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Report

Absorption Enhancement of Rectally Infused Cefoxitin Sodium by Medium-Chain Fatty Acids in Conscious Rats: Concentration–Effect Relationship

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The objective of this study was to assess the relative absorption promoting potency in terms of concentration–effect relationships of the medium-chain fatty acids hexanoic acid, octanoic acid, decanoic acid, and dodecanoic acid in conscious rats, using cefoxitin sodium as the rectally delivered model compound. Rectal uptake of cefoxitin, which was absorbed to a limited extent without enhancer (30 ± 25%), proved to be significantly enhanced by 2.0 *M* sodium hexanoate, 0.69 *M* sodium octanoate, and 0.22 *M* sodium decanoate, resulting in mean bioavailabilities of 102 ± 24, 68 ± 25, and 68 ± 10%, respectively. Thus, increasing fatty acid chain length results in increased enhancing potency from hexanoic acid to decanoic acid. However, using dodecanoate a statistically significant effect could not be reached, because of its limited aqueous solubility. Optimal chain length for absorption enhancement by medium-chain fatty acids is probably determined by interplay of intrinsic effects on mucosal permeability and solubility of the medium-chain fatty acid.

KEY WORDS: rectal absorption enhancement; cefoxitin; fatty acids.

INTRODUCTION

The therapeutic use of very polar drugs, e.g., insulin, peptide drugs, and several cephalosporin antibiotics, is generally restricted to parenteral delivery because of poor passage through epithelial membranes. In order to improve the passage of epithelia, extensive research programs are undertaken to develop more appropriate delivery methods, including intestinal delivery by using absorption enhancing agents. The absorption of the cephalosporin antibiotic cefoxitin was recently reported to be promoted by fractionated coconut oil and by fatty acid emulsions (1), sodium salicylate and Brij 35 (2), acylcarnitines (3), and EDTA and polyoxyethylene-23-lauryl ether (4). Of these, fatty acids are considered to be relatively nontoxic, because of their physiological presence in the gastrointestinal tract. A limited number of studies concerning the effect of fatty acids on drug absorption has been published and reviewed by Muranishi (5). Fatty acids proved to enhance rectal absorption of sodium ampicillin from suppositories (6) and solutions (7) in rats. At the dose tested (0.02 mmol/kg), sodium decanoate proved to be the most effective fatty acid (6). Naloxone penetration through human skin was enhanced by saturated medium-chain fatty acids *in vitro* (8). At the concentration tested (10%), dodecanoic acid showed the strongest effect.

The experimental design of these studies did not allow a comparison of the relative potencies of the fatty acids studied. The effects were determined at a single fatty acid concentration and the use of suppositories (6) and suspensions (8) introduced additional variables in the process of penetration enhancement. The aim of the present study was to establish the relationship between the concentration and the rectal absorption enhancing effect of several medium-chain fatty acid sodium salts in aqueous solutions, using cefoxitin sodium as the poorly absorbed model compound.

MATERIALS AND METHODS

Chemicals

Cefoxitin sodium (Mefoxin) was a gift from Merck, Sharp & Dohme, Haarlem, The Netherlands. Cefazolin sodium (Kefzol) was obtained from Eli Lilly & Co., Utrecht, The Netherlands. Hexanoic acid (98%) was purchased from E. Merck AG, Darmstadt, West Germany. Octanoic acid (99.5–100%) was obtained from Janssen Chimica, Beerse, Belgium. Decanoic acid (99–100%) and dodecanoic acid (99–100%) were supplied by Sigma Chemical Co., St. Louis, Mo. Ethyl acetate (Baker Chemicals, Deventer, The Netherlands) was distilled before use. All other reagents used were of analytical grade.

Drug Solutions

For i.v. administration a solution of cefoxitin sodium (15 mg/ml) in water was used, made isotonic by the addition

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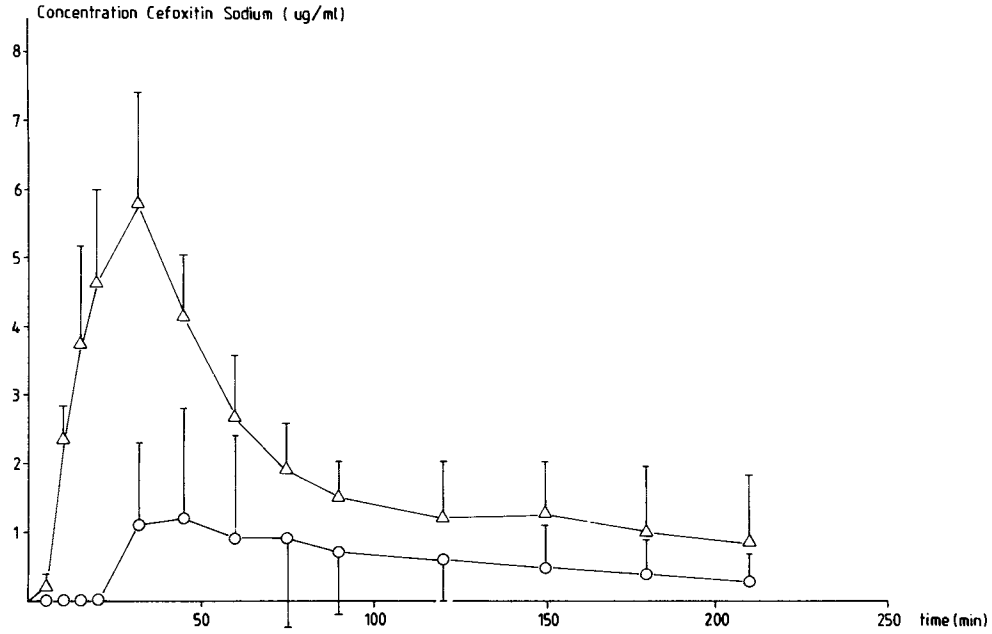

