Colonic absorption of human calcitonin in man

K. H. ANTONIN, V. SAANO, P. BIECK, J. HASTEWELL*, R. FOX*, P. LOWE* and M. MACKAY*
*Human Pharmacology Institute (HPI), Ciba–Geigy GmbH, Tübingen, Federal Republic of Germany, and *Drug Preformulation and Delivery, Ciba–Geigy Pharmaceuticals, Horsham, West Sussex, U.K.*

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1. Human calcitonin was administered into the distal colon and by intravenous infusion in eight healthy subjects in an open, fixed sequence, cross-over bioavailability study.
2. Intravenously infused human calcitonin elicited a standard pharmacokinetic profile in eight healthy subjects with a biphasic elimination with half-lives of 10.2 ± 0.7 min and 37.8 ± 2.5 min.
3. Colonoscopically administered human calcitonin was absorbed across the distal colonic mucosa in low amounts with a bioavailability of 0.00–0.22%.
4. Absorption from the distal colon was impeded by the presence of faecal material in three of the eight subjects.
5. We conclude that human calcitonin crosses the gastrointestinal epithelium of man. This may demonstrate the feasibility of an oral form for clinical use.

INTRODUCTION

Human calcitonin (hCT) is a 32-amino acid hormone produced by the C-cells of the thyroid gland. It lowers blood calcium levels by inhibition of bone resorption and increasing urinary calcium excretion in animal models and in human subjects with diseases where the rate of bone turnover is high [1]. Calcitonin (CT) from several sources, for example, human, salmon (sCT), porcine and an analogue from eel, is marketed in several countries. It has proved to be an effective therapeutic agent in the management of several disorders identified with accelerated bone resorption. These disorders include Paget's disease [2, 3] and postmenopausal osteoporosis [4].

The major challenge associated with current therapy is that subcutaneous and intramuscular injections were the only routes of administration until recently [5]. In addition, side-effects (nausea and facial flushing) are common after injection of sCT and hCT [6]. The efficacy of intranasal administration of hCT and sCT to healthy subjects [7, 8], patients with Paget's disease [9–13] and patients with postmenopausal osteoporosis [14–16] has been demonstrated. Moreover, intranasal hCT was shown to be better tolerated than parenteral administrations [13].

Oral administration would be the favoured mode of delivery for CT. Unfortunately, the physical and chemical properties of CT make it a poor candidate for oral delivery. To date, there are few data which demonstrate the potential availability of CT after oral or intraintestinal administration. We have previously reported the absorption of hCT from the colon of rats *in vivo* [17]. In this study we have determined the absorption of hCT after intracolonic administration to man.

MATERIALS AND METHODS

Subjects and ethical approval

Eight healthy human subjects, four male (weight 70.5 ± 7.2 kg, mean ± SD) and four female (weight 63.0 ± 4.2 kg, mean ± SD), aged between 22 and 27 years (25.6 ± 2.5 years, mean ± SD) 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 were allowed for 2 weeks before the study or during the study period except oral contraceptives. Informed written consent was obtained from each participant. The study was approved by the Ethical Committee responsible for the Human Pharmacology Institute of Ciba–Geigy in Tübingen, whose members are professors of Medicine, Law and Theology at the Universities of Tübingen or Freiburg.

After an overnight fast the subjects received two doses of hCT in an open, fixed sequence, cross-over study. On the day before the study and during the study period the subjects abstained from consumption of alcohol, caffeine and nicotine.

Adverse events

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

Key words: absorption, colon, human calcitonin, oral drug delivery.
Abbreviations: ABV, absolute bioavailability; CT, calcitonin; hCT, human calcitonin; sCT, salmon calcitonin.
Correspondence: Dr Martin Mackay, Drug Preformulation and Delivery, Ciba-Geigy Pharmaceuticals, Wimblehurst Road, Horsham, West Sussex RH12 4AB, U.K.

Study details

On the first study day, 10 mg of hCT was placed into the descending colon (42–75 cm, mean 60 + 13 cm post rectum) during colonoscopy. On the second study day, 0.5 mg of hCT was administered by intravenous infusion. There was a washout period of at least 1 week between the two administrations. Each study day started at 0.800 hours and a standard breakfast (one to three bread rolls, 40 g of butter, 50 g of jam and decaffeinated coffee) was given 150 min after hCT administration.

Blood (4 ml) was collected by venepuncture 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.

Blood and urine analysis

The following clinical chemical parameters were measured using standard procedures: in blood, glucose, creatinine, urea, uric acid, total bilirubin, protein, cholesterol, triglyacylglycerol, glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, alkaline phosphatase, γ-glutamyltransferase, potassium, sodium, calcium and chloride; in urine, pH, nitrite, protein, glucose, ketone bodies, urobilinogen, bilirubin and blood.

