DISPOSITION KINETICS OF ERYTHROMYCIN AFTER INTRAVENOUS ADMINISTRATION IN NORMAL AND ALLOXAN DIABETIC RABBITS

Mahmood Ahmad, R. Nawaz[1] and Muhammad Nawaz[2]
Department of Pharmacy, Islamia University, Bahawalpur,
[1]Department of Chemistry [2]Department of Physiology & Pharmacology,
University of Agriculture, Faisalabad-38040, Pakistan

ABSTRACT

The disposition kinetics was determined after intravenous injection of erythromycin 10 mg/kg b.wt dose in normal rabbits. These rabbits were treated with alloxan 150 mg/kg b.wt intravenously to develop diabetic condition. The body weight and blood pH remained unaltered while significant ($P<0.05$) decrease in packed cell volume, and a highly significant ($P<0.01$) increase in plasma glucose, total lipids, total proteins, albumin and globulin was recorded in alloxan treated diabetic rabbits. The disposition kinetics of erythromycin in alloxan diabetic condition showed a significant ($P<0.05$) increase in the area under curve (AUC total), elimination half-life ($t_{1/2}$), mean residence time (MRT) and a decrease in elimination rate constant (k-terminal) and total body clearance (Cl). An altered steady state of erythromycin in alloxan treated diabetic condition apprise of the need for an adjustment of dosage regimen and proposes similar studies in the "real" clinical condition.

INTRODUCTION

Metabolic change in diabetes are manifested by hyperglycemia, glucosuria, polyuria, lipaemia, ketonaemia, acidemia, polydipsia and polyphagia. These changes in the biochemical *milieu interieur* are likely to influence the biodisposition and fate of drugs which are either weak acids or weak bases. Studies on alloxan diabetic dogs revealed significant changes in haematochemical values and disposition kinetic parameters of sulphadimidine indicating a need for an adjustment of dosage regimen under alloxan diabetic condition in dogs (Nawaz *et al.*, 1982). The disposition kinetics of various drugs in metabolic disorders like diabetes have not been sufficiently investigated. Erythromycin is one of the extensively used broad spectrum macrolide antibiotic of weakly basic nature. The biodisposition of erythromycin is likely to be affected in altered biochemical environments of the body during diabetes. The study therefore, was conducted to determine the disposition kinetics of intravenously injected erythromycin in normal and alloxan diabetic rabbits.

MATERIALS AND METHODS

The disposition kinetics of intravenously injected erythromycin was investigated in normal and alloxan diabetic rabbits.

Animals

Experiments were performed on 12 normal, adult male rabbit of local land-race breed. Before experiments all animals were maintained under similar management for minimum 10 days and were conditioned to the experimental procedures. The rabbits were fed green fodder in morning and afternoon and fresh water was provided all the times in the cages. As a routine clinical examination of the animals was also conducted.

Experimental

The normal rabbits were used for the study of haematochemical and biodisposition kinetic parameters. For haematochemical studies, a blood sample was drawn from the jugular vein of each animal for determination of packed cell volume, blood pH, plasma glucose, total lipids, total proteins and albumin.

Each animal was injected through the marginal ear vein, (Erythromycin lactobionate injectable, batch No. 04-6570-XV, Abbot Laboratories (Pvt. Ltd.) Pakistan as a single dose 10 mg/kg b. wt. Blood samples were collected from vena jugularis at 5, 15, 30, 45, 60, 120, 300, 480, and 20 minutes after drug administration. Blood was collected in heparinized centrifuge tube. Plasma was separated and used fresh for analysis.

After determination of disposition kinetics in normal rabbits, animals were maintained for a wash out period of 10 days for complete elimination of the drug. After 10 days each rabbit was injected 1 percent solution of

alloxan (in sterilized distilled water) at a dosage rate of 150 mg/kg b. wt. in the marginal ear vein. Following alloxan injection, blood glucose of each rabbit was measured daily until the level exceeded 300 mg/dL when the animal was considered hyperglycemic. The blood samples were drawn for the estimation of packed cell volume, blood pH, plasma glucose, total lipids, total proteins and albumin.

The alloxan treated hyperglycemic rabbits showed a non-significant reduction in their body weights and showed clinical signs of polyuria, polydipsia and general weakness. These diabetic rabbits were then used for determination of the disposition kinetics of erythromycin following intravenous drug administration. Procedure for the study of disposition kinetics in the alloxan treated diabetic condition was similar as described above for the normal rabbits.

