Research Article

Relative Bioavailability of Chlorothiazide from Mucoadhesive Compacts in Pigs

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Abstract. The relative bioavailability of chlorothiazide from mucoadhesive polymeric compacts is compared to commercial oral suspension in pigs. A single-dose randomized study was conducted in 12 healthy pigs that are 9–10 weeks old. After overnight fasting, pigs were divided into two groups of six animals. To the first group, a reference product containing 50 mg of chlorothiazide suspension, and in the second group, test product (mucoadhesive compacts) chlorothiazide (50 mg) was administered with 75 mL of water via gastric tubes. Blood samples were collected between 0 to 24 h using catheters inserted into the jugular vein. Plasma was separated by protein precipitation, and chlorothiazide concentrations were determined using a high-performance liquid chromatography method. The mean T_{max} and the C_{max} of chlorothiazide following the administration of oral suspension and mucoadhesive compacts were 0.58 ± 0.20 h and 682.97 ± 415.69 ng/mL and 2.17 ± 0.98 h and 99.42 ± 124.08 ng/mL, respectively. The K_{el} and $T_{1/2}$ of chlorothiazide were found to be 1.06 ± 0.28 h^{-1} and 0.70 ± 0.21 h from suspension and 0.95 ± 1.11 h^{-1} and 2.05 ± 1.90 h from the compacts, respectively. The T_{max} of mucoadhesive compacts were significantly longer ($p < 0.05$; 2.17 h) than the reference products (0.58 h), whereas the C_{max} of compacts were significantly lower (99 ng/mL) than the reference product (683 ng/mL; $p < 0.05$). The area under the curve (AUC) of compacts accounts only 50.15% (404.32 ± 449.93 ng h/mL) of the reference product's AUC (806.27 ± 395.97 ng h/mL). The relative bioavailability of the compacts was lower than that of the suspension, and this may be due to the narrow window of absorption for chlorothiazide.

KEY WORDS: bioavailability; chlorothiazide; mucoadhesive compacts; pigs.

INTRODUCTION

Oral route of administration is the most convenient route for delivering drugs to systemic circulation. Poor bioavailability of some classes of drugs, however, restricts their administration by this route of administration. Drugs that are unstable in the diverse pH conditions of gastrointestinal (GI) tract or that undergo intestinal and first-pass metabolism show poor bioavailability. Narrow absorption window in the gastrointestinal tract can also contributes for poor oral bioavailability. Narrow absorption window refers to the preferential absorption in the upper part of the gastrointestinal tract, especially in the proximal part of small intestine due to the presence of larger gaps in tight junctions and active transporters. Poor oral bioavailabilities of riboflavin (1,2), levodopa (3), and metformin (4,5) have been attributed to their narrow absorption window. Although the duodenum and jejunum are ideal sites for the absorption of these drugs, the shorter residence in these regions limit their absorption. Chlorothiazide, a thiazide derivative, is a non-mercurial diuretic and is used in the treatment of hypertension, congestive heart failure, and other edematous conditions for about 30 years. Although it is safe and effective on oral administration, absorption of chlorothiazide from the gastrointestinal tract is considered to be incomplete and variable (6,7). This could be due to its site specific absorption from a limited segment of the upper gastrointestinal tract. For such drugs, enhancement of gastric residence is considered to be an approach for improving their oral bioavailability (8–11). To achieve consistent gastroretentive results, several formulations have been designed, including altered density systems (12–17), expandable swelling systems (18–20) and bioadhesive systems (21–24). Bioadhesive systems utilize the adhesive properties of some polymers on the mucus linings of various biological tissues for increasing the residence time of delivery devices in a specific location. Such increase in residence time may enhance the bioavailability of drugs (8,22,25).

