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Pharmacokinetics of chloroquine in diabetic rabbits

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Abstract

The pharmacokinetic parameters derived from diabetic rabbits have been compared to those of normal rabbits. Two sets of rabbits were used, normal rabbits and diabetic rabbits. The diabetic rabbits were obtained by inducing diabetes in rabbits using streptozotocin. Chloroquine at a dose of 10 mg/kg was administered to this set of rabbits by oral administration. Blood samples were obtained from the rabbits at suitable time intervals and they were analyzed for chloroquine concentrations. From the plasma-concentration profile, the pharmacokinetic parameters such as the half-life of absorption, the absorption rate constant (k_a), the half-life of elimination ($t½_{el}$) and elimination rate constant (k_{el}), the time lag, (T_{lag}) area under the curve AUC, the volume of distribution (V_d), and the plasma clearance (P_{cl}) were determined and compared. It was found that some of the pharmacokinetic parameters such as volume of distribution (V_d), plasma clearance (P_{cl}) and area under the curve AUC differ significantly ($p \geq 0.05$) in the diabetic rabbits compared to normal rabbits. This is an indication that chloroquine is less metabolized in the diabetic rabbits than in the normal rabbits, therefore indicating impairment of metabolism in the diabetic rabbits.

Keywords: *Pharmacokinetics, Chloroquine, Rabbits, Diabetes*

Introduction

Chloroquine has been a drug of choice in the treatment of malaria for several years since its introduction into therapeutics (Loeb *et al*., 1946). The drug which is used in the treatment of malaria in tropical and sub-tropical region can be administered in malaria cases alone or administered to cure malaria when other diseases are involved. The effect of protein energy malnutrition on the levels of chloroquine in the body has been reported (Adelusi and Salako, 1982). The urinary levels of chloroquine in relation to dietary protein (Adelusi, 1982) and the effect of fasting on the pharmacokinetics of chloroquine (Adelusi, 1982) have also been studied. It is possible to administer this drug (chloroquine) in other diseased states such as diabetes. This investigation is aimed at inducing diabetes in rabbits and comparing the pharmacokinetic parameters from the diabetic rabbits with those from normal rabbits after chloroquine administration.

Rabbits have been used for this study because previous workers used the animal to study the toxic effects produced by chloroquine (Dale *et al.*, 1965; Smith and O' Grady, 1966; Kubasta *et al.*, 1966; Francois and Maudgal, 1967 and Jarzy *et al.*, 2001). Establishing the precise relationships between the pharmacokinetics of chloroquine in normal and diabetic rabbits would provide

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important information relevant to therapeutic uses of the drug.

Experimental

Venous blood was collected from rabbits that have not been given chloroquine into heparinized tubes and immediately spun to separate the plasma from Red Blood Cells (RBC) which were then stored in screw-capped glass bottles until analyzed. Chloroquine phosphate was obtained from May and Baker Plc, Ikeja, Lagos. Rabbits with weights between 1.46 – 2.50kg were purchased from breeders outside the university. The animals were maintained on a standard diet (Ladokun feeds, Ibadan, Oyo State, Nigeria) and had free access to food and water *ad libitum*. Animals were housed in a cage with a twelve hour light-dark cycle.

Glasswares were soaked in 20% nitric acid overnight, washed with distilled water and dried for 8 hours at 100^0C. Blood samples were collected into 5ml plane fluoride oxalate tubes.

Ethical Approval. Approval for use of animals in this work was obtained from the Ethical Committee on the use of Animals for Experiments of the Faculty of Pharmacy, University of Benin, Benin City.

Induction of diabetes mellitus. Ten (10) rabbits were administered with streptozotocin (60mg/kg), through intraperitoneal route. Prior to the administration, blood samples were collected for the determination of blood glucose level. These served as normal values for subsequent comparison with the glucose levels 14 days after streptozotocin administration. Streptozotocin induces animal model of diabetes mellitus within 48 hours of administration, which manifests fully within 14 days. The rabbits were observed for 14 days right from the date of administration of streptozotocin. This monitoring was done in comparison with the normal rabbits with no streptozotocin administered. The following were observed: (1) Body weight changes, (2) Physical appearance (3) Rate of urination and water consumption, (4) Food intake, (5) Activity level. During the 14 day period, the streptozotocin-treated rabbits were all positive for the observation, indicating that diabetes has been induced. After the expiration of the 14-day period, the rabbits were bled through cardiac puncture. The rabbits were each anaesthetized with chloroform in a glass desiccator. Blood samples were withdrawn from the heart with 1ml syringes (insulin syringe), but swapped with 2ml syringe needles, into a fluoride oxalate tube. The fluoride oxalate served as anticoagulant and glucose metabolism inhibitor.