Colonoscopic administration

Colonoscopy [18] was performed with an Olympus Colonoscope (CF-LB 3R; Olympus, Hamburg, Germany) after microenema-induced bowel movement (5 ml of Microklist; Pharmacia Arzneimittel GmbH, Ratingen, Germany). The large intestine was not prepared in any other manner. The instrument was introduced as far as possible into the distal colon compatible with luminal patency. The dose (1 ml of a hCT solution, 10 mg/ml 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 20 ml of saline (150 mmol/l NaCl). The subjects remained in the supine position for at least 30 min after 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 min, 30 min and immediately before and at 10, 20, 30, 45, 60, 90, 120, 150, 180, 240, 300, 420 and 540 min after administration of the dose.

Intravenous administration

hCT (0.5 mg) was dissolved in 1 ml of 3% (w/v) mannitol and the solution was diluted in 500 ml of saline (150 mmol/l NaCl) with 0.1% (w/v) human serum albumin. This solution was infused at a constant rate for 90 min.

Blood was sampled at 60 min, 30 min and immediately before the start of the infusion, at 10, 20, 30, 45, 60, 90 min during the infusion period and at 5, 10, 15, 20, 25, 30, 40, 50, 60, 75, 90 and 105 min after stopping the infusion.

Plasma hCT assay

hCT levels were determined using a commercial immunoassay (Immunoradiometric Assay for hCT, International CIS, High Wycombe, Bucks, U.K.). The samples were suitably diluted 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.

Materials

All chemicals used for the study in the subjects were of European Pharmacopoeial grade. hCT (batches 000390 and 14/501/1) was supplied by Ciba–Geigy, Basle, Switzerland. All other chemicals were of analytical grade.

Expression of results

The absolute bioavailability (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 means ± SD and/or SEM. Results from all eight subjects were included. Plasma levels of hCT are given as pg/ml of plasma. Statistical analysis was performed by Student's *t*-test.

RESULTS

Physiological parameters and adverse events

No drug-related or clinically relevant changes were observed in the blood pressure, pulse rate, ECG or laboratory parameters determined from the blood. Similarly, no changes in the parameters determined from the urine samples were seen.

A variety of adverse events were recorded as a result of hCT administration. After intracolonic administration, one subject reported a headache and abdominal bloating, and a second subject complained of abdominal cramps. After intravenous administration, three subjects reported a burning feeling in the throat, which subsided once the infusion ceased. Facial flushing was observed in four subjects during the intravenous infusion period,

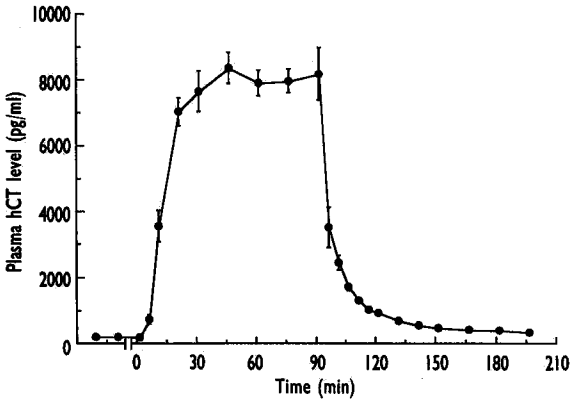

Fig. 1. Plasma hCT levels after intravenous infusion of 0.5 mg of hCT. Values are means ± SEM ($n=8$).

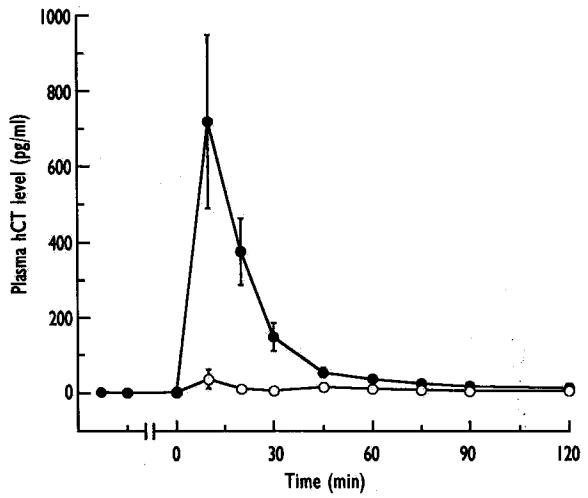

Fig. 2. Semi-logarithmic plot showing the decay phases of hCT after intravenous infusion. ■, First half-life (10.2 ± 0.7 min); ●, second half-life (37.8 ± 2.5 min) ($P<0.0001$ comparing half-lives); ○, data excluded from the regression analysis. Values are means ± SEM ($n=8$).

although only one subject spontaneously reported this effect.

