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Oral availability of a poorly absorbed drug, hydrochlorothiazide, from a bioadhesive formulation in the rat

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Summary

The bioavailability of certain drugs can be limited by the residence time of the formulation in the upper GI tract. The availability of a poorly-absorbed drug, hydrochlorothiazide, was determined from two formulations: a bioadhesive poly(acrylic acid) (Carbopol) formulation, which showed delayed transit to the ileocaecal junction, and a similar non-adhesive formulation. The results showed identical availabilities from the two formulations, suggesting that factors other than GI residence time were affecting drug availability from these formulations.

Introduction

It is known that in certain situations the oral availability of a drug may be limited by the GI transit time of the dose (Hofmann et al., 1983). Firstly, a number of drugs are absorbed only from the small intestine, and then only slowly, with the result that their availability is limited by the residence time of the dose in or upstream of the small intestine. Where the bioavailability of the drug is related to the GI transit time, there is a risk of variable and unpredictable availability. Secondly, the absorption, and possibly the release, of drug from a controlled-release formulation may depend in part on the location of the system within the GI tract. In the case of drugs which are absorbed only from the small intestine, release times of greater than 4–8 h are likely to be ineffective, since the dose will probably have reached the large intestine by this time. In any case, release times in excess of 24–48 h are precluded, since the system is likely to have been voided from the GI tract altogether by this time.

It has been shown that the concomitant intake of food can delay the gastric emptying of the dose, extending the time available for absorption and increasing the fraction of the dose absorbed (Welling and Barbhaiya, 1982; Beermann and Groschinsky-Grind, 1978b). Similarly, the coadministration of propantheline, a drug which slows gastric emptying and small intestinal transit, has been shown to increase the availability of a number of drugs (Manninen et al., 1973; Beermann and Groschinsky-Grind, 1978a).

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A number of techniques have been proposed to modify the GI transit of oral pharmaceutical formulations. One approach is to design a formulation which can adhere to the lining of the stomach or small intestine, thus retaining the dose in the upper GI tract. This utilises the phenomenon of bioadhesion – an adhesive interaction between a polymeric material and a biological surface. Ch'ng et al. (1985) and Longer et al. (1985) investigated capsule formulations containing one such material, polycarbophil, in the rat. They demonstrated that these formulations showed delayed GI transit and gave improved availability of a poorly absorbed drug, chlorothiazide: the relevance of these studies may be questionable, however, given the large doses of polymer administered. More recently, Harris et al. (1989) studied the GI transit of a range of polymeric systems in the rat, by the method of Varga (1976). They showed delays of around 25% in the orocaecal transit of 4% and 5% solutions of Carbopol 934, compared with a non-adhesive control.

The aim of this study was to investigate the oral availability of a poorly absorbed drug, hydrochlorothiazide, from one of these delayed-transit Carbopol formulations and from a non-adhesive control. It was thought that the adhesive formulation could give an increase of up to 25% in the availability of the drug. The adhesive formulation used was 5% Carbopol, and the control was 1.5% hydroxyethyl cellulose (HEC): these formulations had shown orocaecal transit times of 6.5 and 5.2 h, respectively (Harris et al., 1989).

The drug used in this study was hydrochlorothiazide (HCT). It has been shown, both in man and in rat, that the absorption of HCT is site-specific, occurring in the small intestine but not the colon (Beermann et al., 1976; Lynch et al., 1987). In man, HCT is 60–70% adsorbed from an oral dose (Beermann et al., 1976, 1977): absorption is increased by food and propantheline, both of which slow GI transit (Beermann and Groschinsky-Grind, 1978a and b). HCT is mainly excreted unchanged by the kidneys (Beermann et al., 1976). The kinetics of HCT in the rat have been less widely reported. The results of Taylor and Burke (unpublished observations) indicated that the oral availability of HCT is approximately $57 \pm 6\%$ in the rat (mean $\pm$ S.E.M.). Sheppard et al. (1960) studied the kinetics of ^{3}H-labelled HCT: they showed that 40–70% of an oral dose of HCT appeared in the urine and, moreover, that the percentage recovery was related to dose (68.5% of a 0.15 mg/kg dose recovered, cf. 43.4% of a 4.7 mg/kg dose). HCT was eliminated fairly rapidly, with almost 90% of the recovered dose appearing in the urine within 24 h. They were unable to detect any metabolites of HCT.

