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Bioavailability of hydrochlorothiazide: conventional versus freeze-dried tablets

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Abstract

The objective of the study was to evaluate the pharmacokinetic parameters and relative bioavailability of freeze-dried rapidly disintegrating tablets (formulation A) and lyophilized emulsion tablets (formulation B) containing 25 mg hydrochlorothiazide as a model drug. The tablets were produced by freeze-drying an o/w emulsion containing Miglyol 812, maltodextrin and Methocel A15LV or a suspension containing maltodextrin, PEG6000 and xanthan gum in blisters. Dissolution tests were performed on the tablets using a USP XXII paddle method. A content uniformity test was done on the tablets using an HPLC method. Eight healthy volunteers participated in the bioavailability study. Each volunteer received in a randomized cross-over design, 25 mg HCT on three occasions. Serum samples were analysed on HCT concentration using a validated HPLC method. The C_{max} and T_{max} values were determined from the individual serum concentration–time profiles, while the AUC_{0-24h} and $T_{1/2}$ were calculated using a software package. Both the lyophilized dry emulsion tablet and the conventional tablet showed similar release profiles, while the formulation of a freeze-dried rapidly disintegrating tablet containing 6 mg PEG 6000 resulted in an increase in HCT in vitro release rate. The calculated AUC_{0-24h} values were significantly different between the three formulations: the freeze-dried rapidly disintegrating tablet containing 6 mg PEG 6000 showed a significantly higher AUC_{0-24h} in comparison with the other formulations. The C_{max} value of the formulation A was higher compared to the other, although not significantly. The T_{max} values for the freeze-dried formulations were lower compared to the conventional tablet, although not significantly. The serum half life ($T_{1/2}(\beta)$) ranged from 2.1 to 10.5 h for the three formulations. From these results it can be concluded that freeze-dried tablets containing maltodextrin, xanthan gum and PEG 6000 yielded a higher hydrochlorothiazide bioavailability compared to a conventional tablet. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Freeze-dried tablet; Lyophilized emulsion; Hydrochlorothiazide; Maltodextrin

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1. Introduction

There is an increased interest in fast-dissolving dosage forms for buccal, sublingual and oral administration. Oral lyophilized products combine the properties of freeze-dried dosage forms, such as fast reconstitution, good preservation and stability with the advantages of liquid dosage forms for bioavailability (Jaccard and Leyder, 1985). The formulation and production of rapidly disintegrating tablets by lyophilization has been reported by Corveleyn and Remon (1997). These authors demonstrated that the incorporation of PEG 6000 into a freeze-dried tablet resulted in an increase of the in vitro release rate of hydrochlorothiazide (HCT). In an other study, Corveleyn and Remon (1998) reported on the formulation of lyophilized dry emulsion (LDE) tablets with HCT as a model drug. Freeze-dried emulsion tablets might offer advantages in the formulation of drugs with a low water solubility. The current commercially available freeze-dried tablet formulations contain gelatin as a binder. The freeze-dried tablets used in this study were gelatin free, which could be an advantage considering the problems with BSE infections (Marwick, 1997).

In this study the pharmacokinetic parameters of HCT in human volunteers were evaluated after oral administration of lyophilized emulsion tablets and rapidly disintegrating tablets containing 25 mg HCT.

2. Materials and methods

2.1. Materials

The spray dried maltodextrin (C✱PUR01934; Eridania-Beghin Say-Cerestar, Vilvoorde, Belgium) was obtained by enzymatic hydrolysis of corn starch, and had a dextrose equivalent (DE) of 38. Xanthan gum was obtained from Ludeco (Brussels, Belgium). Polyethyleneglycol (PEG 6000) was obtained from Union Carbide (Danbury, CT, USA). Solutions were made in distilled water. Hydrochlorothiazide (HCT) (batch no. 5327 B, Ludeco) was chosen as a model drug. HCT is a diuretic, practically insoluble in water (25°C) and having a solubility of 250 mg/l in 0.1 N HCl (25°C). Methylcellulose (Methocel® A15LV, 2% aqueous solution viscosity 15 mPa·s (20°C)) was provided by Colorcon (Kent, UK). A medium chain triglyceride Miglyol 812 (Federa, Belgium) was used as the oil phase in the emulsion tablets.

