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Formulation and pharmacological studies of a controlled release pentagastrin injection

K. N. WAI, E. E. L. GERRING AND R. D. BROAD

Imperial Chemical Industries Limited, Pharmaceuticals Division, Alderley Park, Macclesfield, Cheshire, U.K.

Four formulations were designed to release pentagastrin at different rates after subcutaneous injection. These formulations were tested *in vitro* for rate of diffusion across a dialysis membrane into pH 7·3 phosphate buffer, and *in vivo* in rats for rate of excretion in the bile and on dogs for pharmacological responses. Good agreement was observed between the *in vitro* data, elimination rate and dose response data for the rate of release and duration of action of the four formulations. The results agreed with the theory that by controlling the rate of release of a short acting drug, one could sustain the pharmacological response to the drug and increase the total response by better utilization of an administered dose.

The synthetic pentapeptide, pentagastrin (Peptavlon ICI 50 123), is active in stimulating gastric secretion (Morley, Tracy & Gregory, 1965). It produces maximal stimulation of gastric secretion after subcutaneous injection in man at a dose of 6 μg/kg dose (Multicentre Pilot Study, 1967). The rate of secretion reaches a maximum between 20 to 40 min after injection and returns to basal levels in 90 to 120 min (Makhlouf, McManus & Card, 1966). Intravenous infusion of a pentagastrin solution at a dose of 0·01 μg/kg min^{-1} in 0·15M sodium chloride solution at a rate of 150 ml h^{-1} produces stimulation which approaches the maximal gastric output in most human subjects (Wormsley, Mahoney & Ng, 1966; Aagaard & Schmidt, 1967). This evidence indicates that the subcutaneous dose of pentagastrin is not fully utilized in the body for the desired function. Most of the subcutaneous dose may pass from the site of injection into the blood stream at a rate faster than that required to produce the maximal pharmacological response and is rapidly eliminated or metabolized. The possibility of controlling the rate of release of pentagastrin by formulation techniques and thus achieving more efficient use of the dose has been investigated.

Theory

Many drugs in solution when injected into subcutaneous or intramuscular sites behave as if their absorption were taking place passively by diffusion in one direction (Sund & Schou, 1964a, b; Ballard & Menczel, 1967; Ballard, 1968). The disappearance rate of the drug from the injection site can be described by Fick's Law. In practice, it may be assumed that the rate of absorption, R_t, of a subcutaneous dose of drug from the site of injection into the blood is proportional to the amount of drug at the site, A_s, and the relation may be expressed as

$$R_t = \frac{dN}{dt} = p(A_s)$$

where dN/dt is the penetration rate and N is the amount of drug penetrating the tissue at time, t. The penetration coefficient, p, depends upon the diffusion coefficient

of the drug in the particular environment, the area of absorption membrane exposed to the solution and the concentration of drug at the site.

Fig. 1 shows graphically an hypothetical exponential relation between the release rate, R, and time, t, after subcutaneous or intramuscular injection of a drug. R_1 represents the rate of release at which a particular drug produces its maximum pharmacological action. A drug diffusing into the blood at a higher rate will not cause a further rise in pharmacological response. R_2 is the release rate required to

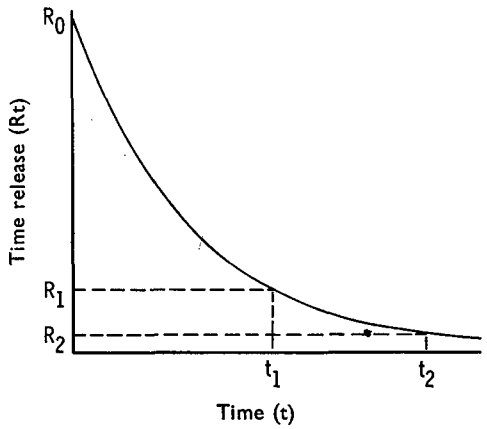


FIG. 1. Exponential relation between rate of release and time after subcutaneous injections.

maintain the threshold blood level for producing a pharmacocological response. In the period t_0 to t_1, the drug is released into the blood at a rate faster than it can be utilized. The total dose is represented by the area under the curve. The total dose, utilized dose and dose efficiency can be calculated as follows:

$$\text{Total dose} = \int_0^{\infty} R(t)dt$$

$$\text{Utilized dose} = R_1 t_1 + \int_1^{2} R(t)dt$$

$$\text{Dose efficiency} = \frac{\text{Utilised dose}}{\text{Total dose}} = \frac{R_1 t_1 + \tau(R_1 - R_2)}{R_0 \tau}$$

The dose efficiency represents the fraction of the dose being used for its pharmacological action. The difference between total dose and utilized dose represents overdosing. τ = time constant.

