of sodium chloride, was used. For rectal administration unbuffered aqueous solutions of APD (0 to 6% w/v) with or without cefoxitin sodium 15 mg mL^{-1} were used. The pH of these solutions was in the range between 5·5 and 6·5, and the ionic strength varied from 0·03 to 0·68, depending on the concentration of APD.

Drug administration and blood sampling. Drug administration and blood sampling were performed as described previously by Van Hoogdalem et al (1988b). In short, 200 μL of drug solution were infused i.v. or rectally over 32 min, or were delivered as rectal bolus in 24 s. Arterial blood samples of 100 μL were collected at regular intervals.

Drug assay. Cefoxitin sodium was assayed in haemolysed blood samples by RP-HPLC as described previously by Van Hoogdalem et al (1988b). Cefazolin was used as internal standard.

Data analysis. Areas under the individual blood concentration-time curves from $t = 0$ to the last sampling point were calculated with the linear-logarithmic trapezoidal rule. I.v. curves were extrapolated to infinity using the individual elimination rate constants, whereas rectal curves were extrapolated using the mean elimination rate constant for i.v. administration, because of an irregular apparent elimination phase of some of the curves.

Systemic cefoxitin clearance was calculated as D/AUC, D referring to the i.v. dose of cefoxitin sodium and AUC representing the total area under the curve. The Wilcoxon rank sum test was used for statistical evaluation of the results. Only groups containing at least six individuals were compared, using the results of rectal delivery without enhancer as control. A comparative error rate of 0·05 was maintained.

Results

Intravenous infusion of 3 mg of cefoxitin sodium resulted in a mean AUC (±s.d.) of 430±70 μg min mL^{-1} and a mean cefoxitin clearance (±s.d.) of 7·1±1·1 mL min^{-1} (n=6) was calculated. Concurrent rectal infusion of 1% w/v of APD resulted in comparable values of 540±80 μg min mL^{-1} and 5·6±0·8 mL min^{-1} (n=3), excluding a relevant interaction of rectally delivered APD with cefoxitin elimination kinetics.

On rectal administration without enhancer, cefoxitin proved to be poorly absorbed after infusion (Fig. 1) as well as after bolus delivery (Fig. 2). With both delivery regimens, co-administration

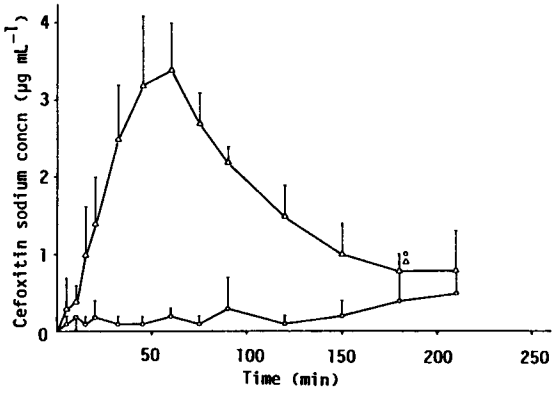

FIG. 1. Mean blood levels of cefoxitin sodium ± s.d. during and after rectal infusion of 3 mg of cefoxitin sodium without (○, n=6) and with 4% w/v of APDNa$_2$ (△, n=6)

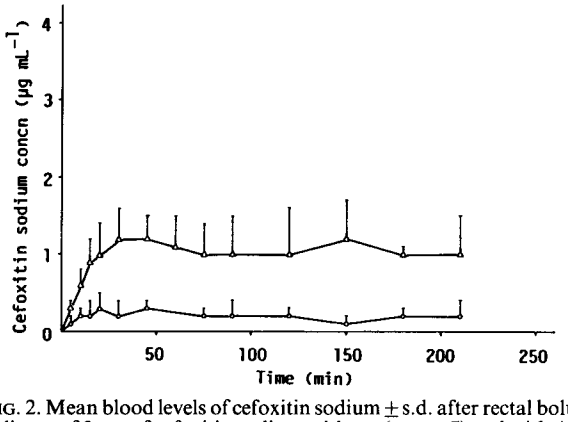

FIG. 2. Mean blood levels of cefoxitin sodium ± s.d. after rectal bolus delivery of 3 mg of cefoxitin sodium without (○, n=7) and with 4% w/v of APDNa$_2$ (△, n=3)

of 4% of APD considerably increased cefoxitin blood levels. The effect on rectal infusion (Fig. 1) was more pronounced than the effect on bolus delivery (Fig. 2). Rectal infusion with 4% of APD resulted in a mean t_{max} (±s.d.) of 55±8 min, whereas on bolus delivery a relatively flat profile was observed (Fig. 2).

The concentration-effect profile of APD showed a promoting action of 0·5 to 6% w/v of APD on rectal cefoxitin bioavailability after both delivery regimens (Fig. 3). A maximal mean cefoxitin bioavailability (±s.d.) of 85±10% was obtained by rectal infusion with 4% w/v of APD. On infusion with 1% w/v of diphosphonate comparable values were obtained, but variability proved to be larger. At all APD concentrations tested, bolus delivery tended to result in lower bioavailabilities than with infusion (Fig. 3).

