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The absorption of human calcitonin from the transverse colon of man

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Abstract

Patients with a loop stoma were used to provide direct access to the transverse colon. The ease of delivery offers the chance to study absorption of human calcitonin (hCT) in a defined region of the large intestine difficult to access in healthy volunteers; i.v. infused hCT elicited a standard pharmacokinetic profile in eight loop stoma patients showing a biphasic elimination with half-lives of 11.5 ± 0.8 min and 33.7 ± 1.8 min. hCT administered via the loop stoma was absorbed across the transverse colonic mucosa in low amounts. The 10-mg dose achieved a mean maximum plasma concentration of 1242 ± 346 pg ml^{-1}, after 5-10 min with an absolute bioavailability of 0.22 ± 0.06%. We conclude that the transverse colon is a better site for the absorption of human calcitonin than the more distal regions of the colon. This could be a function of the transverse colonic epithelium. Alternatively, it could be due to the reduced levels of luminal debris and bacterial colonisation in the stoma patients compared with the previous studies carried out in the distal colon of healthy volunteers.

Keywords: Human calcitonin; Absorption; Oral drug delivery; Loop stoma; Colon; Human study

1. Introduction

Human calcitonin (hCT) is a 32-amino acid hormone synthesised by C-cells of the thyroid gland. hCT is involved in the regulation of blood calcium levels (Austin and Heath, 1981). Calcitonins from several sources are used in the management of disorders identified with accelerated bone resorption (Greenberg et al., 1974; McDermott and Kidd, 1987). Until recently the drug was administered by injection (Stevenson and Evans, 1981). This causes problems of patient compliance and side-effects. Nausea and facial flushing are common due to the high peak plasma levels achieved following injection (Gennari et al.,

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1983). The successful introduction of intranasal salmon calcitonin has demonstrated the legitimacy of non-injectable routes of administration (Reginster et al., 1985; Muff et al., 1990; O'Doherty et al., 1990). The oral route of administration would offer further advantages in terms of delivery and patient compliance.

It has been recognised that there may be absorption windows in different regions of the gastrointestinal (GI) tract (Mackay, 1991; Mackay and Tomlinson, 1993). Moreover, the colon has been proposed as a potential site for peptide and protein absorption due to the presence of fewer proteases (Ritschel, 1991).

We have previously shown that hCT is absorbed across the proximal colon of rats (Hastewell et al., 1992; Hastewell et al., 1994), the descending colon of man (Antonin et al., 1992) and the sigmoid colon of man (Beglinger et al., 1992). In all cases the absolute bioavailability (ABV) was low. In this study we have chosen the transverse colon of man as a potential site for hCT absorption. In the descending colon study (Antonin et al., 1992) there were problems associated with the presence of luminal debris. Access to the transverse colon in healthy volunteers is more difficult. Therefore, we have used loop stoma patients for this investigation. This artifical lesion offers direct access to the transverse colon. In addition, the hCT can be precisely administered.

2. Materials and methods

2.1. Patients and ethical approval

The patients (six male, 67 ± 14 kg; two female, 46 ± 5 kg) aged between 32 and 68 years were hospitalised in the Surgery Department of the University of Leipzig. The loop stomas resulted in seven patients from treatment of carcinomas (one breast, one endometrial, three rectal carcinomas, two sigmoid carcinomas) and one as a result of perforation of the sigmoid colon by a foreign body. In all patients there were no pathological findings in the remaining intestine. Exclusion criteria for the trial were: any clinically relevant abnormal findings (excepting the loop stoma) in the medical examination including physical status, haematology, blood chemistry, urine analysis and ECG; pathological findings in the remaining colon; regular drug intake or abuse; pregnancy and allergic diathesis. The study was performed in accordance with the latest amendment of the World Medical Association's Declaration of Helsinki and Good Clinical Practice Guidelines of the European Community. The trial was approved by the Ethical Committee responsible for the Medical Department of the University of Leipzig. Written and informed consent was obtained from all volunteers.

After an overnight fast the loop stoma patients received two doses of hCT in an open, partly randomised, cross-over study. On the day before the study and during the study period the patients abstained from consumption of alcohol, caffeine and nicotine.

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. Intracolonic administration

hCT (10 mg) dissolved in 1 ml of 0.1% acetic acid (v/v) was applied as a bolus into the distal part of the transverse colon through the stoma. This was followed by a further 20 ml of saline (150 mmol l^{-1}).

2.3.2. Intravenous administration

hCT (0.5 mg) dissolved in 1 ml of 3% mannitol before dilution in 500 ml of saline (150 mmol l^{-1}) with 0.1% human albumin (w/v) was administered by constant rate i.v. infusion over a period of 90 min.

