The Bioavailability and Pharmacokinetics of Mefenamic Acid in Alloxan-diabetic Rabbits

Shadab Qamar*, Nadeem Irfan**, Mahmood Ahmad**, Muhammad Jamshaid*** and Naeem A. Muzafrar***

*Pakistan Council for Scientific & Industrial Research (PCSIR) Laboratories, Lahore, Pakistan.
**Department of Pharmacy, Islamia University, Bahawalpur, Pakistan.
***Faculty of Pharmacy, Punjab University, Lahore, Pakistan.

(Received July 14, 1997; Accepted September 9, 1997)

The bioavailability and pharmacokinetics of mefenamic acid was studied in alloxan-diabetic rabbits. Mefenamic acid in plasma was assayed by high performance liquid chromatography. A paired t-test for normal and alloxan treated rabbits revealed a significant decrease in all the bioavailability and disposition kinetic parameters of mefenamic acid during diabetes was observed in the present study. The altered bioavailability and disposition of mefenamic acid in the diabetic state will require adjustment of the dosage regimen prescribed for diabetes in a clinical setting.

[Key words: Mefenamic acid, Pharmacokinetics, Alloxan diabetes, Bioavailability]

INTRODUCTION

Diabetes is a disorder characterized by excessive urine excretion. Diabetes mellitus, a chronic syndrome of impaired carbohydrate, protein, and fat metabolism, is secondary to an insufficient secretion of insulin. Alloxan diabetes, on the other hand, refers to animal models in which administration of alloxan leads to destruction of the beta cells of the pancreatic islets, resulting in hyperglycemia and ketoacidosis. In addition, Alloxan diabetes of varying degrees of severity is accompanied by increased cholesterol and triglyceride levels (1). The triglyceride levels are increased significantly in alloxan treated animals as compared to controls (2). Nawaz and co-workers (3) investigated the disposition kinetics and urinary excretion of sulphadimidine in normal and alloxan treated dogs and found significant differences between the two groups. Iqbal and others (4) determined the biodisposition kinetics of sulphadiazine in normal and metabolically altered rabbits and reported highly significant differences. Ahmad et al. (5) have studied the effect of experimentally induced diabetes on the pharmacokinetics and bioavailability of erythromycin in rabbits. All of these studies suggest that the diabetic state induced by alloxan influences the bioavailability and disposition kinetics of drugs. The current study was, therefore, designed to estimate the bioavailability and disposition kinetics of mefenamic acid, a commonly used anti-inflammatory agent, in alloxan-treated rabbits.

EXPERIMENTAL

Animals

A total of 9 healthy rabbits of both sexes were maintained under uniform conditions. The body weight of the animals ranged from 1.2-2.4 kgs. The animals were fed fresh green fodder and black gram in the morning and evening; water was provided ad-libitum.

Drug Administration

Mefenamic acid was administered as a single oral dose of 50 mg/kg body weight [PONSTAN suspension, 50 mg/5 ml, Park Davis & Co. Ltd., Karachi Pakistan]. The preparation was administered by oral intubation before and after the induction of diabetes.

Mahmood Ahmad, Assoc. Professor, Department of Pharmacy, Islamia University, Bahawalpur, Pakistan.
Induction of Diabetes

After the determination of bioavailability and disposition kinetics in normal controls, a washout period of ten days was allowed before the induction of diabetes. A diabetic condition was produced by injecting a 1% solution of alloxan (in sterilized water) at the dosage level of 150 mg/kg body weight to each rabbit. After the alloxan injections, the rabbits were monitored daily until glucose levels exceeded 300 mg/dl, when the animals were considered hyperglycemic. At this time, the diabetic animals were used for experimentation.

Sampling Procedure

For the collection of blood samples, the area around the left and right jugular veins were shaved and swabbed with ethyl alcohol. The blood samples (2 ml) were drawn from the jugular vain of the rabbits, in heparinized glass centrifuge tubes. The blood samples were collected just before, and at 0.5, 1.0, 1.5, 2.5, 4.0, 6.0, 8.0 and 12.0 hours after administration of mfenamic acid. After recording blood pH with a pH meter (Hanna instruments, H 18214), and blood glucose by the method of Barham and Trinder (6), the blood samples were centrifuged at 3000 to 4000 rpm for 10 minutes. Plasma was separated and used for the analysis of mfenamic acid.

