European Journal of Biology and Medical Science Research

Vol.1 No.2, pp. 24-30, June 2013

Published by European Centre for Research Training and Development UK (www.ea-journals.org)

SERUM LIVER ENZYMES AS MARKERS IN ASSESSING PHYSIOLOGIC TOLERANCE OF AMALAR, COTECXIN, CHLOROQUINE AND FANSIDAR

E. O. JIMMY[1*] I. F. USOH[2], A. J. EKPO[3], AND I. UMOH[4]

[1]DEPARTMENT OF PHYSIOLOGY,
FACULTY OF BASIC MEDICAL SCIENCE, UNIVERSITY OF UYO, AKWA IBOM STATE, NIGERIA

[2&3]DEPARTMENT OF BIOCHEMISTRY,
FACULTY OF BASIC MEDICAL SCIENCES, UNIVERSITY OF UYO, AKWA IBOM STATE, NIGERIA.

[4]DEPARTMENT OF ANATOMY,
FACULTY OF BASIC MEDICAL SCIENCES, UNIVERSITY OF UYO, AKWA IBOM STATE, NIGERIA.

Abstract: *Colorimetric assay was used in the determination of plasma concentrations of aspartate amino transferase (AST), alanine amino transferease (ALT) and alkaline phosphate (ALP) in the administration of amalar, chloroquine, cotecxin and fansidar. In amalar group the aspartate amino transferase (AST) and alanine amino transferase (ALT) significantly decreased against control, (P<0.05) but there was no significant diference in the decrease of alkaline phosphatase (ALP) (P<0.05) when compared with control. In the chloroquine group there was increase in aspartate amino transferase (AST) and a decrease in alanine amino transferase (ALT) significantly (P>0.05) but the decrease in alkaline phosphatase (ALP) was not significant when compared with control (P<0.05). There was no significant difference in the concentrations of aspartate amino transferase (AST) and alkaline phosphatase (ALP) (P>0.05) in cotecxin group when compared with control but the decrease in alanine amino transferase (ALT) was significant (P<0.05). In fansidar group there was no significant decrease in aspartate amino transferase (AST) (P>0.05) but alanine amino transferase (ALT) showed significant decrease (P<0.05), whereas the alkaline phosphatase decrease (ALP) was not significant (P>0.05) when compared with control. It is observed in this study that chloroquine has damaging effect on the liver by elevating alanine and aspartate amino transferase respectively whereas fansidar lowers the activities of the liver by decreasing alanine amino transferase. It means that chloroquine and fansidar administration endangers liver functions via its enzyme systems dysfunctioning.*

Keywords: Liver enzymes, antimalarials, physiologic tolerance.

INTRODUCTION

Enzymes are proteins endowed with biological catalytic properties such which enhance biochemical reactions and without enzymes the rate of cellular reaction would be impaired and may lead eventually to cells death and subsequent halt to the entire physiologic systems (Leninger, 1993). Enzymes also serve as signals for the detection of clinical abnormalities in the

body. For instance in decreased or elevated situations it often indicates a pathological condition, (Compel, Smith, 1993). But importantly too enzymes play key roles in the metabolism of drugs, Ebadi, 1997.

There are essential tissue enzymes which apart from their metabolic significances are used as indicators for clinical diagnosis of certain diseases. Enzymes like alkaline phosphatase, alanine amino transferase and aspartate amino transferase are very symbolic in disease diagnosis and drugs interactions assessment.

Alkaline phosphatase is hydrolytic in nature, it is found in almost all tissues in the body e.g hepatocytes, epithelial cells of the bile ducts, intestine, placenta and bone forming oesteoblast. It is also found in the kidney particularly in proximal convoluted tubes and also in mammary glands. Its activity is located in the cell membrane with major function of transport of phosphate. It is used in the deferential diagnosis of liver disease and of the key enzymes in liver function test and gall bladder disease, (Lazar 2001). Aspartate amino transferase catalysis the transfer of amino group. It is used in the diagnosis of the diseases of the liver, heart and the kidney and the skeletal tissues but very diagnostic in liver disease. The plasma level is raised in other disease condition e.g. malaria, tumours, sickle cell disease, kwashiorkor and myocardial infarction and in hepatitis.

Alanine amino transferase is found in the liver, kidney, heart and skeletal muscle and pancreas, but the concentration is more in the liver. It is very sensitive in deferential diagnosis particularly between acute and chronic hepatitis and hepatic necrosis.

