Biochemical Changes in Experimentally Induced Metabolic Conditions in Rabbits

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(Received 11th December 2007, accepted in revised form 12th July 2008)

Summary: Biochemical and physiological parameters like body weight, blood pH, blood glucose, total lipids, total protein, globulin, albumin and albumin/globulin ratio were determined in two groups of rabbits, (*i.e.* febrile and diabetic) each comprised of twelve animals. Statistically significant differences were identified when the comparison was made between normal rabbits. Blood glucose and globulin levels increased significantly in febrile rabbits, where as packed cell volume, albumin and albumin/globulin ratio decreased significantly in febrile animals when compared with control animals. Body weight, blood pH, total protein and total lipids showed no change. In Diabetic rabbits blood glucose, total proteins, total lipid and globulin increased significantly, where as body weight, packed cell volume, albumin and albumin/globulin ratio decreased significantly as compared to the normal rabbits. These differences in the physiological and biochemical parameters in disease induced conditions indicated the necessity for analyzing the changes in the pharmacokinetics parameters like absorption, distribution metabolism and excretion leading to alteration in the pharmacokinetics of drugs.

Introduction

So far science is advancing with production of various important drugs to care major and minor problems of animals including humans. The drugs normally are helpful with having some side effects and need care in their administration. Direct applications of such drugs are not permitted in humans and require the application in animals and bacterial for validation, effects and side effects, to avoid possible problems.

Rabbits are the best animals to be tested for drugs/chemical under control conditions. Experiments carried out on rabbits in induced disease condition are important for accessing biological changes and the influences obtained from such results can be extrapolated for application in real sick conditions in human. Consequently, these changes may be observed in the form of physiological/ biochemical markers and may alter the bioavailability and pharmacokinetics of the drug. Fever and diabetes are the common example of frequently encountered in clinical practice.

Rise in body temperature (fever) is manifested in several infectious diseases and climatic changes like hay fever and heat strokes, which leads to an altered homeostasis and enhanced basal metabolic rate as well as acceleration of enzymatic reactions [1]. Various studies have been carried out to check the effect of these conditions on various physiological and biochemical parameters in animals. Occurrence of fever in various disease states causes potential therapeutic problems. It alters various physiological and biochemical parameters [2, 3]. Alloxan diabetic dogs revealed significant changes in haematochemical values and disposition kinetic parameters of sulphadimidine [4]. These studies indicated a need for an adjustment of dose and dosage regimen under such conditions to achieve the desired therapeutic effects.

Ciprofloxacin is a flouroquinolone derivative with outstanding antimicrobial activity against gram-negative and some gram-positive bacteria in animals. Quinolones exhibit favorable pharmacokinetic properties; their apparent volume of distribution is indicative of substantial tissue penetration [5, 6].

The present study was carried to investigate the changes in physiological/biochemical parameters in experimentally induced, febrile and diabetic conditions in rabbits.

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Results and Discussion

Normal and Febrile Condition

Ciprofloxacin 20 mg/kg body weight was administered orally to hyperpyrexic rabbits. The physiological and biochemical parameters determined 2 hr after the administration of drug are presented in Table-1. Febrile condition in the presence of ciprofloxacin did not cause any significant (P<0.05) change in the body weight, blood pH, total protein and total lipids. However a significant difference was observed in packed cell volume (18.96 %), blood glucose (9.85 %), albumin (14.28 %), globulin (25.35 %) and albumin globulin ratio (31.69 %) in febrile rabbits as compared to values of normal animals.

Table-1: Values [Mean ± SEM (n = 12)] for the physiological and biochemical parameters in normal and febrile rabbits.

Parameters	Groups		Ratio	% Differ	Paired t-test
	Normal	Febrile			
Body Weight (Kg)	1.37 ± .17	1.35 ± .14	1.01	-1.46	ns
Blood pH	7.38 ± .22	7.35± .05	1.00	-0.4	ns
Packed Cell Volume (%)	36.60 ± 3.21	29.66± 2.62	1.23	-18.96	**
Blood Glucose (mg/dl)	110. 35± 4.06	121.23±10.93	0.91	+9.85	**
Total Proteins (g/dl)	6.90 ± .24	7.04 ± 1.86	0.98	+2.02	ns
Total Lipid (mg/dl)	300.73± 13.08	307.56±10.07	0.97	+2.27	ns
Albumin (g/dl)	4.06 ± .24	3.48 ± .10	1.16	-14.28	**
Globulins (g/dl)	2.84 ± .14	3.56 ± .24	0.79	+25.35	**
A/G Ratio	1.42 ± .11	0.97 ± .01	1.46	-31.69	***

ns = non- significant difference
** = significant difference (P < 0.05)
*** = highly significant difference (P < 0.01)
__ = decrease
\+ = increase
A/G = Albumin/Globulin

The decrease in packed cell volume may be attributed to increased cell destruction due to endotoxin (*E. coli*) induced pyrexia. These findings are also supported by the similar type of studies conducted in rabbits using different drugs in the past [2, 3, 7].

