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Biochemical Changes Induced in Some Rabbit Tissues on the Administration of an Antimalaria Drug, Fansidar

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Abstract. The effect of Fansidar (40 mg/kg), a widely used antimalaria drug, was investigated on enzyme activities and some other biochemical constituents in some selected rabbit tissues. The enzymes assayed were alanine transaminase, aspartate transaminase and alkaline phosphatase. Total protein and glucose contents in the tissues were also determined. The results obtained showed a decrease in the activities of alanine transaminase and aspartate transaminase in liver and heart when the drug was administered. This indicates tissue damage, which was complicated with an increase in the activities of these enzymes in the blood due to cell leakage. There was a significant elevation of alkaline phosphatase activity in liver, heart and blood, on the third day of drug administration, which continued upto the seventh day only in the heart. This shows that prolonged usage of the antimalaria drug, Fansidar, may lead to cell destruction and degradation.

Keywords: antimalaria drug, Fansidar, alanine transaminase, aspartate transaminase, tissue damage, alkaline phosphatase

Introduction

Malaria is widely distributed, manifesting itself in different populations in different ways due to differences in the various disease determining factors in different localities, such as the strain of parasite, vector, prevalent environment, and the humans themselves. The disease burden in Africa rests predominantly on young children and pregnant women, whereas in Asia, adults are affected as much as are the children. In Africa, the disease is characterized by death as the most significant end-result, whereas in other parts of the world, physical debility and loss of economic productivity are the predominant end-results (Fansidar Monograph, 1998; McComark and Morgan, 1987; Muto *et al.,* 1971). Several reports have been published on the successful treatment of acute attacks of falciparum malaria in adults with sulfadoxine pyrimethamine combination since the middle sixties todate, both in chloroquine sensitive and chloroquine resistant areas. The earlier reports were mainly focused on efficacy, tolerance and the dose determining studies (Walker *et al.,* 1993; Weidekamn *et al.,* 1987; Walter *et al*., 1986; Waxman and Herbert, 1969).

Fansidar, an antimalaria drug, is composed of two active ingredients: sulfadoxine (N´-(5,6-dimethoxy-4-pyrimidinyl)-sulfanilamide and pyrimethamine (2,4-diamino-5-(*p*-chlorophenyl)-6-ethyl pyrimidine) in a ratio 20 : 1 (sulfadoxine 500 mg and pyrimethamine 25 mg). It is available in tablets, syrup and ampoule (Fansidar Monograph, 1998). The mode of action is based on the reciprocal potentiation of its two components. The antimalaria action is accomplished by sequential blockade of the two enzymes involved in the biosynthesis of folic acid within the parasites. Sulfadoxine is a structural analogue of para-aminobenzoic acid (PABA), and it competitively inhibits the enzyme, dihydropleroate synthetase, which is responsible for the incorporation of PABA into dihydrofolic acid and decrease in the amount of metabolically active tetrahydrofolic acid, a cofactor for the synthesis of purines, thymidines and DNA. Pyrimethamine binds to, and reversibly inhibits, the protozoal enzyme dihydrofolate reductase, selectively blocking the conversion of dihydrofolic acid to its functional form, tetrahydrofolic acid. When pyrimethamine is administered concurrently with sulfadoxine, synergism occurs, which is attributed to the inhibition of tetrahydrofolate production at two sequential steps in its biosynthesis (Fansidar Monograph, 1998).

The main objective of the present was to investigate the effect of Fansidar on transaminase activities, so as to ensure a prompt cure of malaria without any undue adverse effects.

Materials and Methods

The antimalaria drug. Fansidar tablets were obtained from SwissPharma, Nigeria Limited (formerly Roche), Lagos State, Nigeria. All other reagents used were of analytical grade and were prepared in double glass-distilled water.

