International Journal of Pharmaceutics, 76 (1991) R1–R4

ADONIS 037851739100357A

IJP 10036

Rapid communication

Absorption of thyrotropin-releasing hormone (TRH) and a TRH prodrug in a human intestinal cell line (Caco-2)

S. Lundin [1], J. Møss [3], H. Bundgaard [3] and P. Artursson [2]

[1] *Department of Clinical Pharmacology, Lund University Hospital, Lund (Sweden),*
[2] *Department of Pharmaceutics, Biomedical Centre, Uppsala (Sweden) and*
[3] *Department of Pharmaceutical Chemistry, The Royal Danish School of Pharmacy, Copenhagen (Denmark)*

(Received 16 July 1991)
(Accepted 16 August 1991)

Key words: Thyrotropin-releasing hormone; Cell culture; Prodrug; Intestinal absorption

Summary

The transepithelial transport of TRH and its prodrug (*N*-octyloxycarbonyl) derivative was studied using the human colorectal carcinoma cell line Caco-2. No intact TRH prodrug was transported across the cells. Instead, TRH released from the prodrug was absorbed at rates comparable to those of labelled and unlabelled TRH. No metabolites of TRH could be detected. It can be concluded that the increased lipophilicity of the TRH prodrug is without effect on its transport characteristics.

Thyrotropin-releasing hormone (TRH) is a peptide of potential clinical value for the treatment of various neurologic and neuropsychiatric disorders (Metcalf and Jackson, 1989). This tripeptide hormone was reported to be rapidly inactivated in plasma, thereby limiting its clinical utility (Hickens, 1983; Møss and Bundgaard, 1990a). The low lipophilicity displayed by TRH is unfavourable for penetration across biological membranes (Banks and Kastin, 1985). On the other hand, the low molecular mass (362 Da) of the peptide is within the range believed to be rather well absorbed across the nasal and gastrointestinal mucosae (McMartin et al., 1987; Donovan et al., 1990). In fact, an active transport mechanism of TRH across the intestinal mucosa has been suggested in the dog and the rat (Yokohama et al., 1984) thus utilizing the intestinal peptide carrier(s) (Wilson et al., 1989). The problems of rapid enzymatic inactivation and poor lipophilicity of TRH may be overcome by bioreversible derivatization of the peptide (Bundgaard and Møss, 1990). The derivatives developed are *N*-alkoxycarbonyl derivatives of TRH formed by *N*-acylating the imidazole group of the histidine residue with various chloroformates. These derivatives are totally resistant to cleavage by the TRH-degrading pyroglutamyl aminopeptidase serum enzyme but are readily bioreversible, releasing TRH by spontaneous or plasma esterase-catalyzed hydrolysis. The *N*-alkoxycarbonyl prodrug derivatives possess greatly increased lipophilicity relative to TRH (Bundgaard and Møss, 1990). Taken together, these properties may ren-

Correspondence: S. Lundin, Department of Clinical Pharmacology, Lund University Hospital, S-221 85 Lund, Sweden.

der the prodrug forms more capable of penetrating various biological membranes. Thus, successful transdermal penetration of the highly lipophilic *N*-octyloxycarbonyl derivative of TRH was recently reported (Møss and Bundgaard, 1990b). The possibility of improved intestinal absorption by some prodrugs of TRH was studied in Ussing Chambers using isolated intestinal segments of rabbit and rat (Møss et al., 1990). The prodrugs did not improve the penetration of TRH across jejunal, ileal and colonic segments of the rat. In addition, the prodrugs were susceptible to enzymatic degradation, most likely due to the action of prolyl endopeptidase and non-specific esterases.

In the last few years, attempts have been made at developing cell-culture systems which may serve as models for the small intestinal epithelium (Neutra and Louvard, 1989). One of the most promising cell lines to date is derived from a human colon carcinoma, designated Caco-2. Caco-2 cells differentiate in cell culture to polarized monolayers with a morphological appearance similar to that of small intestinal enterocytes. The monolayers share several features with the small intestinal epithelium and are therefore useful for studies of both epithelial transport and metabolism (Lundin and Artursson, 1990; Artursson and Karlsson, 1991).

The purpose of the present study was to determine the rate of permeation of TRH and its *N*-octyloxycarbonyl prodrug derivative across Caco-2 monolayers.

