ELSEVIER

International Journal of Pharmaceutics 143 (1996) 101–106

international journal of pharmaceutics

Notes

Absorption enhancement of captopril in the rat colon as a putative method for captopril delivery by extended release formulations

Amnon Sintov[a,*], Mark Simberg[b], Abraham Rubinstein[b]

[a] *Ben-Gurion University of the Negev, The Institutes for Applied Research, Ernst David Bergmann Campus, P.O. Box 653, Beer-Sheva 84105, Israel*

[b] *The Hebrew University of Jerusalem, Faculty of Medicine, School of Pharmacy, P.O. Box 12065, Jerusalem 91120, Israel*

Received 17 March 1996; revised 17 July 1996; accepted 29 July 1996

Abstract

The absorption of captopril from aqueous solution and solid dosage form was compared in the jejunum and colon of the anesthetized rat, with and without a mixture of oleic acid and sodium oleate (OASO). Captopril was absorbed better in the jejunum than in the rat colon ($32.5 \pm 7.5\%$ compared with $9.0 \pm 4.6\%$ of the administered dose, respectively). The addition of 1.0 or 2.5% of OASO significantly enhanced captopril colonic absorption, but did not affect captopril absorption from the rat jejunum. $AUC_{0-5\,h}$ values were increased two-fold after colonic administration of captopril-OASO solid formulations. The colonic absorption with or without OASO was characterized by flat drug plasma profiles. It is concluded that colonic delivery of captopril together with OASO could be used to sustain captopril input into the body for a prolonged period of time.

Keywords: Captopril; Absorption enhancers; In situ absorption; Colon; Controlled drug delivery

1. Introduction

For the improvement of patient compliance after per-oral administration of captopril (an ACE inhibitor with elimination half-life of 1.7 h, Creasey et al., 1988), it is suggested to administer it once daily in a sustained release manner. This requires that captopril would be absorbed in the colon, where drug residence time is the longest. Hu and Amidon (1988) found that, in the rat, at pH 7, captopril was poorly absorbed in the colon

* Corresponding author.

0378-5173/96/$15.00 © 1996 Elsevier Science B.V. All rights reserved
PII S0378-5173(96)04682-0

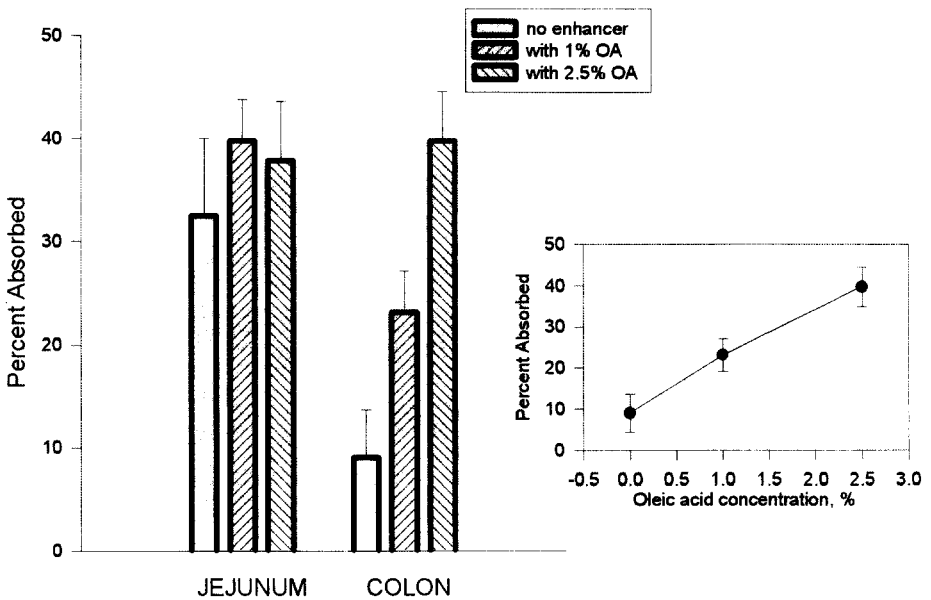

Fig. 1. Captopril absorption in the jejunum and colon of the rat after intra-jejunal or intra-colonic administration of a captopril solution with (1 or 2.5% w/v) and without OASO, as calculated from the 1 h captopril effluent levels. Shown are the mean values ± S.D.; $n = 10$. Inner plot shows the concentration-dependent absorption enhancement of captopril in the colon.