The enzyme could be used in recognition of early hepatitis. The liver is a very important organ in the body with vascular function as the formation of lymph and hepatic phagocytic function. It is very strategic in the metabolism of carbohydrate, lipids and proteins, (Oyebola, 2002) also perform secretory and excretory functions in respect of bile synthesis and secretion, Garret, 1999). The liver is the major organ in drug metabolism, Ebadi, 1997.

It is this last role that actually attracted the interest in the study particularly the role of this organ in schizonts-parasite formation before the entry into the blood stream and the development of schizonticides i.e the preventive treatment drugs against this stage of parasite development. The liver activities are actually affected by the presence of malaria parasites due to the presence of hepatocytes receptors. (Basnzigar et al, 1980). Such effects by the parasites would be aggravated if any antimalaria drugs spell any toll on the liver with its enzyme systems that catalysis the functioning roles of the liver. Fluctuation in liver enzyme levels means damage to the liver organ and impaired physiologic function and since the liver metabolizes drugs the negative interactive tendencies are possible particularly with antimalarials that are frequently used in the treatment of malaria. Thus, the use of liver enzymes as markers in determining the tolerance of anti malarial drugs is very imperative at this period of drug pressure due to increase malaria attacks and high persistence of drug-resistant *plasmodium falciparum* in Tropical Africa. The key enzyme like alkaline phosphatase which is very sensitive in liver function test and other liver enzymes could be used to screen antimalaria drugs which physiologic implications are enough indicators in

assessment of antimalaria drugs. These physiologic indicators e.g liver damage and cirrhosis likely to be induced by antimalaria drugs should be paramount in policy implementation of roll back malaria with morbidity and mortality roll back. This is because at the moment it appears only the direct effects of malaria has been used in determining deaths and morbidity rate. This strategy need be amplified with cell-drugs interactions for a more effective approach in realizing the goals of roll back malaria.

The study is aimed at projecting and identifying antimalaria drugs with lethal effects on the liver enzymes. Antimalaria drugs are indiscriminately consumed in Tropical Africa because majority of the health seekers are on self medication ie they choose the drugs types and dosages without consulting medical doctors, Jimmy,2000. The study has put in place comaparative assessment of the new and old antimalaia drugs effects on a target organ; the liver. It is known that malaria infection leads to hepatocytes changes that results in the leaking of enzymes into the circulatory system, Burtis 2001. Our study is aimed at highlighting on a more severe aspect; antimalarials effects on the hepatocytes enzymes which are more clinically disastrous in the management of malaria. The study is also aimed at creating health awareness on the use of certain antimalaria drugs particularly the organ friendly ones with less toxic properties. Importantly, biomarkers and hepatoxicity which is the focus of this study are research fields that holds lots of clues in future studies relating chemotherapy of malaria disease and renal failure particularly in Tropical Africa.

MATERIAL AND METHOD

Animals: A total of thirty albino rats of average weight 75-148g obtained from Faculty of Pharmacy animal house, University of Uyo, Nigeria were used for the study. The animals were fed with food pellets and water. The right to use the animals was not obtained as there is no animal right where the study was done but the animals were not tortured during the course of the study. The animals were grouped into four drugs group with each group having six animals including control. The animals were kept in a well ventilated room.

Drug Administration: Four drugs, amalar®, coteсxin®, chloroquine® and fansidar® were used for the study. The methods of Bertram, 2004, Robert et al, 1979 were used for the administration of the drugs. But briefly drugs were given per weights of the animals using the average weight of man (70kg) for derivation. The drugs were also administered based on the curative and preventive dosages and regimen, but were given orally using canula by-passing the Oesophagus and delivered into the stomach. The Liver enzymes levels were monitored for 28 days adopting WHO 1982 model for antimalaria drugs, efficacy monitoring and malaria parasite clearance but malaria parasites were not given to the animals.

Blood and serum Collection: The animals were given chloroform anaesthesia by inhalation to enable unclotted blood to be collected by cardiac puncture, (Thompson, 1983). The collected blood was allowed to clot and spun at 1,200rpm for 10mins to obtain serum for the determination of enzyme levels.

Assay of liver enzymes: The liver enzymes, alkaline phosphates, alanine amino transferase and aspartate amino transferase were determined by enzymatic colorimetric method of Reitman and

Frankel, 1957, Schmidtt et al, 1963. In alanine amino transferase, the catalytic concentration was determined from the rate of decrease of NADH measured at 340nm by means of the lactate dehydrogenase (LDH) completed reaction. For alkaline phosphatase, in its presence and P-nitrophenyl phosphate and water, P – nitrophenyl phosphate is formed but P-nitrophenol has strong absorbance at 450nm and so the rate of the increase absorbance will be proportional to the activity of alkaline phosphatase. In the estimation of aspartate amino transferase, in its presence aspartate oxoglutavate generates oxaloacetate aspirate enzyme activity at 340nm wave length. The determinations were based on authors and manufacturers instructions.