Analysis

Packed cell volume was determined after centrifugation of fresh heparinized blood in capillary tubes for 15 minutes at 3000 rpm and the values were read on the hematocrit scale (Benjamin 1978). Blood pH was measured with the help of a pH meter (Hanna instruments, H 18214) at 37°C. Plasma glucose was measured by GOD/PAP reaction (Barham and Trinder, 1972) involving sulfophosphovanillin reaction. Plasma levels of total lipids was determined by E. Mercks kit method of Zollner and Kirsch (1962). Total proteins were estimated by biuret reaction (Gornall *et al.*, 1949), albumin with bromocresolgreen to form a complex (Doumas *et al.*, 1971) and globulin by subtracting the concentration of albumin from the concentration of total proteins.

The concentration of erythromycin in plasma was determined by the microbiological assay (Arret *et al.*, 1971), using *Sarcina lutea* as test organism obtained from the Pakistan Council of Scientific and Industrial Research Laboratories, Lahore, Pakistan. Plasma levels of erythromycin in normal and metabolically altered rabbits were used to compute disposition kinetics parameters using the non-compartmental model of analysis (Gibaldi, 1984). Mean values and their standard error (SE) were calculated for each parameter and the differences between the two conditions were ascertained by Students t-test using a computer programme M-Stat (Nissen, 1985).

RESULTS

Body weight, Packed Cell Volume and Haematochemical Parameters

Body weights, packed cell volume and haematological parameters in normal and alloxan diabetic condition of rabbits have been compared statistically in Table 1. A significantly ($P<0.05$) higher packed cell volume was recorded in the normal than in the alloxan diabetic rabbits. In addition, plasma glucose, total lipids, total proteins, albumin and globulin revealed significantly ($P<0.01$, <0.05) lower levels in the normal than in the alloxan diabetic rabbits.

Disposition Kinetics

The mean values for the plasma concentration of erythromycin on a semilogarithmic scale against time for the normal and alloxan diabetic rabbits are shown in Fig. 1. The concentration of erythromycin in plasma of normal rabbits was significantly ($P<0.01$, <0.05) higher from 5 minutes to 60 minutes followed by significantly ($P<0.01$, <0.05) lower from 120 to 720 minutes when compared with the alloxan treated diabetic condition of rabbits.

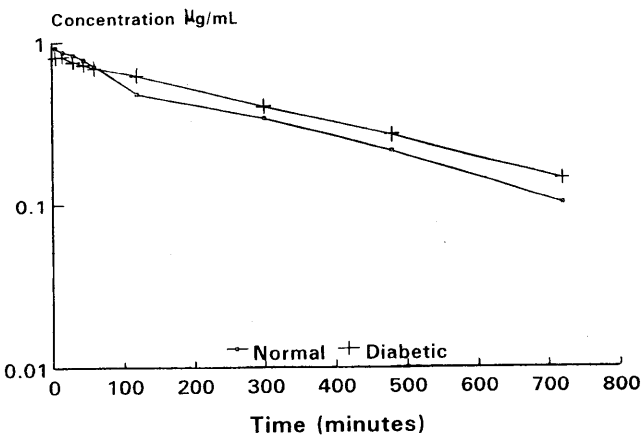

Fig. 1: Mean (n=12) plasma concentration after intravenous dose of erythromycin 10 mg/kg b.wt in rabbits.

Mean values for the disposition kinetic parameters of intravenously administered erythromycin in normal and diabetic condition of rabbits have been presented in Table 2. Statistical analysis revealed significantly ($P<0.05$) lower values for the total area under concentration versus time curve and total area under the first moment curve in normal rabbits. The elimination ($P<0.05$) higher in normal rabbits while biological constant and total body clearance were significantly rate half-life and mean residence time were significantly ($P<0.05$) higher in alloxan diabetic animals. Apparent volume of distribution and apparent volume at steady state were statistically similar in both the normal and alloxan diabetic conditions.