Pharmacokinetic evaluations in animal models of bioadhesive gastro retentive dosage forms (GRDF) may have different implications when GRDFs input drug continuously for prolonged periods. It could improve the bioavailability of certain drugs, e.g., riboflavin (26). Animal models are valuable for the evaluation of the gastrointestinal absorption of new chemical entities and the performance of novel dosage forms. Pig is considered to be the a suitable nonprimate animal model since it resembles the human situation better

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than any other nonprimate animal species with regard to eating behavior, anatomy, and physiology of the gastrointestinal tract. Pig is currently being used for the evaluation of a range of pharmaceutical dosage forms in bioavailability studies (27). Additionally, pigs are most suitable animals for bioavailability studies for amount and frequency of sampling. Mucoadhesive compacts containing a marker dye (phenylazoaniline, PAA) when administered to pigs showed variable, but prolonged, residence in the stomach over a period of 1–4 h (28). Several biopharmaceutical processes such as disintegration, dissolution, solubility, and pH are related to absorption of the drug in the gastrointestinal tract, and these factors may act concomitantly. Therefore, to study the influence of these factors on drug absorption, an *in vivo* study was conducted in pigs to compare the bioavailability of chlorothiazide from mucoadhesive polymeric compacts with its oral solution.

MATERIALS AND METHODS

Materials

Chlorothiazide (Lot no. 124 K0950) and hydroflumethiazide (Lot no. 122F0832) were obtained from Sigma Chemicals, St. Louis, MO, USA. The reference product, Diuril, containing 50 mg/mL of chlorothiazide (Lot no. P0168, Merck) was purchased from a local pharmacy. All other chemical and reagents were of analytical and/or high-performance liquid chromatography (HPLC) grades. The test product was formulated in the industrial laboratory of University of the Pacific, Stockton, CA, USA. Chlorothiazide compacts that are circular-shaped with 6.35 mm internal diameter, flat surfaced, and weighing 65 mg were prepared. Each compact contained 25 mg of chlorothiazide, 16 mg of polyox, and 24 mg of polyvinylpyrrolidone 90F. Their mean hardness was 4.77 ± 0.32 kp and coated to 3% weight gain with hydroxypropyl methylcellulose before animal use to avoid sticking to the esophagus while administering.

Animals

A single-dose randomized study was conducted in 12 healthy pigs (Yorkshire cross swine) of either sex that are 9–10 weeks old, weighing from 20 to 24 kg. The study was conducted in two phases. The day before each phase of the study, the animals were anesthetized using isoflurane (4%) by inhalation, and the external jugular vein was cannulated with an intravenous catheter for blood sampling. The sampling port of the catheter was placed over the dorsal side of neck and closed for ease of sampling. The animals were placed in individual cages and were carefully observed until regaining consciousness. The study was carried out at the research facilities of Pork Power Farms, Turlock, CA, USA, and the protocol were approved by Institutional Care and Use Committee of University of the Pacific.

Dosing and Sampling

After overnight fasting, the animals were examined for any physical and behavior abnormalities. In the first phase, six animals were administered with the reference product (2 mL/kg, p.o.), and in the second phase, another six animals were administered with the test product containing an equivalent dose of chlorothiazide reference product (two compacts, each containing 25 mg of chlorothiazide). In both the phases, the reference and test products were administered with 75 mL of water via orogastric tubes. Care was taken to prevent the entry of tube into the trachea or puncture the esophagus. Blood samples (5 mL) were collected from jugular vein using disposable syringes at 0 (predosing), 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, and 24 h postdosing. Samples were transferred into citrated centrifugal tubes and centrifuged at 3,500 rpm for 10 min to separate plasma. The plasma samples were stored at $-20°C$ until analysis. Animals had free access to water throughout the study and were fed 3 h postdosing. They were also observed for their normal behavior and/or any possible clinical adverse effects during the study. At the end of 24 h, animals were sacrificed and disposed as per the approved protocol.

HPLC Analysis of Chlorothiazide

Extraction

The drug from plasma was separated by protein precipitating method using perchloric acid. Briefly, to 700 μL of plasma, 100 μL of internal standard solution (hydroflumethiazide, 100 μg/mL) was added and vortexed for 2 min. To this, 50 μL each of perchloric acid (14%, *v/v* aqueous solution) and mobile phase were added to make the volume 1 mL. The dispersion was vortexed for 2 min and centrifuged at 10,000 rpm for 30 min. The supernatant was collected and 50 μL was injected into HPLC. The extraction procedure was carried out at room temperature (25°C). Standard curve solutions were prepared with blank plasma in the range of 30 to 5,000 ng/mL chlorothiazide.