Intravenous administration

The plasma concentration profile for hCT after the 90 min intravenous administration is shown in Fig. 1. Constant plasma levels of hCT were reached after 30 min and were maintained until the end of the infusion. The decay of the plasma levels was rapid. Fig. 2 shows the semi-logarithmic plot of the decay curve with the elimination of hCT being accounted for by a biphasic process. The half-lives were 10.2 ± 0.7 min and 37.8 ± 2.5 min, respectively. The elimination rate constant for the first half-life accounted for 89% ± 1% of hCT loss from the body.

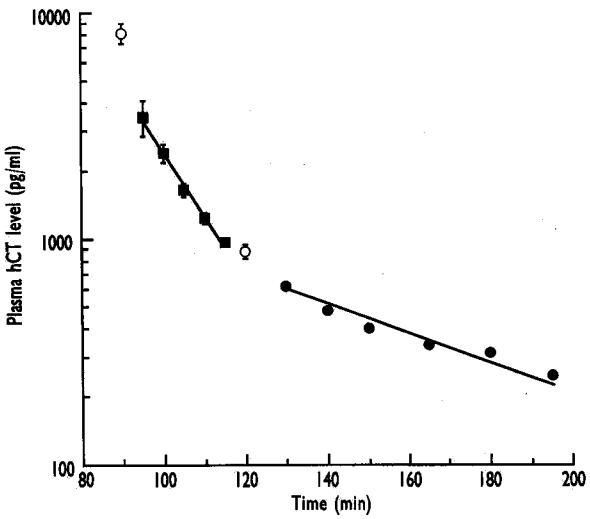

Fig. 3. Plasma hCT levels after colonoscopic administration of 10.0 mg of hCT in group A ($n=5$, ●) and group B ($n=3$, ○). Values are means ± SEM.

Intracolonic administration

From a consideration of plasma hCT levels, it appeared that the subjects fell into two distinct groups. Group A (five/eight) where hCT absorption occurred, and group B where it did not. On referring to the notes made during the administration, it was observed that in the group B subjects the microenema-induced bowel movement failed to clear the distal colon of faecal material, whereas the distal colon of the subjects in group A was free from faecal material. As a result of this, the data presented from the colonoscopic administration were divided into two groups.

The plasma concentration profile for hCT after the intracolonic bolus administration of hCT is shown in Fig. 3. In group A, the appearance of hCT is rapid with maximum plasma levels at 10 min (the first time point blood was sampled at). As in the case of the intravenous dose, the decay of the plasma profile was biphasic (Fig. 4) with the elimination rate constant for the first half-life (9.3 ± 1.3 min) accounting for the majority (92 ± 3%) of hCT loss from the body. The half-life for the second phase was 44.2 ± 6.1 min. There was no significant ($P>0.2$) difference between the half-lives after intravenous infusion or colonoscopic administration.

Bioavailability

Bioavailability data are presented in Table 1, which presents the results in the individual subjects, the group mean and the means as a result of dividing the subjects into the two groups. The results indicate that although there was no influence

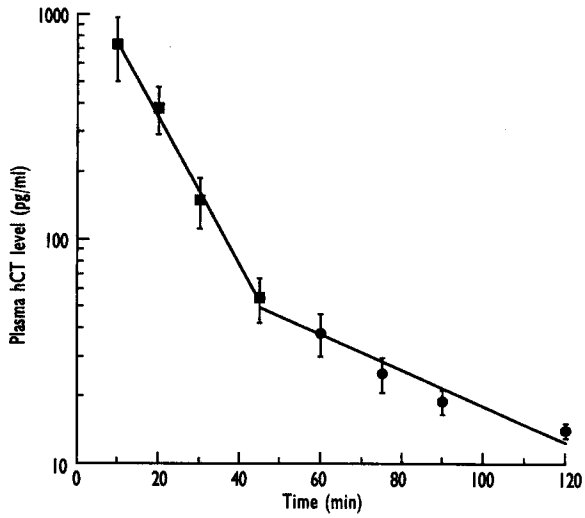

Fig. 4. Semi-logarithmic plot showing the decay phases of hCT after colonoscopic administration. ■, First half-life (9.3 ± 1.3 min); ●, second half-life (44.2 ± 6.1 min) ($P < 0.001$ comparing half-lives). Only data from group A are presented. Values are means ± SEM ($n = 5$).

Table 1. Determination of ABV of hCT after intracolonic administration. Abbreviations: $C_{max.}$, maximum plasma concentration; AUC, area under curve. Statistical signidicance: *$P < 0.01$, †$P > 0.3$ compared with group B.

