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Absorption Enhancement of a Hydrophilic Model Compound by Verapamil After Rectal Administration to Rats

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Abstract

The use of verapamil as an absorption enhancer for the paracellular route in-vivo was studied using FITC-labelled dextran (molecular weight 4000) (FD-4) as a hydrophilic model compound for transport enhancement.

The kinetics of FD-4 after intravenous doses of 1 or 10 mg could be described by a two-compartment model with a systemic clearance of approximately 2 mL min^{-1} and a terminal plasma half-life of approximately 36 min. Rectal administration to rats, performed as a rectal infusion of 10 mg FD-4 together with 7 mM verapamil, resulted in a 10-fold increase in the percentage of the dose absorbed over a 5-h period compared with the control and a 6-fold increase compared with a bolus administration, although the total amount absorbed remained relatively low (approx. 3% maximum). Large inter-animal variation in effect values were noted.

The data indicate that although verapamil is able to enhance the absorption of hydrophilic compounds in-vivo, practical application of verapamil for this purpose does not seem feasible.

The passive absorption of hydrophilic compounds, such as peptide and protein drugs, from the lumen of the intestine to the blood is restricted to the paracellular pathway, in which the tight junctions between the epithelial cells lining the lumen form a major impediment for transport.

Among the factors known to be involved in maintaining this paracellular barrier are the intra- as well as the extra-cellular calcium concentrations. Manipulation of this barrier may occur through lowering the intracellular calcium concentration, e.g. by verapamil (Wróbel & Michalska 1977; Gafter et al 1990), and a subsequent stimulation of the water transport, and thus of enhanced solvent drag of hydrophilic compounds across the epithelial cell layer (Donowitz & Asarkof 1982; Donowitz et al 1985; Madara & Pappenheimer 1987; Pappenheimer & Reiss 1987; Lu et al 1992).

In recent experiments it was shown that verapamil induces the transport of hydrophilic model compounds across Caco-2 cell monolayers (Sakai et al 1994), which recently have been shown to be a good model for human intestinal epithelium (Artursson 1990; Hilgers et al 1990; Artursson & Karlsson 1991). However, the results found in this cell-culture system should be verified in-vivo (Noach et al 1994), as we did in the present investigations in rats. The hydrophilic model compound FITC-dextran (molecular weight 4000) was used, since it is restricted in its transport to the paracellular transport route. Furthermore, this compound was chosen for its considerable size in view of the fact that success in enhancing the transport of this molecule would most likely also suggest enhancement of the transport of smaller compounds.

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Materials and Methods

The protocol for these experiments was approved by the Leiden University ethics committee for animal experimentation.

Materials

(±)-Verapamil was a kind gift from Dr A. A. T. M. M. Vinks (Apotheek Haagse Ziekenhuizen, The Hague, The Netherlands). Fluorescein-sodium and fluorescein isothiocyanate labelled dextran (molecular weight 4000) (FD-4) were purchased from Sigma Chemical Co., USA.

Animals

Male Wistar rats (Breeding facility of the Sylvius Laboratory, Leiden University, The Netherlands), 175–195 g, were fasted for 16 h before the experiment and had free access to tap water.

Experimental

On the day of the experiment the animals were cannulated under ether anaesthesia with a polyvinylchloride C1E (0·5 mm i.d. × 1·0 mm o.d.) cannula filled with heparinized saline in the right carotid artery. The cannula was pulled subcutaneously, emerging from the nape of the neck, to avoid destruction by the rat. The animals were allowed to recover for at least 2 h after the surgery and were allowed to move freely during the experiment. Blood samples of 200 μL were