Animal groupings. Eight rabbits, both sexes, weighing 256 to 550 g, were obtained from the Animal Unit of the Anatomy and Physiology Department, University of Ibadan,

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Ibadan, Nigeria. The animals were divided into four groups of two rabbits each. Rabbits in group one served as the control. They were fed with pellets and water *ad libitum.*

Drug administration. Fansidar tablets (8 g), liquified in 100 ml distilled water, were orally administered to the rabbits at a dose of 40 mg/kg body weight. Rabbits in group two were given only one dose, those in group three were administered two doses at 24 h intervals, while those in group four were administered three doses at 24 h intervals and were kept alive for four days. The animals were dissected on the respective completion of one, two, and three dose regimens after two, three and four days of the administration of the respective drug doses to collect the tissue samples. The control group was given distilled water, instead of the drug.

Collection of tissues. The rabbits were bled, while under anaesthesia, into a clean, dry beaker and serum was prepared as described by Akanji and Ngaha (1989). The blood was kept frozen at -20 °C, until required. Animals in each group were killed 24 h after the completion of each dose regimen. They were killed while still under anaesthesia by cervical dislocation and were quickly dissected. Liver and heart were removed into ice-cold 0.25 M sucrose solution. The tissues were decapsulated and washed free of blood, before weighing. Each organ tissue was cut very thin with a clean sterile blade and then homogenized in ice-cold 0.25 M sucrose solution (1 : 5 w/v). The homogenates were kept frozen at -20 °C overnight, before use for enzyme analysis. This was to ensure the maximum release of the enzymes located in the cell organelles (Akanji and Ngaha, 1989; Ngaha, 1984).

Enzyme and protein determinations. Alkaline phosphatase, alanine transaminase and aspartate transaminase activities were determined, using appropriate buffer systems. Alkaline phosphatase activity was determined by measuring the *p*-nitrophenol liberated from *p*-nitrophenyl phosphate at 400 nm (Wright *et al*., 1972). Protein content of the tissues were determined by the biuret method (Plummer, 1978). Spectrophotometric method was used to determine alanine and aspartate transaminases (Kings, 1960). Alanine transaminase was measured by monitoring the concentration of pyruvate hydrazone formed with 2,4-dinitrophenylhydrazine at 546 nm. Aspartate transaminase was determined by monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitro-phenylhydrazine at 546 nm.

Results and Discussion

Table 1 illustrates the changes in the activities of aspartat transaminase, alanine transaminase, and alkaline phosphatase in some selected rabbit tissues, following the daily administration of Fansidar. There were significant changes in the liver and heart tissues ($p < 0.05$) throughout the duration of drug administration, as compared with the control values. Aspartate transaminase activity reduced in the liver after the administration of the first dose ($p < 0.05$). The reduction continued until the third day of the drug administration. The activity of this enzyme in the liver recovered by the seventh day, however showing a small increase in the aspartate transaminase activity as compared with the control values. The alanine transaminase activity, on the other hand, was noted to reduce throughout the study period. Alkaline phosphatase activity increased on the third and seventh day, as compared with the control value. In the heart tissue, activities of all the enzymes under investigation reduced ($p < 0.05$) after the first dose of drug administration, but later increased appreciably on the third day. The increase in alanine transaminase and alkaline phosphatase activities in the heart tissue lasted throughout the duration of the drug administration period.

Table 1. Effect of Fansidar (40 mg/kg animal body weight) on some enzyme activities* in the rabbit tissues

Tissues	Days after injection	Group	Aspartate transaminase	Alanine transaminase	Alkaline phosphatase
Liver	0	1(control)	142.00±2.83	217.10±2.41	23.40±14.14
	1	2 (one-dose)	62.20±1.00	28.60±0.89	1988±16.97
	3	3 (two-dose)	11.10±0.28	28.60±1.67	3748.80±16.97
	7	4 (three-dose)	62.00±2.12	17.10±1.34	221.5±4.95
Heart	0	1 (control)	144.45±7.85	100.00±1.41	3976.00±33.94
	1	2 (one-dose)	79.98±0.03	34.29±1.71	170.40±13.58
	3	3 (two-dose)	106.65±0.64	51.42±0.82	5026.80±32.81
	7	4 (three-dose)	66.66±0.48	120.00±13.44	7071.60±26.02

*enzyme activities are expressed as specific activity (unit per litre); ± = standard deviation; statistical significance was tested using student's t-test, compared with control value $p < 0.05$

