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Pharmacokinetics of trefoil peptides and their stability in gastrointestinal contents☆

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ABSTRACT

Trefoil factor family (TFF) peptides are considered promising for therapeutic use in gastrointestinal diseases, and there is a need to explore the fate of injected TFF and the stability of the peptides in the gastrointestinal tract. We studied the pharmacokinetics of intravenously (i.v.) administered hTFF2 in mice and rats and of hTFF3 administered i.v., intramuscularly, intraperitoneally, and subcutaneously in mice, and estimated by ELISA the decay of the peptides added to rat and human gastrointestinal contents. We found that i.v. injected hTFF2 and hTFF3 were cleared from the circulation within 2–3 h, exhibiting comparable pharmacokinetic profiles. In contents from the rat stomach, hTFF levels remained unchanged for up to 6 days. In the small and large intestine of rats, the hTFF levels decreased markedly after 4 and 1 h, respectively. In small intestinal contents from humans, the levels remained stable for more than 24 h. We conclude that systemically administered hTFF2 and hTFF3 are rapidly eliminated from the circulation and that the stability of hTFF2 and hTFF3 in GI contents appeared higher in the gastric and small intestinal milieu than in the large intestine and feces, suggesting a higher stability toward gastric acid and digestive enzymes than toward microbial degradation.

1. Introduction

The trefoil factor family (TFF) 1–3 comprises a group of small peptides (7–12 kDa) sharing a common structure termed the trefoil domain. TFF2 contains two trefoil domains due to genomic duplication of the motif, while the two domains in TFF1 and TFF3 are linked by disulfide bonds via a seventh cysteine residue (reviewed in [18]). The peptides are produced

☆ SK, PT, and LT are employed at Novo Nordisk a/s.

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Abbreviations: ELISA, enzyme-linked immunosorbant assay; GI, gastrointestinal; HPLC, high-performance liquid chromatography; ID, injected dose; i.m., intramuscular; i.p., intraperitoneal; i.v., intravenous; %ID, percentage of injected dose; PK, pharmacokinetic; s.c., subcutaneous; TFF, trefoil factor family

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AUC	area under the curve
$AUC_{\%Extrapol}$	extrapolated percentage of the molecule left in serum after last measured time point
C_0	start concentration in serum
C_{max}	maximal serum concentration
CL	clearance
F	bioavailability
MRT	mean residence time
$T_{1/2}$	half-life of molecule in serum
T_{max}	time to maximal serum concentration
V_z	volume of distribution
V_{ss}	volume of distribution at steady state
λ_z	terminal elimination rate constant

by mucus-secreting cells, especially in the gastrointestinal (GI) tract in a site-specific manner for each peptide, i.e., TFF1 is mainly produced by gastric surface epithelial cells, TFF2 by gastric mucous neck cells and pyloric glands, and in the duodenal Brunner's glands, whereas TFF3 is mainly produced by the intestinal goblet cells and the antral mucous cells [6]. Upregulation and ectopic expression of the peptides have been reported in GI tissues from patients with chronic GI inflammation [24], and all three peptides have shown a therapeutic effect in experimental models of GI disease [1,5,12–14]. Earlier in vitro studies reported that TFF2 was extremely resistant to protease and acid, based on the findings that TFF2 incubated with HCl or trypsin was only slightly degraded [7]. Likewise, ^{125}I-hTFF2 seemed stable following incubation in small intestinal or gastric juice or in HCl- and GI protease-containing solutions [12]. Our previous studies using ^{125}I-labeled TFFs administered intravenously (i.v.) to rats suggested that the peptides were rather slowly cleared from the circulation and that TFF2 was very resistant toward degradation in vivo [16]. These findings in concert with the compact structure of the TFF peptides have led to the assumption that all TFFs were generally highly resistant toward degradation [12,21].

Pharmacological effects of TFFs have been obtained after systemic as well as local administration, and the aim of this study was therefore to study in more detail the distribution and elimination in vivo by examining the pharmacokinetics of human TFF2 and TFF3 (hTFF2 and hTFF3) in mice and rats after systemic administration and to study the stability of TFFs added to different types of rat GI contents (to mimic the conditions following local administration) using enzyme-linked immunosorbant assays (ELISA) specifically developed for hTFF2 or hTFF3. Finally, this paper presents the results of studies on the levels of hTFF2 and hTFF3 in human bowel contents from different parts of the gut, and the effect of storage at 37 °C on the concentration of these peptides.

2. Materials and methods

2.1. Animals

Female BALB/cAnNTac mice (20–23 g) and female Wistar Hannover GALAS Rats (HanTac:WH) (250–300 g) (Taconic M&B, Ry, Denmark) were housed at the Panum Institute (University of Copenhagen, Denmark) or Novo Nordisk a/s. The animal studies were approved by the Danish National Committee for Animal Studies (i.e., the Animal Experiments Inspectorate).

2.2. Subjects

From humans, samples of normal stool ($n = 5$) were obtained as well as wet ostomy samples from jejunostomies ($n = 8$), ileostomies ($n = 5$), and colostomies ($n = 5$). None of the subjects had active disease at the time of sampling. The samples were collected after lunch (the subjects were not fasted), were frozen immediately after collection, and stored at −20 °C until analysis. The local scientific ethics committee approved all procedures. All participants gave informed consent.

2.3. Peptides

hTFF2 and dimeric hTFF3 were produced as described previously [20,21]. For the study of the molecular forms of TFF (Section 2.7), both peptides were labeled at Novo Nordisk a/s with sodium ^{125}iodide ($Na^{125}I$) using lactoperoxidase to a radiochemical purity of ~98%. The specific radioactivity was 2.2 μCi/pmol (hTFF3) and ~0.12 μCi/pmol (hTFF2). The tracers were diluted with 0.9% saline to obtain the final concentrations for injection (Section 2.7).

2.4. Pharmacokinetics of hTFF2 and hTFF3 in mice and rats

hTFF2 or hTFF3 was administered to mice and rats i.v., intraperitoneally (i.p.), intramuscularly (i.m.), or subcutaneously (s.c.) according to Table 1. Prior to the i.v. injection, the mice were anesthetized with a mixture of fentanyl (0.15 mg/kg), droperidol (9.8 mg/kg), and midazolam (1 mg/kg) administered s.c. The rats were anesthetized with methohexital (50 mg/kg) i.p. (buprenorfin 0.1 mg/kg s.c. for analgesia). The i.v. injection into the inferior vena cava was performed via a midline incision, which was closed with 3–0 silk sutures following injection. For collection of urine, the urethra was ligated when the hTFF was administered. The animals were sacrificed in groups of three to five at the time points specified in Table 1. Prior to sacrifice, blood and urine sampling was performed under general anesthesia. Blood was collected from the orbital venous plexus and urine was directly aspirated from the bladder. The blood was allowed to

Table 1 – Dosing regimens for pharmacokinetic analysis

Species	Peptide	Route	Dose (mg/kg)	Time (min)
Mouse	TFF3	i.m.	1	6, 15, 30, 60, 120, 180
Mouse	TFF3	i.p., s.c.	1, 5	15, 30, 60, 180
Mouse	TFF3	i.v.	5	2, 6, 15, 45[a], 120[a], 240
Mouse	TFF2	i.v.	5	2, 6, 15, 45[a], 120[a], 240[a]
Rat	TFF2	i.v.	5	2, 6, 15, 45[a], 120[a], 240[a]

[a] Simultaneous collection of urine.