and much better absorbed in the jejunum. The jejunal absorption of captopril was dose-dependent, suggesting carrier-mediated transport, accompanied by a passive, pH-dependent absorption. The lack of colonic absorption of captopril was verified in humans by Wilding et al. (1992). Similar results with the ACE inhibitors benazepril and CGS 16617 were observed in vitro by Kim et al. (1994). Since a major cause of low colonic drug absorption is the existence of non-leaky tight junctions, the use of absorption enhancers is reasonable in the design of prolonged release dosage forms.

The goal of this study was to measure the effect of a mixture of sodium oleate and oleic acid on captopril absorption in the jejunum and colon of the rat after dosing with aqueous solution and solid formulation of the drug.

2. Rat preparation and administration of captopril in buffer solutions or capsules

After an overnight fast (water ad libitum), nine male Sabra rats (250–300 g) were anesthetized by an i.p. injection of Equitensine solution (equivalent to 6 mg/100 g body weight sodium phenobarbitone). The jejunum (15 cm long segment, 10 cm^2) and the colon (7–8 cm long segment, 10 cm^2) were exposed through a midline incision. The pre-rinsed segments were ligated at both ends to create closed pouches. Then 1.5 ml (into the jejunum) or 1.0 ml (into the colon) of 10 mM freshly prepared solutions of captopril (Selog AG, Germany) in isotonic Sorensen buffer solution (SBS, pH = 7) were injected. In separate experiments the same captopril solutions were injected together with pre-prepared mixtures of oleic acid and sodium oleate (OASO), adjusted with NaOH to pH 7.3–7.5, at two different total oleate concentrations: 1.0% w/w and 2.5% w/w (35 and 88 mM as oleic acid). Exactly 1 h after the administration, the ligated small intestine and colonic segments were cut open and their fluid contents, as well as the mucosal tissues (homogenized), were assayed for captopril. Each animal was used for both colonic and jejunal absorption studies.

Water uptake during the absorption studies was measured gravimetrically (Martin-Villodre et al., 1986) and did not exceed a mean value of 0.5%. Captopril in SBS (pH 7) was found to be stable for at least 1 h when perfused in a closed loop system through the jejunum or colon of the rat. Tissue captopril metabolism was studied in homogenates prepared separately from jejunal and colonic mucosal tissues. Similar captopril degradation values in the jejunum and colon of the rat were observed: after 1.5 h of incubation with mucosal homogenates $18.2 \pm 0.81\%$ and $20.9 \pm 0.33\%$ of captopril degraded in the colon and jejunum, respectively.

A different rat preparation was used for the captopril capsule administration. A single ligature was made in the proximal end of the jejunum or the colon of the anesthetized rats. This was followed by a 2 mm incision through which a No. 3 gelatin capsule was immediately administered. After the administration, each intestinal segment was ligated underneath the incision. Each capsule contained 5 or 10 mg of captopril (16.7 and 33.4 mg/kg), 3.25 mg of freeze-dried OASO (aqueous solution for freeze-drying was prepared as described above), and lactose to a total weight of 135 mg. Control capsules were prepared without the OASO dry mixture. In vitro dissolution tests showed that captopril was released from the capsules within 5 min in SBS, pH 7. Blood samples (4 ml) from the anesthetized rats were taken at predetermined time intervals and assayed for captopril. Each rat was used as a single time point in each study.

Table 1
Captopril mucosal levels in the rat jejunum and colon after jejunal or colonic administration of captopril in SBS expressed as % of dose per tissue area (cm^2) (values are mean ± S.D.; $n = 6$)

	Jejunum	Colon
No enhancer	3.88 ± 0.50	0.17 ± 0.02
OASO (2.5% w/v)	3.27 ± 0.71	2.83 ± 0.46

3. Histological analysis

The effect of OASO mixtures (concentrations of 0.5, 1.0, and 2.5% w/w) on the jejunal or colonic mucosa was assessed histologically against control tissues (no enhancers) using hematoxylin/eosin staining. In all cases no damage to the colonic or jejunal tissues could be observed (data not shown).