Table 2. Effect of Fansidar (40 mg/kg aninmal body weight) on some blood serum enzyme activities* in rabbits administerad the antimalaria drug

Days after injection	Group	Aspartate transaminase	Alanine transaminase	Alkaline phosphatase
0	1 (control)	20.00±0.74	17.10±0.42	153.00±1.00
1	2 (one-dose)	13.30±0.28	5.70±0.28	70.40±2.97
3	3 (two-dose)	62.20±0.51	22.90±0.42	454.40±8.49
7	4 (three-dose)	24.40±0.14	32.28±1.02	284.00±5.66

*enzyme activities are expresed as specific activity (unit per litre); ± = standard deviation; statistical significance was tested using student's t-test, compared with control value $p < 0.05$

The level of enzyme activities in the serum are shown in Table 2. There was an increase in the activity of all the enzymes under investigation on the third day. Aspartate transaminase and alkaline phosphatase activities reduced ($p < 0.05$) on the seventh day, whereas alanine transaminase activity was noted to increase, till the termination of the drug administration period.

The pattern of variations in protein and glucose contents in the rabbit tissues is shown in Table 3. Decrease in protein and glucose contents occurred in the liver, which lasted for the duration of drug administration period. A decrease in protein contents was observed in the heart, immediately after the administration of the first dose as opposed to an increase in the glucose contents. The heart tissue showed a continuous increase in the protein contents until the termination of the experiment. Oral administrations of 40 mg/kg animal body weight of Fansidar were tolerated by the rabbits and adverse drug reaction was not observed.

The results of the present study indicate that Fansidar administration (40 mg/kg) resulted in a significant reduction in the activities of aspartate transaminase, alanine transaminase and alkaline phosphatase in the rabbit liver and heart. The reduction in the activities of these enzymes in the liver and heart may be attributed to the loss of membrane components in the tissues, resulting in the release of biochemicals including the enzymes, in to the extracellular environment. This may be due to destruction of the lysosomal membrane of the liver and heart by excess dosage of Fansidar (i.e., on the 3rd day), which leads to the loss of enzymes from these tissues to the extra-cellular environment, i.e., in to the blood, thus increasing the level of these biochemicals in the serum.

Elevated alkaline phosphatase activity observed in the heart on the seventh day, viz., on termination of the experiment, may have resulted from increased synthesis of plasma membrane proteins during the repair of the damages caused by the drug molecules (Wright *et al*., 1972; Brain and Kay, 1927). It may also be due to the increase in the functional activity of the organs (Brain and Kay, 1927). The lack of further significant increase in aspartate transaminase activity in the heart, with increasing number of doses, may be due to a reduction in the effect of the drug, or as a sign of cell recovery (Ngaha, 1984).

Table 3. Effect of Fansidar (40 mg/kg animal body weight) on protein and glucose contents of rabbit tissues

Tissues	Days after injection	Group	Protein (g/l)	Glucose (mg/100 ml)
Liver	0	1(control)	239.00±1.00	403.36±4.75
	1	2(one-dose)	45.50±1.00	365.77±8.16
	3	3(two-dose)	32.80±3.96	232.43±10.71
	7	4(three-dose)	13.20±1.98	194.59±7.65
Heart	0	1(control)	119.50±0.71	1144.14±5.66
	1	2(one-dose)	49.20±4.40	1702.70±3.82
	3	3(two-dose)	92.70±3.82	567.57±10.71
	7	4(three-dose)	219.45±0.78	464.86±21.41
Serum	0	1(control)	51.90±2.55	209.01±1.40
	1	2(one-dose)	38.20±0.28	335.14±6.87
	3	3(two-dose)	38.20±0.57	219.82±0.25
	7	4(three-dose)	50.50±0.71	227.47±1.41

± = standard deviation; statistical significance was tested using student's t-test, compared with control value $p < 0.05$

In conclusion, the results obtained from this study have revealed that enzyme activities decreased with increased dose of Fansidar in the tissues studied, as opposed to an increase in the blood (especially on the 3rd day). On the basis of these observations, it may be suggested that prolonged usage and high dosage of the drug (Fansidar) can lead to damage of organs such as liver and heart.

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