2.2. Methods

2.2.1. Formulations

Two lyophilized tablet formulations were evaluated in vivo. Formulation A was a freeze-dried rapidly disintegrating tablet and consisted of maltodextrin DE38 80 mg, PEG 6000 8 mg, xanthan gum 8 mg and HCT 25 mg. Formulation B was a lyophilized dry emulsion tablet which consisted of Miglyol 812 160 mg, maltodextrin DE38 80 mg, Methocel A15LV 16 mg and HCT 25 mg. Esidrex® 25 (batch no. 96B06AS) was used as a conventional reference formulation (Ciba, Basel, Switzerland).

2.2.2. Preparation of the tablets

For the manufacturing of formulation A, 12.5 g of HCT was suspended in 400 ml of a solution containing maltodextrin in a concentration of 20% (w/v), PEG 6000 in a 1% (w/v) concentration and xanthan gum 1% (w/v) concentration.

For the preparation of the emulsion formulation B, an aqueous solution was prepared containing 20% (w/v) maltodextrin and this solution was emulsified with Miglyol 812 using 2% (w/w) Methocel A15LV. HCT (12.5 g) was mixed with the emulsion. The ratio water phase/oil phase was 80/20 (w/w). The emulsion was prepared using a Silverson mixer (Silverson Machines, Waterside, UK) according to a standardized production protocol as described by Kiekens et al. (1997).

The freeze-dried tablets were prepared as follows: 0.8 g of the emulsion or the suspension were placed in PVC blisters with a diameter of 15 mm and a depth of 6 mm. The blisters were placed on the shelves of the freeze-dryer (Amsco-Finn Aqua GT4, Amsco, Brussels, Belgium). The samples were frozen to −45°C at a rate of 0.5°C/min and kept at this temperature for 1.5 h. Primary drying

was performed by keeping the blisters for 8 h at a pressure of 1 mbar, a shelf temperature of −10°C and a condensor temperature of −60°C. Secondary drying was carried out by reducing the pressure to 0.1 mbar and increasing the shelf temperature to 25°C. Secondary drying time was 6 h. Lyophilization was terminated by venting the drying chamber with air.

2.2.3. Dissolution testing

Dissolution testing was performed on tablets containing 25 mg hydrochlorothiazide in distilled water and in 0.1 N HCl (37°C) using the paddle method (USP XXII) at a rotational speed of 100 rpm (Van Kel dissolution testing station, Van Kel, Hornchurch, UK). Samples of 5 ml were withdrawn at regular time intervals, replaced by fresh medium and spectrophotometrically analysed at 273 nm (Perkin-Elmer Lambda 12 spectrophotometer, PE, Brussels, Belgium) after filtration through a porous metallic filter (pore diameter, 2 μm). All dissolution tests were performed in triplicate.

2.2.4. Bioavailability testing

Eight healthy Caucasian male volunteers, aged 23–30 years and weighing between 69 and 113 kg, participated in the study after giving informed consent. The physical state of all volunteers was examined before they were allowed to participate in the study. The study was approved by the Ethical Committee. The subjects were instructed to take no drugs for 1 week prior to and during the study. Each volunteer was given, in a randomized cross-over design, 25 mg hydrochlorothiazide on three occasions, once as a tablet and twice as a freeze-dried tablet (formulation A and formulation B). The interval between the intakes was 1 week. The drug was administered orally at 08:00 h with 200 ml water after overnight fasting. A standard breakfast (sandwiches, butter, marmalade) was given 2 h after administration of the dosage form. A lunch was taken at 12:00 h. No consumption of alcoholic beverages and nicotine was permitted 12 h before and 24 h after drug intake. Venous blood samples were collected in glass tubes before administration of the drug, and at 15, 30, 45 min and 1, 1.5, 2, 2.5, 3, 3.5,4, 6, 8, 10, 12 and 24 h after drug administration. Serum was separated from the blood cells by centrifugation at 3000 rev/min and stored at −20°C until analysis.