There was a washout period of at least 5 days between the two administrations. Each study day started at 8 a.m. 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, the plasma collected and stored at −20°C for analysis. For the intracolonic administration blood was sampled at 60 and 30 min, and immediately before dosing, and at 5, 10, 20, 30, 45, 60, 90, 120, 150, 180, 240, 300, 420 and 540 min after administration of the dose. In the case of the i.v. infusion blood was sampled at 60 and 30 min, and immediately before the start of the infusion, samples were taken at 10, 20, 30, 45, 60 and 90 min during the infusion period, and at 5, 10, 15, 20, 25, 30, 40, 50, 60, 75, 90 and 105 after stopping the infusion.

2.4. 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 and blood.

2.5. 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 interassay coefficient of variation ranged between 2.3 and 10.7% and the intraassay 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}.

2.6. Deviations from protocol

The 5-min time point after colonic dosing was only taken for five of the eight patients. In some patients there was evidence of pathologies associated with primary diseases or concomitant treatment as evidenced by elevated liver enzyme activity in the blood. However, there were no histological or functional pathologies observed in the transverse colon.

2.7. Materials

All chemicals used for the study were of European Pharmacopoeial grade. hCT (batches 000390 and 145011) was supplied by Ciba Pharmaceuticals, Basle, Switzerland. All other chemicals were of analytical grade.

2.8. 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 $\pm$ standard deviation of the mean (S.D.) and/or standard error of the mean (S.E.M.). Results from all eight loop stoma patients 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. Parameters and adverse events

No drug-related or clinically relevant changes were observed in 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 adverse events were recorded as a result of hCT administration. After intracolonic administration one patient reported a feeling of mild nausea and a heavy stomach and a second patient had mild facial flushing. After i.v. administration three patients developed facial flushing, one patient complained of a headache, abdominal disturbance and generally feeling unwell, and three patients reported feeling hot or cold or a combination of both.