4. Captopril and captopril disulfide HPLC analysis

In all experiments, samples were immediately quenched by HCl solution to pH 1.0 to prevent captopril oxidation (Hu and Amidon, 1988). Captopril and captopril disulfide concentrations in SBS and tissue homogenates were separated on a reverse-phase column (LiChrospher 60 RP select B, 5 μm, 125 × 4 mm, Merck). The mobile phase was water:methanol:phosphoric-acid (45:55:0.05), the flow rate was 1 ml/min and detection was performed at 220 nm with Merck-Hitachi apparatus consisting of 6200 A pump, AS-4000 autosampler and L-4250 UV-Vis-detector. A different procedure was taken for captopril detection in blood samples as follows: 4 ml of blood were collected into a heparinized test-tube. After centrifugation, 0.5 ml of plasma was added immediately into a glass test-tube containing 0.03 ml of 1 mg/ml of *p*-bromophenacyl bromide in acetonitrile. The test-tubes were vortexed for 30 s and placed at room temperature without shaking for 20 min. Then, 0.1 ml of 1 N HCl was added and the test-tubes were vortexed for 15–20 s, followed by a further addition of 2 ml of 1:1 mixture of ethyl acetate:benzene containing 0.01 mg/ml of benzyl benzoate as an internal standard. After centrifugation, 2 ml of the organic phase were collected, evaporated to dryness under nitrogen stream, and reconstituted in 0.2 ml acetonitrile. Aliquots of 20 μl were injected into the HPLC system equipped with a Partisil 10 ODF-1 column (Whatman, 250 × 4.6 mm). The mobile phase was water:acetonitrile:glacial acetic acid (45:55:0.04). The flow rate was 1 ml/min. Detection was performed at a wavelength of 260 nm.

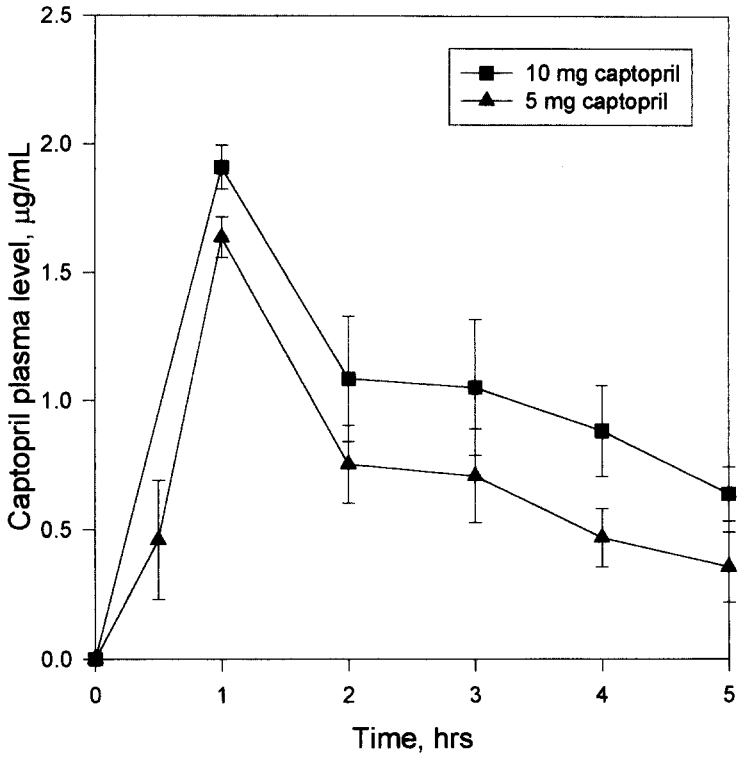

Fig. 2. Captopril plasma levels obtained after jejunal administration of captopril capsules containing 5 or 10 mg of captopril. Shown are the mean values ± S.D.; $n = 9$.

