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The colonic absorption of human calcitonin: the effects of increasing local concentration and co-administration with a protease inhibitor

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Abstract

Attention has recently focused on the colon as a potential site to target therapeutic peptides and proteins for systemic absorption. This paper describes the colonic absorption of human calcitonin (hCT) in man. Low concentrations of human hCT are rapidly degraded by human faecal material. This degradation can be retarded by addition of the protease inhibitor aprotinin. The absolute bioavailability (ABV) of 10 mg hCT administered in a high concentration to the descending colon of nine healthy volunteers was 0.22 ± 0.07%. The maximum plasma concentration was 1208 ± 357 pg ml^{-1}, observed after 5 min. The co-administration of aprotinin with 10 mg hCT decreased the ABV to 0.11 ± 0.03%, and the plasma profile gave a maximum concentration of 442 ± 115 pg ml^{-1}, observed after 10 min. High local concentrations of hCT in the colon improve absorption, but do not alter the plasma profile of the peptide.

Keywords: Human calcitonin; Colonic absorption; Oral drug delivery; Aprotinin

1. Introduction

Oral drug administration is favoured by patients, medical practitioners and the pharmaceutical industry for reasons of compliance, ease and economics. However, this route of administration is only of value if the drugs are bioavailable in a reproducible manner from the gastrointestinal (GI) tract or if the treatment is for a local indication in the GI tract. This is true whether we are considering conventional low molecular weight drugs or the therapeutic peptides and proteins that the biotechnology revolution has recently provided (Mackay, 1991).

In considering therapeutic peptides and proteins, biopharmaceutical scientists have lived in the shadow of insulin. This peptide has been a

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focus of drug delivery for 60 years, however, the most effective formulations are still administered by injection. Whilst type I insulin-dependent diabetes melitus patients have been poorly served, in terms of user friendly formulations, their dependence upon insulin for longevity has supported the need for parenteral formulations. Other therapeutic peptides and proteins indicated for non-life threatening diseases will not have the same degree of patient loyalty. For this reason it is recognised that the immense potential of therapeutic peptides and proteins will not be realised until effective routes of administration are established.

In recent years attention has turned to the colon as an important site for the delivery of therapeutic peptides and proteins (Ritschel, 1991; Mackay and Tomlinson, 1993). To judge the potential of the colon, three significant factors must be considered: (i) accessibility to the colon and specificity of release in this region of the GI tract; (ii) the environment and its influence on the degradation of therapeutic peptides and proteins; (iii) the permeability of the epithelium to therapeutic peptides and proteins.

Each of these factors presents different opportunities within the overall challenge of oral delivery via the colon. However, in the case of therapeutic peptides and proteins it was believed that the product of these factors when compared with other regions of the GI tract created a window of opportunity for systemic delivery that was not present elsewhere in the GI tract (Table 1).

However, even with this optimistic view of the colon as the site for systemic delivery of therapeutic peptides and proteins it is important to realise the significance of other key factors before this route is pharmaceutically acceptable. Any drug taken for routine therapeutic purposes should ideally have a reproducible bioavailability to help design effective dosing regimes. It is also important that the bioavailability is sufficiently high that the cost of the therapy does not become limiting or, indeed, that in choosing to administer the drug orally the large doses needed does not reduce the number of people with access to the therapy.

Table 1
General features of the GI tract influencing the delivery and absorption of therapeutic peptides and proteins

Factor	Stomach	Jejunum	Ileum	Colon
Accessibility	Good	Good	Good	Poor
Retention	Variable	Short	Short	Long
Luminal hydrolases	Harsh	Harsh	Harsh	Mild
Cellular hydrolases	Mild	Harsh	Mild	Mild
Permeability	Low	Low	Low	Low