2.2.5. Chromatography

HCT concentrations were determined using a validated HPLC described by Vervaet and Remon (1997). A RP-C18 column (250 × 4 mm × 5 μm) (Lichrospher 100, Merck, Darmstadt, Germany) equipped with a precolumn (RP-C18 18, 4 × 4 mm × 5 μm) was used. Both were kept at a constant temperature of 40°C. The mobile phase was 0.2 M phosphate buffer (pH 7.0)/tetrahydrofuran/acetonitrile (85/10/5, v/v/v). The UV detector was set at a wavelength of 273 nm. Hydroflumethiazide (HFMT) (Sigma, St. Louis, MO, USA) was used as the internal standard (IS). Serum (500 μl), 100 μl 1.25 μg/ml HFMT and 5 ml methyl *tert*.-butylether (Sigma) were pipetted into borosilicate glass tubes. After vortexing for 2 min, and 5 min centrifugation at 2700 × *g*, the organic phase was transferred into a new borosilicate glass tube and evaporated until completely dry under a nitrogen stream. The residue was dissolved in 200 μl water, followed by the addition of 3 ml toluene (Vel, Leuven, Belgium). The bulk of the toluene layer was discarded after 2 min of vortexing and 10 min centrifugation at 2700 × *g*. After evaporation of the water fraction under a nitrogen stream, the residue was dissolved in 200 μl mobile phase. A 100-μl aliquot of the homogenized solution was injected into the HPLC system.

2.2.6. HPLC validation

The HCT recovery (10–1000 ng/ml) varied between 91.0 and 100.7%.

While 94.5% of the internal standard was recovered. The calibration line was linear between 0 and 1000 ng/ml ($r^2 = 0.9992$) ($n = 8$). The within-day variability was 1.8% in the 10–1000-ng/ml range, while the intra-day variability for the same concentration range was determined at 0.2%. The detection and quantification limit in serum were 2.6 and 8.6 ng/ml, respectively.

2.2.7. Content uniformity

Ten tablets were each weighed to obtain the mean tablet weight. An accurately weighed portion of the homogenized powder corresponding with 25 mg hydrochlorothiazide (HCT) and 25 mg of hydroflumethiazide (HFMT) (Sigma) as the

