In Vivo Buccal Delivery of the Peptide Drug Buserelin with Glycodeoxycholate as an Absorption Enhancer in Pigs

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Purpose. To study the potential of buccal delivery of the peptide drug in pigs.

Methods. Intravenous administration and buccal delivery without and with 10 mM sodium glycodeoxycholate (GDC) as absorption enhancer were investigated as a randomised cross-over study in six pigs. The buccal delivery device consisted of an application chamber with a solution of buserelin and was attached to the buccal mucosa for 4 hours using an adhesive patch.

Results. Buccal administration of buserelin resulted in rapidly reached steady state plasma levels. The absolute bioavailability of the peptide after buccal delivery for 4 hours could be increased from 1.0 ± 0.3 to 5.3 ± 1.1% (mean ± S.D.) by co-administration of 10 mM GDC (0.45% w/v)).

Conclusions. The results of this study demonstrate that buccal administration with the use of absorption enhancers is a useful approach for the delivery of peptide drugs such as buserelin.

INTRODUCTION

Peptides are of great therapeutic interest because of their high potency and generally low toxicity. Peptide drugs, however, can often not be administered perorally, because they are highly susceptible to metabolism by gastro-intestinal enzymes, and because of their poor absorption properties. Therefore, alternative routes of administration have to be developed (1). Buccal delivery of peptide drugs circumvents the degradation in the gastro-intestinal tract and avoids the hepatic first-pass effect (1–3).

In this study, the efficiency of buccal delivery of a highly active luteinizing hormone-releasing hormone (LHRH) agonist, D-Ser(Bu^t)^6-LHRH(1–9)-nonapeptide-ethylamide (buserelin) was investigated. The clinical applications of LHRH agonists extend from precocious puberty, endometriosis and leiomyomas to hormone-dependent tumours, and to male and female contraception, covering a wide dose range. LHRH agonists have been administered via injections, nasal sprays or subcutaneous implantations, and other routes of administration have been and are being evaluated (4). Sandow and Petri (5) have found that nasal delivery of LHRH agonists was more efficient than other non-parenteral routes, requiring a 100-fold multiple of the doses by either intravenous or subcutaneous injection. The inconvenient vaginal or rectal administration was 4–6 times less efficient. Peroral delivery of LHRH analogues resulted in a very low bioavailability, 30–100 times lower than the nasal bioavailability. Buccal administration of LHRH analogues, using an adhesive polymer film containing 5 mg of the drug, resulted in a low rate of absorption across the buccal mucosa (5). Coadministration of absorption enhancers could change the permeability of the buccal epithelium and makes this route of delivery applicable for the delivery of hydrophilic macromolecular drugs (6). Moreover, the robust nature of the buccal epithelium and the fact that the oral mucosa is routinely exposed to harmful substances make the buccal route a suitable site for the use of absorption enhancers. Bile salts, such as sodium glycodeoxycholate (GDC), have proven to be successful in promoting the buccal absorption of hydrophilic high molecular weight compounds (7,8). Bile salts are thought to act by solubilizing epithelial lipids, possibly via micellization, thereby increasing the mucosal permeability. The effect on the permeability barrier has been reported to be reversible and dependent on the concentration of the bile salts, and no gross morphological changes have been observed on non-keratinized buccal epithelium in-vivo (9–11).

The aim of this study was to examine the possibility of buccal delivery of peptide drugs by investigating the pharmacokinetics of buccal absorption of buserelin in pigs, without and with the use of an absorption enhancer, GDC.

The porcine cheek was chosen as a model for the human buccal mucosa, based on the similarity in permeability and morphology of their non-keratinized epithelia (12).

MATERIALS AND METHODS

Materials

The nonapeptide buserelin was kindly provided by Hoechst AG (Frankfurt, Germany). For radioimmunoassay (RIA) of the peptide in plasma samples, labelling with ^{125}I (Amersham, 's Hertogenbosch, The Netherlands) was performed using iodogen beads (Pierce, Rockford, IL, USA) as oxidizing agent. Separation of free ^{125}I from the labelled peptide was carried out by solid phase fractionation with Seppak C18 cartridges (Waters Ass., Etten-Leur, The Netherlands) equilibrated with 1% (w/v) trifluoracetic acid (13). The labelled peptide was stored at −20°C until used in the RIA. A specific antibody for buserelin was raised in rabbits and kindly provided by Hoechst AG (Frankfurt, Germany). The second antibody used in the RIA for precipitation purposes was a sheep-anti-rabbit-IgG, purchased from Sigma (St. Louis, MO, USA).