We have taken human calcitonin (hCT) as a therapeutic peptide to explore the value of the colon for the systemic delivery of peptides and proteins after oral administration. hCT is a 32-amino acid hormone involved in the regulation of blood calcium levels (Austin and Heath, 1981). Calcitonins from several sources are used in the management of a variety of disorders identified with accelerated bone resorption (Greenberg et al., 1974; McDermott and Kidd, 1987). Until recently, the drug was administered by injection. The successful introduction of intranasal salmon calcitonin has demonstrated the potential of non-injectable routes of administration for other calcitonins (Muff et al., 1990; O'Doherty et al., 1990). We have previously shown that hCT is absorbed across the proximal colon of rat (Hastewell et al., 1992, 1994), the descending colon of man (Antonin et al., 1992) the sigmoid colon of man (Beglinger et al., 1992) and the transverse colon of man (Antonin et al., 1995). In all cases the absolute bioavailability (ABV) was low. In this study, we have delivered hCT to the descending colon of man in the presence of the protease inhibitor aprotinin. In addition, we report the effect of increasing the local concentration of hCT on the absorption from the colon of man.

2. Materials and methods

2.1. Faecal degradation studies

2.1.1. Materials

Freshly passed faeces, 5 g, was made up to 20 ml volume with phosphate-buffered saline (150 mM NaCl; 5 mM Na_2HPO_4; pH 7.4). The solids were dispersed and the suspension centrifuged at 1500 × *g* for 15 min. The supernatant was

collected and used for degradation studies. hCT solutions were prepared in 0.1% acetic acid. A commercial preparation of aprotinin (25000 kallidinogenase inactivator units ml^{-1}) in 0.9% saline with benzyl alcohol was used in the inhibitor studies. ^{125}I-hCT was added to yield a final concentation of 0.3 μCi ml^{-1}.

2.1.2. Degradation studies

All solutions were pre-warmed to 37°C before the reaction was initiated by addition of the faecal extract to the premixed hCT solutions, including aprotinin at 24000 units ml^{-1} if required. The overall dilution was 1:2 faecal extract to reagents. The reaction was quenched by the addition of 100 μl of the incubation medium to 100 μl of 40% trichloroacetic acid (0°C). The precipitate was left to form at 0°C (1 h) before centrifugation. The supernatant and pellet were counted separately. Degradation was determined as the appearance of counts in the supernatant fraction.

2.2. Volunteer study

2.2.1. Subjects and ethical approval

Nine healthy human subjects (two male, 68 ± 4 kg, standard deviation; seven female, 60 ± 8 kg) aged between 18 and 36 years (24 ± 5 years, standard deviation) participated in the study. All subjects underwent medical examinations that included physical status, haematology, blood chemistry, urine analysis and ECG, and all were shown to be healthy. Pregnant women were excluded from the study. No drugs, except oral contraceptives, were allowed for 2 weeks prior to the study or during the study period. Informed written consent was obtained from each participant. The study was approved by the Ethical Committee responsible for the Human Pharmacology Institute of Ciba Pharmaceuticals in Tübingen, whose members are Professors of Medicine, Law and Theology at the Universities of Tübingen or Freiburg.

2.2.2. Adverse events

Subjects reported spontaneously adverse events and they were monitored (by observation) for the known side-effects of hCT.

2.3. Study details

2.3.1. Design

After an overnight fast the volunteers received three doses of hCT in a randomised open, cross-over, single centre study with at least a 3-day washout period between administrations. On the day before the study, and during the study period, the volunteers abstained from consumption of alcohol, caffeine and nicotine. Each study day started at 08:00 and a standard breakfast (one to three bread rolls, 40 g of butter, 50 g jam and decaffeinated coffee) was given 150 min after hCT administration.

Blood (4 ml) was collected by venipuncture from an antecubital vein and placed into EDTA-charged tubes. The samples were centrifuged immediately, the plasma collected and stored at − 20°C for analysis. Urine was collected before drug administration and at hourly intervals, after the administration period, for 8 h. Samples were stored at − 20°C for analysis.

2.4. Colonoscopic administration

Colonoscopy was performed with an Olympus Colonoscope (CF-LB 3 R, Olympus, Hamburg, Germany) 45 min after an enema-induced bowel movement (YAL®solution; Medalsdorf, Alsdorf, Germany). The large intestine was not prepared in any other way. The instrument was introduced as far as possible into the distal colon, compatible with luminal patency. The dose (1 ml of a 10-mg ml^{-1} hCT solution in 0.1% (v/v) acetic acid) was applied as a bolus through a catheter passed down the instrument channel of the colonoscope. This was rinsed through with either 5 ml of saline (150 mmol l^{-1}) or 5 ml of aprotinin solution (Trasylol, 20000 kallidinogenase inactivator units ml^{-1}, Bayer, Germany). The volunteers remained in the supine position for at least 30 min following the administration after which the remaining dose was voided. Bowel movements were restricted for 4 h after the dose was administered. Blood was sampled at 60 and 30 min, immediately prior to, and 5, 10, 15, 20, 30, 45, 60, 90, 120, 150, 180, 210, 240 and 300 min after administration of the dose.