RESULTS

Table 1: Effects of Amalar®, Cotectxin®, Chloroquine® and Fansidar® on Serum liver Enzymes (Summary of Results)

	LIVER ENZYMES			
Drugs	**ALP**	**ALT**	**AST**	**P Value**
Amalar	30.55±5.51	16.98±14.87	10.94±1.41	P<0.05 in AST&ALT P>0.05 in ALP
Cotecxin	28.59±5.87	18.87±14.96	13.76±5.3	P<0.05 AST&ALP P<0.05 in ACT
Chloroquine	30.69±1.19	18.59±14.39	18.83±7.16	P<0.05 in AST & in ALT
Fansidar	30.44±4.07	22.99±13.04	12.73±1.21	P<0.05
Control	32.50±1.29	28.46±5.02	14.96±4.03	P<0.05 in ALT P>0.05 in AST & ALP

ALP: Alkaline phosphatase
ALT: Alanine amino transferase
AST: Aspartate amino transferase

The results showed that amalar drug decrease the concentration of aspartate amino transferease (AST) and alanine amino transferase (ALT), significantly 10.94.±1.41, 16.98±14.87, (P<0.05) but the decrease in alkaline phosphatase (ALP), 30-.55±.5.51 was not significant (P>0.05) when compared with control, table 1. Chloroquine effected increase in aspartate amino transferase and decrease in alanine amino transferase 18.83±7.16, 18.59±14.39 (P<0.05), but the decrease in alkaline phosphatase, 30.69±1.19 was not significant, table 1.

Cotecxin did not effect decrease or increase in aspartate amino transferase, alkaline phosphates, but decrease in alanine amino transferase 13.76±5.31, 28.59±5.87, (P>0.05), 18.87±14.96 (P<0.05), when compared with control, table 1.

Fansidar decrease aspartate amino transferase, 10.94±1.41 but was not significant (P>0.05) but it decreases alanine amino transferase, 22.99±13.04 significantly (P<0.05) whereas alkaline

phosphatase decrease with fansidar, 30.44±4.07 was not significant, (P>0.05) when compared with control, table 1.

DISCUSSION

From the study, it is shown that each antimalaria drug has effect on the liver enzymes though such effects may not be significant to induce serious pathology. It is clear from this observation that there is the interaction between antimalarial drugs and the liver enzymes. This is expected as the liver serves as the major conduit for drug metabolism, (Ebadi, 1997). However, what is peculiar in our study is the degree effects of interactions which one antimalaria drug exhibits and not observed in another. Also, the general observation is that almost 90% of the antimalaria drugs has affinity for aspartate and alanine transferases whereas alkaline phosphatase is less affected. The implication here is that there is the specificity of the antimalarials with the liver enzyme system, a kind of that exhibited in enzyme substrate reaction. It also means that such antimalarials compete inhibitively to the functions of the enzymes thereby either increasing or decreasing its activities pathologically.

Chloroquine for instance increased aspirate amino transferase but decreased alanine amino transferase significantly but its decrease effect in alkaline phosphatase was also observed though not enough to significantly alter physiologic activities of the liver. This different actions of one antimalaria drug on the liver enzymes also explains that the actions of chloroquine is not directly on the liver as the enzymes are also affected. This is in line with the general trend of raised liver enzymes in acute hepatoxicity and decreased activity with prolonged intoxicity as prolonged cellular damage decrease liver tissue mass, Nosten, et al 1993.

Particularly, the activity of aspartate amino transferase in the liver is observed to increase with chloroquine indicating the possibility of induction of mycardiac infection and liver cirrhosis in the drug administration. This study has re-confirmed raised aspartate and decrease alanine amino transferease as pathologic indicators of liver damage, (Sood, 1999). Here chloroquine has demonstrated marker quality in assessing its physiologic tolerance between aspartate amino transferase and alanine amino transferase and alkaline phosphatase on the other hand. Chloroquine is one of the cheapest and highly available antimalarial drugs judging in comparison with other anitmalarials as it is safest. But with the observations in this study definitely such good evaluation may not be upheld completely, hence, the dire need for physiologic imputs in the monitoring of drug efficacy to be scored higher than just pharmacologic imputs.