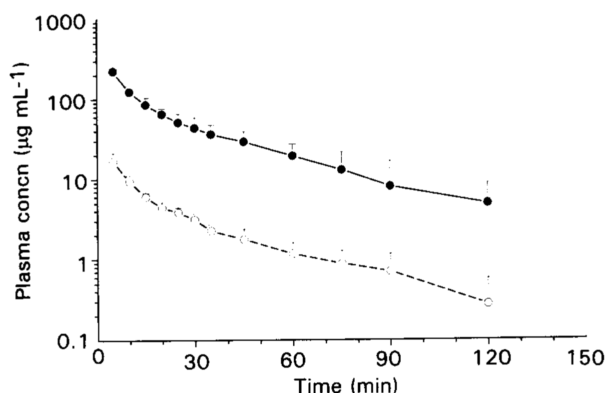

FIG. 1. Log plasma concentration vs time profiles of FD-4 after intravenous administration of 10 mg (●) and 1 mg (○). n = 6 for each group.

taken at frequent time intervals. In experiments in which the kinetics of the model compound were investigated, FD-4 was administered as a bolus administration via a cannula in the left jugular vein. To ascertain that the rectal administration of verapamil did not influence the plasma kinetics of FD-4, experiments were performed in which FD-4 was given intravenously, while at the same time, 7 mM verapamil in saline was administered rectally as a bolus or as an infusion.

For rectal administration, a stainless-steel infusion device was inserted into the rectum and fixed in the anal canal to avoid leakage of the infused solution out of the rectum. This device was connected to a polyvinylchloride cannula containing the FD-4 solution to be infused. The cannula was pulled subcutaneously to emerge from the nape of the neck and was connected to the infusion pump. Two hundred microlitres of the solution was infused either in 24 s to simulate bolus administration or in 32 min at a constant rate.

After the experiment, blood samples were centrifuged and 100 µL supernatant was frozen at −30°C until analysis. To precipitate plasma proteins, 0·5 mL 5% (w/v) trichloroacetic acid was added. After centrifugation the supernatant was evaporated using a vortex vacuum evaporator (Büchler Instruments Inc, USA) at 50°C. The residue was dissolved in 150 µL 0·15 M carbonate buffer, pH 10·5. After addition of 25 µL internal HPLC standard (fluorescein-Na 150 ng mL^{-1}), 140 µL of the sample was injected into the HPLC. The size exclusion HPLC system was as previously described (Hurni et al 1993).

Areas under the individual plasma concentration–time curves (AUCs) were calculated using the trapezoidal rule.

Table 1. Plasma kinetics of FD-4 after intravenous administration.

Dose	1 mg	10 mg
Terminal half-life (min)	35·5 ± 17·9	37·0 ± 17·6
Clearance (mL min^{-1})	2·31 ± 0·41	1·81 ± 0·29

Data for the 1 and 10 mg doses were not statistically different ($P > 0·05$). n = 6 for each dose.

Intravenous curves were extrapolated to infinity using the individual elimination rate constants. Systemic clearance was calculated as Dose/AUC. Kinetic parameters were calculated using the SIPHAR v3.3 (SIMED, France) program.

Results and Discussion

Fig. 1 shows the results of the intravenous kinetic study of FD-4 after doses of 1 and 10 mg. The plasma concentration–time profiles showed the characteristics of a two-compartment pharmacokinetic model. The clearance values of FD-4 after 1 and 10 mg doses are presented in Table 1. From these data it is clear that the plasma kinetics of FD-4 are linear in the anticipated concentration range for the absorption-enhancement experiments, although the values are somewhat different from recently published results (Mehvar & Shepard 1992). Simultaneous administration of FD-4 and verapamil did not result in significant changes in the FD-4 kinetics (data not shown).

In a model for studying the effect of drug absorption enhancers (van Hoogdalem 1989), rectal administration of FD-4 in saline together with 7 mM verapamil resulted in