These results indicated that HCT was a suitable candidate drug for this kind of investigation, being incompletely absorbed, principally from the small intestine. They also confirmed that 24 h urine collection, with HPLC analysis, was a good measure of HCT availability in the rat.

Materials and Methods

Materials used

The materials used in these studies were as follows:

Carbopol 934 (mol.wt. 3×10^6), BF Goodrich, U.K.

Hydroxyethyl cellulose (Natrosol 250HHX, mol. wt. ca. 10^6), Hercules Inc., U.S.A.

Hydrochlorothiazide, Sigma Chemical Co., U.S.A.

Hydroflumethiazide, Sigma Chemical Co., U.S.A.

Hypersil, 5 μg ODC, Shandon Southern Products Ltd., U.K.

Ethyl acetate, reagent grade, FSA Laboratory Supplies, U.K.

Acetonitrile, HPLC grade, FSA Laboratory Supplies, U.K.

Trifluoroacetic acid, Fluorochem Ltd., U.K.

The mean particle size of the HCT was determined by optical microscopy to be 12 μm. HCT is a weak acid ($pK_a = 8.8$) and is only poorly soluble in water (0.5 to 2.0 mg/ml).

All ethyl acetate used in the assay procedure was redistilled before use.

Preparation of formulations

HCT (25 mg) was accurately weighed and dispersed in 4.5 g distilled water. Appropriate quantities of polymer were accurately weighed and added gradually, while constantly mixing by mag-

netic stirrer. The system was made up to 5.0 g by the addition of distilled water. All formulations were prepared 24 h before using, to allow the polymer to fully hydrate and to allow trapped air bubbles to clear. The viscosities of these systems were such that there was no settling of HCT from suspension during the time-span of the study.

Rheological studies

The viscosities of 5% Carbopol 934 solutions with and without 5 mg/ml HCT were measured, to determine whether the presence of HCT affected the rheological properties of Carbopol 934 solutions. These studies were carried out using a Contraves Rheomat 135 (Contraves AG, Switzerland) interfaced with a microcomputer (Hewlett Packard 85B, Hewlett Packard Co., U.S.A.). The test suspensions were prepared in a similar manner to the formulations described above. The pH of the system was measured using a calibrated pH meter and the viscosity was measured. The pH was adjusted by the addition of either 2 M hydrochloric acid or 2 M sodium hydroxide and the pH and viscosity were again measured. These steps were repeated over the pH range 3–7, and viscosity was measured between shear rates of 1 s^{-1} and 10 s^{-1}. Viscosity at a shear rate of 2 s^{-1} was then plotted against pH for each system.

Bioavailability studies

Shortly before dosing, a quantity of the prepared formulation was transferred to a 1 ml gastight syringe (Hamilton Bonaduz AG, Switzerland). This was fitted with a 100-mm-long round-tipped steel needle and a mechanical syringe driver, which could accurately deliver small doses in increments of 20 μl (Hamilton Co., U.S.A.). The formulations were dosed by passing the dosing needle down the oesophagus and into the stomach of the conscious rat, and activating the syringe driver. A 60 μl dose of 5 mg/ml HCT suspension was thus administered to each rat, delivering a dose of 300 μg HCT. This amounted to a dose of approximately 1.2 mg/kg, which is within the usual dose range in man.

Male rats were used in this study, of approximately 250 g in weight (Wistar-derived A.P. strain). They were fasted for 18 h prior to the study, but were allowed free access to a 10% dextrose solution throughout. After dosing, the animals were housed in individual metabowls for 24 h and were given free access to food from 4 h after dosing.

A pilot study was carried out, to ascertain that measurable quantities of drug were excreted in the urine and to confirm that 24 h urine collection was sufficient to recover virtually 100% of the total urinary HCT output. Each formulation was then dosed to 20 rats, and the 24-h urine outputs from each animal were collected and analysed individually.

