Gastrointestinal loss of galanthamine hydrobromide in rats in-situ

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Abstract—The disappearance kinetics of the acetylcholinesterase inhibitor galanthamine hydrobromide from the gastrointestinal tract of male Wistar rats, 200–250 g, in-situ has been examined. After 30 min the galanthamine loss was 16% in the stomach (pH 2), 54–85% in the duodenum and the successive small intestinal segments (pH 6·8), 43% in the colon and 76% in the rectum. Compared with the other segments, the disappearance rate was higher in the terminal ileum (0.38×10^{-2} mg cm^{-1} min) and in the rectum (1.27×10^{-2} mg cm^{-1} min). In the proximal jejunum, terminal ileum and rectum the disappearance rate was linearly dependent on the galanthamine dose (range 0·5–4 mg, 2–16 mg kg^{-1}). The results suggest that when administered orally, rapid galanthamine absorption could be expected all over the gastrointestinal tract due mainly to the passive diffusion mechanism.

The interest in the biodistribution and pharmacokinetics of the anticholinesterase agent galanthamine has increased in recent years because of its predicted effectiveness in the treatment of Alzheimer's disease (US Patent 1987; Sweeney et al 1989). Different animal species, including man, have been involved in the pharmacological and pharmacokinetic study of galanthamine and its metabolites (Mihailova et al 1985, 1989; Mihailova & Yamboliev 1986; Westra et al 1986; Bickel et al 1991). Mihailova & Yamboliev (1986) reported that following oral administration of galanthamine to rats, first-order absorption kinetics is consistent with the plasma concentration-time data with absolute bioavailability reaching 65%. However, the absorption kinetics in healthy volunteers indicated that the rate of absorption varied along the gastrointestinal tract and based upon the data obtained, a two-stage absorption process was proposed (Mihailova et al 1989).

The aim of the present study was to investigate the rate of loss and its dependence on galanthamine concentration by different segments of the gastrointestinal tract of the rat in-situ.

Materials and methods

Materials. Galanthamine hydrobromide was supplied by Pharmachim (Bulgaria).

Animals. Male Wistar rats, 200–250 g, were deprived of food for at least 12 h before the experiment, but were allowed free access to water.

Methods. The rats were anaesthetized with ethylcarbamate (urethane, 1 g kg^{-1}, i.p.) and surgery was commenced about 30 min later. The animals were fixed to a thermostatic table and the gut exposed after a midline abdominal incision.

The animals were divided into eight groups (six rats each) and successive portions of the gastrointestinal tract were selected as follows: stomach, duodenum (about 12 cm long), proximal and distal jejunum, proximal and terminal ileum (15 cm each), colon (10–12 cm) and rectum (4–5 cm). Each portion was then fitted with the entrance and exit cannulae. The entrance and exit cannulae for the stomach were inserted and ligated into the oesophagus and pylorus. The stomach was washed completely

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free from food residues by perfusing with isotonic 0·01 M HCl at 37°C until the affluent liquid was clear. The preparation was completed by a puff of air and gentle treatment with the fingers in order to remove the remaining perfusion fluid. The entrance cannula for the duodenum was ligated about 1 cm apart from the bile duct and the exit cannula about 10–12 cm below. The segment was rinsed with warm physiological saline followed by isotonic phosphate buffer, pH 6·8. This was expelled by a current of warm air. The cannulation of the jejunal and ileal segments, the colon and the rectum was similar to that of the duodenum—the exit cannula from the rectum being fixed into the anus.

The gut and its cannulated segments were then replaced into the cavity taking care not to injure the mesenterial blood supply and the abdominal wall was then sutured. A cotton wad was placed on the suture and kept continuously wet with physiological saline in order to prevent drying of the tissue. The temperature in the operating compartment ($37 \pm 2°C$) was maintained by a heating lamp.

Galanthamine hydrobromide (12·8 mg equivalent to 10 mg galanthamine base) was dissolved in 0·5 mL isotonic 0·01 M HCl for the gastric experiments and in 0·5 mL isotonic phosphate buffer, pH 6·8, for intestinal experiments. A 0·1 mL portion was injected into each of the respective segments through the entrance cannula and in order to prevent any loss of drug the cannula was washed through with 0·2 mL buffer solution.