Fig. 1. Mean blood levels of cefoxitin sodium ± SD after rectal infusion of 3 mg of cefoxitin sodium in 200 μl of sodium chloride solution (○; $N = 6$) and in 200 μl of 2.0 M sodium hexanoate solution (△; $N = 6$).

of sodium chloride. Solutions for rectal administration, containing fatty acid with or without cefoxitin, were prepared by neutralizing the mixture of fatty acid and water by adding NaOH, 1 or 10 N ($6.5 < pH < 8.5$), whereupon cefoxitin sodium (15 mg/ml) was added. The ionic strength was adjusted to 0.75 by adding sodium chloride. The ionic strength of solutions with a fatty acid concentration higher than 0.69 M was not adjusted, because the high concentration of fatty acid resulted in an ionic strength higher than 0.75. The maximal ionic strength was 2.53, occurring in solutions containing 2.5 M sodium hexanoate. Dodecanoate solutions were not adjusted, because the addition of NaCl precipitated sodium dodecanoate. The ionic strength of these solutions did not exceed 0.06.

Animal Experiments

Male Wistar rats of laboratory breed, weighing 175–200 g, were used. Intravenous and rectal infusion of cefoxitin sodium solutions and blood sampling were performed as described previously (9).

Drug Assay

Cefoxitin sodium was assayed in hemolysed blood by reversed-phase high-performance liquid chromatography as described previously (9).

Data Analysis

The areas under the individual blood concentration–time curves were calculated using the linear-logarithmic trapezoidal rule, as described previously (9). Systemic clearance of cefoxitin sodium was calculated as D/AUC, where D is the administered i.v. dose of cefoxitin sodium and AUC is the total area under the curve. The Wilcoxon rank-sum test was used for statistical evaluation of the results. AUC values were compared to control, maintaining a comparisonwise error rate of 0.05. Using the Bonferroni inequality (10), a multicomparison analysis of the maximal effects of the fatty acids was performed, maintaining an experimentwise error rate of 0.05.

RESULTS

Intravenous infusion of 3 mg of cefoxitin sodium resulted in a mean AUC ± SD of 433 ± 74 μg · min/ml, and a systemic clearance value ± SD of 7.1 ± 1.1 ml/min ($N = 6$) was calculated. Concurrent rectal infusion of 2.0 M sodium hexanoate, 0.69 M sodium octanoate, and 0.22 M sodium decanoate resulted in clearance values of 5.4 ± 1.0 ml/min ($N = 3$), 5.5 ± 0.7 ml/min ($N = 3$), and 6.6 ± 1.1 ml/min ($N = 3$), respectively, excluding an effect of rectally delivered medium-chain fatty acids on cefoxitin elimination kinetics.

Rectal infusion of cefoxitin sodium without absorption enhancer resulted in relatively low blood concentrations (Fig. 1) and AUC values (Fig. 2). Coadministration of increasing concentrations of sodium hexanoate did not result in significantly higher AUC values, until a concentration of 2.0 M was used (Figs. 1 and 2). At a concentration of 2.5 M, no further increase was observed. Using sodium octanoate, a significant increase in AUC was obtained at 0.69 M (Fig. 2). Coadministration of cefoxitin with increasing concentrations of sodium decanoate resulted in enhanced AUC values at 0.22 M (Fig. 2). Infusion with 0.22 M decanoate resulted in significantly lower AUC values, compared with 2.0 M hexanoate. Sodium dodecanoate, applied at concentrations up to 0.03 M, was unable to cause a significant increase in AUC (Fig. 2). Due to the low solubility of sodium dodecanoate the effect of higher concentrations could not be evaluated.