Assay procedure

The 24-h urine collections were assayed for HCT by extraction into ethyl acetate and analysis by HPLC, using a HFM internal standard and UV detection. The procedure for this assay was as follows.

The volume of each 24-h urine collection was measured and recorded, and made up to 100 ml with distilled water. 1 ml of this was transferred to a test tube. 1 ml of pH 7 buffer (EIL, Analytical Instruments, U.K.) and 100 μl of a 20 μg/ml solution of HFM in methanol were added and the tube was vortexed. 10 ml redistilled ethyl acetate was added and the tube was stoppered and mixed by inversion for 15 min at 50 rpm. The tube was centrifuged for 5 min at 1500 rpm and 10°C (Mistral 6L centrifuge, M.S.E., U.K.). The ethyl acetate layer was transferred to a fresh tube and 1 ml of a 0.1 M sodium hydroxide solution was added. The tube was stoppered, mixed by inversion and centrifuged as before. The ethyl acetate layer was again transferred and evaporated to dryness at 55°C under a stream of nitrogen. The residue was dissolved in 200 μl of the HPLC solvent and transferred to a glass sample tube for HPLC analysis.

The HPLC system used consisted of a 10 cm column (internal diameter 4.6 mm) of 5 μm Hypersil with a moving phase of 15% acetonitrile and 0.1% trifluoroacetic acid in distilled water. The solvent was sonicated prior to use to degas it. The pump used was an LKB Bromma HPLC pump (LKB-Produkter AB, Sweden), operating at a flow rate of 1.3 ml/min. 50 μl samples were injected by an automatic sample injector (WISP 710B, Waters

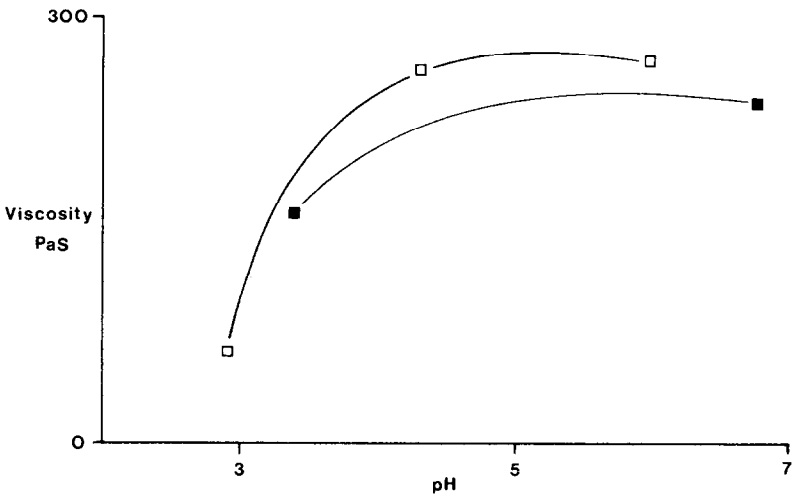

Fig. 1. Viscosity-pH profiles for 5% Carbopol 934, at shear rates of 2 s^{-1}, with (closed squares) and without (open squares) 5 mg/ml hydrochlorothiazide.

Associates Inc., U.S.A.). Detection was by UV absorbance at a wavelength of 270 nm (Spectromonitor III Model 1204A, LDC/Milton Roy, U.S.A.). Output was by means of a chart recorder (BD8 multirange, Kipp and Zonen, Holland).

The HCT and internal standard (HFM) peaks were distinct and well-separated, with retention times of approximately 1.8 and 4.0 min, respectively. A calibration line was constructed for each HPLC run, in terms of peak height ratio (HCT/HFM) against HCT concentration. This assay system gave a lower limit of quantification of not less than 33 μg HCT per 100 ml urine. Two replicate samples were run for each 24-h urine collection: for each replicate pair of samples, the mean HCT urine concentration was calculated.