Caco-2 cells (Fogh et al., 1977) were obtained from the American Cell Culture Collection (Rockville, MD). The cells were cultivated on polycarbonate filters (Transwell cell culture inserts; mean pore diameter 0.45 μm) as described elsewhere (Artursson, 1990). Cells of passage no. 85–95 were used throughout. The integrity of the monolayers was routinely checked by measurements of transepithelial electrical resistance and by determination of the permeability of the hydrophilic marker [^{3}H]mannitol (M_w: 182). The integrity of the Caco-2 cell monolayers remained intact in the presence of TRH and its prodrug as reflected in unchanged P_{app} values for [^{3}H]mannitol (3.42 ± 0.2 and 3.34 ± 0.43, respectively). [^{3}H]TRH was obtained from New England Nuclear (specific activity: 3.7 TBq/mmol), TRH was purchased from Bachem, Basel, Switzerland, and *N*-octyloxycarbonyl TRH was synthesized as described previously (Bundgaard and Møss, 1990).

For the study of peptide transport 10^{-4} M solutions of TRH, [^{3}H]TRH and the TRH prodrug dissolved in incubation medium were added to the apical chamber. After various times samples were collected from the apical and basolateral chamber and were immediately frozen at $-70\,^{\circ}$C. Control samples were prepared by incubating the prodrug in cell culture medium (HBSS) for the same period of time. Analyses of incubated peptides were performed using HPLC (Bundgaard and Møss, 1990). Quantitation of the compounds was carried out by measuring the peak heights in relation to those of standards chromatographed under the same conditions. The sensitivity of the assay was about 0.5 μg ml^{-1} of TRH and its prodrugs. Apparent permeability coefficients (P_{app}) were calculated as described previously (Artursson, 1990).

The transport rates of TRH, [^{3}H]TRH and TRH generated from its prodrug are listed in Table 1. The HPLC analysis revealed that no measurable amount of intact TRH prodrug was transported across the Caco-2 cell monolayers. The P_{app} values for [^{3}H]TRH and TRH agreed

TABLE 1

Molecular weights (M_w), logarithms of partition coefficients (log P) and permeability coefficients P_{app} of labelled and unlabelled TRH and TRH generated from its prodrug in Caco-2 cell monolayers

	M_w	log P [a]	P_{app} ($\times 10^6$ cm s^{-1})
TRH	362	−2.46	1.93 ± 1.04
[^{3}H]TRH	362	−2.46	1.41 ± 0.08 [b]
N-octyloxycarbonyl TRH	518	1.88	-
TRH (from prodrug)			2.73 ± 0.95

[a] P is the partition coefficient between octanol and phosphate buffer of pH 7.40. The values were taken from Bundgaard and Møss (1990).

[b] $N = 3$–10, means ±S.D.

The apparent permeability coefficients for TRH released from the prodrug was calculated using the peptide concentration at each time point ($n = 5$) in the basolateral chamber.

TABLE 2

Comparison of TRH prodrug concentrations (μM) when the prodrug (100 μM) was incubated for various times with Caco-2 cell monolayers or HBSS

Time (min)	Cells	HBSS
0	94.4	90.2
20	87.1	83.5
30	83.5	71.1
60	67.2	75.7
150	71.4	67.5

well with the values obtained for the TRH prodrug (measured as TRH flux). There were no significant differences between TRH prodrug concentrations when incubated with Caco-2 cell monolayers and cell culture medium (Table 2), indicating that the prodrug underwent hydrolysis in solution and that esterase activity was not present. As previously shown, the TRH prodrug is degraded with a half-life of 17.5 h in aqueous solution of pH 7.4 at 37 °C. Based on the formation of TRH from its octyloxycarbonyl derivative, it could be calculated that the degradation half-times of the prodrug were 16.6 and 16.5 h in cell monolayers and incubation medium, respectively. Previous penetration studies using isolated rabbit and rat intestinal segments showed that the penetration of the prodrug derivative was not superior to that of intact TRH (Møss et al., 1990). No metabolites of TRH could be detected using the present HPLC system. The similarity of the P_{app} values for TRH and [^{3}H]TRH therefore indicates that the peptide escapes degradation by the intracellular enzyme prolyl endopeptidase (Wilk, 1983). If so, this would indicate that TRH is transported across Caco-2 cell monolayers by a paracellular route as can be expected by its high hydrophilicity, and not by the intestinal dipeptide/tripeptide carrier system. A dipeptide carrier for cephalosporins has recently been identified in Caco-2 cell monolayers grown on impermeable plastic supports (Danzig and Bergin, 1990). The possible expression of this carrier by the filter-grown cell monolayers used in this study does not influence the interpretation of the present results, since the carrier is reported to be inactive at the neutral pH used in this study.