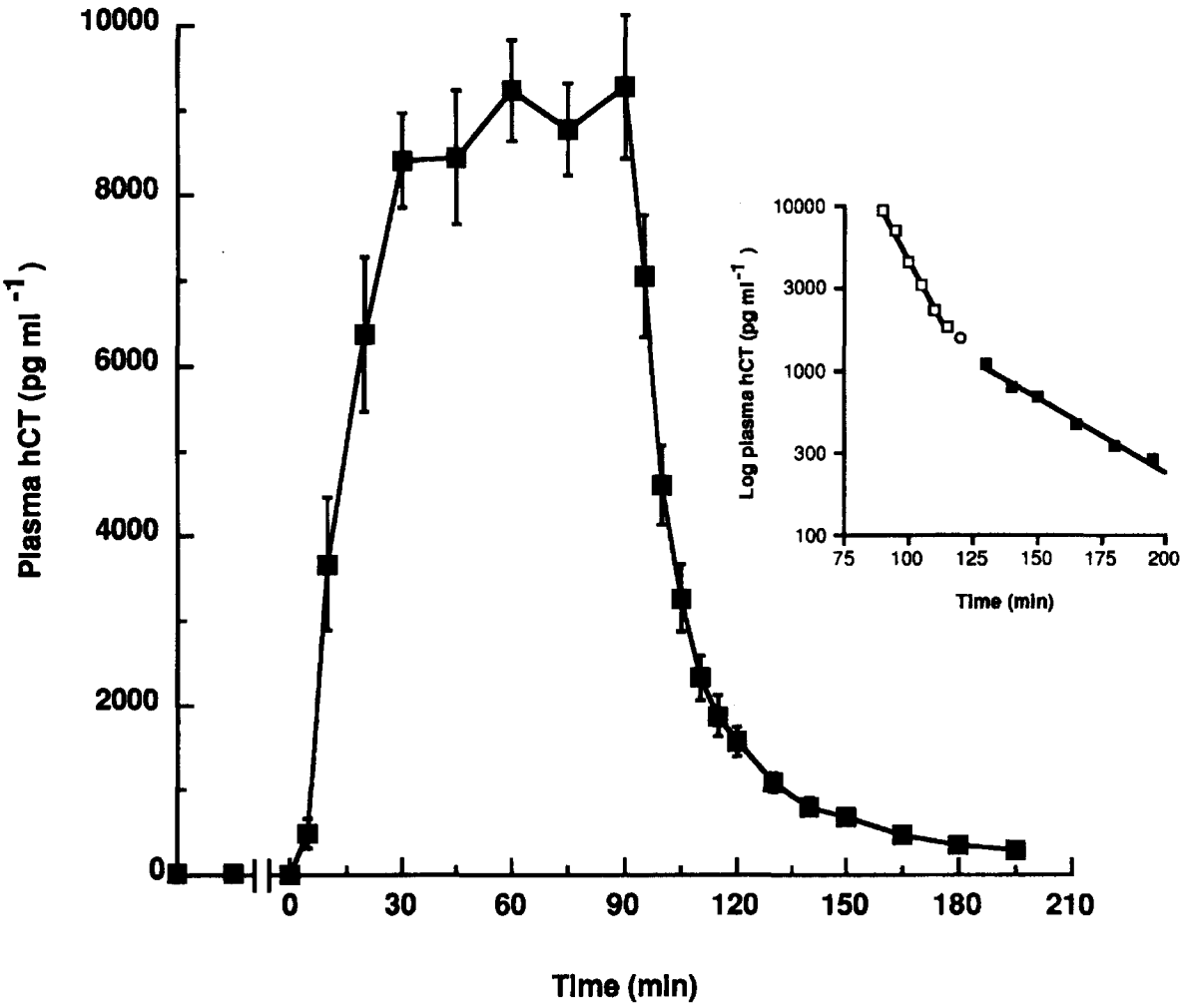

Fig. 1. Plasma hCT levels after intravenous infusion of 0.5 mg of hCT. Inset semi-logarithmic plot showing the decay phases for hCT elimination (n = 8, means ± S.E.M.).

3.2. Pharmacokinetics

The mean plasma hCT profiles after i.v. infusion are presented in Fig. 1, with a semi-logarithmic plot of the elimination phase inset. The mean plasma hCT profiles after administration via the loop stoma are presented in Fig. 2, with a semi-logarithmic plot inset. The pharmacokinetic parameters are presented in Table 1. The rapid absorption of hCT from the colon indicates that the earliest time points are crucial for determining the C_{max}. Because the 5-min data are missing in three of the eight patients the average C_{max} is calculated from the first blood sample after dosing. Therefore the value is derived from a combination of 5- and 10-min points. The C_{max} is 1242.0 ± 346 pg ml^{-1}. This value is not significantly ($p > 0.1$) lower than the observed 5-min value in Fig. 1 (1433.0 ± 524 pg ml^{-1}). The data show maximum absorption occurred 5-10 min after administration.

The ABV of hCT administered via the loop stoma is 0.22 ± 0.06% (data range, 0.02-0.51%). A significant plasma profile was observed in each patient, indicating that hCT absorption occurred in all cases albeit to a variable extent (C_{max} range 140-2711 pg ml^{-1}).