Table 2
Time for loss of 50% of the original dose of hCT in the presence of faecal extract

hCT conc. (mg ml^{-1})	Control (min)	With aprotinin (16000 units) (min)
0.1	2.0±0.1	3.4±0.4*
0.2	3.3±0.4	5.0±0.6*
0.5	6.9±0.4	9.8±0.5*
1.0	14.2±1.5	17.5±0.9*
2.0	25.9±1.8	30.6±2.4*

Data shown as mean±SEM. *$p<0.001$ with control data

2.5. Intravenous administration

hCT (0.25 mg) was dissolved in 1 ml of 3% mannitol and the solution was diluted in 500 ml of saline (150 mmol l^{-1}) with 0.1% (w/v) of human serum albumin. This solution was infused at a constant rate for 45 min. Blood was sampled at 60 and 30 min, immediately prior to the start of the infusion, and at 5, 10, 20 and 30 min during the infusion period, and 5, 10, 20, 30, 45, 50, 55, 60, 65, 75, 85, 95, 105, 120, 135, 150 and 165 min after stopping the infusion.

2.6. Blood and urine analysis

The following clinical chemical parameters were measured using standard procedures. From blood: glucose, creatinine, urea, uric acid, total bilirubin, protein, cholesterol, triglycerides, aspartate amino transferase, alanine amino transferase, alkaline phosphatase, γ-glutamyl transferase, potassium, sodium, calcium and chloride. From urine: pH, nitrite, protein, glucose, ketone bodies, urobilinogen, bilirubin, blood.

2.7. Plasma hCT assay

hCT levels were determined using a commercial immunoassay (Immunoradiometric assay for hCT, International CIS, High Wycombe, UK). The samples were suitably diluted in 4% human serum albumin (Blood Products Laboratory, Elstree, UK) before analysis. All assays were run as duplicates with a standard curve for each assay. The inter-assay coefficient of variation ranged between 2.3 and 10.7% and the intra-assay coefficient of variation ranged between 3.1 and 13.7% over the concentration range assayed. The minimum detection limit was 10.2 pg ml^{-1}.

2.8. Materials

All chemicals used for the volunteer study were of European Pharmacopoeial grade. hCT was kindly supplied by Finlay Skinner, Ciba Pharmaceuticals, Basle, Switzerland. All other chemicals were of analytical grade.

2.9. Expression of results

The ABV of hCT after intracolonic administration of 10 mg was determined by comparison of the area under the curves from the two administration routes. All results are expressed as mean ± standard error of the mean (SEM). Results from eight volunteers were included. hCT plasma levels are given as pg ml plasma^{-1}. Statistical analysis was carried out by Student's t-test.

3. Results

3.1. Faecal degradation studies

The degradation of hCT by the faecal extract was rapid, Table 2 shows the extrapolated time for 50% degadation of various concentrations of hCT. Data generated by linear extrapolation of rates, ($n \geq 4$). In all cases aprotinin significantly reduced the rate of degradation $p < 0.001$.

3.2. Volunteer study

3.2.1. Physiological parameters and adverse events

No drug-related or clinically relevant changes were observed in the volunteers' blood pressure, pulse rate, ECG or laboratory parameters determined from the blood. Similarly, no changes in the parameters determined from the urine samples were observed. A variety of mild adverse events, commonly associated with hCT, were recorded as a result of i.v. administration.