In conclusion, this study shows that the TRH prodrug was not transported across the cells despite its high lipophilicity. This indicates that lipophilicity, as defined by octanol/water partition coefficients, is of limited value in mediating peptide absorption, across the intestinal epithelium. Other parameters, such as molecular weight and the number of hydrogen bonds, may also be important (Ho et al., 1990). On the other hand, TRH remained intact and is most likely transported across the Caco-2 cells by the paracellular route.

Acknowledgement

The authors wish to express their gratitude to Ms Anette Persson for preparing the manuscript.

References

Artursson, P., Epithelial transport of drugs in cell culture. I: A model for studying the passive diffusion of drugs over intestinal absorptive (Caco-2) cells. *J. Pharm. Sci.*, 79 (1990) 476–482.

Artursson, P. and Karlsson, J., Correlation between oral drug absorption in humans and apparent drug permeability coefficients in human intestinal epithelium (Caco-2) cells. *Biochem. Biophys. Res. Commun.*, 175 (1991) 880–885.

Banks, W.A. and Kastin, A.J., Peptides and the blood-brain barrier: Lipophilicity as a predictor of permeability. *Brain Res. Bull.*, 15 (1985) 287–292.

Bundgaard, H. and Møss, J., Prodrugs of peptides 6. Bioreversible derivatives of thyrotropin-releasing hormone (TRH) with increased lipophilicity and resistance to cleavage by the TRH-specific serum enzyme. *Pharm. Res.*, 7 (1990) 885–892.

Danzig, A.H. and Bergin, L., Uptake of the cephalosporin, cephalexin, by a dipeptide transport carrier in the human intestinal cell line, Caco-2. *Biochim. Biophys. Acta*, 1027 (1990) 211–217.

Donovan, M.D., Flynn, G.L. and Amidon, G.L., Absorption of polyethyleneglycols 600 through 2000: The molecular weight dependence of gastrointestinal and nasal absorption. *Pharm. Res.*, 7 (1990) 863–868.

Fogh, J., Fogh, J.N. and Orfeo, T., One-hundred and twenty seven cultured human tumor cell lines producing tumors in mice. *J. Natl. Cancer Inst.*, 59 (1977) 221–226.

Hickens, M., A comparison of thyrotropin-releasing hormone with analogs: Influence of disposition upon pharmacology. *Drug Metab. Rev.*, 14 (1983) 77–98.

Ho, N.F.H., Day, J.S., Barsuhn, C.L., Burton, P.S. and Raub, T.J., Biophysical model approaches to mechanistic transepithelial studies of peptides. *J. Controlled Release*, 11 (1990) 3–24.

Lundin, S. and Artursson, P., Absorption of a vasopressin analogue, 1-deamino-8-D-arginine vasopressin (dDAVP), in a human intestinal cell line, CaCO-2. *Int. J. Pharm.*, 64 (1990) 181–186.

McMartin, C., Hutchinson, L.E.F., Hyde, R. and Peters, G.E., Analysis of the structural requirement for the absorption drugs and macromolecules from the nasal carity. *J. Pharm. Sci.*, 76 (1987) 535–540.

Metcalf, G. and Jackson, I.M.D., Thyrotropin-releasing hormone. Biomedical significance, *Ann. NY Acad. Sci.*, 553 (1989) 1–631.

Møss, J. and Bundgaard, H., Kinetics and pattern of degradation of thyrotropin-releasing hormone (TRH) in human plasma. *Pharm. Res.*, 7 (1990a) 751–755.

Møss, J. and Bundgaard, H., Prodrugs of peptides 7. Transdermal delivery of thyrotropin-releasing hormone (TRH) via prodrugs. *Int. J. Pharm.*, 66 (1990b) 39–45.

Møss, J., Buur, A. and Bundgaard, H. Prodrugs of peptides 8. In vitro study of intestinal metabolism and penetration of thyrotropin-releasing hormone (TRH) and its prodrugs. *Int. J. Pharm.*, 66 (1990) 183–191.

Neutra, M. and Louvard, D., Functional epithelial cells in culture. In Matlin, K.S. and Valentich, J.D. (Eds), *Modern Cell Biology*, vol 8, A.R. Liss, New York, 1989, pp 363–398.

Wilk, S., Prolyl endopeptidase. *Life Sci.*, 33 (1983) 2142–2157.

Wilson, D., Barry, J.A. and Ramaswamy, K., Characteristics of tripeptide transport in human jejunal brush-border membrane vesicles. *Biochim. Biophys. Acta*, 986 (1989) 123–128.

Yokohama, S., Yoshioha, T., Yamashita, K. and Kitamori, N., Intestinal absorption mechanisms of thyrotropin-releasing hormone. *J. Pharm. Dyn.*, 7 (1984) 445–451.