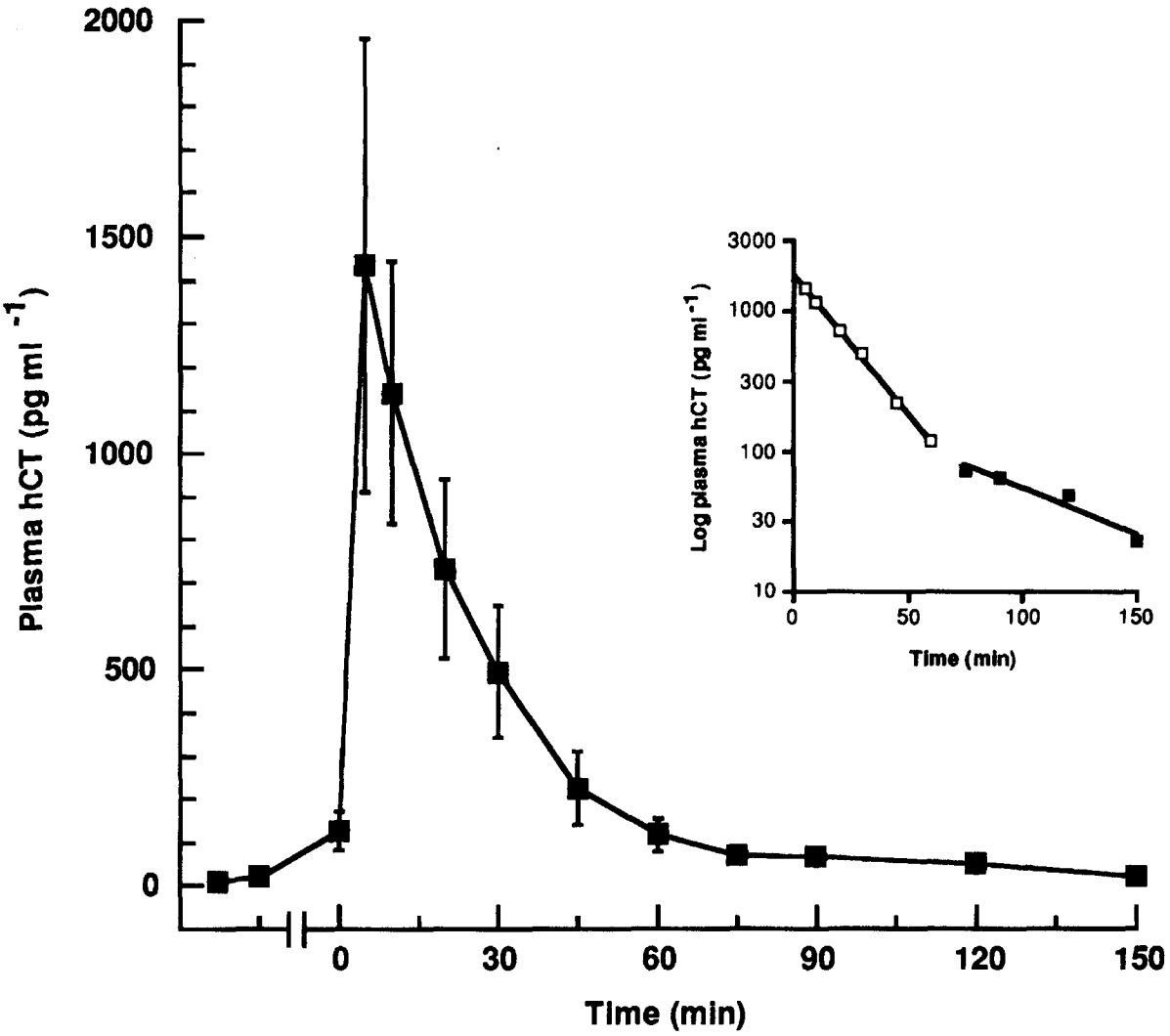

Fig. 2. Plasma hCT levels after bolus administration of 10 mg hCT to the transverse colon via the loop stoma. Inset: semi-logarithmic plot showing the decay phases for hCT elimination (n = 8, means ± S.E.M).

4. Discussion

The data show that hCT is absorbed from the human transverse colon. The absorption is rapid (T_{max} 5-10 min). Both the ABV (0.22%) and the C_{max} (1242 pg ml^{-1}) achieved after intracolonic administration to the transverse colon were significantly ($p < 0.01$) higher than that reported in a previous study in which hCT was dosed to the descending colon (Antonin et al., 1992) as shown in Table 2.

This suggests that the transverse colon is more permeable to hCT than the descending colon. However, it should be noted that stoma patients have reduced luminal contents and bacterial colonisation (Moran and Jackson, 1990) compared with healthy volunteers (Cummings et al., 1990). For this reason the increased absorption from the transverse colon could be a consequence of the different luminal environment. This is supported by the previous study, where it was observed that faecal material in the descending colon

Table 1
Pharmacokinetic parameters after i.v. and i.c. administration of hCT

Parameter	i.v. administration	i.c. administration
$t_{0.5}$initial (min)	11.5 ± 0.8 (2.2)	15.1 ± 1.3 (3.7)
$t_{0.5}$final (min)	33.7 ± 1.8 (5.1)	46.2 ± 5.1 (14.3)
$ABV_{0-180\ min}$ (%)		0.22 ± 0.06 (0.16)
C_{max} (pg ml^{-1})		1242.0 ± 346.0 (980.0)

Data shown as mean ± S.E.M. with (S.D.).

prevented hCT absorption (Antonin et al., 1992). However, the comparison between the transverse and descending colon is complicated by two further factors. Firstly, the loop stoma patients dosed in the transverse colon study were significantly older than the subjects in the distal colon study (see Table 2). Secondly, there is a risk that the loop stoma induces changes in the transverse colon that influences its absorption characteristics. The exclusion criteria for the study suggest that mucosal pathology could not account for the differences. Therefore, the data indicate that the transverse colon of man is a better site for hCT absorption than the descending colon. From this study it is not clear if this results from increased mucosal permeability or is a consequence of the changed luminal environment.

The two-fold increase in absorption for hCT found in the transverse colon only yields an ABV of 0.22%. This combined with the difficulties in dosing to this region of the intestine suggests that without considerable enhancement colonic peptide delivery is not economically relevant for the pharmaceutical industry. Data from animal studies (Hastewell et al., 1994) indicate that enhancement regimes could yield an ABV of 2-5% for peptides dosed to the large intestine. At present, however, there is no systematic understanding of the potential for absorption enhancers systems. The next stage will be to study the enhanced absorption of peptides across the GI tract of man.

Table 2
Comparison of basal human calcitonin absorption in the transverse and descending colon

Region dosed	Transverse colon	Descending colon
ABV (%)	0.22 ± 0.06[a]	0.08 ± 0.03[a]
C_{max} (pg ml^{-1})	1242.0 ± 346.0[a]	525.0 ± 170.0[a]
Age (years)	55.0 ± 10.0[b]	25.0 ± 2.0[b]

Data for descending colon from Antonin et al. (1992). [a]Mean with S.E.M. [b]Mean with S.D.

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