HPLC Analysis

Chlorothiazide was quantified using a modified HPLC method by a Beckman Coulter HPLC system with autosampler connected to a UV detector. A reverse phase C18 column (Zorbax) with 4.8×150 mm and 5 μm dimensions was used as stationary phase and a mixture of acetonitrile (A) and water (B) was used as mobile phase. Samples equivalent to 50 μL were injected into the column using an auto-injector and eluted using gradient mode, where the composition of solvent B (water) was 100% for 0–3 min, 80% between 3 and 10 min, 50% of between 10 and 25 min, and 100% between 25 and 40 min. The samples were eluted at 1 mL/min, and the analytes were monitored for 40 min at 228 nm using a UV detector.

Pharmacokinetic and Statistical Analysis

Plasma concentrations *versus* time profiles were analyzed by noncompartmental pharmacokinetic model using Win-NonLin software. Pharmacokinetic parameters peak height concentration (C_{max}), the time to peak concentration (T_{max}), half-life ($T_{1/2}$), elimination rate constant (K_{el}), and area under the curve (AUC_{0-24} and $AUC_{0-\alpha}$) were calculated. The C_{max} and T_{max} were determined by visual inspection of the individual chlorothiazide profiles. The elimination rate constant (K_{el}) was determined by linear regression of the

Time (h)	Plasma concentration of chlorothiazide (ng/mL)						
	A1	A2	A3	A4	A5	A6	Average±SD
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	939.50	1369.91	476.68	427.08	149.59	668.62	671.90±431.21
1	521.57	635.74	203.50	208.83	216.02	475.16	376.80±190.67
1.5	290.63	299.40	119.80	90.93	142.09	230.46	195.55±90.10
2	160.26	151.23	61.50	45.63	90.09	169.91	113.10±54.13
3	76.99	93.48	56.99	64.72	46.85	120.61	76.60±26.96
4	25.70	11.20	6.40	32.63	12.87	2.05	15.14±11.71
6	0.00	0.00	0.00	0.00	0.00	0.00	0.00
8	0.00	0.00	0.00	0.00	0.00	0.00	0.00
24	0.00	0.00	0.00	0.00	0.00	0.00	0.00

terminal linear portion of the plasma concentration *versus* time curve. The apparent terminal elimination half-life ($T_{1/2}$) was calculated using elimination rate constant values. Area under the plasma concentration *versus* time curve from time 0 to the time of the last quantifiable concentration (AUC_{0-24}) was calculated by trapezoidal integration, and it was extrapolated to infinity to obtain $AUC_{0-\infty}$.

Statistical Analysis

Statistical analysis was carried out using GraphPad Prism. The unpaired *t* test was performed to compare any significance differences between the various parameters of the reference and test groups.

RESULTS

HPLC Analysis

The HPLC method was found to be linear in the concentration range of 30–5,000 ng/mL. Under the elution conditions, the retention times of chlorothiazide and hydroflumethiazide were found to be 13 and 17 min, respectively.

Bioavailability Study

The plasma concentration profile of chlorothiazide and the pharmacokinetic parameters of the reference and test groups are shown in Tables I, II, and III and Fig. 1.

Reference Group

Measurable levels of drug were observed in plasma after administration of oral suspension in all animals within 0.5 h. The peak plasma concentration (C_{max}) was achieved within 0.5 h in all animals, except in animal A5, which showed a C_{max} of 1 h. The mean T_{max} and the C_{max} of chlorothiazide following the administration of oral solutions were 0.58± 0.20 h and 682.97±415.69 ng/mL, respectively. Chlorothiazide concentrations were detected until 4 h in all animals, and no detectable drug was observed in plasma after 6 h. The area under the plasma concentration–time curve (AUC_{0-24} and/or $AUC_{0-\infty}$) was 806.27±395.97 ng h/mL. The elimination rate constant (K_{el}) and elimination half life ($T_{1/2}$) were found to be 1.06±0.28 h^{-1} and 0.70±0.21 h, respectively.