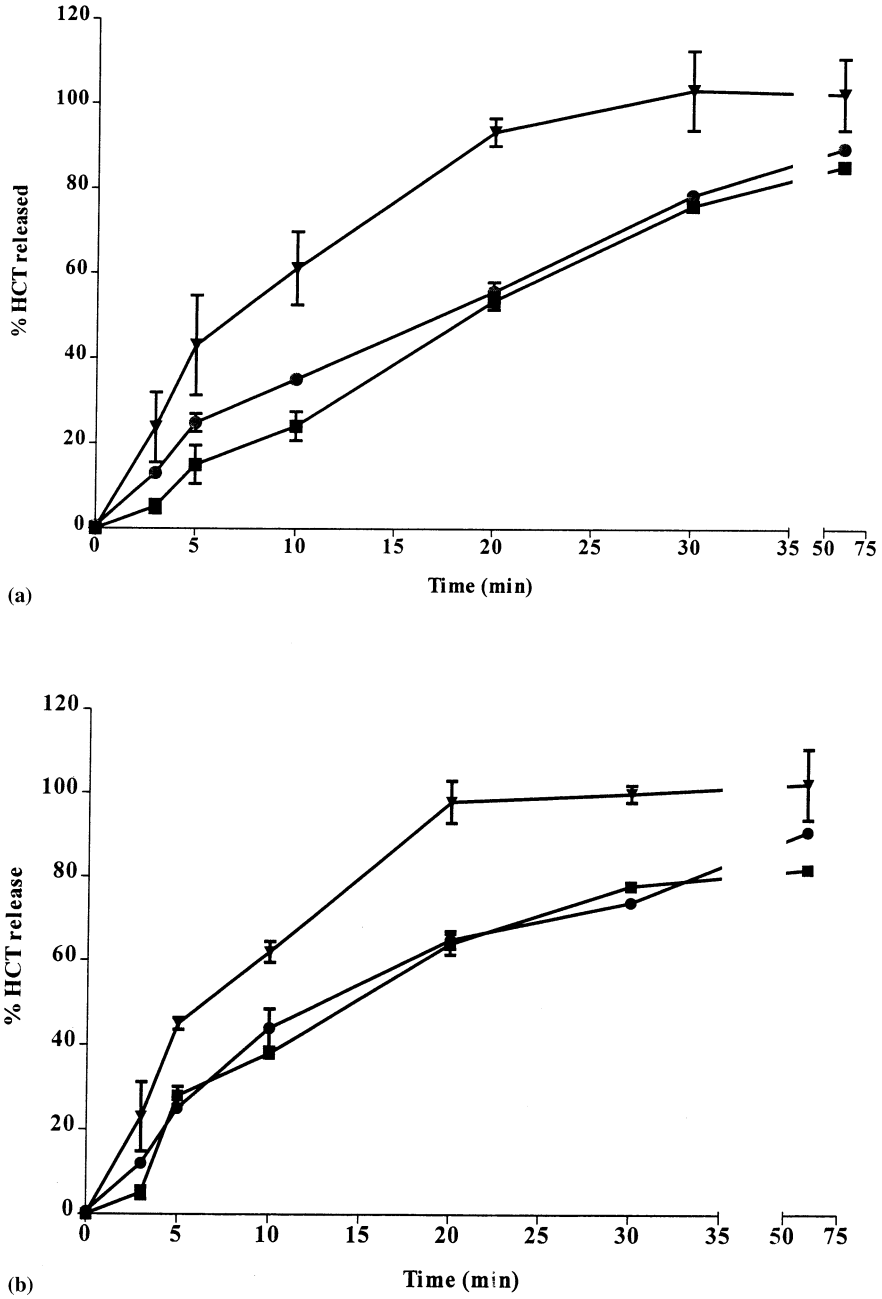

Fig. 1. In vitro release of hydrochlorothiazide (25 mg) from a freeze-dried rapidly disintegrating tablet (▼); a lyophilized emulsion tablet (●) and a reference tablet Esidrex® 25 (■) in water (a) and in 0.1 N HCl (b). Error bars are S.D.

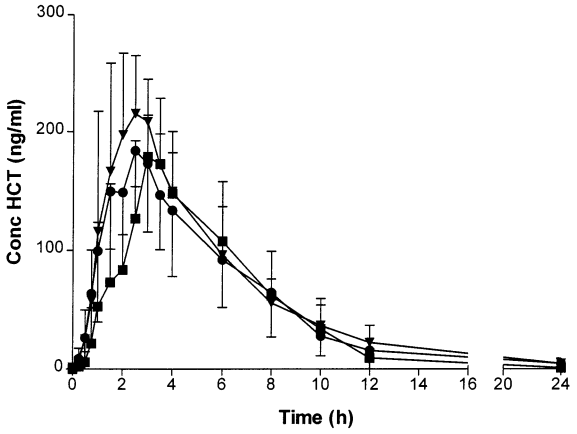

Fig. 2. Mean serum hydrochlorothiazide (25 mg) concentration vs time profile after oral administration of: a freeze-dried rapidly disintegrating tablet (▲); a lyophilized emulsion tablet (●) and a reference tablet Esidrex® 25 (■). Error bars are S.D.

internal standard (IS) were treated with HPLC-grade methanol (Merck, Darmstadt, Germany) for the formulation A tablets, or with diluted sodium hydroxide (NaOH 0.2 M) (Vel, Leuven, Belgium) for the formulation B tablets, next they were put in the ultrasonic generator for 1 h and diluted to 50 ml with the same solvent. Next, 10 ml of the solution was filtered through a 0.2-μm membrane filter (Schleicher & Schuell FP030/3) and the first 5 ml of the filtrate was discarded. The filtrate (2.5 ml) was diluted with mobile phase to 50.0 ml, providing a theoretical concentration of 25 μg/ml hydrochlorothiazide and 25 μg/ml IS. This solution (100 μl) was injected in the HPLC system.

2.2.8. Pharmacokinetic analysis

A model-independent analysis was used. The C_{max} and T_{max} values were determined from the individual serum concentration–time profiles, while the AUC_{0-24h} and $T_{1/2}(\beta)$ were calculated using the MW/Pharm software package (v. 3.0; Mediware 1987–1991, Utrecht, The Netherlands) as described by Proost and Meijer (1992). A mixed integration algorithm with different criteria for choosing the appropriate numerical integration method was used to calculate the AUC_{0-24h} values.

2.2.9. Statistical evaluation

The non-parametric Friedman test (Siegel and Castellan, 1988) was used to evaluate the pharmacokinetic parameters on the three formulations with a significance level of $p < 0.05$. Dunn's multiple-comparison test was used for a 2×2 comparison of the data. All calculations were done using GraphPad Prism TM (v. 2.0, San Diego, CA).