The plasma samples were analyzed using glucose reagent (kit), by incubating a mixture of 0.05ml of standard glucose or plasma for 10 minutes at 37^0C in a water bath. The absorbance of the standard (As) and the plasma sample (Ap) were measured at 500 nm within 60 minutes using spectrophotometer, Spectronic 21D (Milton Roy, Rochester, N.Y.). The reagent blank incubated for 10 minutes at 37^0C is used as control.

Glucose concentration in plasma was calculated thus:

Glucose concn = (Ap/As) *x* 100 *mg/dl* (or *mg/100ml*)

The concentration obtained was corrected for dilution and subjected to statistical treatment. Blood samples were taken from the normal rabbits. A comparison was made using statistical analysis X (means), SD (Standard deviation), Sx (Standard error), and t-test. Apart from the fact that the figures were high for the streptozotocin-treated rabbits, the t-test showed significant difference in the means (sets of data) for normal and diabetic ones. This is in addition to the fact that the values of glucose level are higher for the streptozotocin-treated rabbit samples ($p \geq 0.05$). That is, diabetes had been induced and manifested. This was confirmed by the glucose level test and other physical manifestations earlier outlined.

Administration of chloroquine to normal and diabetic rabbits. Twenty rabbits were procured and kept in animal cages for a period of 14 days with regular supply of food and water for normal growth and development. The body weights of the rabbits were recorded on a daily basis to ascertain whether the condition prevalent in the laboratory is conducive for normal growth, health and development of the animals.

Within this period of acclimatization, a steady increase in body weight was recorded in all rabbits. This was done prior to commencement of all experimental investigations. Subsequently, the rabbits were divided into 2 groups of 10 rabbits each as follows:

Group 1: Normal rabbits for normal dose of chloroquine treatment (10mg/kg) to be given by oral administration.

Group 2: Diabetic rabbits for normal dose of chloroquine 10mg/kg to be given by oral administration.

Prior to each investigation, the rabbits (i.e. each group) were kept in cages for 48 hours to the commencement of investigative studies. While in the metabolic cages, the rabbits were well supplied with water and feed to ensure stable conditions. In each case investigated, dose of chloroquine (prepared using saline) was administered accordingly through oral route using standard stomach tube.

Collection of blood samples from the rabbits. About 2.5ml of venous blood was taken from each rabbit before administering the drug. Eight further blood samples were taken in the first 24 hours after administering chloroquine, and blood samples were collected at intervals ½, 1, 2, 3, 4, 6, 8, 12 and 24 hours. Blood was withdrawn into a lithium heparin bottle and about 2ml was immediately spun to separate the plasma from the blood cells. The plasma samples were stored in screw-capped glass bottles and kept frozen until analyzed.

Drug analysis. Chloroquine was determined in the blood sample by the spectrofluorimetric methods of Rubin *et al.* (1965) as modified by Adelusi and Salako, 1980. A 1.0ml of plasma was used for the analysis without dilution.

Results

Table 1 shows the volume of urine passed by the control rabbits and streptozotocin treated rabbits. From this table, it shows that the rabbit treated with streptozotocin passed more urine than the normal rabbits ($p \geq 0.05$) which is one of the symptoms of diabetes mellitus. They showed negative fluctuating body weight changes and general loss of weight. Their activity level was reduced considerably and they appeared frail, in the long term.

The fact that rabbits are diabetic was supported by Table 3 which shows the level of chloroquine in the blood of diabetic rabbits compared to the control rabbits. The total of 10 rabbits was used in each case and the sugar blood levels for the control rabbits and rabbits treated with streptozotocin were compared using student t-test. The result showed that there is a significant difference between the two values ($p \geq 0.05$) which is a further confirmation that the rabbits are diabetic.