5. Captopril absorption enhancement – results

The absorption of captopril from SBS in the jejunum and colon of the rat is summarized in Fig. 1. Captopril absorption was higher in the jejunal pouch than in the colonic one (32.5 ± 7.5% and 9.0 ± 4.6% of the administered dose, respectively). Adding OASO did not improve captopril absorption in the rat jejunum (39.8 ± 4.0% with 1%, and 37.8 ± 5.8% with 2.5% enhancers mixture). These results verify the observations of Hu and Amidon (1988) that, in the rat, captopril is absorbed mainly via a carrier-mediated mechanism. However, when OASO was added into the SBS containing captopril in the colon, a profound, dose-dependent enhancement in its absorption was observed (23.2 ± 4.0% and 39.8 ± 4.8% of administered dose for 1%, and 2.5% enhancers mixture, respectively). At a pH range of 7.3–7.5 the absorption enhancement of the OASO mixture caused more reproducible enhancement compared with the absorption enhancement caused by oleic acid only (data not shown), probably because of the creation of a buffered (oleate) system. The observation that OASO was effective in the colon and not in the jejunum indicates that paracellular permeation for captopril is dominant in the former organ (Tomita et al., 1988). This was verified by mucosal captopril analysis in both organs (Table 1). As expected, no significant difference in captopril tissue levels was found in the jejunum with and without 2.5% OASO, while in the colon, the addition of the same OASO concentration caused a 16-fold increase in captopril mucosal levels. The inability to enhance captopril absorption in the rat jejunum may also be a result of the thicker mucus lining covering the epithelium of this organ (Rubinstein and Tirosh, 1994).

In the second part of the study two doses (5 or 10 mg) of solid captopril were administered, in

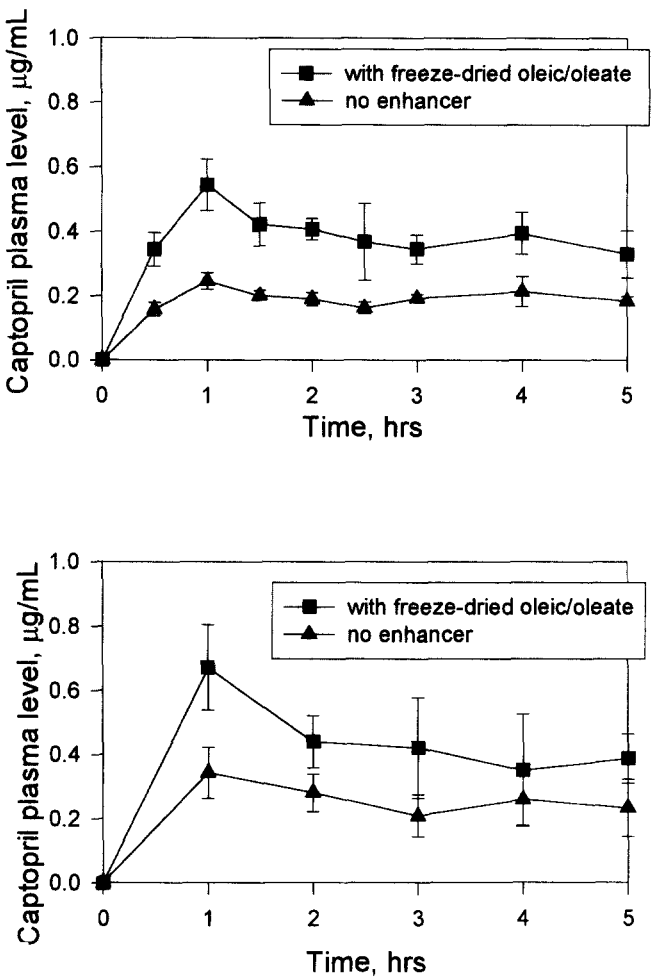

Fig. 3. Captopril plasma levels obtained after colonic administration of captopril capsules containing 5 mg (a) or 10 mg (b) of captopril with and without OASO. Shown are the mean values ± S.D.; $n = 9$.

different studies, to the jejunum or the colon of the rat, with or without OASO. The stationary conditions of the study were aimed at mimicking a physiological situation in which a solid colonic delivery system arrives at the colon intact. Captopril plasma levels after jejunal administration of the various formulations are summarized in Fig. 2 and captopril plasma levels after colonic administration of the various formulations are summarized in Fig. 3. Table 2 summarizes the values of the $AUC_{0-5\,h}$ of captopril plasma concentration versus time profiles after the various administrations. Although OASO almost doubled captopril bioavailability after colonic administration, the bioavailability values were still higher after jejunal administration of captopril capsules without en-