As a result of decrease in albumin and increase in globulin fraction, a decrease in albumin/ globulin ratio was observed which is supported by the Kaneko [8], who finds that the alpha globulin was increased frequently in febrile disorder. An increase in alpha globulin level has been reported by Canterow and Schepart [9], which may be due to the increase in the alpha acid glycoprotein [10].

The induction of pyrexia by endotoxin in normal rabbits might have impaired the protein synthesis from liver, particularly decreasing the albumin. The increased synthesis of globulin seems to be associated with an increased susceptibility to endotoxin. Plasma albumin and globulin were subjected to different influences and their concentrations may therefore, vary independently of one another reported by Goodman, Ahmad *et al.,* [2, 3].

Normal and Diabetic Condition

Table-2 shows the physiological and biochemical parameters of normal and diabetic rabbits. A significant (P<0.05) difference of weight was observed between normal and diabetic rabbits, but there was no significant (P<0.05) difference between the blood pH in two conditions. A highly significant (P<0.01) decrease in packed cell volume (7.5 %) occurred in diabetic rabbits, while blood glucose, total proteins, total lipids and globulin increased (P<0.01) significantly.

Table-2: Values [Mean ± SEM (n = 12)] for the physiological and biochemical parameters in normal and diabetic rabbits.

Parameters	Groups		Ratio	% Difference	Paired t- test
	Normal	Diabetic			
Body Weight (Kg)	1.58 ±.22	1.42 ± .04	1.11	-10.12	**
Blood pH	7.48 ±.31	7.33 ± .15	1.02	-2.00	Ns
Packed Cell Volume (%	34.76 ±.30	32.15 ± .47	1.08	-7.50	***
Blood Glucose (mg/dl)	105.67±2.12	353.55±3.73	0.29	+234.57	***
Total Proteins (g/dl)	6.19 ± .20	7.66±.28	0.80	+23.74	***
Total Lipid (mg/dl)	205.76±12.87	485.73±12.31	0.42	+136.06	***
Albumin (g/dl)	4.05 ±.16	3.82±.12	1.06	-5.67	**
Globulins (g/dl)	2.14 ±.13	3.84±.19	0.55	+79.43	***
A/G Ratio	1.89 ±.07	0.99±.04	1.90	-47.61	***

ns = non- significant difference
** = significant difference (P < 0.05)
*** = highly significant difference (P < 0.01)
__ = decrease
\+ = increase
A/G = Albumin/Globulin

The blood glucose level increased from 105.67 ± 2.12 to 353.55 ± 3.73 mg/dl indicating more than 3 times increase in severe diabetic condition. This increase in glucose level may be due to administration of alloxan which can destroy the beta cells of pancreas, therefore interfering with the normal insulin production. After inducing diabetes the total reduction in weight was 10.12 %, which might be due to the excessive production of ketone bodies. The ketone bodies are neutralized by buffers in body fluid up to certain threshold, above which ketonemia causes a decrease in blood pH [11]. Enhanced catabolic activities may also contribute towards these changes. These results are in line with

previous observations in diabetic dogs [12] and rabbits [13, 14].

The total proteins and total lipids showed significant increase ($P<0.01$) in diabetic animals. The possible cause of increased protein seems to be impairment of protein metabolism in liver; the other reason may be increased urination due to diabetes resulting in an increased plasma volume and consequently increased plasma protein. Similar findings have also been reported in previous studies [14, 15]. The reason for increased total lipid in diabetic state was studied by Kantardzhran [16], who correlated the lipid metabolism, increased cholesterol and triglyceride level with decreased insulin production. Increase in total lipid may also be due to the mobilization of lipid for energy purposes. As a reverse of triglyceride storage in fatty tissues, lipolysis and additional fat mobilization are responsible for the rise in plasma lipid contents [17]. In severe diabetes excessive mobilization of body fat depots may results in the appearance of neutral fat in tissues and lipemia may occur [8]. A similar increase in triglyceride was also observed in previous studies [4, 18-20].

A significant ($P<0.05$) decrease in albumin level was observed in diabetic rabbits probably due to the impaired liver function or excretion of albumin in urine. On the other hand, globulin level was increased ($P<0.01$) significantly in diabetic condition, which may be related to the increased catabolic activity during metabolic disorder.