Sodium glycodeoxycholate (GDC) was also from Sigma. Propofol (2,6 diisopropylphenol, Diprivan®) was from Zeneca BV (Ridderkerk, The Netherlands), being an aqueous isotonic emulsion containing 10 mg propofol/ml.

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Formulations

The solution for intravenous (i.v.) bolus injection of buserelin contained 1 mg in 5 ml physiological saline (0.15 mM). The buccal delivery devices consisted of a small application chamber (Hill Top Biolabs, Cincinnati, OH, USA; diffusional area of 0.87 cm^2) and an adhesive backing (Orahesive®, Squibb, UK). Shortly before administration, a fresh solution of 50 mg/ml (39 mM) buserelin in 0.9 % NaCl was prepared, with and without 10 mM GDC, and 200 µl of this solution was pipetted on a cotton disk in the Hill Top chamber.

Outline of the Absorption Study

A randomised cross-over study was performed in six male pigs:

I. intravenous bolus injection of buserelin,

II. 4 h application of a buccal delivery device with buserelin,

III. 4 h application of a buccal delivery device with buserelin and coadministration of GDC.

The interval between each administration in one pig was at least 36 hours.

In Vivo Protocol

Six male pigs (29–35 kg) from the University of Utrecht breeding farm were housed in individual cages. Food and water intake as well as rectal temperatures were recorded daily. Three days before the study one jugular vein of each pig was cannulated (14). For that purpose, the animals were sedated with a subcutaneous ketamine (Aescoket®, Aesculaap BV, Boxtel, The Netherlands) injection and anaesthetised with a mixture of halothane (2–3%), nitrous oxide and oxygen.

To determine the i.v. pharmacokinetics of buserelin in pigs, 5 ml of the 0.15 mM solution was administered into the jugular vein, followed by 5 ml of 0.9 % physiological saline to ensure injection of the complete dose. Blood samples (10 ml/sample) were collected in (heparinized) Monovet® tubes at −5, 2, 4, 6, 10, 14, 20, 30, 40, 50, 60, 75, 90, 105, 120, 150, 180, 240, 300, 360, and 420 min, and placed on ice.

Prior to the buccal delivery experiments, blank blood samples were collected from the jugular vein. The pigs were sedated with an i.v. bolus injection of propofol and subsequently connected to an i.v. infusion of propofol (infusion rate, 30 ml/h) to maintain anaesthesia throughout the 4 hours of buccal administration of buserelin. The pigs were placed on their side on surgery tables and covered with an aluminium foil blanket to maintain their body temperature. Buccal delivery of buserelin was performed by attaching the delivery device to the inside of one of the cheeks. For this purpose, the mouth of the pigs was opened with a clamp, the buccal mucosa was gently wiped with a tissue and the device was held in place manually for 1 min.

After 4 hours of buccal delivery, the devices were removed and the anaesthesia was terminated. The condition of the mucosa was visually assessed for signs of tissue irritation after removal of the delivery device. The pigs were moved to their cages and blood sampling was continued for another 4 hours. Blood samples were collected at −5, 10, 20, 30, 40, 50, 60, 75, 90, 105, 120, 150, 180, 210, 240, 255, 260, 275, 300, 315, 330, 345, 360, 390, 420, 450 and 480 min. Plasma was obtained by centrifugation, and stored at −20°C until analysis.

Analysis of Buserelin

Plasma concentrations of buserelin were measured in triplicate by specific RIA as described previously (15). To avoid inter-assay variation, all samples were processed in one assay, using one batch of labelled peptide and one batch of antiserum.

Pharmacokinetic Analysis

The plasma profiles of buserelin were fitted using a non-linear least squares regression program Siphar (Simed SA, Creteil, France). The plasma profile after i.v. dosing was fitted according to:

$$C_t = A_1 \cdot e^{-\alpha_1 \cdot t} + A_2 \cdot e^{-\alpha_2 \cdot t}$$

in which C equals the plasma concentration of buserelin at time t and A_1, A_2, α_1 and α_2 are the coefficients and exponents of the equation. The pharmacokinetic parameters were calculated as described in Gibaldi and Perrier (16) and determined for each animal. The areas under the individual plasma concentration-time curves (AUC) were calculated with the linear trapezoidal rule. The steady state concentrations and the time to reach steady state after buccal administration were determined from the individual graphs. Buccal bioavailabilities were calculated according to:

$$F = \frac{AUC_{buc} \cdot D_{i.v.}}{AUC_{i.v.} \cdot D_{buc}} \times 100\%$$

in which F is the bioavailability and D the administered dose. The data were evaluated for statistically significant differences by a one way analysis of variance (ANOVA). The probability level was set at 5%. All data are presented as mean ±S.D.