Thirty minutes later the segment was connected to a recirculation system and 20 mL fresh buffer solution circulated for 5 min at a flow rate of about 4 mL min^{-1}. This was to recover all the remaining galanthamine from the segment. The concentration of the drug in the circulating fluid was determined spectrophotometrically at 288 nm (Kolusheva & Valkova 1966) using a spectrophotometer Specord UVVIS (Karl Zeiss, Jena). The accuracy of this method was confirmed by using non-absorbable phenol red as a quantitative marker in an identical procedure.

The quantities of galanthamine not recovered during the recirculating process were expressed as percent of the initial dose. At the end of each determination the different segments were excised, loaded with 2 g for 10 min and their lengths measured. This enabled the disappearance rate of the galanthamine to be calculated as mg cm^{-1} min.

To investigate the relationship between the dose of galanthamine and the rate of its disappearance, three portions of the gastrointestinal tract were used: proximal jejunum, terminal ileum and rectum. Different galanthamine doses (range 0·5–4 mg, 2–16 mg kg^{-1}) dissolved in 0·1 mL phosphate buffer were introduced into the segments and the procedure followed as described above.

Results

Table 1 shows the percentage of galanthamine disappearing during 30 min and its rate of disappearance. It can be seen that the quantitative disappearance of galanthamine varied from segment to segment in the range of 16% (stomach) to 84·5% (terminal ileum). The disappearance rates were of similar magnitude in the small and large intestines (0.24–0.32×10^{-2} mg cm^{-1} min) but were higher from the terminal ileum (0.38×10^{-2} mg cm^{-1} min) and from the rectum (1.27×10^{-2} mg cm^{-1} min).

Fig. 1 presents the relationship between the disappearance

Table 1. Rate of loss of galanthamine hydrobromide in the rat gastrointestinal tract in-situ.

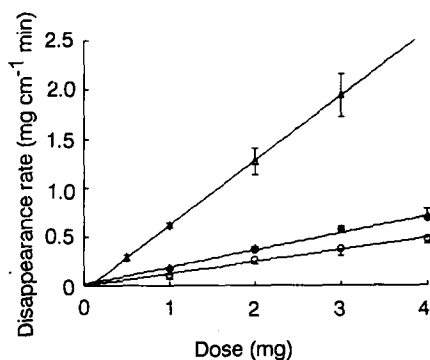| Segment	Galanthamine lost over 30 min (%)	Galanthamine rate of loss (mg cm^{-1} min) $\times 10^2$
Stomach	16·3 ± 4·2*	—
Duodenum	58·2 ± 11·9	0·32 ± 0·07
Proximal jejunum	59·8 ± 10·5	0·27 ± 0·05
Distal jejunum	54·4 ± 8·1	0·24 ± 0·04
Proximal ileum	62·3 ± 6·0	0·28 ± 0·03
Terminal ileum	84·5 ± 7·0	0·38 ± 0·03
Colon	43·1 ± 6·1	0·24 ± 0·03
Rectum	76·2 ± 8·6	1·27 ± 0·14

*Means ± s.d. of 5–8 measurements.

Fig. 1. Relationship between the disappearance rate and the dose of galanthamine introduced in the proximal jejunum (O), terminal ileum (●) and rectum (△) of rats in-situ. Means ± s.d., n = 5–8.

rate and the galanthamine dose. In the dose range investigated (1–4 mg in the proximal jejunum and 0·5–3 mg in the terminal ileum and rectum) there is a linear relationship between the rates of loss and the quantity of galanthamine introduced.

Discussion

To diminish the influence of the concentration gradient on the disappearance kinetics, equal galanthamine doses were introduced into the segments at about 1/10 of the oral LD50 (Paskov et al 1964). Although the pH changes along the intestine, the pH of the introduced galanthamine solution was kept constant (pH 6·8) in order to maintain a constant galanthamine fraction ionized. Only the gastric disappearance experiments were performed at pH 2.

Our observations on the gastric galanthamine loss showed that about 16% of the introduced dose disappeared within 30 min. Galanthamine is a weak base with pK$_a$ 8·32 (Yamboliev & Mihailova 1983), meaning that in the stomach (pH 2) all the drug molecules are ionized and the diffusion across the gastric wall is quite slow, if at all. Due to the large molecular size of the galanthamine molecule (mol. wt = 287) water-channel diffusion is negligible. Although other mechanisms could also mediate the transmembrane galanthamine passage (Turnheim & Lauterbach 1980; Ruifrok 1981; Ruifrok & Mol 1983; Seki et al 1985; Tsubaki & Komai 1986) the elucidation of their nature still requires further investigation.