Experimental

Material, Chemicals and Instruments

Microbial stain: *E. coli.* ATTC/11775 (A Coli Migula); Nutrient Broth: Difco; Aloxan monohydrate: Sigma Germany; Ciprofloxacin Tablet, Bayer Pharmaceuticals Karachi, Pakistan; Neubaur scale, China; Electric Shaver, Germany; Centrifuge machine, China; Glucometer, Glucomen; (Farmaceuticha Riunite Sridiagnostic Florence, Italy), Spectrophotometer, Japan (U-2000 Hitachi); Randox Diagnostic Kit, U.K.

Animals

Experiments were performed on 24 normal healthy white albino rabbits of either sex with their body weights ranging 1.2-1.8 kg. White albino rabbits obtained from Veterinary University Lahore, were checked periodically by a Veterinary Doctor before and during the experiments. Fresh green fodder and black gram were used for feeding purpose after checking for any contamination. They were kept under similar feeding and managemental conditions in the animal house of University College of Pharmacy, University of the Punjab, Lahore. The animals were divided into two groups (*i.e.* rabbits having induced febrile conditions and rabbits having induced diabetic conditions). They were fed twice a day with fresh green fodder and black gram in the morning and evening while water was available ad-libitum.

Protocol of Study

Weights of all the rabbits were recorded individually and their blood pH, blood glucose, total lipids, total plasma protein, albumin, globulin and albumin/globulin ratio were determined. Then the disease conditions were induced in both the groups. The blood samples of all the rabbits were collected to investigate the changes in biochemical and physiological parameters.

Drug Administration

To induce metabolically altered conditions in rabbits, Ciprofloxacin (500 mg) tablet (Bayer) was dissolved in distilled water and administered as a single dose of 20 mg/kg of body weight orally with the help of a feeding needle attached to a 10 ml syringe.

Induction of Disease Conditions

Febrile Condition

To induce fever in the rabbits, a suspension of *Escherichia coli* (*E. coli*) in sterilized saline water was injected into the marginal ear vein at the dose rate of 0.01 ml/kg body weight [21, 22]. Suspension was prepared by inoculating the pure culture of *E. coli* in nutrient broth and a ten fold dilution of the suspended broth had 130×10^7 microbes/ml was prepared. Counting was performed on Neubauer scale, after serial dilution of the suspension. Rectal temperatures were recorded before and after *E. coli* injection at a regular interval during the experiments.

Diabetic Condition

For the induction of diabetes, 1 % solution of alloxan in normal saline at a dose level of 150

mg/kg body weight in divided doses of 20-30 mg for 3-4 consecutive days were injected in the marginal ear vein of the rabbits. Following the injection of alloxan, blood glucose level of each rabbit was recorded daily until the level exceeded 300 mg/dl, when the rabbits were considered as hyperglycemic.

Sampling Procedure

The blood samples were collected through the juggler vein of the rabbits that were shaved completely with electric shaver. Blood samples (2-3 ml) were drawn in heparinized glass centrifuge tubes with the help of sterilized disposable plastic syringes. After recording the blood pH and blood glucose, the blood samples were centrifuged at 3000 rpm for 10 minutes. The plasma was separated for physiological and biochemical analysis.

Physiological/ Biochemical Analysis

Determination of Packed Cell Volume

The packed cell volume of the heparinised blood was determined in wintrobe hematocrit tube with the help of wintrob pipette and was centrifuged at 3000 rpm for 15 minutes [2, 23].

Determination of pH

The pH of the fresh blood samples of both the normal and sick rabbits were recorded with pH meter (WTW 740, Germany).

Determination of Plasma Glucose

For determination of plasma glucose of normal and experimental rabbits, fresh blood samples were taken and analyzed by Glucometer (Gluco Men, Italy).

Total Lipids

Total lipids in the blood were determined by the method of Ahmed *et al.,* [3] which is based on the sulpho-phospho-vanillin reaction, determined spectrophotometrically, using Randox diagnostic kit at 530 nm wave length.

Total Proteins

The total proteins in blood sample were determined by using the kit method of Randox Laboratories. The results were read spectrophotometrically at 546 nm [24].

Albumin

To analyze the total amount of albumin in plasma samples of normal and experimental rabbits, colorimetric procedure of bromocresol green was used, by employing the kit method of Randox Laboratories and the absorbances were measured at 630nm spectrophotometrically [25].

Globulin

The concentration of globulin in plasma was determined by subtracting the concentration of albumin from the concentration of total protein.

Statistical Analysis

All the values are expressed as the mean ± standard deviation (SD) of twelve animals in respective groups. The physiological/biochemical parameters obtained for the normal and experimental rabbits, were compared using a Paired-t-test, at ($P< 0.05$).

Conclusion

The results revealed that fever and diabetes have the potential of modifying the physiological and biochemical parameters of rabbits. Such results changes can influence the bioavailability and pharmacokinetic of drugs to a great extent. Thus it is suggested that therapies used in the presence of these conditions should be used cautiously and dose adjustment be made accordingly.

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