3.2.2. Pharmacokinetic profiles

The data presented are the mean of eight of the nine volunteers, one data set was excluded because the volunteer did not complete the study. The plasma concentration profile for hCT following the 60-min i.v. administration is shown in Fig. 1. Constant plasma levels of hCT were reached after approximately 30 min, and maintained until the end of the infusion. The decay of the plasma levels was rapid. The plasma hCT profiles after colonic administration in the presence of saline and aprotinin are shown in Fig. 2 and Fig. 3, respectively. The ABV of 10 mg hCT administered in a high concentration to the descending colon was 0.22 ± 0.07%. The maximum plasma concentration was 1208 ± 357 pg ml^{-1}, observed after 5 min. The co-administration of aprotinin with 10 mg hCT decreased the bioavailability to 0.11 ± 0.03%, and the plasma profile gave a maximum concentration of 442 ± 115 pg ml^{-1}, observed after 10 min.

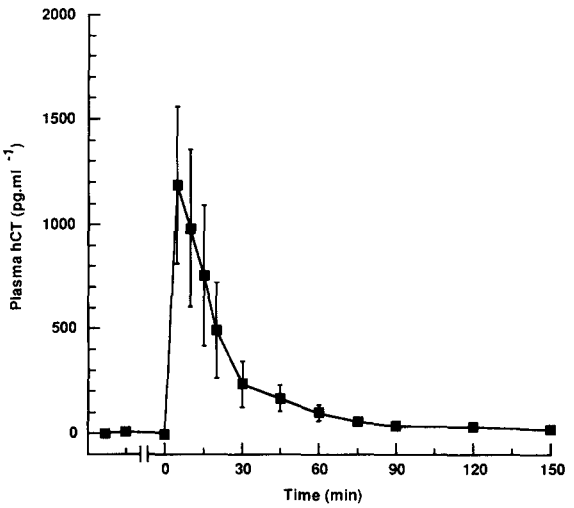

Fig. 2. Plasma hCT levels colonoscopic administration of 10 mg hCT to the descending colon. Values are mean ± SEM ($n = 8$).

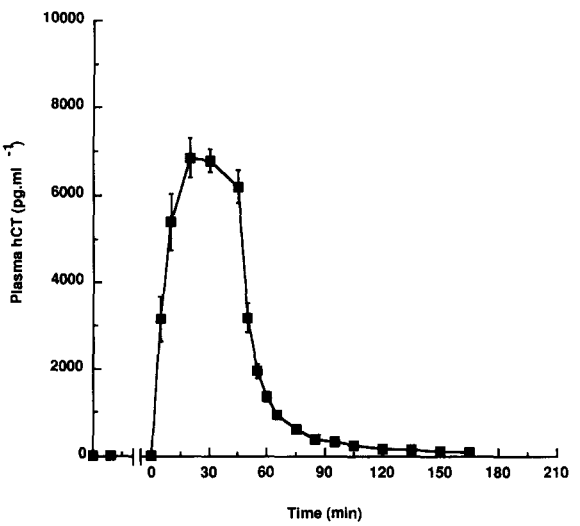

Fig. 1. Plasma hCT levels after i.v. infusion of 0.25 mg hCT. Values are mean ± SEM ($n = 8$).

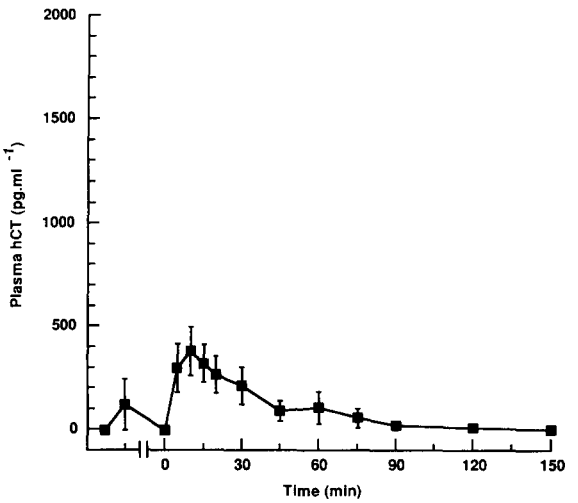

Fig. 3. Plasma hCT levels colonoscopic administration of 10 mg hCT to the descending colon in the presence of aprotinin. Values are mean ± SEM ($n = 8$).