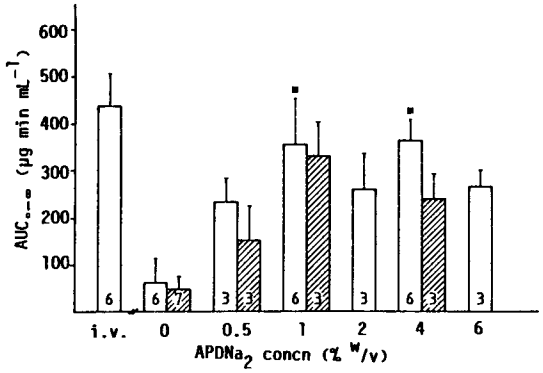

FIG. 3. Histogram of the mean AUC (0−inf) ± s.d. of cefoxitin sodium on i.v. infusion and after rectal administration of 3 mg of cefoxitin sodium as infusion (open bars) and as bolus (hatched bars) with various concentrations of APDNa$_2$; *: significantly different from infusion without enhancer ($P<0·05$, Wilcoxon rank sum test)

Discussion

On rectal infusion as well as on bolus delivery APD exerted a substantial promoting effect on rectal cefoxitin bioavailability. The effective concentration range of APD as assessed in this study corresponds well with the EDTA concentration range of 1–7% enhancing colonic drug absorption (Nishihata et al 1985; Ishizawa et al 1987; Suzuka et al 1987). As diphosphonates are able to form stable complexes with calcium and magnesium ions (Irani & Moedritzer 1962) it is tempting to suggest a paracellular absorption promoting mechanism of APD by binding of calcium, as described for EDTA (Cassidy & Tidball 1967).

Remarkably, rectal infusion resulted in concentration-time curves with a clear c_{max} (Fig. 1), whereas on bolus delivery a more protracted absorption phase was observed (Fig. 2). The latter observation is in contrast with the effects of bolus-delivered salicylate (Van Hoogdalem et al 1988b) and medium chain glycerides (Van Hoogdalem et al 1988a) on rectal cephalosporin absorption, both inducing a sharp increase in blood concentrations, resulting in relatively short t_{max}-values.

This behaviour of bolus-delivered APD might be caused by a reversible interaction of APD with contents of the intestinal lumen, resulting in attenuation of the initial effects on blood levels (Fig. 2). Since on rectal infusion drug and enhancer are delivered to a smaller mucosal area in a longer period of time (Van Hoogdalem et al 1988b), the interaction with luminal contents is expected to be less, resulting in a stronger initial effect of APD (Fig. 1). It is conceivable that this interaction also causes the slightly lower cefoxitin bioavailabilities observed after bolus delivery, compared with infusion (Fig. 3). In addition, a modifying action of the amount of enhancer delivered per mucosal area may be involved, as described for medium-chain glycerides (Van Hoogdalem et al 1988a). As on rectal infusion the amount of enhancer is delivered to a smaller mucosal area, the amount per unit of area will be higher and, consequently, the effect may be stronger.

As no data are available on the safety of APD as rectal absorption promoter, its action on rectal mucosal integrity requires assessment.

The authors wish to thank Dr P. Vermeij, Ph.D., Pharm.D. (University Hospital, Leiden, The Netherlands) and Merck, Sharp & Dohme (Haarlem, The Netherlands) for generously supplying APD and cefoxitin, respectively.

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J. Pharm. Pharmacol: 1989, 41: 341–342
Communicated February 29, 1988

Continuous shear rheometry of o/w emulsions; control of evaporation in cone/plate geometry

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Abstract—Volatile solvents may evaporate during cone/plate viscometry so that false rheograms develop. This surface evaporation was prevented in a cod-liver oil-in-water emulsion stabilized with zanthoxylum gum by layering a film of cod-liver oil on the exposed surface of the emulsion test sample. The oil layer effectively prevented evaporation and did not alter significantly the rheological behaviour of the test material.

A disadvantage of a cone and plate viscometer is the evaporation of solvent from the exposed surface of the material under test. Such evaporation can increase because of the heat generated at high shearing speeds (Mckennell 1956) or because of long sweep-times (Davis et al 1968; Barry 1974). As a result, for materials such as those which contain gums, the apparent viscosity increases and a clockwise hysteresis loop forms under cyclic testing even for time independent materials (Davis et al 1968).

Zanthoxylum gum is the gummy exudate from the tree, *Zanthoxylum tessmannii* (Engl.) Waterm., family Rutaceae. In the course of studying the emulsifying properties of the gum, cod-liver oil-in-water emulsions were prepared. A Ferranti-Shirley cone and plate viscometer was used to determine the viscosity of the emulsions. The emulsions exhibited apparent shear thickening with hysteresis as indicated by a clockwise loop. The area of the loop and the apparent viscosity at maximum shear rate, however, increased significantly when the sample was left for some time on the viscometer and when the anti-evaporation hood was not used. It was then suspected that the clockwise hysteresis loop probably arose from evaporation of water from the exposed surface of the sample under test, which increased the apparent viscosity of the sample with time.

Method

A 50% w/w emulsion of cod-liver oil-in-water, stabilized with 4·17% w/w zanthoxylum gum was prepared in a stainless steel cup using the Silverson mixer. Mixing was for 5 min and the temperature of the emulsion was not allowed to exceed 25°C during mixing by circulating water at 12°C around the stainless steel cup. The emulsion was passed 5 times through the "Q.P." hand homogenizer; packed in glass bottles and stored at 25°C for 2 days. A Ferranti-Shirley viscometer fitted with a cone of radius 3·5 cm and a cone angle of 0·005852 radians was used to study the flow properties of the emulsions. Water at 25 ± 0·1°C was circulated through the plate. A maximum speed of 100 rev min^{-1} was used. About 1 mL of the emulsion was used for each test. With a sweep time of 600 s, the cone and plate assembly was employed, respectively, without the anti-evaporation hood, with the anti-evaporation hood, and with cod-liver oil carefully layered on the exposed surface of the extruded emulsion between the cone and plate, using a 2 mL syringe with a needle (Fig. 1).