Analytical Technique

Plasma concentrations of mfenamic acid were determined by a modification of the method of Hind and Underwood (7). To 0.5 ml of plasma in a 10 ml test tube was added 0.5 ml of methanol solution containing ibuprofen (5µg/ml), to serve as an internal standard. After acidification with 0.25 ml of 1 N HCl, 3 ml of diethylether were added, the mixture shaken gently for 3 minutes, then centrifuged at 2000 rpm for 5 minutes. The top organic layer was transferred to conical glass tubes by pasteur pipette. The organic-ether layer was evaporated to dryness at 37°C under a stream of nitrogen. The residue was dissolved in 1 ml of an HPLC mobile phase containing 2% dimethylsulphoxide. an aliquot of 20 µl was injected into the HPLC system (Shimadzu, Japan) by means of a graduated microsyringe (Hamilton, USA) after filtration through a membrane filter of 0.45 µm pore size (Milipore, HVLP 01300). The HPLC system consisted of an injection valve (Rheodyne, USA) fitted with an injection loop of 20 µl capacity, a multi wave length detector (UV-SPD-6AV, Shimadzu, Japan) with an absorbance of 0.001-2.56 aufs, and a solvent metering pump (LC-9A, Shimadzu, Japan) working at about 200 kgf/cm² pressure. Chromatographic separation was achieved on a reverse phase ODS-C18 column 15 cm x 4.6 mm (Shimadzu, Japan) fitted in a column oven (CTO-6A, Shimadzu, Japan) to keep the column at ambient temperature.

A degassed mixture of acetonirile and 0.05M phosphate buffer (35:65 v/v) at pH 7.5 was used as the eluting solvent. The eluate was pumped through the analytical column at a

![Fig. 1 Mefenamic acid plasma concentrations after a single oral dose of 50 mg (n=9).](image-url)
flow rate of 1.0 ml/minute and monitored at a 225nm wavelength with a full scale deflection of 0.02 aufs. Data was recorded on a CR 4A chromatopac (Shimadzu, Japan), run at a chart speed of 10mm/min. To control the detector pumps and column, an oven system controller (SCL 6B, Shimadzu, Japan) was used.

The quantitation of mefenamic acid was achieved by comparing the peak height ratios of mefenamic acid/ibuprofen in plasma to those prepared by spiking blank plasma samples with various concentrations of mefenamic acid (0.5–0.8 μg/ml) and 5 μg/ml ibuprofen.

Data Analysis

To analyze the disposition kinetic parameters of mefenamic acid, a one-compartment model of analysis, employing a PK II (8) computer software program for calculation of pharmacokinetic parameters, was used.

Statistical Analysis

The bioavailability and pharmacokinetic parameters obtained from normal and diabetic rabbits were subjected to a paired t-test to evaluate the differences between the normal and metabolic altered condition. Mean values and standard error of the mean were calculated for each parameter using a program of SPSS for windows 6 (9). A value of P<0.05 was considered to be statistically significant.

RESULTS AND DISCUSSIONS

Mean plasma concentration versus time, after a single oral dose of mefenamic acid (50 mg/kg) in normal and diabetic rabbits, is presented graphically in fig. 1. The maximum plasma concentrations of 3.323 ±0.061 μg/ml and 2.39±0.057 μg/ml was attained in normal and diabetic rabbits, respectively, 2.5 hours after treatment. From the mean plasma concentration data, a highly significant (P<0.01) decrease was observed in the diabetic animals. The lower values in the diabetic binding of the drug. Other reasons for the low plasma concentration may be a reduced absorption of the drug from the gastrointestinal tract, or a reduction in blood pH might have favored passage of the drug across the biomembranes. Similar results were reported by Iqbal et al. (4).