Results and Discussion

Rheological studies

Plots of viscosity against pH for 5% Carbopol solutions with and without 5 mg/ml HCT are presented in Fig. 1. As can be seen from these results, the presence of 5 mg/ml HCT had only a small effect on the rheological properties of the Carbopol solution; viscosity was reduced by 10–15% by HCT over the pH range studied. This suggested that the inclusion of HCT did not greatly alter the structure of the polymer gel.

Bioavailability studies

The accuracy of the administered dose was determined by weighing a number of doses: the dose delivered was found to be 59.4 ± 0.25 mg in weight (mean $\pm$ S.E.M., $n = 6$), which was close to the nominal dose of 60 μl.

The pilot investigation carried out in one rat indicated an availability of 45% of the administered dose, with adequate urine concentrations for the assay employed and 95% of the recovered HCT excreted in the first 12 h.

The results of the full HCT bioavailability studies on the two formulations are presented in Table 1. It can be seen from the results in the table that

TABLE 1

Results of bioavailability studies on bioadhesive and non-adhesive hydrochlorothiazide formulations in the rat (mean $\pm$ S.E.M., n = 20)

Formulation	5% Carbopol with 5 mg/ml HCT	1.5% HEC with 5 mg/ml HCT
Rat weight (g)	259 $\pm$9	255 $\pm$4
Urine HCT (μg)	160.8 $\pm$ 5.6	160.8 $\pm$ 4.6
% Availability	53.6 $\pm$ 1.9	53.6 $\pm$ 1.5

the oral bioavailability of HCT from these formulations, in terms of 24-h cumulative urinary excretion, was exactly 53.6% in each case. This suggested either that the transit of the dose was not affected by the formulation, or that the absorption of the drug from the formulation was otherwise impeded.

(It is possible, using the data in the table, to estimate the smallest difference between the means which could have been resolved. By this calculation, this study would have been able to determine a significant difference of 5 percentage points or greater between the percentages absorbed from the two formulations (two-tailed t-test, $P < 0.05$).)

It was thought unlikely that the dose of HCT would have separated entirely from the formulation in the GI tract. The HCT was of similar particle size to the ^{51}Cr microspheres employed in the earlier study (Harris et al., 1989) and could therefore be expected to behave similarly. The GI transit of the entire formulation may have been modified by the inclusion of HCT. This does not seem likely, however, since HCT would represent only a small particulate load to the system ($<$ 0.5%) and, being poorly soluble and a weak acid, would make a negligible contribution to the ionic strength of the system. The rheological studies on a Carbopol/HCT system support this view, showing HCT to have only a minor effect on the viscosity of the system. The other possibility is that the viscosity of the system in some way retarded the delivery of drug to the absorbing surface. This effect was suggested by Levy and co-workers (Levy and Jusko, 1965; Hewitt and Levy, 1971; Ashley and Levy, 1973), who studied the absorption of a number of drugs from viscous alginate and methylcellulose solutions. They suggested a number of possible mechanisms for this effect, including: (a) that the rate of movement of drug in solution from the bulk of the viscous solution to the absorbing surface was slowed; (b) that the ability of the viscous intestinal content to penetrate and come into contact with the epithelial surface was reduced; (c) that, in the case of a drug in suspension, the dissolution rate of the solid drug was decreased by retardation of the movement of dissolved drug away from the boundary layer.

It is suggested that, in these studies, the transit of the Carbopol formulation was indeed delayed compared with that of the control. The absorption of HCT from this formulation was subsequently hindered, however, as the viscosity of the formulation increased with the rise in pH encountered in the small intestine. Since HCT is poorly soluble and was administered as a suspension, it was thought that the most likely cause of this was the effect of viscosity on drug dissolution, although other mechanisms may also have influenced drug availability.

Conclusions

In conclusion, therefore, the overall effect was probably an increase in the residence time of the dose in or above the absorbing region, but impaired absorption of drug from the formulation for much of this time. If this hypothesis is correct, then it highlights an important consideration in the development of bioadhesive formulations of this kind. For such formulations to be effective, they must be capable of delaying the GI transit of the dose, but must do so in such a way that the absorption of drug from the formulation is not substantially retarded.

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