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The influence of components on the rectal absorption of cefazolin in rats

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Abstract—A study has been made to identify the component(s) responsible for the absorption-promoting effect of MGK medium chain glyceride preparation commercially available as a mixture of glyceryl-1-monooctanoate, glyceryl-1,3-dioctanoate, glyceryl-1,2-dioctanoate, glyceryl trioctanoate, octanoic acid and glycerol. The action of the individual constituents has been evaluated on the rectal absorption of cefazolin in conscious rats. The results indicate that the action of MGK can be completely explained by the effect of glyceryl-1-monooctanoate, which both enhanced the extent and rate of cefazolin uptake.

The medium chain glyceride preparation MGK (Nikho, Tokyo, a commercially available mixture of glyceryl-1-monooctanoate, glyceryl-1,2-dioctanoate, glyceryl-1,3-dioctanoate, glyceryl trioctanoate, glycerol and octanoic acid) has been reported to be an effective enhancer of the absorption of cefmetazole sodium from the rat rectum (Sekine et al 1984) and from various intestinal segments in dogs (Sekine et al 1985a). This effect was increased by coadministration of non-ionic surfactants (Sekine et al 1985c). Higaki et al (1987) reported on enhancing effect of the preparation on the intestinal absorption of phenol red in rats. The reported low oral acute toxicity of MGK and the absence of local irritation (Sekine et al 1985b) suggest that a preparation is a potentially useful enhancer of intestinal absorption for poorly absorbed drugs, e.g. peptides, proteins and antibiotics.

To better understand the absorption-enhancing action of MGK and to further develop absorption enhancing compounds, it is necessary to elucidate to what extent each of the components contribute to the effect of the mixture. Using the separated glycerides from the preparation as well as mixtures of several commercial preparations, Sekine et al (1984) observed that the action of MGK could in part be ascribed to glyceryl monooctanoate. It was suggested that a certain component ratio of mono-, di- and trioctanoate was of primary importance for the promoting effect (Sekine et al 1984). However, the results of that study are not unequivocal, because the comparisons were made with mixtures which differed in concentration of more than one component. Furthermore in the study of the effects of the separate glycerides, it was not reported which isomers of mono- and diglycerides had been used. The fact that glyceryl-1,2-dioctanoate could affect membrane permeability by stimulating phosphorylation (Eichberg et al 1986), illustrates the importance of studying the effects of the individual isomers present in MGK.

Our aim was to evaluate the effect of the individual components of the preparation on the rate and extent of rectal absorption of the polar model compound cefazolin sodium in conscious rats and thereby identify the component(s) responsible for the absorption promoting action.

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Materials and methods

Chemicals. MGK was a gift from Nikko Chemicals Co. Ltd. (Tokyo, Japan) and contained glyceryl-1-monooctanoate 55–57% w/v, glyceryl-1,2-dioctanoate 9% w/v, glyceryl-1,3-dioctanoate 20% w/v, glyceryl trioctanoate 3% w/v, octanoic acid 3% w/v and glycerol 8% w/v (Manufacturer's data). Glyceryl-1-monooctanoate was a gift from Tramedico (Weesp, The Netherlands). Glyceryl-1,2-dioctanoate, glyceryl-1,3-dioctanoate and glyceryl trioctanoate were obtained from Sigma Chemical Co. (St. Louis, USA). Octanoic acid was from Janssen Chimica (Beerse, Belgium), glycerol was purchased from J. T. Baker Chemicals B.V. (Deventer, The Netherlands). Cefazolin sodium (Kefzol) was a gift from Eli Lilly Nederland (Utrecht, The Netherlands), cefoxitin sodium (Mefoxin) was a gift from Merck, Sharp & Dohme (Haarlem, The Netherlands). All chemicals used were of analytical grade. Ethyl acetate was distilled before use.

Animals. Male Wistar rats of laboratory breed, 170–200 g, were used. The rats were fasted for 16 h before the experiments, but water was freely available. Experiments were performed in groups of 5 to 8 animals.

Drug preparations. For i.v. infusion a solution of cefazolin sodium 15 mg mL^{-1} was made isotonic by the addition of sodium chloride.

For rectal administration without enhancer, a solution was used containing cefazolin sodium 15 mg mL^{-1} in 0·067 M phosphate buffer pH 7·4. The preparation with MGK contained cefazolin sodium 15 mg mL^{-1} MGK: water (13:1 w/w). Preparations of the individual components of MGK contained cefazolin sodium 15 mg mL^{-1} and the component, at a percentage corresponding to that in the MGK:water mixture containing MGK, glyceryl-1-monooctanoate, octanoic acid or glycerol were clear which octanoic acid was dissolved by

addition of M NaOH to pH < 8. The triglyceride and diglycerides were immiscible with water, resulting in o/w emulsions on whirlmixing with water. The emulsion of glyceryl-1,2-dioctanoate was prepared immediately before administration, to prevent loss of compound by isomerization during recovery.