Overdosing frequently happens in the administration of short-acting drugs. In order to produce a desirable duration of action, a drug is often given at excessively high dose. By maintaining the rate of diffusion of the drug from the site of injection at a constant level or by prolonging the diffusion half-life, the dose efficiency can be improved and this is demonstrated by a prolongation of pharmacological action without increase in dose.

EXPERIMENTAL

Material. Pentagastrin (n-t-butyloxycarbony-β-Ala.Try.Met.Asp.Phe.NH_2). The equilibrium solubilities of pentagastrin are 7·5 ppm in 0·1 N hydrochloric acid and

$4{\cdot}0 \times 10^3$ ppm in isotonic phosphate buffer of pH 7·3 at 25° as determined by the method of Martin (1960). Radioactive pentagastrin (0·6 μCi/mg) [^{14}C] labelled at the methylenyl carbon of the tryptophan moiety was synthesized for these experiments and its purity was confirmed by electrophoresis and thin-layer chromatography. Propylene glycol B.P. was distilled at 0·5 mm mercury pressure. All other reagents were analytical quality.

Preparation of injections. To conserve the labelled pentagastrin, it was diluted to a specific activity of 0·24 μCi/mg with unlabelled material. Four types of injectable formulation were prepared using this material for the *in vitro* testing and for the determination of absorption and excretion kinetics. The same formulations using unlabelled pentagastrin were used in the studies of gastric response in dogs. These formulations were as follows:

Formulation I. Pentagastrin (10 mg) was dissolved in 0·1N ammonia (0·04 ml) and adjusted to 10 ml with normal saline. The injection was filtered through a sterile 0·22 μm Millipore filter.

Formulation II. Pentagastrin (10 mg) was dissolved in 0·1N ammonia (0·04 ml), diluted with propylene glycol (4 ml) and a sufficient quantity of normal saline was added to make 10 ml of solution which was filtered through a sterile 0·22 μm Millipore filter.

Formulation III. Propylene glycol (4 ml) and normal saline (4 ml) were added to pentagastrin (10 mg) dissolved in 0·1N ammonia (0·04 ml) and then filtered. An equivalent quantity of 0·1N acetic acid was added to neutralize the ammonia. The solution was stored for 24 h at 4° to allow crystallization of pentagastrin in its acid form to be completed. Sterile normal saline was then added to adjust the volume to 10 ml.

Formulation IV. Crystals were produced as in Formulation III. To the suspension, 0·1M aluminium potassium sulphate (0·32 ml) was added and the preparation adjusted to pH 5·0 with 0·1N sodium hydroxide the volume being made up to 10 ml with normal saline.

In vitro *release rate.* An apparatus (Fig. 2) was designed to study passive diffusion in one direction. Compartment A, constructed from Perspex, was clamped to compartment B but separated from it by a Millipore filter (H.A. 02560, 0·45 μm) exposing an efficient diffusion area of 3·14 cm^2. Before use, the diffusion membrane was soaked in the formulation for 24 h and rinsed twice with 50 ml portions of distilled water. One ml of Formulations I to IV, prepared with labelled pentagastrin, was separately placed in compartment A and was allowed to diffuse through the diffusion

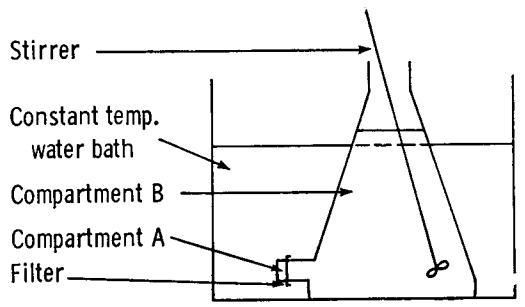


FIG. 2. Schematic diagram of apparatus for the study of drug release by diffusion.

membrane into 1000 ml isotonic phosphate buffer of pH 7·3 in compartment B which was stirred at 750 rev/min with a glass paddle. The apparatus was at 37° in a water bath. One ml samples were removed from compartment B over 8 h. The amount of pentagastrin diffusing through the membrane was calculated from the radioactivity detected in the samples. Two tests were run on each formulation and the average results of the two runs were reported.

Biliary excretion in rats after subcutaneous injection. Polyethylene cannulae were inserted into the common bile duct of four anaesthetized rats (Alderley Park strain), each to receive a different formulation prepared with labelled pentagastrin. Two h after recovery from the anaesthetic, the rats, housed in restraining cages, were injected with one ml of the formulation. Bile samples were collected hourly for 24 h, diluted to 5·0 ml with normal saline and the radioactivity of 0·10 ml of each aliquot measured.

