ELSEVIER

International Journal of Pharmaceutics 222 (2001) 1–6

international journal of pharmaceutics

www.elsevier.com/locate/ijpharm

In vivo evaluation of hydrochlorothiazide liquisolid tablets in beagle dogs

Khaled A. Khaled [a,*], Yousif A. Asiri [b], Yousry M. El-Sayed [a]

[a] *Department of Pharmaceutics, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Saudi Arabia*
[b] *Department of Clinical Pharmacy, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Saudi Arabia*

Received 12 June 2000; received in revised form 21 February 2001; accepted 26 February 2001

Abstract

This study was carried out to evaluate the absorption characteristics of experimentally developed hydrochlorothiazide liquisolid tablets using six male beagle dogs. Comparison with reference commercial tablets was made. As no bibliographic data were found for the pharmacokinetic parameters of the drug in dogs, an intravenous drug administration was included in the study. The drug was administered orally as a single 25 mg dose of commercial and liquisolid tablets on two occasions in a randomized two-way crossover design. The pharmacokinetic parameters of the drug post intravenous dosing were reported for the first time. The results of the oral administration revealed statistically significant differences between the liquisolid and the commercial tablets in the area under the plasma concentration–time curve, the peak plasma concentration, and the absolute bioavailability. On the other hand, no significant differences were observed between the two formulations with regard to the mean residence time, the mean absorption time, and the rate of absorption. The absolute bioavailability of the drug from the liquisolid tablets was 15% higher than that from the commercial one. The parametric 90% confidence intervals for the different parameters were higher than the commonly expected intervals for bioequivalency, indicating greater bioavailability of the liquisolid tablets. © 2001 Published by Elsevier Science B.V.

Keywords: Hydrochlorothiazide; Liquisolid tablets; Bioavailability; Pharmacokinetic

1. Introduction

Hydrochlorothiazide has been used, for a long period of time, as a diuretic and antihypertensive agent. Because of its limited aqueous solubility, the drug has a potential for poor GI tract absorption. The drug was included in a list of drug entities described as having 'known or potential bioequivalency or bioavailability problems' (Shah and Needham, 1979). Hydrochlorothiazide is absorbed from GI tract and apparently not metabolized and excreted unchanged in urine. At least 61% of the drug is reportedly eliminated from the body when excretion is essentially completed within 24 h post administration. The oral bioavailability of the drug was reported to be 60–80% of the administered dose (Sietsema, 1989).

* Corresponding author. Present address: Department of Pharmaceutics, Assiut University, Assiut, Egypt..

0378-5173/01/$ - see front matter © 2001 Published by Elsevier Science B.V.
PII: S0378-5173(01)00633-0

The technique of liquisolid preparation is used to formulate drug solution in solid dosage forms. Drug solution is, generally, prepared by dissolving the drug in non-volatile water-miscible solvent. Even though the drug is in a tablet form, it is held in solution. Accordingly, the dissolution step, a pre-requiste for drug absorption, may be bypassed (Liao and Jarowski, 1984) and better bioavailability of poorly soluble drugs achieved (Spireas et al., 1992; Spireas, 1995). In order to overcome the limited solubility of the drug, hydrochlorothiazide was formulated as liquisolid tablets. The method of preparation of hydrochlorothiazide liquisolid tablets as well as the effect of various formulation and processing variables on the preparation and the release properties of the tablets are described in details elsewhere (Khaled, 1998).

The objective of this study was to evaluate the in vivo absorption characteristics of hydrochlorothiazide liquisolid tablets. Comparison with commercially available conventional tablets was also performed. Literature review showed that no bibliographic data were cited for the pharmacokinetic parameters of the drug in dogs. This fact has been reported by others (Reppas et al., 1998). Therefore, it was an essential part of this study to assess these parameters via the intravenous administration of the drug.