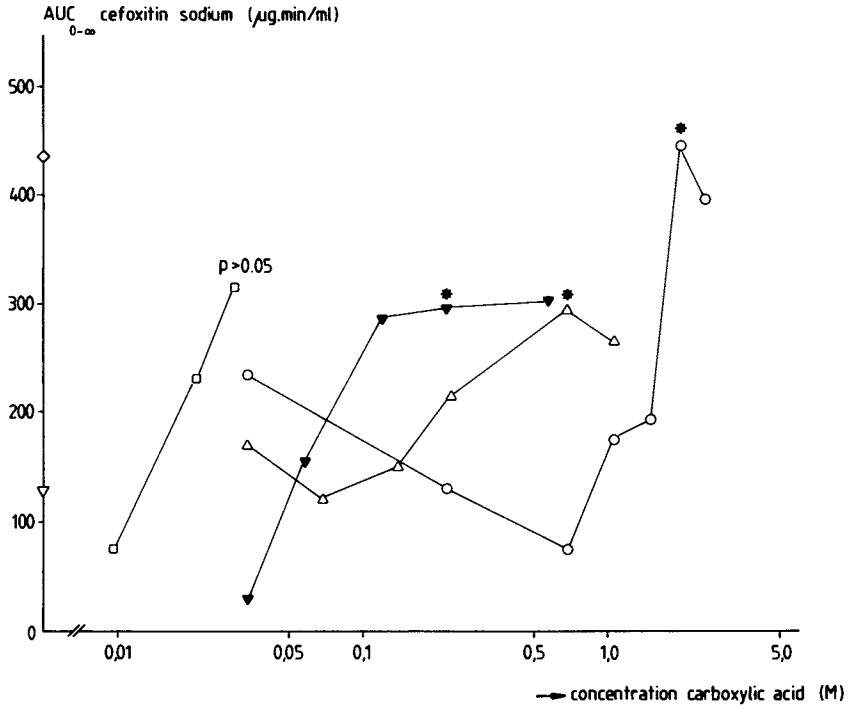

Fig. 2. Graph of the mean AUC ± SD of cefoxitin sodium after i.v. infusion of 3 mg of cefoxitin sodium (◇) and after rectal infusion without enhancer (▽) and with various concentrations of hexanoic acid (○), octanoic acid (△), decanoic acid (▼), and dodecanoic acid (□) as sodium salt. Symbols represent the mean values of three to six rats. (*) Significantly different from control value without enhancer ($P < 0.05$, Wilcoxon rank-sum test).

DISCUSSION

Sodium salts of the medium-chain fatty acids hexanoic acid, octanoic acid, and decanoic acid proved to exert an enhancing action on the absorption of rectally infused cefoxitin. The potency of the fatty acids, defined in terms of concentration giving a statistically significant increase in cefoxitin bioavailability, increased with increasing chain length. Maximal effects of octanoate and decanoate proved to be comparable, whereas that of sodium hexanoate was stronger, resulting in complete absorption of the model compound. Because the sodium ion concentration has been shown to potentiate the effect of absorption enhancers (11,12), it is conceivable that the high ionic strength of the hexanoate solution contributes to the observed maximal effect. Compared with sodium decanoate a further increase in chain length results in a low water solubility, thus limiting the assessment of the concentration–effect relationship of sodium dodecanoate to relatively low concentrations. Probably the interplay of intrinsic effects on mucosal permeability and water solubility resulted in sodium decanoate being the most effective promoter of rectal ampicillin absorption from suppositories (6).

Both a transcellular and a paracellular absorption enhancing mechanism may bring about the promoting effect of medium-chain fatty acids on drug absorption. Sallee and Dietschy reported increased intestinal uptake of medium chain fatty acids with increasing chain length (13). During the uptake process fatty acids seem to increase transcellular permeability by perturbing membrane lipids (14–16), their disordering potency increasing with chain length (15). On the other hand, increased paracellular enhancing effect with increasing chain length could be elicited by an increasing affinity for calcium (17), resulting in widening of tight junctions.

Sodium salts of fatty acids are able to form micelles in aqueous solutions. The critical micelle concentration (CMC) of these systems decreases with increasing chain length (18). The effective concentrations of hexanoate, octanoate, and decanoate reasonably correspond with their theoretical CMC at 20°C, being 2.2, 0.56, and 0.14 *M*, respectively, calculated according to Davis *et al.* (18). True CMC values in the rectum will differ from calculated values, because of influences of temperature, luminal contents, and solute absorption on micelle formation. These results are in agreement with the model describing an increase in the absorption enhancing effect with increasing concentrations of free enhancer. Above the CMC the free monomer concentration will remain constant, resulting in a plateau of the concentration–effect curve, provided that the model compound is not included into micelles (19).

The present study demonstrates the effectiveness of medium-chain fatty acids as absorption promoting agents. Further studies should be directed to optimization of formulation parameters such as physicochemical properties of the vehicle and ionic strength in relationship to properties of the individual fatty acid. Also, the rate of delivery could be an important parameter of the absorption enhancing effect of medium-chain fatty acids, as was observed when using sodium salicylate (9) and medium-chain glycerides (20) as ab-

sorption promoters. The effect of the delivery rate on the absorption enhancing action of fatty acids will be the subject of further studies.

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