Measurement of radioactivity. Radioactivity was assayed in a Packard Tri-Carb Automatic Liquid Scintillation Spectrometer. The samples were counted in a mixture of 6·6 ml of 0·6% Butyl-PBD [2-(4′-t-butylphenyl)-5-(4″-biphenyl)-1,3,4-oxdiazole] in toluene and 3·3 ml Triton X-100.

Stimulation of gastric secretion in dogs. The two dogs used had been provided with an innervated pouch of the oxyntic gland area of the stomach several months previously. The dogs were deprived of food, but not water for 18 h before the test. Basal secretion, if any, was collected for 15 min before the injection of the test formulation. Each formulation was then given by subcutaneous injection and the gastric juice produced in each 15 min interval was collected until the secretion had returned to the basal level or to the end of $4\frac{1}{2}$ h period. The volume of secretion was measured, and aliquot titrated against 0·1N sodium hydroxide to the phenolphthalein end point and total acid output was computed for each time period. In each test, Formulation I was compared with one of the other formulations on the same dog in duplicate runs.

RESULTS AND DISCUSSION

Formulations I and II were solutions of pentagastrin whereas, Formulations III and IV contained crystalline pentagastrin. Comparative studies were conducted on these four formulations to illustrate the effects of 40% propylene glycol (Formulations II, III and IV), crystallinity (Formulations III and IV) and a protective coating of aluminium hydroxide (Formulation IV) on the rate of release of the drug from the site of injection. The crystals of Formulation III and IV had a similar appearance under the microscope. The needle-shaped crystals have a particle size distribution of 3–20 μm equivalent spherical diameter and a mean diameter of 8·7 μm as analysed by a Coulter Counter using an 140 μm orifice.

The results of the *in vitro* diffusion study are summarized in Fig. 3. Formulations I and II followed Fick's Law in contrast to Formulations III and IV which showed an initial period of constant release rate. The observed release pattern for Formulation III is believed to be due to the combined effects of dissolution and diffusion, with dissolution dominating the initial stage. Dissolution factors also govern the initial release rate of Formulation IV. On coming into contact with the buffer solution at pH 7·3 a gel of aluminium hydroxide is formed around the crystals. This gel provides a barrier controlling the rate of release of pentagastrin at a relatively steady level.

Using the same sets of data, the *in vitro* rates of release of the four formulations at various times were determined. Since the *in vitro* release of Formulations I and II followed first order kinetics, their rates were determined with the aid of a digital

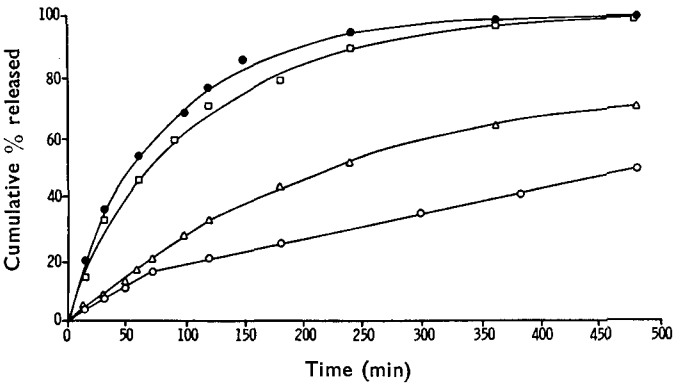
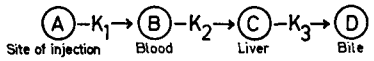


FIG. 3. *In vitro* release of pentagastrin from Formulation I (●), Formulation II (□), Formulation III (△), Formulation IV (○).

computer. The release rates of Formulations III and IV at various times were determined by graphical approximation. Fig. 4 compares the *in vitro* release rates, R_t, of the four formulations over the test period. Formulations I and II show a theoretical initial release rate of 11·7 and 9·8 μg/min respectively. Formulation III and IV gave gradual release of the drug at rates not exceeding 2·4 μg/min over a long period of time.

Fig. 5 shows the total ^{14}C excreted in the bile of rats after subcutaneous dosing of the four radio-labelled formulations. The absorption and elimination may be represented by the following model:



The blood level half-life of pentagastrin is less than 1 min as determined by tracer technique in this laboratory. Therefore it may be taken that K_2 is very high in comparison to K_1 and K_3. In practice, the absorption and elimination kinetics can