Test Group

A delay of detectable drug concentrations into systemic circulation was observed after administration of chlorothiazide mucoadhesive compacts. All the test group animals (A7 to A12) showed detectable blood drug levels starting from 1 to 1.5 h, showing delayed T_{max}. The time for maximum concentration (C_{max}) varied from 1 to 3 h. The T_{max} and C_{max} of chlorothiazide following the administration of the test group were 2.17±0.98 h and 99.42±124.08 ng/mL, respectively. A greater variability in the blood drug levels was also observed following test product administration. Animals A8 and A10 showed prolonged blood levels for about 24 h,

Time (h)	Plasma concentration of chlorothiazide (ng/mL)						
	A7	A8	A9	A10	A11	A12	Average±SD
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	0.00	23.35	0.00	345.81	0.00	39.87	68.17±136.99
1.5	0.00	30.20	0.00	182.71	14.03	7.53	39.08±71.25
2	21.71	38.02	0.00	119.33	22.55	3.75	34.23±43.93
3	29.61	103.18	55.47	74.57	7.99	0.00	45.14±40.00
4	18.05	58.07	50.50	56.57	0.20	0.00	30.56±27.72
6	0.00	38.22	31.27	42.45	0.00	0.00	18.66±20.75
8	0.00	41.85	0.00	25.13	0.00	0.00	11.16±18.09
24	0.00	4.49	0.00	12.70	0.00	0.00	2.86±5.14

Table III. Comparison of Pharmacokinetic Parameters of the Reference and Test Products Containing Chlorothiazide

Product	T_{max} (h)	$T_{1/2}$ (h)	C_{max} (ng/mL)	K_{el} (h^{-1})	AUC$_{0-t}$ (ng h/mL)	AUC$_{0-\infty}$ (ng h/mL)
Suspension (reference)	0.58±0.20	0.70±0.21	682.97±415.69	1.06±0.28	806.27±395.97	806.27±395.97
Compacts (test)	2.17±0.98	2.05±1.90	99.42±124.08	0.95±1.11	348.02±422.25	404.32±449.93
Test/reference ratio	3.74	2.92	0.15	0.87	0.43	0.50

animals A7, A9, and A11 showed drug levels for about 4 to 6 h, whereas animal A12 did not show any drug in the plasma after 2 h. This animal showed distress after the administration of the test product and observed to spit frequently. The mean area under the plasma concentration–time curve (AUC$_{0-24}$ and AUC$_{0-\infty}$) of this group was 348.02±422.25 and 404.32± 449.93 ng h/mL, respectively. Their mean elimination rate constant (K_{el}) and elimination half life ($T_{1/2}$) were 0.95± 1.11 h^{-1} and 2.05±1.90 h, respectively.

Statistical Analysis

The statistical analysis was carried out by using Graph-Pad Prism software. The unpaired *t* test was used to test the significance between groups. The T_{max} of mucoadhesive compacts were significantly longer ($p<0.05$; 2.17 h) than the reference products (0.58 h), whereas the C_{max} of compacts were significantly lower (99 ng/mL) than the reference product (683 ng/mL; $p<0.05$).

DISCUSSION

A delay in the T_{max} (2.17 from 0.58 h) and decrease in C_{max} (99 from 683 ng/mL) were observed following the administration of mucoadhesive compacts when compared to the reference suspension. Being a nondisintegrating dosage form, the test product (polymeric compacts) was anticipated to show slower dissolution and/or absorption rates in comparison with the reference product (suspension), where the drug is readily available for dissolution. Factors such as initial surface wetting, entry of GI fluids into the polymer matrix, dissolution/diffusion of the drug through the polymer networks, and limited surface area of the compacts could have limited the dissolution and diffusion processes from compacts under *in vivo* conditions. This was also evident from the *in vitro* drug release study of the test and reference

Fig. 1. Plasma concentration and time profile of reference and test products of chlorothiazide

products. About 90% of the drug was released (T_{90}) within 15 min from suspension, whereas the T_{90} value of compacts was about 12 h. This slow and sustained release of compacts is mainly due to the rate controlling property of polyethylene oxide (polyox), which is the mucoadhesive component of the compacts (28). The nature of dosage form and presence of rate controlling mucoadhesive component are therefore considered the probable reasons for delayed T_{max} and lower C_{max} of chlorothiazide in plasma after administering compacts. On the other hand, the elimination rate constants of both formulations were not significantly different as this is the inherent property of chlorothiazide.