A more drastic effect likely is that of the preventive antimalarials e.g fansidar and amalar which are schizonticides by their actions and the choice of the schizonts parasites to prefer the liver organ as host amidst all other organs in the body. In our study, fansidar only decrease alanine amino transferase whereas amalar significantly decrease both aspartate amino transferase and alanine amino transferase. Such decrease explains prolonged cellular intoxication and damage which further clarify the relationship between prevention drugs and long cumulative effects as per administration. The striking observation between amalar and fansidar is in the pattern of these drugs reaction. Both have sulphadozine/pyrimethamine of same concentration 525mg/kg and yet effects on the enzymes are different, perhaps these two drugs are indeed different or

rebranded version or adulterated. Here, the issue of marker indicators and physiologic tolerance are observed. But it appears amalar seems to be more deleterious in its effects on the liver enzyme. However, hepatic granulomas effects from fansidar is reported, (Laazard, 2001). But amalar drug is a highly patronized preventive therapy and such negative effects would be aggravated in hepatic stage of malaria parasite development. However, cotecxin only decline alanine amino transferase which also implied prolong cellular damage of the liver organ. The comparative study of old antimalarials, chloroquine, fanisdar and new ones; amalar, cotecxin has shown a rather worrisome physiologic trend, that of intolerance signally impending and inevitable cells, tissue, organ and organ systems damage by the effects of antimalaria drugs. However, the new drugs particularly cotecxin has partially proven its alternative to chloroquine and fansidar which are not very effective against resistant Strain, P. Falciparum, (Rowland, 1997).

But such alternative should be reflected physiologically. The trend of new antimalarias effects on the body system need be monitored. This is because there is unprecedented insurgence of new antimalaria drugs leading to drug pressure and the likely tendency of physiologic damage. The monitoring assessment is important to forestall more morbidity and deaths accruing from malaria therapy than malaria disease. The results of our study has shown that the work is innovative as no study on comparative serum enzymes markers with Amalar, cotecxin, chloroquine and fansidar has been done. The study has also shown the effects of drugs pressure on the liver enzymes as in chloroquine and fansidar based on their affordability and availability in the country of study. The study has created impacts on the development of transpecies biomarkers as in our study with animals which can be used to determine target organ toxicities for clinical application in man.Such approach will encourage and expand the scope of drug safety research. The study has shown predictive pattern of antimalarials activities used in the investigation and their related enzymopathies detected . The study has demonstrated novel enzyme markers and their potentials in detecting hepatoxicity induced antimalaria drugs.

REFERENCES

Bertram, G. (2004). In Basic and Clinical Pharmacology, 9th Edition.B Burtis, C, E. Ashwood, B, Barker, B. Border,(2001), Liver functions In;Tietz fundamentals of clinical chemistry 5th ed. Saunders company , Philadelphia. PP748-770

Ebadi, M. (1997). In Core Concepts in Pharmacology, first edition, USA.

Jimmy, E. O. ,A, Emeka and S orji.(2000). Pattern of antimalaria Dispensing by patent medicine dealers in rural settlement in Nigeria. Publich Health 114;282-285 DO1:1038/sj.ph1900657

Lazaar, H. P. Heligreen, U. (2001). Fansidar and Hepatic Granulomas, Annual Intentional Journal of Pharmacology, 102:722.

Leninger, A. L., Nelson, D. L. Cox. M. N. (1993): In Principle of Biochemistry, 2nd Edition North Publishers, New York.

Nosten, F. Terkuile, F. O., Luxemburger, C. Woodrow, C. Kuyle, D. Chong, E. Suphajusidhhi T. (1993). Cardiac effects of antimalaria treatment with chloroquine. Lancet Pumed. 341:1054-56.

Oyebola, D. O. (2002). In Essential Physiology, Vol.1 Nhort, Ibadan, Nigeria.

Reitman, S. Frankel S. (1957). Colorimetric determination of electrolytes, *American Journal of Clinical Pathology*, 3:6.

Robert, G. (1979). Gastric Cytoprotective Property of Prostaglandins. *Gastroenterol* 77:762-769

Rowland, M. Dunami, N. Hewitts and Sandorp E. (1997). Resistance of falciparum malaria to chloroquine and Sulphadoxine-Pyrimethamine in Afghan refuge settlements in Western Pakistan: Survey by general health services using a simplified invivo test. *Tropical Medicine and International Health*. Vol. 2 No.11, 1049-1056.

Good, R. (1999): In Medical Laboratory Technology and Interpretation. 5th Edition, Brokers Medical Publishers, New Dehli.

Thompson, A. R. Harker, L. A. (1983): In Manual of Haemostasis and Thrombosis 3rd Edition, F. A. Davies Company, Philadelphia.

WHO (1982): In Modern Design of Antimalaria drugs proceedings of a meeting held in Bethesda, Maryland USA.