Table 1: Mean ± SE (n = 12) of body weight, packed cell volume and haematochemical parameters in normal and alloxan diabetic rabbits

Parameters	Units	Normal	Diabetic
Body weight	kg	1.71±0.11	1.64±0.07ns
Packed cell volume	%	34.58±1.05	31.41±1.11*
Blood pH		7.62±0.05	7.51±0.04ns
Glucose	mg/dL	115.00±2.32	368.00±9.05**
Total lipids	mg/dL	205.00±30.83	580.00±21.06**
Total protein	mg/dL	5.54±0.56	7.69±0.48**
Albumin	g/dL	3.68±0.27	4.57±0.10**
Globulin	g/dL	1.86±0.30	3.12±0.41*
A/G Ratio	g/dL	2.44±0.29	2.03±0.45NS

NS = non-significant difference * = significant difference ($P<0.05$), ** = significant difference ($P<0.01$)

Table 2: Mean ± SE (N = 12) of disposition kinetics of erythromycin following intravenous administration of 10 mg/kg dose in normal and alloxan diabetic rabbits

Parameters	Units	Normal	Diabetic
AUC total	mcg.h/ml/kg	4.57±0.26	5.63±0.32*
AUMC total	mcg.h^2/ml/kg	26.29±3.08	41.41±5.03*
K-terminal	hr^{-1}	0.190±0.013	0.148±0.010*
$t_{1/2}$	hr	3.81±0.25	4.93±0.34*
MRT	hr	5.53±0.36	7.06±0.48*
Cl	ml/h/kg	2.27±0.14	1.84±0.11*
Vd	L/kg	11.98±0.19	12.60±0.25NS
Vss	L/kg	12.05±0.21	12.52±0.23NS

NS = non-significant difference, *significant difference ($P<0.05$)

DISCUSSION

Clinically, diabetes mellitus is characterized by polyuria, glycosuria, hyperglycemia and a prolonged glucose tolerance test (GTT). The cause of diabetes mellitus is an absolute or relative lack of insulin, but other factors such as pancreatitis, trauma, stress, neurogenic changes, obesity, infection, hereditary and hormonal changes may lead to diabetes. Alloxan is a well known hyperglycemic agent (Lehninger, 1975), therefore, in the present study, diabetes was produced by administering alloxan to rabbits.

Body Weight, Packed Cells Volume and Hematochemical Parameters

A slight decrease (non-significant) in body weight of diabetic rabbits noted in the present study was similar to the findings in dogs showing a non-significant reduction in the body weight of alloxan treated animals (Nawaz *et al.*, 1982). The alloxan treated rabbits showed a significant ($P<0.05$) decrease in packed cell volume as compared with untreated rabbits.

The mean value for the blood glucose in normal rabbits was 115 mg/dL which increased three fold showing a mean value 368 mg/dL following treatment of alloxan. After alloxan induced diabetic condition, a four fold and a three fold increase in blood glucose levels has been reported in dogs (Nawaz *et al.*, 1982) and rabbits (Iqbal *et al.*, 1989).

A highly significant ($P<0.01$) increase in plasma lipids in diabetic rabbits may be attributed to an excessive mobilization of lipids. Reversal of triglyceride storage in fatty tissues, lipolysis and additional fat mobilization are responsible for the rise in plasma lipid contents (Guyton, 1971). In severe diabetic states excessive mobilization of body fat depots may result in the appearance of neutral fat in circulation, and frank lipaemia may occur (Kaneko and Cornelius, 1971). A similar increase in triglyceride level has been recorded in diabetic dogs (Nawaz *et al.*, 1982) and rabbits (Iqbal

et al., 1989). Total proteins, albumin and globulin showed a significant ($P<0.01$, <0.05) increase in plasma levels of alloxan treated rabbits. The difference in plasma concentration of proteins before and after alloxan treatment may be due to an impairment of protein metabolism in liver (Mohan-Chari *et al.*, 1980).

Disposition Kinetics

Plasma concentration of intravenously administered erythromycin in rabbits revealed lower values in diabetic state from 5 minutes to 60 minutes and higher values from 120 to 720 minutes indicating a shift in the steady state after the establishment of disposition equilibrium.

Increased levels of plasma proteins in diabetic rabbits leads to increased protein binding reducing the availability of free microbiologically active drug. The percentage of erythromycin binding depends upon the concentration of α-1-acid glyco-protein in plasma (Prandota *et al.*, 1980). Using sulphadiazine, Iqbal *et al.* (1989) reported a decrease in the plasma levels of the drug after alloxan induced diabetic condition in rabbits.