The K_{el} values of chlorothiazide compacts (0.95± 1.11 h^{-1}) and reference suspension (1.06±0.28 h^{-1}) found to be comparable. This is anticipated as elimination rate is the inherent property of chlorothiazide and is independent of the dosage form. But the $T_{1/2}$ values were found to be different for reference suspension and compacts. It can be attributed to variable absorption, and release rates form two different formulations.

The sustained release chlorothiazide compacts showed lower oral bioavailability than that of the suspension, which was evident from the lower area under the plasma concentration *versus* time curve (AUC). The AUC of test product accounts only 50.15% (404.32±449.93 ng h/mL) of the reference product's AUC (806.27±395.97 ng h/mL). Higher intersubject variability in gastric residence could be the most probable reason for reduced bioavailability of chlorothiazide from bioadhesive compacts (Table III). This may be due to various reasons. The GI tract is composed of several regions differing in anatomy, biochemical environment, microbial flora, expression of transporters, and absorption characteristics. There are several processes that may occur simultaneously following drug release from a dosage form in the GI tract, including chemical/enzymatic/bacterial degradation, absorption (passive and/or active), precipitation, efflux by P-glycoprotein pump, and metabolism by Cyp450 enzymes. As a consequence, the pharmacokinetic profile of a drug may be influenced by its delivery site (29). An earlier study evaluating the gastric residence of compacts containing a poorly soluble dye, PAA, in pigs showed residues of the compacts in the stomach region of only two animals (out of six animals) during 2 to 4 h postadministration. In the remaining four animals, no parts of the compact were found in any other part of GI tract (28). As these compacts hydrate and forms a gel-like mass and maintain their physical integrity for at least 6–8 h in 0.1 N hydrochloric acid under *in vitro* conditions, dissolution of entire compact matrix was unlikely. Absence of compacts in the GI tract of four animals was therefore correlated with biological reflux actions and evacuation of hydrated compact matrix from stomach to mouth and the resultant chewing or spitting actions. If a similar trend in gastroretention of compacts is repeated (with a success rate of
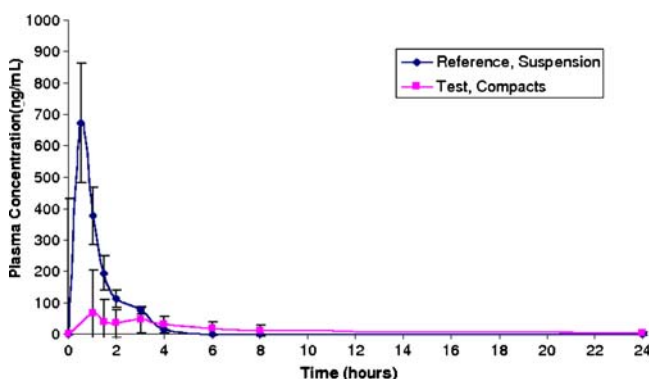
only 33%), it is probable that at least two out of six animals used in bioavailability studies should show comparable or improved bioavailability than oral suspension. It was interesting to note that the mean AUC of two test group animals A8 and A10 (947.24 ng h/mL) was about 17% higher than the mean of reference group animals (806.27 ng h/mL). Based on this observation, it was assumed that the reduced bioavailability of chlorothiazide from the test compacts is due to its variable gastric residence time.

CONCLUSION

The relative bioavailability of chlorothiazide from mucoadhesive polymeric compact designed to improve the gastric retention of chlorothiazide is compared to its oral suspension. The results demonstrated sustained release of chlorothiazide from polymeric compacts in pigs. The relative bioavailability of chlorothiazide is lower than its oral suspension. Large variations in the gastric residence in pigs and the reported narrow absorption window of chlorothiazide seemed to result in the reduced bioavailability from mucosal compacts.

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