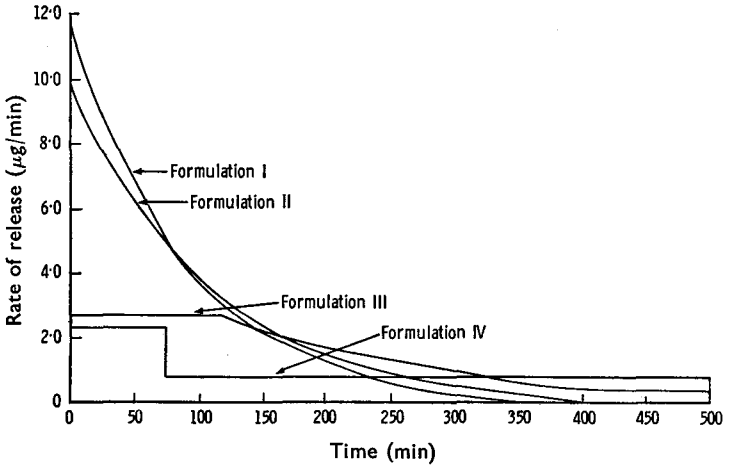


FIG. 4. *In vitro* rate of release of pentagastrin formulations.

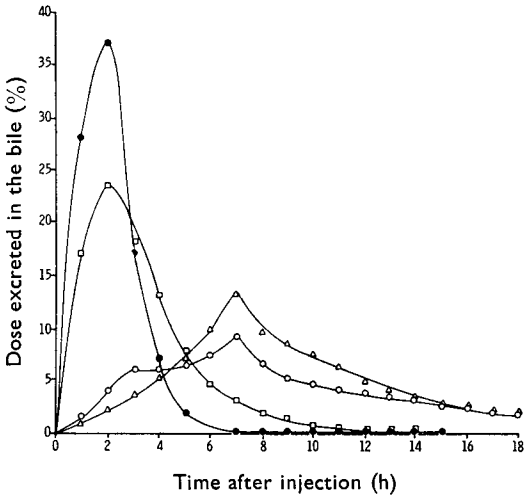


FIG. 5. Biliary excretion in rats of labelled pentagastrin after subcutaneous dosing. Formulation I (●), formulation II (□), formulation III (△), formulation IV (○).

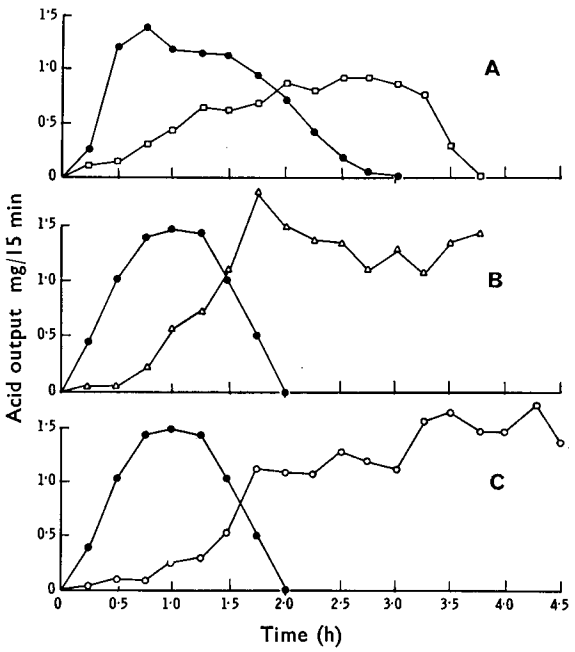
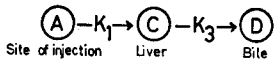


FIG. 6. Secretion of gastric acid in dogs after subcutaneous injection of various pentagastrin formulations. (A) Comparison of formulations I (●) and II (□). (B) Comparison of formulations I and III (△). (C) Comparisons of formulations I and IV (○).

be simulated using the following simplified model:



An analogue computer was used to evaluate K_1 and K_3 based on the biliary excretion data. Very close approximations to the biliary excretion results were achieved with Formulations I and II when a time lag of 0·3 h was chosen for the onset of action and 93·5% was taken as the maximum excretion of ^{14}C. The K_1 and K_3 values for Formulation I were 1·0 h^{-1} and 5·0 h^{-1} respectively and the corresponding figures for the experiment on Formulation II were 0·45 h^{-1} and 5·0 h^{-1} respectively. The calculations demonstrated that the K_1 values for Formulation III and IV do not follow first order kinetics.

Fig. 6 compares the gastric secretory response in the dog of Formulations II, III and IV against Formulation I after subcutaneous injection at 10 μg/kg. Each point is the average mean acid output from two experiments on the same dog. There is good agreement between the *in vitro* data, elimination data and dose-response data for the duration of action of the formulations. The maximum secretion can be sustained by controlling the rate of release. This agrees with the theoretical implication that by controlling the rate of release of a short-acting drug, the maximum response to the drug can be sustained and the total response increased by better utilization of an administered dose.

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