Tables 4 and 5 show the treated data for the calculation of the residual concentration (C'-C) as described before for the normal rabbits (Table 4) and diabetic rabbits (Table 5). The table 6 shows the pharmacokinetic parameters that were calculated for the normal and diabetic rats, from the tablet, the T_{lag} the time that absorption started is the same, also the maximum concentration C_{max} at the maximum time of absorption T_{max} are all the same ($p \leq 0.05$). The blood levels of chloroquine in the diabetic rabbits were higher than normal rabbits ($p \geq 0.05$). Other parameters that differ are the half-life of absorption ($t½_a$) and

that of elimination ($t½_{el}$) and absorption rate constants (ka) in two cases showed significant difference ($p \geq 0.05$). Also the volumes of distribution (Vd) for the diabetic rabbits are lower than those of normal rabbits.

Table 1: Urinary volume of control and streptozotocin treated rabbits

Time interval (h)	Urinary volume (mL)	
	Control rabbits	Streptozotocin-treated rabbits
0 – 12	15.36	19.54
12 – 24	13.80	18.48
24 – 48	15.00	19.45
48 – 72	14.80	18.80
72 – 96	15.15	20.10
Mean ± S.E.M	14.82 ± 1.56	19.27 ± 1.62

Table 2: Blood glucose levels in normal and Streptozotocin treated rabbits (mg /dl)

Normal rabbits (mg /dl)	Streptozotocin-treated rabbits
87.5	132.8
63.8	161.6
87.5	133.3
99.0	161.6
72.0	111.1
66.6	161.5
76.2	182.0
88.0	172.0
76.2	161.0
61.0	194.0
Mean ± S.E.M. = 77.96 ± 5.18	156.26 ± 11.02

Table 3: Plasma concentration (μg/ml) of chloroquine after oral administration in normal and diabetic rabbits

Time (h)	Plasma concentration (μg/ml) ± SD	
	Normal rabbits	Diabetic rabbits
½	0.05 ± 0.006	0.07 ± 0.014
1	0.14 ± 0.012	0.158 ± 0.203
2	0.168 ± 0.014	0.0172 ± 0.025
4	0.155 ± 0.017	0.0172 ± 0.020
6	0.138 ± 0.017	0.156 ± 0.018
8	0.115 ± 0.013	0.133 ± 0.015
10	0.108 ± 0.015	0.128 ± 0.014
24	0.049 ± 0.012	0.048 ± 0.016

Table 4: Normal rabbits

Time (h)	Plasma conc., C, (μg/ml)	Extrapolated conc., C', (μg/ml)	Residual conc., C'-C, (μg/ml)
½	0.05	0.205	0.155
1	0.147	0.195	0.048
2	0.168	0.180	0.048
4	0.155	0.155	-
6	0.138	0.138	-
8	0.115	0.115	-
10	0.108	0.108	-
24	0.049	0.049	

Table 5: Diabetic rabbits

Time (h)	Plasma conc., C, (μg/ml)	Extrapolated conc., C', (μg/ml)	Residual conc., C'-C, (μg/ml)
½	0.07	0.215	0.145
1	0.158	0.208	0.05
2	1.185	0.190	0.005s
4	0.172	0.172	-
6	0.156	0.156	-
8	0.133	0.133	-
10	0.128	0.128	-
24	0.048	0.048	

Table 6: Pharmacokinetic parameters of chloroquine in normal and diabetic rabbits after oral administration

Pharmacokinetic parameters	Normal rabbits	Diabetic rabbits
$t_{½\ a}$	0.45 ± 0.021h	0.55 ± 0.026
$t_{½\ e}$l	11.5 ± 1.34h	10 ± 1.29hr
K_a	1.54 ± 0.15h^{-1}	1.26 ± 1.29
K_{el}	0.060 ± 0.001h^{-1}	0.069 ± 0.011h
T_{max}	2 ± 0.32h^{-1}	2 ± 0.40h^{-1}
C_{max}	0.185 ± -.24μg/ml	0.185 ± 0.24μg/ml
T_{lag}	0.0	0.0
V_d	31.29 litres	21.5 ± 2.3 litres
P_{cl}	1.87 ± 0.25 litre/hr	1.48 ± 0.13 litre/hr
AUC	2.397 ± 0.1μ/hr	2.696 ± 0.19μ/hr

Discussion

This study shows the effects of diabetes mellitus on the pharmacokinetics of chloroquine. The fact that the chloroquine level in the diabetic rabbits is higher than that of normal rabbits is an indication of impairment of metabolism signifying the fact that chloroquine is less metabolized in the diabetic rabbits. Such impairment of metabolism in a disease state had been previously reported in the literature (Wharton and McChesney, 1970).