RESULTS

The recorded amounts of food and water intake as well as the rectal temperatures were normal and did not change during the course of the study. Initial pilot experiments in which the i.v. administration of two doses of buserelin was investigated without and with anaethesia with propofol, showed that the plasma concentration time profiles and the pharmacokinetic parameters after i.v. dosing were not different from each other. It was concluded that the anaesthesia did not influence the pharmacokinetics of buserelin in the pig. Visual inspection of the buccal mucosa before and after buccal delivery did not indicate any abnormalities or signs of irritation.

Pharmacokinetics of Buserelin After I.V. Injection

Figure 1 shows the mean plasma concentrations after an i.v. bolus injection of 5 ml of a 0.15 mM solution of buserelin (1 mg peptide/pig). Plasma concentration versus time profiles after i.v. dosing were analysed in terms of a two-compartment model. The pharmacokinetic parameters are given in Table 1. Plasma levels of buserelin after i.v. injection of 1 mg revealed a bi-exponential decline, with a mean initial half-life of 11 min, followed by an elimination half-life of 103 min. The average clearance was 2.0 ml/(min*kg) and the volume of distribution 304 ml/kg.

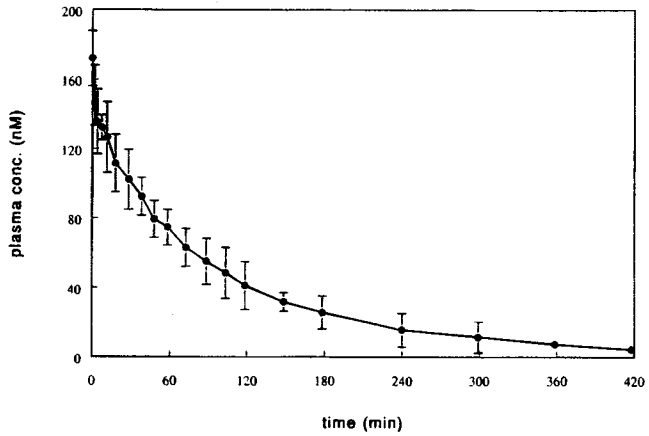

Fig. 1. Plasma concentrations after i.v. injection of buserelin (1 mg in 5 ml saline; 0.15 mM), avarage values ±S.D. in 6 pigs.

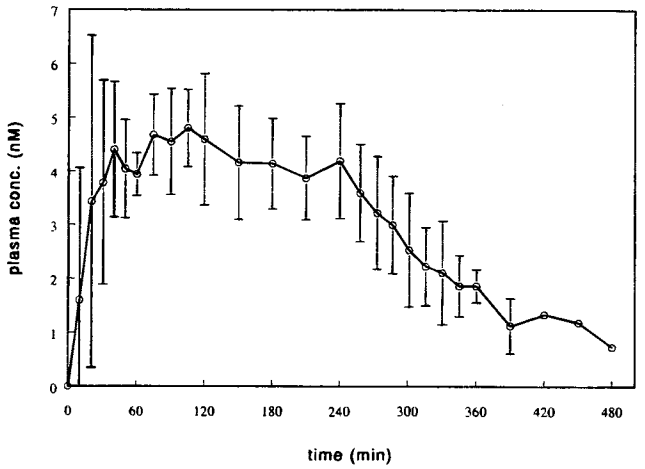

Fig. 2. Plasma concentrations after buccal delivery of buserelin (200 µl of a 39 mM solution in saline) for 4h, average values ± S.D. in 5 pigs.

Buccal Absorption of Buserelin

Plasma levels after buccal administration of 200 µl of a 39 mM solution in 0.9% physiological saline and after buccal administration of 200 µl of a 39 mM buserelin solution in 0.9% physiological saline containing 10 mM of the absorption enhancer GDC are shown in Figures 2 and 3 (10 mg peptide/pig). Table 2 lists the pharmacokinetic parameters of the 4-hours buccal administration of buserelin. Buccal delivery of buserelin, both with and without GDC, showed a rapid absorption by the buccal mucosa, resulting in steady state plasma levels until 240 min, the time at which the delivery device was removed. Buccal administration of buserelin without GDC in pig number 6 did not result in measurable plasma levels. During buccal delivery of buserelin with GDC in pig number 2, the delivery device did not stay attached very well; therefore, the data obtained in this pig were not included for calculating the mean pharmacokinetic parameters.