The finding is consistent with our results obtained previously in-vivo, when oral administration of galanthamine solution to intact rats resulted in peak plasma concentrations within 15–20 min of dosing (Mihailova & Yamboliev 1986). This time course of galanthamine could be attributed to fast absorption from the stomach soon after the drug intake.

In the intestinal solutions (pH 6·8) greater un-ionized galanthamine fraction is available (about 5%). In addition, other factors, such as the larger absorption surface and intestinal peristalsis, suggest an enhancement of the galanthamine loss compared with the stomach. Different mechanisms could be responsible for the faster disappearance rate from the lower segments. The existence of a capacity-saturable transport mechanism was experimentally checked. For this purpose the disappearance rates of galanthamine were determined in the terminal ileum and rectum and also in the proximal jejunum (Fig. 1). The analysis of the data suggests that the disappearance rates are proportional to the galanthamine doses (range 2–16 mg kg^{-1}) and no carrier-mediated mechanism is involved in galanthamine transport across the gut wall.

The anticholinesterase activity of galanthamine (Irwin & Smith 1960; Mikhailova 1965; Thomsen & Kewitz 1990), could be expected to result in differences in the mechanical activities of the various segments. Our preliminary investigations confirmed a dose-dependent galanthamine-induced increase both in the basal tone and in the spontaneous phasic activity being more pronounced in the terminal ileum than in the proximal jejunum (unpublished data). The galanthamine-intensified intestinal peristalsis enhances the turbulence of the intestinal content thus facilitating the loss of galanthamine predominantly from the ileum rather than from the jejunum.

According to Varga (1976), after oral administration of drugs or drug formulations to rats, the mean transit time is about 0·4 h for the upper and 2·5 h for the lower small intestine. After oral administration of the galanthamine solution to rats, absorption is almost completed in the first hour after dosing, indicating that the absorption is located in the stomach and in the upper intestine and negligible amounts would reach the terminal ileum and be absorbed there (Mihailova & Yamboliev 1986). However, when a retard galanthamine formulation is administered the response could be different. The longer transit time in the lower intestine combined with the higher rate of loss in the ileum could unpredictably influence the plasma concentration-time curve of galanthamine, and its subsequent pharmacological effects.

Data on rectal drug absorption suggest that effective secretory mechanisms are involved in the pH-control of rectal solutions (Crommelin et al 1979) so that only a few minutes would be sufficient to alter the pH of the galanthamine solutions introduced in the rectum in our experiments (0·3 mL) from 6·8 to 7·4. Thus, the un-ionized galanthamine moiety would reach 10%, i.e. twice that in the initial solution, thus enabling some increase in the rate of loss.

In conclusion, the present investigation shows that the loss of galanthamine from different segments along the rat gastrointestinal tract occurs at comparable rates and most probably by passive diffusion, and—with the possible exception of the rectum—with site-specific disappearance of the drug playing an insignificant role.

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Definitive IUPAC Recommendations

The following definitive recommendations on nomenclature, terminology, and symbolism have been published since January 1992:

1. Glossary for chemists of terms used in biotechnology. Pure Appl. Chem. (1992) 64: 143
2. Nomenclature, symbols, units and their usage in spectrochemical analysis-XII. Terms related to electrothermal atomization. Pure Appl. Chem. (1992) 64: 253
3. Nomenclature, symbols, units and their usage in spectrochemical analysis-XIII. Terms related to chemical vapour generation. Pure Appl. Chem. (1992) 64: 261
4. Selection of terms, symbols and units related to microbial processes. Pure Appl. Chem. (1992) 64: 1047
5. Quantities and units for metabolic processes as a function of time. Pure Appl. Chem. (1992) 64: 1569

Comments on these recommendations would be welcomed, addressed to the originating IUPAC Commission (for addresses see the appropriate issue of Pure Appl. Chem.), with copies to Dr A. D. McNaught, Secretary, Royal Society of Chemistry Nomenclature Committee, Thomas Graham House, Science Park, Milton Road, Cambridge CB4 4WF, UK.