Chloroquine as a drug has been found to be the best drug for the treatment of malaria in the tropics and subtropics. Since its introduction into therapeutics by (Loeb *et al*., 1946) in 1946, no resistance was noticed until 1987, 41 years of after its introduction into therapeutics. This means that the resistance developed by chloroquine against *Plasmodium falciparum* might be caused by low amount of active ingredient in the formulation, either as tablet, injection or syrup.

This study was carried out in animal but there is the need to carry out the same study in man. The purpose of studying the pharmacokinetics of drug in any diseased state is to find out whether such disease will have significant effect on the absorption, distribution and elimination of the drug. In a case where significant differences are noticed in the parameters considered, then there should be an appropriate adjustment of the required dosage regimen.

Conclusion

The study reveals the pharmacokinetic parameters of chloroquine in normal and diabetic rabbits. It also showed the different rates of metabolism of chloroquine in normal and diabetic rabbits with the former showing less metabolism than the latter.

References

Adelusi, S.A. (1982). Urinary levels of chloroquine in relation to dietary protein. *Experimentia*, 38, 1326 – 1327.

Adelusi, S.A. (1992). Pharmacokinetics of Chloroquine in rabbit. Effect of starvation. *Pk. J. Sci. Ind. Res.* S, 242 – 243.

Adelusi, S.A. and Salako L.A. (1982). The Effect of Protein-Energy Malnutrition on the absorption, Distribution and Elimination of Chloroquine in the rat. *Gen. Pharmac.* 13, 505 – 509.

Adelusi, S.A. and Salako, L.A. (1980). Improved fluorimeric assay of chloroquine in biological samples. *J. Pharm. Pharmacol.* 32, 711 – 712.

Dale, A.J., Parkhill, E. M. and Layon, D.D. (1965). Studies on chloroquine retinopathy in rabbits. *J. Am. Med. Ass.* 193, 241 – 243.

Francois J. and Maudgal, M.C. (1967). Experimentally induced chloroquine retinopathy in rabbits. *Am. J. Ophthal* 64, 886 – 893.

Jarzy, R., Kiersztan, A., Lisowa, O. and Brya, J. (2001). The inhibition of gluconeogenesis by chloroquine contributes to its hyoplycaemic action. *Env. J. Pharmacol.* 428, 381 – 388.

Kubasta, M., Vykydal, M., Zmeskal, A. and Gikalovova, I. (1966). Effect of chloroquine on the heart. *Acta Univ. Olumuc. Fac. Med.* 42, 105 – 133.

Loeb, R.F., Clark, W.M., Coatney, R.R., Cogneshall, L.T., Dieuaide, F.R. Dochez, A.R., Hakanson, E.G., Marshall, E.K. (Jr), Marvel, V.W. McCoy, O.R., Sapero, J.J., Sebrell, W.H., Shannon, J.A. and Garden G.A. Jr. (1946). Activity of a new antimalarial agent chloroquine (SN – 7618). Statement approved by the board for coordination of malarial studies. *J. Am. Med. Ass.* 30, 1069 – 1070.

Rowland, M. and Tozer, T.N. (1980). Clinical Pharmacokinetics: Concepts and Applications, pp. 293 – 296 Lea & Febiger, Philadelphia.

Rubin, M., Zvaifler, N., Bemstein, H. and Mansour, A. (1965). Chloroquine toxicity. Proceedings of the 2nd Int. Cong. of Pharmacology. Pergamon Press, Oxford; pp. 467 – 486.

Smith, B. and O-Grady, F. (1966). Experimental Chloroquine myopathy. *J. Neurol Neurosurg. Psychiat.* 29, 255 – 258.

Wharton, B.A. and McChesney, E.W. (1970). Chloroquine metabolism in kwashiorkor. *J. Trop. Pediatr.* 16, 130 – 132.