	Intravenous (0.5 mg)	Intracolonic (10 mg)		
	AUC (ng min ml^{-1})	$C_{max.}$ (pg/ml)	AUC (ng min ml^{-1})	ABV
Group A				
1	702.6	1094.0	17.4	0.123
2	551.3	633.0	10.7	0.097
3	569.1	1306.0	25.4	0.223
4	816.1	545.0	10.1	0.061
5	667.9	474.0	11.7	0.087
Mean	661.4	810.4	15.1	0.118
SD	107.6	367.8	6.5	0.063
SEM	48.1	164.5	2.9	0.028
Group B				
6	634.5	27.2	0.5	0.004
7	755.7	40.0	1.2	0.008
8	861.5	82.2	1.4	0.008
Mean	750.6†	49.8*	1.0*	0.007*
SD	113.6	28.8	0.5	0.002
SEM	65.6	16.6	0.3	0.001
Combined data				
Mean	694.8	525.2	9.8	0.076
SD	111.5	482.2	8.8	0.075
SEM	39.4	170.5	3.1	0.026

of these divisions on the intravenous parameters, there was a significant influence on the intracolonic parameters (Table 1). The ABV ranged from 0.00 to 0.22 (0.08 ± SEM 0.02) when all subjects were considered, and from 0.09 to 0.22 (0.12 ± SEM 0.03) when only group A was considered.

DISCUSSION

Opportunity windows have been identified for transmucosal administration of medium-sized peptides such as CT that have a relatively wide therapeutic index [19]. For example, nasal sCT is now a marketed product in many countries and the efficacy of intranasally administered hCT has been demonstrated [12, 13]. Other transmucosal routes, such as the rectal route [8, 20], have also been considered as alternative modes of delivery. However, given the indications for CT, the favoured route of administration would be oral. It has been recognized that specific regions of the gastrointestinal tract may offer potential in terms of macromolecular absorption. For example, the colon possesses less degradative enzymes and may offer a less harsh milieu to peptide and protein drugs before absorption [21–23]. Dosage forms that deliver peptide drugs to specific regions of the gastrointestinal tract such as the colon have been proposed based on either a 'trigger' mechanism [24, 25] or a timed-release element.

We have previously demonstrated in rats that intracolonically administered hCT was absorbed and over the dose range 0.1–5.0 mg/kg resulted in a dose-dependent reduction in plasma calcium levels [17]. In extending these studies to man we concentrated on pharmacokinetic parameters because only a small, transient hypocalcaemic response is elicited even when pharmacological doses of CT are administered to healthy subjects. This is a consequence of the low rate of bone turnover in normal subjects [5]. The comparatively small dose of 10 mg, which corresponds to 0.15 mg/kg, was used. Doses of this order administered intranasally have previously been shown to be therapeutically active [12, 13]. Even at this dose, hCT is absorbed from the human colon, albeit in low amounts. The bioavailability of hCT in man (0.1%) is similar to that observed in rats (0.2%) at equivalent doses based on body weight (0.1 mg/kg) [17]. The results from this study suggest that the pharmacokinetic parameters associated with hCT elimination from the body are independent of the route of administration. This observation is in keeping with the transient pharmacokinetic profile generated after the intracolonic dose, which suggests that the absorption was rapid and not sustained over a period of time. The lack of any depot effect of the dose in the colon may be a consequence of rapid metabolism of hCT. The observation that faecal material in the colon resulted in low hCT absorption may be explained by increased proteolytic activity associated with the luminal contents or binding of hCT to the faecal material, thus preventing absorption. This may exclude the descending (distal) colon as a site for optimal peptide absorption.

Recent literature suggests that the luminal environment of the colon varies along its length [26]. The ascending (proximal) colon provides a liquid environment with considerably reduced levels of proteolytic enzymes [23]. Therefore, this region of the large intestine could be more favourable for peptide absorption, giving increased bioavailabilities over other regions of the gastrointestinal tract. This view is supported by the work of Saffran *et al.* [25], who have shown that oral insulin targeted to the proximal colon is therapeutically effective in diabetic dogs.

Clearly, the bioavailability of hCT needs to be increased. The use of gastrointestinal absorption enhancers for therapeutic peptides and proteins [27–29] would increase bioavailability, although questions remain concerning the safety of many of the systems described in the literature. However, this study shows that in the absence of absorption enhancers and contrary to current dogma, the amounts of hCT detected in the plasma after intracolonic administration are significant in some subjects. The feasibility of clinically effective oral forms of hCT will depend in part on the extra costs demanded because of low bioavailability, and the acceptance of this low bioavailability by regulatory authorities. Our studies continue with an aim to increase the absorption of hCT in man in a safe and pharmaceutically acceptable manner.

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