2. Materials and methods

2.1. Materials

The following materials were used as received: Hydrochlorothiazide and Aerosil (Winlab Limited, Berkshire, UK), Avicel PH 101 and Avicel PH 102 (FMC Corp., Princeton, NJ), light magnesium carbonate (Evans Medical Ltd., UK). All other chemicals were analytical grade and other solvents were HPLC grade. In addition, Esidrix tablets (25 mg, batch # 017700, Ciba-Giegy Limited, Switzerland) were used as a reference formulation.

2.2. Preparation of hydrochlorothiazide liquisolid tablets

An accurately measured volume of hydrochlorothiazide solution in PEG200 (400 mg ml^{-1}) was triturated with the specified amount of Aerosil. Other absorbents (Avicel PH 101, Avicel PH 102, and light magnesium carbonate) were, then, added and the mixture was further triturated. Finally, talc and magnesium stearate were added. The resulted mixture was sieve sized to the 80-mesh range. The final mixture was remixed by tumbling for 3 min using tumbling mixer (Erweka, G.m.b.H., type S2V, Germany). Tablets were made by direct compressing the mixture using a single punch machine (EKO, Erweka, Germany) provided with a 0.64 cm flat face punch and die set. Tablets containing 25 mg of the drug and weighing 0.5 g were produced at a hardness level of 10 ± 1.0 Kp. The tablets were subjected to weight variation, content uniformity and dissolution testing, and were found to fulfil the USP requirements.

2.3. Preparation of hydrochlorothiazide intravenous injections

The intravenous injections of hydrochlorothiazide were prepared under sterile conditions in air laminar flow cabinet. An appropriate amount of the drug was dissolved in an isotonic 0.1 M sodium bicarbonate aqueous solution prepared using a sterile water for injection. The final concentration of the drug was 0.75 mg ml^{-1}. The drug solution was filtered using bacterial filters (Milleex-GS, 0.22 μm bacterial filter, Millipore Corp., Bradford, MA, USA). The solution was prepared freshly and used within 12 h of its preparation.

2.4. Animals

Six male beagle dogs, weighing between 9 and 14 kg (11 ± 1.5 kg, Mean $\pm$ SD) were used in the study. Hydrochlorothiazide was administered orally on two occasions separated by 2 week washout period between each treatment. One month later, the same dogs received an intra-

venous injection of the drug. The animals remained in good health through the entire period of the experiment. The dogs were starved for about 18 h prior to the experiment and continued fasting until 4 h post dose, but allowed water ad libitum. During the first 4 h of the experiment, each dog was placed in the upright position in the restrainer stand. On the other hand, during the rest of the experiment the dogs were set free in their individual cages and re-restrained 10 min before sampling time. The legs were shaved and a cephalic vein was canulated using 18-gauge cannula. The cannula was used for intravenous administration and blood sampling.

2.5. Study design and plasma samples

The study design was a single 25 mg dose, two-treatment, two-period, two-sequence crossover study with a 2 week washout period between phase I and II dosing. Dogs were randomly divided into two groups and assigned to one of the two sequences of administration. Each dog was given hydrochlorothiazide tablets in two occasions. Once as commercial tablets and in the second occasion as liquisolid tablets. The tablets were administered by gastric intubation and washed down the tube using 50 ml of water. Multiple blood samples (2.5 ml) were collected in evacuated glass tubes (heparinized vacutainers, Becton and Dickinson, CA, USA) before and at 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 10.0, 12.0, and 24.0 h after dosing for both iv and liquisolid tablets; and before and at 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 10.0, 12.0, and 24 h after dosing for commercial tablets. The plasma was separated after centrifugation of blood samples for 10 min at 3000 rpm and stored frozen at -20°C pending analysis.

2.6. Analysis of plasma samples

Concentrations of hydrochlorothiazide in plasma were determined using HPLC, with flumethiazide as internal standard (Barrhaiya et al., 1981).