Table 3
Comparison of pharmacokinetic data after various dosing regimes to the descending colon

Colonic concentration	ABV (%)	C_{max} (pg ml^{-1})
0.4 mg ml^{-1} hCT*	0.08±0.03	525.0±170.0
1.7 mg ml^{-1} hCT	0.22±0.07	1208.0±357.0
1.7 mg ml^{-1} hCT with aprotinin	0.11±0.03	440.0±116.0

Data shown as mean±SEM. *Data from Antonin et al. (1992).

4. Discussion

A previous study (Antonin et al., 1992) suggested that faecal material in the colon reduced the absorption of hCT. This could result from either increased luminal degradation or adsorption of hCT to the faecal material. Both phenomena effectively reducing the free concentration of hCT in the lumen available for absorption. The in vitro degradation studies suggest that faecal material contains considerable degradative capabilities. It is not possible to predict the degradative capabilities of the descending colon lumen from these data. However, in the volunteer study (Antonin et al., 1992) the luminal concentration of hCT after colonoscopic administration was in the region of 0.4 mg ml^{-1}, estimated from 1 ml of a 10-mg ml^{-1} hCT dosed as a bolus diluted with a 20-ml saline rinse. It is therefore valid to say that degradation could have been the limiting factor during absorption because of the susceptibility of this low concentration of hCT to degradation. The in vitro degradation studies show that either increasing the concentration of hCT in the colon or the coadministration of aprotinin would effectively increase the luminal residence time of hCT.

The volunteer study was designed to reduce the quantity of saline used to flush the hCT bolus out of the colonoscope to 5 ml compared with 20 ml used in the previous study (Antonin et al., 1992). This should increase the luminal concentration of hCT to 1.7 mg ml^{-1}. This 4-fold greater luminal concentration would be expected to increase the residency 3–4-fold with a concommitant increase in hCT absorption. The hypothesis was further tested with 5 ml of aprotinin to directly inhibit degradation.

The data (Fig. 2 and Table 3) show that elevating the luminal concentration of hCT 4-fold increased the ABV 2.5-fold to 0.22%. However, the plasma profile against time was unchanged from that observed in the previous study with the increased bioavailability being explained by a pro rata increase in C_{max} without any extension of the plasma profile. Interestingly, the increased residency time for hCT in the high luminal concentration part of the study would be expected to prolong the plasma profile due to the extended opportunity for absorption. This suggests that there are other factors beyond degradation that limit the absorption window for hCT in the descending colon.

It was expected that the presence of aprotinin would further increase the absorption of hCT. However, this was not observed with the data showing a significant decrease in bioavailability ($p < 0.001$) returning the level of absorption observed to the low luminal concentration (Table 3). There is little explanation of this beyond the observation that at high concentrations of hCT (5–10 mg ml^{-1}) aprotinin causes rapid precipitation of the hCT (data not shown). It is possible that the hCT dose could have precipitated in the instrument and been delivered to the colon in a form that would considerably limit absorption. It is also tempting to speculate that luminal debris in the colon could have a similar effect on the administered hCT reducing the effective absorption window. Our rat and volunteer studies have revealed that hCT is absorbed from the colon (Antonin et al., 1992; Beglinger et al., 1992; Hastewell et al., 1992, 1994; Antonin et al., 1995) However, absorption is low and unless absorption enhancers are used (Hastewell et al., 1994) rarely exceed an ABV of 1%. In this study we show that increasing the local concentration of hCT results in an increase in the ABV in man. We conclude that for the effective absorption of hCT from the colon, absorption enhancers will need to be used. However, the long-term toxicity implications of absorption enhancers can only be assessed in chronic studies. Our previous study (Hastewell et al., 1994) showed that there is no acute toxicity as a result of administration of lipidic enchancers. This is consistent with data from other laborato-

ries (Richardson et al., 1991) who showed that 0.5% L-α-lysophosphatidylglycerol caused little epithelial damage after 24 h contact with the vaginal epithelium of rats. However, concerns still exist with regard to chronic use of absorption enhancers as well as the potential for co-absorption of other substances from the lumen of the GI tract. For this reason, a better understanding of how absorption enhancers work is needed before pharmaceutically relevant formulations can be developed for improving the GI tract delivery of therapeutic peptides and proteins in a safe and selective manner.

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