About 1h after application of the buccal delivery device (both with and without 10 mM GDC), steady state plasma concentrations were reached. Coadministration of 10 mM GDC resulted in an increase in steady state plasma concentrations from 4.4 to 23.2 nM. After removal of the delivery device, the mean elimination half-lives for buserelin were 77 and 54 min, without and with GDC, respectively. The absolute bioavailability of buserelin after buccal administration for 4h without GDC was 1.0 ± 0.3% and increased substantially by coadministration of 10 mM GDC to 5.3 ± 1.1%.

Table 1. Pharmacokinetic Parameters of I.V. Administered Buserelin in Pigs (n = 6)

	mean	S.D.
weight (kg)	32	2
$t^1/_2$ dist. (min)	11	11
$t^1/_2$ elim. (min)	103	20
Cl (ml/min*kg)	2.0	0.4
V_d (ml/kg)	304	112

Note: $t^1/_2$ distr. = Distribution half-life; $t^1/_2$ elim. = Elimination half-life; Cl = Clearance; V_d = Volume of distribution.

DISCUSSION

The buserelin plasma concentrations in the pig after i.v. administration corresponded well with a bi-exponential model. After a distribution half-life of about 11 min, buserelin was cleared from the blood stream with an mean elimination half-life of 103 min. The observed two-compartment model in pigs, differs from the previously reported multi-exponential decline of buserelin plasma levels after i.v. injection in rats and dogs (4). However, adequate determination of the elimination profile of this peptide may require monitoring plasma concentration longer than the 3h reported in dogs and rats after i.v. dosing. It has been reported that LHRH agonists are eliminated via the kidneys, both the intact peptides and their metabolites (4). From the antibody-specificity data it is evident that both intact buserelin and its inactive metabolites, sharing the C_{6-9} sequence of the parent peptide, are included in the measured concentration (4). The clearance and volume of distribution in pigs, as determined in the present study, indicated that buserelin and its

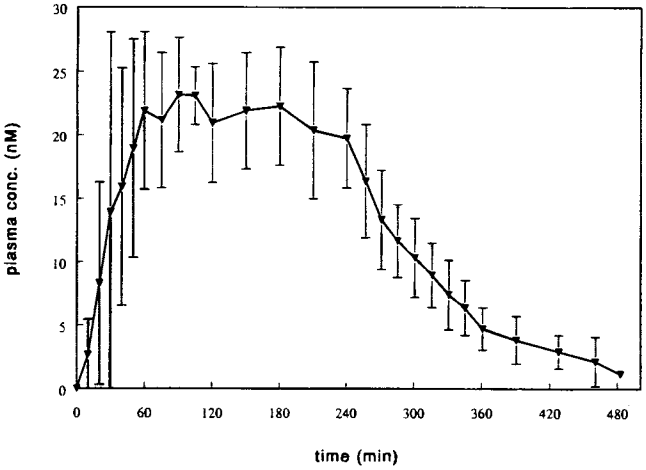

Fig. 3. Plasma concentrations after buccal delivery of buserelin (200 µl of a 39 mM solution in saline) for 4h with coadministration of 10 mM GDC, average values ± S.D: in 5 pigs.

Table 2. Pharmacokinetic Parameters of Buccal Administration of Buserelin in Pigs

pig	weight (kg)	$t^1/_2$ elim. (min)	C_{ss} (nM)	t_{ss} (min)	F (%)
BUCCAL WITHOUT ENHANCER					
1	34	113	4.5	50	1.3
2	32	52	5	50	1.2
3	35	82	5	90	1.0
4	33	87	4.5	50	1.0
5	31	50	3	70	0.7
6	29	#	#	#	#
mean	32	77[@]	4.4[@]	62[@]	1.0[@]
S.D.	2	29	0.8	18	0.3
BUCCAL WITH ENHANCER					
pig		$t^1/_2$ elim. (min)	C_{ss} (nM)	t_{ss} (min)	F (%)
1		70	23	60	6.5
2		49	15	90	3.0
3		59	23.5	75	4.4
4		53	19	75	4.0
5		50	24.5	60	5.6
6		46	26	60	5.9
mean		54*	23.2[$]**	66[$]	5.3[$]**
S.D.		10	2.6	8	1.1

Note: #: Plasma concentrations were below the detection limit; [@]: n = 5 Excluding pig number 6; [$]: n = 5, Excluding pig number 2; $t^1/_2$ elim. = Elimination half-life; C_{ss} = Steady state plasma concentration; t_{ss} = Time to reach steady state plasma concentration; F = Absolute bioavailability; * = Significantly different from i.v. ($p < 0.005$); ** = Significantly different from buccal administration without GDC ($p < 0.005$).

metabolites are mainly cleared from the circulation by urinary excretion.