Drug administration and blood sampling. Drug administration and blood sampling were as described by Van Hoogdalem et al (1988). The preparation of the drug (200 μL) was delivered by constant rate infusion for 32 min into the right jugular vein, or into the rectum, at a distance of 1 cm from the anus. For intravenous drug administration, a polyvinyl chloride cannula (length 125 cm, i.d. 0·5 mm, o.d. 1 mm) containing the preparation to be delivered, was used. Because of its high viscosity, the preparation containing glyceryl-1-monooctanoate was delivered via a Teflon cannular of larger internal diameter (0·9 mm). Blood samples (100 μm) were taken from a cannulated carotid artery at regular intervals after starting the experiment.

Assay of cefazolin sodium. Cefazolin sodium was quantified in arterial blood samples by reversed phase high performance liquid chromatography as described by Van Hoogdalem et al (1988). Cefoxitin sodium (24 μg mL^{-1}) was used as internal standard.

Data analysis. The area under the individual blood concentration time curve was calculated using the linear-logarithmic trapezoidal rule. Bioavailabilities were calculated as $(AUC_{rect}/AUC_{i.v.}) \times (D_{i.v.}/D_{rect}) \times 100\%$, D being the dose of cefazolin sodium, the subscripts $_{rect}$ and $_{i.v.}$ referring to rectal and i.v. infusion, respectively.

Statistical moment theory (Gibaldi & Perrier 1982) was used to calculate mean residence time (MRT) after i.v. and rectal administration of cefazolin sodium. The mean absorption time after rectal delivery was calculated as the difference between the mean MRT after i.v. infusion and the individual MRT after rectal infusion.

Comparison of results between groups was by the Wilcoxon rank sum test, maintaining an error-rate of 0·05.

Results and discussion

Rectal infusion of cefazolin in phosphate buffer resulted in a low mean cefazolin bioavailability (±s.d.) of 17±14%, which was significantly enhanced by coadministration of MGK and glyceryl-1-monooctanoate (GMO) to 104±7% and 108±20%, respectively (Fig. 1). Coadministration with other components of MGK did not result in significantly enhanced bioavailabilities (Fig. 1).

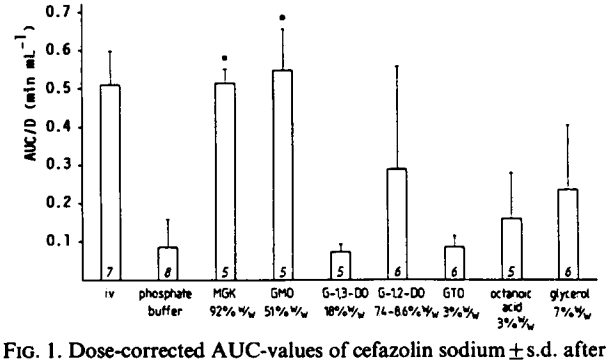

FIG. 1. Dose-corrected AUC-values of cefazolin sodium ± s.d. after i.v. infusion and after rectal infusion of 3 mg of cefazolin sodium in phosphate buffer, with MGK and with the individual MGK -components glycerl-1-monooctanoate (GMO), glyceryl-1,3-dioctanoate (G-1,3DO), glyceryl-1,2-dioctanoate (G-1,2-DO), glyceryl trioctanoate (GTO), octanoic acid and glycerol. *Significantly different from control infusion in phosphate buffer ($P < 0·05$, Wilcoxon rank sum test).

GMO significantly reduced the mean absorption time (MAT) of cefazolin, compared with delivery in phosphate buffer or with MGK, and MGK did not have a statistically significant effect, although a decreasing trend was observed (Fig. 2). Coadministration with other components of MGK resulted in MAT-values which were comparable with those after delivery in phosphate buffer and which were significantly longer than the mean MAT-valuers obtained after co-delivery with MGK (Fig. 2).

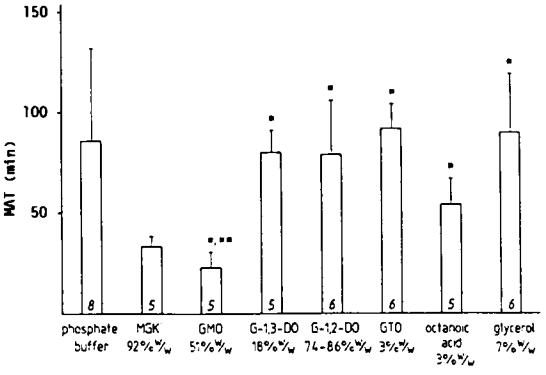

FIG. 2. Mean absorption time (MAT) values of cefazolin sodium after rectal infusion of 3 mg of cefazolin sodium in phosphate buffer, with MGK and with the individual components of MGK (abbreviations as in Fig. 1). * Significantly different from infusion with MGK; ** Significantly different from infusion in phosphate buffer $P < 0·05$, Wilcoxon rank sum test).

The results show that GMO enhanced cefazolin absorption as effectively as MGK. Its effect on the absorption rate was even greater. This indicates that the other components in MGK are capable of interfering with the action of GMO on cefazolin absorption rate, possibly by reducing the rate of release of cefazolin or GMO from MGK solution.