The concentration data of mefenamic acid in both normal and diabetic animals generated biphasic curves and, according to the Akaike Information Criterion (AIC) values (10), the one-compartment open model best fits the data in each case. The bioavailability and disposition kinetic parameters of mefenamic acid are furnished in table 1. It is clear from the table that significant (P<0.01) decreases in the areas under the plasma concentration time curve (AUC) and under the first moment curve (AUMC) occurred the diabetic rabbits. As dia-

| Table 1 Bioavailability and disposition kinetics of mefenamic acid in normal and alloxan diabetic rabbits (50 mg/kg body weight) |
| Pharmacokinetic Parameters | Cmax μg/ml | tmaxhours | AUC0-∞ μg.hr/ml | AUMC0-∞ μg.hr2/ml | MRT hours | t1/2abs hours | t1/2elim hours | Kaabs hr-1 | Kelmhr hr-1 | VdL | Cl ml/hr/kg | Relative bioavailability |
| Rabbits | Normal | Diabetic | Normal | Diabetic | Normal | Diabetic | Normal | Diabetic | Normal | Normal | Normal | |
| Statistical Significance | | | | | | | | | | | | | |
| | | | | | | | | | | | | |
| Normal | 3.316±0.063 | 2.500±0.000 | 14.808±0.295 | 61.957±1.512 | 4.183±0.049 | 0.774±0.041 | 1.945±0.069 | 0.912±0.045 | 0.359±0.013 | 14.093±0.634 | 83.777±2.446 | 100% |
| Diabetic | 2.39±0.058 | 2.500±0.000 | 10.055±0.098 | 37.994±0.559 | 3.798±0.033 | 0.977±0.043 | 1.475±0.063 | 0.758±0.055 | 0.475±0.018 | 15.586±0.668 | 122.64±2.38 | 67% |
| Statistical Significance | ns | ns | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | |
| ns=not significant | *=statistically significant at P<0.05 | **=statistically significant at P<0.01 |
betes may reduce the absorption of mefenamic acid into the general circulation, there is not surprising a lower value i.e., 10.03±0.098 µg.hr/ml of AUC as compared to 14.89±0.285 µg.hr/ml in the normal animals. Similarly, a significant (P<0.01) decrease in mean residence time (MRT) was observed in the diabetic rabbits. The mean residence time provides a quantitative estimation of the time of persistence of the drug in the body. The values of MRT were 4.183±0.049 hours and 3.762±0.033 hours in the normal and diabetic rabbits, respectively. The mean ± SEM value for the absorption half-life was 0.774±0.041 hours and 1.028±0.043 hours in the normal and diabetic animals, showing a statistically significant (P<0.05) difference. A higher absorption half-life value in the diabetic animals further suggests a slower absorption of the drug. The overall absorption rate constant of mefenamic acid was decreased significantly (P<0.05), while the value for the elimination rate constant was increased significantly (P<0.01) in the diabetic animals. This resulted in a shorter elimination half-life of 1.412±0.063 hours compared with 1.856±0.069 hours in diabetic vs. normal animals. Similar results have also been described by Watkins and co-workers (11).

A significant (P<0.01) increase in total body clearance of mefenamic was observed in the alloxan treated rabbits compared with normal controls, i.e., 123.08±2.38 ml/min/kg to 83.527±2.44 ml/min/kg. The possible cause of this increase may be due to excessive urination by the diabetic animals. These results are similar to observations made by Nawaz et al. (3). In the alloxan treated and metabolically altered rabbits, a decrease in pH is favorable for conjugation of the drug. The conjugated drug can easily cross the biomembranes of drug-eliminating organs. Assuming non-significant alteration by the urinary pH, the higher flow of urine prevented any possible back diffusion and caused greater elimination in total body clearance. The volume of distribution (Vd) of mefenamic acid following oral administration was significantly increased (P<0.05; 14.093±0.634 in the diabetic rabbits vs. 15.05±0.668 liters in the controls). This higher value of Vd in the diabetic rabbits suggests better tissue penetration of mefenamic acid. This effect of diabetes on the increased volume of distribution was also reported by Iqbal et al. (4) and Ahmed et al. (5). It has been shown by the present results that diabetes has distinct effects on the disposition kinetics of mefenamic acid in rabbits. Considering the AUC of the normal rabbits as 100%, the relative bioavailability of mefenamic acid in the diabetic rabbits was 67%. Hence, the current body of data reveals that the metabolic modifications caused by diabetes distinct affects the bioavailability and disposition of mefenamic acid. In turn, these data demonstrate the necessity for regulating the dosage regimen to be prescribed to patients under clinical conditions.

REFERENCES
9) SPSS (computer program) version 6, MS-Windows© SPSS Inc. USA. 1993.