Buccal delivery of buserelin resulted in mean steady state plasma levels of 4.4 and 23.2 nM, without and with coapplication of 10 mM GDC, respectively. These steady state concentrations were already reached at 1h after attaching the buccal delivery device, both without and with GDC present in the buccal dosage form. The relatively rapid onset of absorption following buccal administration, as observed for buserelin in this study, has also been reported for other compounds, such as buprenorphine (17), low molecular weight heparin (9) and fluorescein isothiocyanate labelled dextran 4000 (18). It seems therefore likely that, when the buccal mucosa is exposed to a drug delivery system, the drug diffuses into the tissue within the first hour, and fills up this reservoir. An equilibrium between the buccal mucosa and the blood compartment is established after the maximum tissue concentration has been reached.

GDC increased both the absorbed amount of buserelin and the rate of absorption across the buccal mucosa. The first hour of buccal absorption was further investigated by making a Loo-Riegelman plot to determine the absorption rate constant. However, the absorption rate was not constant over time, and could therefore not be determined. This observation is very similar to our previous results on the buccal delivery of FITC labelled dextran 4400 (18).

In order to determine whether the mechanism of action of GDC is based on an increased diffusivity of GDC in the buccal epithelium or an altered pathway across the buccal mucosa, more detailed monitoring of the plasma concentrations is necessary.

The elimination half-life of buserelin after removal of the buccal delivery device was similar to that observed after i.v. injection. A depot effect in the buccal mucosa, as previously suggested for buprenorphine (17) and FITC-labelled dextran 4400 (18), could not be observed for buserelin.

The coadministration of the absorption enhancer GDC, in a concentration of 0.45% (w/v), resulted in an increase of the bioavailability of 4-hours buccal delivery by a factor 5 (mean absolute bioavailability of 5.3%). In addition, much higher steady state plasma levels were reached within same time period. A relatively low concentration of GDC, 10 mM (0.45% (w/v)), is already effective in promoting buccal absorption of a hydrophilic macromolecular compound in pigs.

Absorption enhancement by a bile salt, as reported in this study, corresponds well with the increase in bioavailability found by Ebert et al. (9) in dogs, and by Oh and Ritschel (19) and Nakada et al. (11) in rats. Ebert et al. (9) reported a higher absorption enhancement effect using sodium taurocholate in dogs (with a non-keratinized epithelium, similar to pigs) than obtained in the present study with GDC. It should be noticed, however, that the concentration of taurocholate was higher and the surface area was larger than in this study. These two aspects are expected to result in a higher enhancement. In a previous in-vitro study from our laboratory the effects of various concentrations of different bile salts on the structure of porcine buccal epithelium were investigated. A high concentration (100 mM, 5% w/w) of bile salt resulted in loss of outer epithelial cell layers, disruption of the connection between epithelium and the underlying connective tissue, and a disorganisation of the intercellular and membrane lipids (20). No mucosal damage was observed for bile salts, neither in the present study, nor by Ebert et al. (9). Zhang et al. (10) proposed that bile salts like sodium taurocholate and GDC transiently alter the permeability barrier. Both the lower concentration used and the fact that the latter study was performed in-vivo (supply of nutrients by the supporting tissue and blood stream and possible recovery) are expected to lead to less profound effects of bile salts on the buccal mucosa. We therefore suggest that the bile salt GDC is able to increase the absorption buserelin by altering the buccal permeability barrier, possibly by solubilizing epithelial lipids and thereby facilitating diffusion of the hydrophilic peptide drug.

In conclusion, the present study indicates that buccal administration is a suitable route of delivery for peptide drugs, such as the LHRH agonist buserelin. Dependent on the therapy, the bioavailability of buccally delivered peptides can be increased by increasing the concentration of the absorption enhancer and/or the application time of delivery device.

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