Other components of MGK did not exert a statistically significant action on rate or extent of cefazolin absorption. However, compared with delivery in phosphate buffer, a trend of increased bioavailabilities was observed with glyceryl-1,2-dioctanoate, octanoic acid and glycerol, suggesting that higher concentrations of these components may effectively enhance the extent of cefazolin absorption. The absorption-promoting properties of fatty acids have been discussed by Nishimura et al (1985), who reported a promoting action of 4·4% octanoic acid sodium salt on rectal absorption of ampicillin.

The response to coadministration of the drug with glyceryl-1,2-dioctanoate displayed a large inter-animal variability: from negligible to maximal (Fig. 1). Variation in release of cefazolin and glyceryl-1,2-dioctanoate from the inhomogenous system, and erratic penetration of the unstirred water layer by the poorly water soluble diglyceride may have partly contributed to this variable performance. The addition of an emulsifying agent may allow the preparation of a stable emulsion and could facilitate the passage of the diglyceride through the stagnant water layer, thus reducing variability. However, the applicability of glyceryl-1,2-dioctanoate as possible absorption enhancer will be limited by the poor stability of 1,2-diglycerides (Dueul 1951) and by its relatively high cost.

The results indicate that the effect of MGK on rectal cefazolin absorption may be completely accounted for by the action of the component GMO. However, an action of other MGK constituents, which are inactive as single components, on glyceryl-1,2-dioctanoate cannot be excluded. Contrary to the reported action of MGK on cefmetazole absorption (Sekine et al 1984), in the present study no indications were found for a potentiating action

of a particular component ratio. This discrepancy might be attributed to the fact that Sekine et al (1984) did not evaluate the action of the individual components, as discussed above. Besides, differences in technique of the animal experiments and the use of different antibiotics might contribute to the discrepancy.

The absorption promoting effect of GMO may be mediated by interference with the orientation of membrane phospholipids, as has been described for glycerylmonooleate (Muranushi et al 1981). Since GMO has been reported to solubilize cholesterol (Lillemoe et al 1982), an additional transcellular absorption-enhancing effect may be caused by extraction of the membrane stabilizing agent cholesterol from the epithelial membranes. The effectiveness of GMO suggests that further exploration of the absorption promoting properties of monoglycerides, should be interesting.

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4-Methylpyrazole alters phenobarbitone hypnotic concentrations in rats

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Abstract—The liver alcohol dehydrogenase inhibitor, 4-methylpyrazole, has been tested for its ability to change the hypnotic concentrations of phenobarbitone (phenobarbital) in rats. Following a single dose of 1 mmol kg^{-1} i.v., administered 60 min before phenobarbitone, 4-methylpyrazole shortened the onset time and reduced the dose of phenobarbitone required to produce loss of righting reflex. Consistent with this, phenobarbitone concentrations in serum (both total and free), brain and in cerebrospinal fluid at onset of hypnosis were about half in 4-methylpyrazole compared with saline-treated rats. These results suggest that acute 4-methylpyrazole pretreatment increases the central nervous system sensitivity to phenobarbitone and presumably other barbiturates; an effect apparently distinct from its inhibition of liver alcohol dehydrogenase.

Pyrazole and 4-methylpyrazole (4-MP) are potent competitive inhibitors of mammalian liver alcohol dehydrogenase (Li & Theorell 1969), with 4-MP being more useful clinically, presumably due to its low toxicity and high specificity against the enzyme (Inoue et al 1985).

Potentiation of the action of central nervous system (CNS) depressants by the pyrazoles has been taken as evidence for the involvement of liver alcohol dehydrogenase in their metabolism (Schultz & Weiner 1979). However, pyrazole potentiates the effect of trichloroethanol which is not a substrate for this enzyme (Owen & Taberner 1980). In addition both pyrazole and 4-MP increase sleep time in mice following chloral hydrate, pentobarbitone, barbitone, temazepam and halothane, but not diethyl ether (Taberner & Unwin 1987). In the same study, 4-MP produced a shortening of the latency to loss of righting reflex (LRR) following barbitone which is not metabolized. This suggests that the effect of these pyrazoles and in particular 4-MP is not specific to the inhibition of liver alcohol dehydrogenase and that it may potentiate the CNS activity of barbiturates via a pharmacodynamic mechanism.

The aim of the present study was therefore to examine in detail the reported interaction between 4-MP and barbiturate sensitivity. Specifically, the effect of acute 4-MP pretreatment on phenobarbitone concentrations at the onset of hypnosis or LRR, was examined. Phenobarbitone (instead of barbitone or pentobarbitone) was used since phenobarbitone is optically pure, has a long half life, thus minimizing possible 4-MP effect on its pharmacokinetics and since its cerebrospinal fluid (CSF) concentrations at onset of LRR reflect effective concentrations at its biophase or effector site (Danhof & Levy 1984). Rats (rather than mice) were used to allow a comprehensive investigation with measurement of both free (unbound) and total phenobarbitone concentrations in arterial serum, CSF and brain tissue at LRR.