2.7. Pharmacokinetic analysis

Pharmacokinetic parameters for hydrochlorothiazide following oral administration were determined from the plasma concentration–time data. The maximum plasma concentration (C_{max}) and the corresponding time (T_{max}) were obtained directly from the plasma concentration–time data. The area under the concentration–time curve (AUC) and the area under the first moment curve (AUMC) were estimated according to the linear trapezoidal rule and extrapolated to infinity using standard techniques (Gibaldi and Perrier, 1982). The apparent elimination rate constant (k_{el}) was calculated by the technique of least-squares regression from the data of the last four points of each plasma concentration–time curve. The data of plasma hydrochlorothiazide concentrations after intravenous administration were analyzed by a linear two-compartment open model with elimination from the central compartment. The mean residence time of the drug in the body (MRT), mean absorption time (MAT) and the absolute bioavailability (F) of the oral tablets were calculated using the following equations:

$$\mathrm{MRT} = (\mathrm{AUMC})/(\mathrm{AUC}),$$

$$\mathrm{MAT} = \mathrm{MRT}_{\mathrm{oral}} - \mathrm{MRT}_{\mathrm{i.v.}},$$

$$F = ((\mathrm{AUC})_{\mathrm{oral}}/(\mathrm{AUC})_{\mathrm{i.v.}})(\mathrm{dose}_{\mathrm{i.v.}}/\mathrm{dose}_{\mathrm{oral}}),$$

where $\mathrm{MRT}_{\mathrm{oral}}$ is the mean residence time after oral administration and $\mathrm{MRT}_{\mathrm{i.v.}}$ denotes the mean residence time after i.v. administration. The rate of absorption was also evaluated, indirectly, by means of the ratio of $C_{max}/\mathrm{AUC}_{0\rightarrow\infty}$ (Reppas et al., 1995).

2.8. Statistical analysis

The pharmacokinetic characteristics of hydrochlorothiazide following oral administration of conventional tablets and the prepared liquisolid tablets were evaluated statistically using two-way analysis of variance (ANOVA) for crossover design. Formulations, periods, sequence effects, and dogs were the variables. Differences between two related parameters were considered statistically significant for $P \leq 0.05$. Parametric 90% confi-

dence intervals based on the ANOVA of the mean [(test (liquisolid))/(reference (commercial))] ratios (T/R ratios) of the AUC, C_{max}, and C_{max}/AUC were computed under the assumption of a multiplicative model. All analyses of data were performed with a statistical software package (Statistical Analysis System, SAS Institute, Inc., Cary, NC, USA).

3. Results and discussion

The results of the i.v. bolus administration of hydrochlorothiazide (Fig. 1) indicated that the disposition of the drug could be adequately described using a two-compartment open model with an average terminal half-life of 8.9 ± 1.4 h. A summary of the pharmacokinetic parameters obtained is shown in Table 1.

The mean plasma concentration–time curves following the oral administration of 25 mg single dose of hydrochlorothiazide from the commercial and the experimental liquisolid tablets are shown in Fig. 2. It is clear from the figure that the mean plasma concentrations obtained with the liquisolid tablets are, generally, higher during the course of the experiment. It should be mentioned that, hydrochlorothiazide was measurable at the last sampling time (24 h) in all dogs following the administration of either tablets. The mean pharmacokinetic parameters of the drug after the oral administration of the tablets are listed in Table 2.

Table 1
Pharmacokinetic parameters of hydrochlorothiazide following an intravenous bolus injection of 0.417 mg kg^{-1} dose in dogs[a]

Parameter	Value[a]
Alpha, h^{-1}	1.61 ± 0.39
Beta, h^{-1}	0.079 ± 0.011
Beta half-life, h	8.9 ± 1.37
$AUC_{0\rightarrow\infty}$, ng h ml^{-1} kg^{-1}	1468 ± 466
Volume of distribution, ml kg^{-1}	352.8 ± 74.6
Clearance, ml kg^{-1} h^{-1}	34.0 ± 9.3
MRT, h	10.8 ± 2.8

[a] $n = 6$, values given are mean $\pm$ SD.