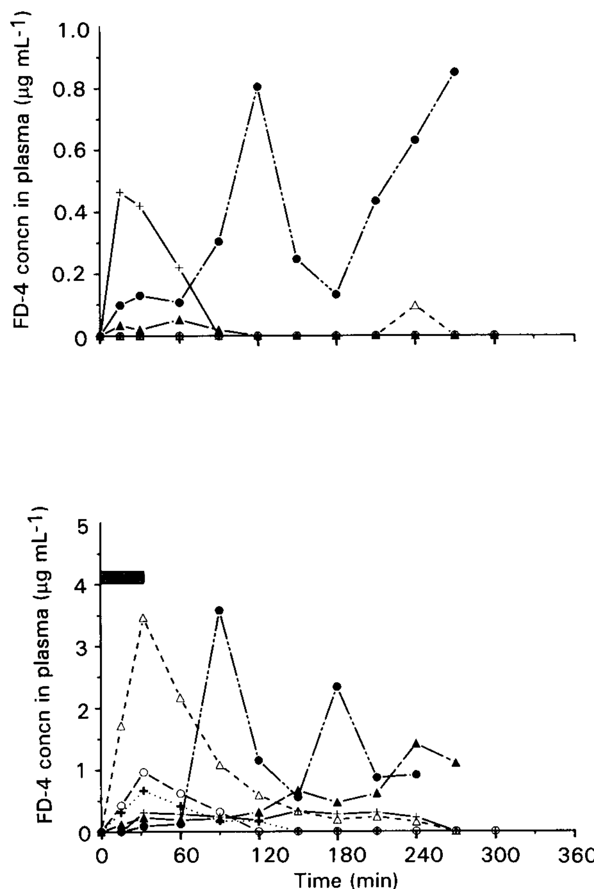

FIG. 2. Plasma concentration vs time profiles of FD-4 after rectal bolus administration (upper panel) or after rectal infusion (lower panel) of 10 mg FD-4. Six individual curves are shown in each graph.

Table 2. Percentage of the dose absorbed after rectal administration of 10 mg FD-4 without or together with 7 mM verapamil.

Administration	Absorption (%)	
	Control	Verapamil
Bolus (24 s)	0·280 ± 0·418	0·475 ± 0·881
Infusion (32 min)	0·306 ± 0·101	3·046 ± 1·735[ab]

[a] $P < 0{\cdot}05$ compared with control infusion. [b] $P < 0{\cdot}01$ compared with bolus with verapamil.

plasma concentration vs time profiles which were very different after bolus administration compared with the data obtained after infusion (Fig. 2, note the difference in y-axis scale). Large inter-animal variation in effect values were found and no pharmacokinetic modelling of the concentration vs time profiles was possible. For that reason the percentages of the dose which were absorbed in the time period 0–300 min were calculated with a custom-made APL program (SCSI, USA) which calculated these percentages via deconvolution with the point-area method (Vaughan & Dennis 1978) using the mean coefficients and exponents of the intravenous experiments as input function. The results of these calculations are presented in Table 2, showing no enhancement of the uptake of FD-4 after bolus administration. After infusion, a 10-fold increase in percentage of the dose absorbed was found compared with control, while the increase compared with bolus administration with 7 mM verapamil was 6-fold (Table 2). However, the actual percentage absorbed was still very low at about 3% maximum.

As an explanation for the high degree of inter-animal variation in effect, a large variability in the degree of spreading of the infused solution in the rectal area may be proposed.

Substantial differences in the degree of spreading between bolus administration and infusion, and thus a different effect on absorption enhancement, have been reported (van Hoogdalem et al 1988a,b), resulting in a variable topical concentration and thus variable effectiveness of the enhancer. Furthermore, the correlation between the concentration–effect relationship of the enhancer and the absorption rate of the drug is an important factor (de Boer et al 1990), which possibly may explain part of the large variability in effect that was found. Moreover it is not known if the susceptibility of colon and rectal tissue to verapamil is different, so that spreading of the infused solution over a larger area could result in different degrees of uptake.

The concentration range in which verapamil can be used safely as an absorption enhancer, as found in our in-vitro model, is very narrow (≈700 μM) (Sakai et al 1994). It is thus possible that we are not able to reach this effective concentration range in-vivo, possibly due to the presence of a mucus layer on the cell surface, which also could be of influence on the effective concentration of the enhancer at the tissue surface (Artursson 1991).

In conclusion, it can be stated that relatively high concentrations of verapamil are needed in-vivo to exert an absorption-enhancing effect. Furthermore, a high inter-animal variation in effect was observed. This makes the practical applicability of this compound as an absorption enhancer unlikely.

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