3. Results and discussion

The results of the content uniformity analysis of the freeze-dried tablets were 25.3 ± 0.3 and 24.6 ± 0.7 mg for formulation A and B, respectively.

The in vitro release of hydrochlorothiazide from both lyophilized formulations was compared with the release from the conventional tablet (Fig. 1a,b). Both the lyophilized dry emulsion tablet (formulation B) and the conventional tablet showed similar release profiles: 76.2 and 74.6% HCT was released in water within 30 min for formulation B and the conventional tablet, respec tively. The formulation of a freeze-dried rapidly disintegrating tablet containing 8 mg PEG 6000 (formulation A) resulted in an increase in HCT in vitro release rate in water as 99.4% HCT was released within 30 min.

Table 1
Pharmacokinetic parameters of hydrochlorothiazide in human volunteers, after administration of a conventional tablet (Esidrex® 25) and two lyophilized dosage forms

Formulation	AUC_{0-24h} (ng/h per ml)	C_{max} (ng/ml)	T_{max} (min)	$T_{1/2}$ (h)
Reference	1009.5 ± 399.8	200.1 ± 34.9	183.7 ± 40.7	5.8 ± 2.3
Formulation A	1843.4 ± 476.2[a]	244.2 ± 44.3	142.5 ± 47.4	5.4 ± 1.8
Formulation B	1072.8 ± 368.6	201.8 ± 38.5	135.0 ± 35.8	5.2 ± 2.2

[a] Significantly higher than the reference and formulation B ($p < 0.05$; Dunn's multiple comparison test).

This could be due to the formation, at least partially, of a solid dispersion of the HCT in the PEG 6000. Solid dispersions of high-molecular weight polyethyleneglycols are reported to increase the in vitro release rate and bioavailability of poorly soluble drugs (Chiba et al., 1991). It was reported that the formulation of solid dispersion with PEG 6000 resulted in an increase of the in vitro release rate of HCT (Simonelli et al., 1994).

The bioavailability of the three formulations was evaluated: the commercially available tablet (Esidrex® 25 mg) and two freeze-dried formulations, one rapidly disintegrating tablet containing 8 mg PEG 6000 and one lyophilized dry emulsion tablet. The mean serum hydrochlorothiazide concentration–time profiles are shown in Fig. 2. The pharmacokinetic parameters after administration of the three formulations are shown in Table 1. The calculated AUC_{0-24h} values were significantly different ($p < 0.05$; Friedman test) between the three formulations: the freeze-dried rapidly disintegrating tablet containing 8 mg PEG 6000 (formulation A) showed a significantly higher AUC_{0-24h} value ($p < 0.05$; Dunn's multiple-comparison test) in comparison with the other formulations. The C_{max} value of the formulation A tablets (244.2 ± 44.3 ng/ml) was higher compared to the other tablets (200.1 ± 34.9 and 201.8 ± 38.5 ng/ml for the reference tablet and formulation B, respectively), although not significantly. These values were in accordance with the plasma C_{max} values (range 97–247 ng/ml) after oral administration of 25 mg HCT reported by Beermann and Groschinsky-Grind (1977). The T_{max} values for the freeze-dried formulations were lower compared to the conventional tablet, although not significantly. The serum half life ($T_{1/2}(\beta)$)was 5.8 ± 2.3, 5.4 ± 1.8 and 5.2 ± 2.2 h for the reference formulation, formulation A and formulation B, respectively. Beermann and Groschinsky-Grind (1977) reported a plasma half-life in the range 0.86–14.81 h, with a high inter-individual variability, which was also observed in this study. The relative bioavailability (F_{rel}) of formulation A was 187.6% compared to the conventional tablet, whereas the F_{rel} of formulation B was 118.9%. Several authors reported the existence of an absorption window of HCT in the gastro-intestinal tract, since the HCT uptake mainly takes place in the duodenum and the first part of the jejenum and to a small extent in the stomach (Beermann et al., 1976; Beermann and Groschinsky-Grind, 1977). The higher bioavailability of the rapidly disintegrating freeze-dried tablet containing PEG 6000 could be due to the fast disintegration of the tablet and the high dissolution rate of HCT, resulting in a higher availability.

From these results it can be concluded that freeze-dried tablets containing maltodextrin, xanthan gum and PEG 6000 yielded a higher hydrochlorothiazide bioavailability compared to a conventional tablet.

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