The results of the analysis of variance of the pharmacokinetic parameters, following the oral administration of the drug, indicated that none of these variables showed significant differences with regard to dogs between the two treatments. In addition, no period effect was found. However, there were significant inter-dog variabilities, as estimated from the coefficients of variation (CV, %), on all parameters. This may be expected in view of the wide inter-dog variations in these parameters probably due to inter-dog variabilities in drug clearance.

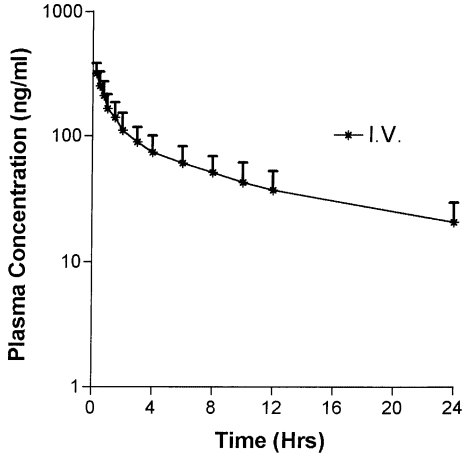

Fig. 1. Plasma concentration versus time profile of hydrochlorothiazide after intravenous bolus injection (3.75 mg) in dogs ($n = 6$).

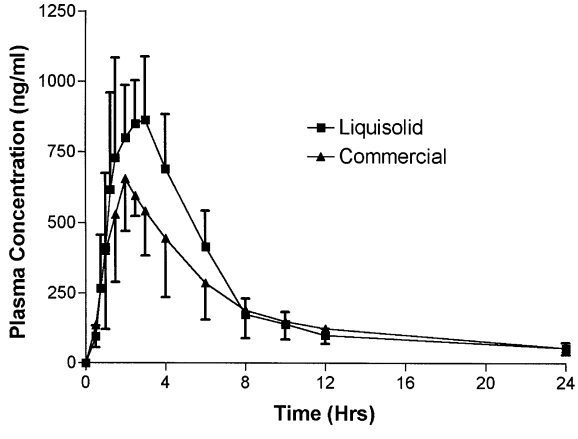

Fig. 2. Mean plasma concentration–time profiles ($\pm$ SD) obtained after the intake of 25 mg hydrochlorothiazide commercial or liquisolid tablets to six dogs.

Table 2
Mean pharmacokinetic parameters of hydrochlorothiazide after oral administration (25 mg) of commercial and liquisolid tablets to six dogs[c]

Parameter	Commercial tablets[a]	Liquisolid tablets[a]	P-value[b]
AUC_t, ng h ml^{-1}	4692 ± 1333	5622 ± 1039	0.0124 S
$AUC_{0\rightarrow\infty}$, ng h ml^{-1}	5435 ± 1627	6373 ± 1201	0.0363 S
C_{max}, ng ml^{-1}	734 ± 116	970 ± 161	0.0252 S
T_{max}, h	2.4 ± 0.9	2.1 ± 0.7	0.3939 NS
k_{el}, h^{-1}	0.074 ± 0.008	0.073 ± 0.006	0.3548 NS
$t_{1/2}$, h	9.4 ± 0.99 (10.5)	9.6 ± 0.78 (8.1)	0.2474 NS
C_{max}/AUC, h^{-1}	0.143 ± 0.037 (25.8)	0.156 ± 0.024 (15.3)	0.4284 NS
MRT, h	11.1 ± 1.39	10.33 ± 1.67	0.2742 NS
MAT, h	2.5 ± 1.3 (52.0)	1.58 ± 0.7 (44.3)	0.1956 NS
F, %	72.6 ± 22.9	83.7 ± 15.8	0.0442 S

[a] Mean $\pm$ SD of six dogs.
[b] P-value of the analysis of variance between treatments.
[c] S, significant; NS, not significant; CV, % in parenthesis.