Table 2
$AUC_{0-5\,h}$ values (ng/ml·h) of plasma captopril levels depicted in Fig. 3 (values are mean ± S.D., $n = 9$)

Dose (mg)	Jejunum	Colon	
		Without OASA	With OASO
5	3748 ± 376	928 ± 51	1859 ± 30
10	5260 ± 485	1208 ± 112	2075 ± 241

hancer. Yet, it is expected that captopril bioavailability can be increased even more with increase in colonic residence time under real conditions.

The differences in the results obtained after the administration of liquid captopril with OASO (Fig. 1) and solid captopril with OASO (Table 2) are related to the different models used to assess absorption. They can be related also to the slower and the non-synchronous release rate of captopril and OASO once formulated into a solid dosage form, indicating the need for a dosage form that would be able to supply constant levels of captopril and OASO (such as an osmotic pump or erodible hydrogel) (Rubinstein et al., 1997). The flat plasma captopril profile after colonic administration (Fig. 3), as opposed to the regular pharmacokinetic profiles obtained after jejunal administration of captopril capsules (Fig. 2) is noteworthy. The flat nature of the profiles, which was maintained also after the addition of OASO to the solid formulation, indicates that the colonic epithelium is a homogeneous barrier to captopril absorption and that the effect of OASO was homogeneous along the entire study, attributed, probably, to the 'closed compartment' conditions of the rat colon. These conditions result from the low motility and chyme movement in that organ. The prolonged drug blood levels after colonic administration, which has been demonstrated in a number of studies (Rubinstein, 1995), highlights the importance of the colon in the design of controlled release formulations of drugs, such as captopril, to lower blood drug fluctuations possible.

References

Creasey, W.A., Morrison, R.A., Singhvi, S.M. and Willard, D.A., Pharmacokinetics of intravenous captopril in healthy men. *Eur. J. Pharmacol.*, 35 (1988) 367–370.

Hu, M. and Amidon, G.L., Passive and carrier-mediated intestinal absorption components of captopril. *J. Pharm. Sci.*, 77 (1988) 1007–1011.

Kim, J.S., Oberle, R.L., Krummel, D.A., Dressman, J.B. and Fleisher, D., Absorption of ACE inhibitors from small intestine and colon. *J. Pharm. Sci.*, 83 (1994) 1350–1356.

Martin-Villodre, A., Pla-Delphina, J.M., Moreno, J., Pereg-Buendia, D., Niralles, J., Collado, E.F., Sanchez-Moyano, E. and Del-Pozo, A., Studies on reliability of a bihyperbolic functional absorption model, I — Ring substituted anilines. *J. Pharmacokin. Biopharm.*, 14 (1986) 615–633.

Rubinstein, A., Approaches and opportunities in colon-specific drug delivery. *Crit. Rev. Ther. Drug Carr. Sys.*, 12 (1995) 101–149.

Rubinstein, A. and Tirosh, B., Mucus gel thickness and turnover in the gastrointestinal tract of the rat, responses to cholinergic stimulus and implication for mucoadhesion. *Pharm. Res.*, 11 (1994) 794–799.

Rubinstein, A., Tirosh, B., Baluom, M., Nassar, T., David, A., Gliko-Kabir I., Radai, R. and Friedman, M., The rationale for peptide drugs delivery to the colon and the potential of polymeric carriers as effective tools. *J. Contr. Rel.*, (1997) in press.

Tomita, M., Shiga, M., Hayashi, M. and Awazu, S., Enhancement of colonic drug absorption by the paracellular permeation route. *Pharm. Res.*, 5 (1988) 341–346.

Wilding, I.R., Davis, S.S., Bakhshaee, M., Stevens, H.N.E., Sparrow, R.A. and Brennan, J., Gastrointestinal transit and systemic absorption of captopril from pulsed-release formulation. *Pharm. Res.*, 9 (1992) 654–657.