Total area under plasma erythromycin concentration versus time cure and total area under first moment curve were significantly ($P<0.005$) lower in normal than in alloxan diabetic condition. The mean value for the biological half-life and mean residence time of erythromycin in diabetic rabbits was significantly ($P<0.05$) longer than in normal rabbits. In clinical terms, reabsorption of a drug by non-ionic diffusion depends on the pH of urine and pKa of the drug and indicates that weak acids are excreted more rapidly in alkaline urine and weak bases in acid urine (Reindenberg, 1971). In diabetes, acidosis is fundamentally related to ketonaemia ultimately making the urine basic. Thus, it may be suggested that the reabsorption or back diffusion of weakly basic unionized erythromycin from the renal tubules might be responsible for the longer half-life and mean residence time in alloxan treated diabetic condition.

The rate of drug elimination relative to the amount of drug in the body is called the fractional rate of drug elimination, or elimination rate constant and its value depends upon the values of both clearance and volume of distribution and vice versa. In the present study, the value for elimination rate constant was significantly ($P<0.05$) lower in diabetic condition. A significantly ($P<0.05$) lower total body clearance in alloxan treated animals may be due to the lower value of K-terminal.

The present study show that the metabolic alteration of the alloxan induced diabetes after intravenous administration of erythromycin influenced the disposition kinetics which apprise of the need for an adjustment of dosage regimen under such conditions. Clinical implications of this study await verification in "real" diabetic condition.

REFERENCES

Arret, B. P., Diana, Johnson and A. Kirshbaum, 1971. Outlines of details of microbiological assay of antibiotics. 2nd Revision. J. Pharm. Sci., 660: 1689-1694.

Barham, D. and P. Trinder, 1972. Kit method for the analysis of plasma glucose. Analyst. 97: 142.

Benjamin, M., 1978. Outline of Veterinary Clinical Pathology. 3rd Ed. The IOWA Stat University Press, Ames. IOWA, USA. pp. 52.

Doumas, B. T., W. A. Watson and H. G. Briggs, 1971. Kit method for the analysis of albumin in plasma. Clin. Chem. Acta., 31: 87.

Gibaldi, M., 1984. Biopharmaceutics and Clinical Disposition Kinetics. 3rd Ed. Lee and Febiger. Philadelphia.

Gornall, A. G., C. J. Bordawill and M. M. David, 1949. Determination of serum proteins by means of biuret reaction. J. Biol. Chem. 177: 751-766.

Guyton, A. C., 1971. A Textbook of Medical Physiology. 4th ed., W. B. Saunders and Co. Philadelphia, pp: 915-928.

Iqbal, T., R. Nawaz, A. Illahi and M. Nawaz, 1989. Disposition kinetics of sulphadiazine in normal and diabetic rabbits. J. Pak. Med. Assoc., 39: 50-53.

Kaneko, J. J. and C. E. Cornelius, 1971. Clinical Biochemistry of Domestic Animals. 2nd Ed/Vol. 1. Academic Press Inc. III. Fifth Avenue, New York, 10003, pp. 99.

Lehninger, A. L., 1975. Biochemistry 2nd Ed. Diabetes Mellitus, Worth Publ. New York. pp. 847-849.

Mohan-Chari, V. Neeraja, K. Inelira and K. B. Sawami, 1980. Alloxan induced. Metabolic changes in liver. Curr. Sci., 49: 699-701.

Nawaz, M., S. Akhtar and A. S. Hashmi, 1982. Disposition Kinetics and urinary excretion of sulphadiazine in normal and alloxan diabetic dogs. Acta. Pharmacol. et. Toxicol., 51: 63-68.

Nissen, O., Mstat Version 3.00/Em. Copyrighted June 1985, Michigan State University. Revised 9/1/95 by Deptt. of Crop and Soil Sci. and Deptt. of Agri. Economics.

Prandota, J., J. P. Tillement, P. D. Athis, H. Campos and J. Barre, 1980. Binding of erythromycin base to human plasma proteins. J. Int. Med. Res., 8: 11-18.

Reindenberg, M. M., 1971. Renal function and Drug Action, 1st Ed., W. B. Saunders and Co., Philadelphia.

Zollner, N. and K. Kirsch, 1962. Kit method for the determination of total lipids in plasma. Ges. Exp. Med., 135: 545.