Significant differences were found between the two formulations with regard to AUC_t, AUC_∞, C_{max}, and F parameters. Liquisolid tablets consistently showed higher values of the aforementioned parameters. On the other hand, no significant differences were observed between the formulations regarding k_{el}, $t_{1/2}$, T_{max}, MRT, MAT, and rate of absorption (C_{max}/AUC_∞). It is worth mentioning that the sampling period covered more than 80% of the total AUC for both tablets. The results indicate that the elimination half-life of the drug is similar to that of i.v. bolus as statistical analysis revealed no significant differences between values of the $t_{1/2}$ of the three treatments.

The mean values of the absolute bioavailability of hydrochlorothiazide from the commercial and the liquisolid tablets were found to be 72.6 and 83.7%, respectively and significantly different. This finding reflects an increase in the bioavailability of the drug by $\sim$15% when formulated as liquisolid tablets. The C_{max} value achieved following the administration of the liquisolid tablets was $\sim$32% higher than that of the commercial tablets.

Although the time to maximum plasma concentration (T_{max}) for liquisolid tablets is shorter than that for commercial tablets, this difference was found to be statistically not significant. As the principal site for hydrochlorothiazide absorption is in the proximal part of the intestine, T_{max} may be expected to be not affected by changing the formulation (Reppas et al., 1998). The mean absorption time for the liquisolid tablets was 1.58 h, which was 55 min shorter than the commercial tablets (2.5 h). Although this difference was not statistically significant, this may reflect the rapid absorption of the liquisolid tablets.

The CV % values for (C_{max}/AUC), and MAT were markedly lower in case of liquisolid tablets. This may indicate less inter-dog variations in these parameters in case of liquisolid tablets.

The parametric 90% confidence intervals of the mean values of AUC_t, AUC_∞, and C_{max} were 107.5–132.2, 102.8–131.7, and 110.7–153.7%, respectively. These intervals are, generally, higher than the expected interval range for bioequivalency (80–125%, using log–transformed data). This should be expected as liquisolid tablets showed greater extent of absorption than the commercial tablets.

In conclusion, hydrochlorothiazide liquisolid tablets showed significantly greater extent of absorption than the commercial tablets. Therefore,

this formulation of the drug has the potential to be considered for human study in order to be manufactured on a large scale.

Acknowledgements

The authors gratefully acknowledge the technical assistance of Dr B. Al-Hadia.

References

Barrhaiya, R.H., Philips, T.A., Welling, P.G., 1981. High-pressure liquid chromatographic determination of chlorothiazide and hydrochlorothiazide in plasma and urine: preliminary results of clinical studies. J. Pharm. Sci. 70, 291–295.

Gibaldi, M., Perrier, D., 1982. Pharmacokinetics. Dekker, New York.

Khaled, K.A., 1998. Formulation and evaluation of hydrochlorothiazide liquisolid tablets. Saudi Pharm. J. 6, 39–46.

Liao, C.C., Jarowski, C.I., 1984. Dissolution rates of corticoid solutions dispersed on silicas. J. Pharm. Sci. 73, 401–403.

Reppas, C., Lacey, L., Keene, O., Macheras, P., Bye, A., 1995. Evaluation of different metrics as indirect measures of rate of drug absorption from extended release dosage forms at steady state. Pharm. Res. 12, 103–107.

Reppas, C., Eleftheriou, G., Macheras, P., Symillides, M., Dressman, J.B., 1998. Effect of elevated viscosity in the upper gastrointestinal tract on drug absorption in dogs. Eur. J. Pharm. Sci. 6, 131–139.

Shah, A.S., Needham, T.E., 1979. Correlation of urinary excretion with in vitro dissolution using several dissolution methods for hydrochlorothiazide formulations. J. Pharm. Sci. 68, 1486–1490.

Sietsema, W.K., 1989. The absolute oral bioavailability of selected drugs. Int. J. Clin. Pharmcol. Ther. Toxicol. 27, 179–211.

Spireas, S.S., Jarowski, C.I, Rohera, B.D., 1992. Powdered solution technology: principles and mechanisms. Pharm. Res. 9, 1351–1358.

Spireas, S., 1995. Bioavailability improvement of clofibrate using liquisolid compact technology, APHA Annual Meeting, pp. 142–161.