International Journal of Current Research and Academic Review

ISSN: 2347-3215 Volume 3 Number 11 (November-2015) pp.73-78

www.ijcrar.com

Lipase Activity in Albino Rats Treated with an Antimalarial Drug

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KEYWORDS

Pancreas,
Serum,
Malaria,
Total protein,
Toxicity

A B S T R A C T

Malaria is a common disease caused by *Plasmodium* parasites, which infect red blood cells through the bites of an infected female anopheles mosquito. Anti-malaria drugs have been reported to show various side effects. The present research was set up to examine the activity of lipase in serum of albino rats as a measure of possible toxic effect of an antimalarial drug. The albino rats were placed in four (4) groups, A, B, C, and D, with five rats per group. Groups A, B, and C were treated with oral doses of 8.0, 16.0, and 32.0mg/kg body weight respectively of the drug solution, while group D served as the control. The treatment lasted for seven (7) consecutive days. There was a general decrease in average body weight, feed and water intake of the groups treated with the drug relative to the control. The treatment of the animals did not produce any significant difference ($P > 0.05$) in total protein concentration between test and control. The lipase activity in the serum of the albino rats in the test groups was found to be significantly higher ($P < 0.05$) than in the control. This effect was found to increase with the doses. The findings in this research are indicative that an antimalarial drug may be toxic to the pancreas.

Introduction

Malaria is a mosquito-borne infectious disease of humans and other animals caused by protists (a type of microorganism) of the genus *Plasmodium* [1]. It begins with a bite from an infected female *Anopheles* mosquito, which introduces the protists through saliva into the circulatory system. In the blood, the protists travel to the liver to mature and reproduce.

Malaria causes symptoms that typically include fever and headache, which in severe cases can progress to coma or death [2]. The disease is widespread in tropical and subtropical regions in a broad band around the equator, including much of Sub-Saharan Africa, Asia, and the Americas [3].

Five species of *Plasmodium* can infect and be transmitted by humans. The vast majority of deaths are caused by *P. falciparum* and *P. vivax*, while *P. ovale*, and *P. malariae* cause a generally milder form of malaria that is rarely fatal. *P. falciparum* is the principal parasite in sub-Saharan Africa and accounts for approximately 90% of all deaths worldwide from malaria. Most fatal cases affect children aged under 5 years, where levels of acquired immunity to the parasite are often insufficient to allow survival beyond infancy [4]. The zoonotic species *P. knowlesi*, prevalent in Southeast Asia, causes malaria in macaques but can also cause severe infections in humans. Malaria is prevalent in tropical and subtropical regions because rainfall, warm temperatures, and stagnant waters provide habitats ideal for mosquito larvae. Disease transmission can be reduced by preventing mosquito bites by distribution of mosquito nets and insect repellents, or with mosquito-control measures such as spraying insecticides and draining standing water.

Antimalarial drugs are classified into tree according to site of action in malaria parasites. These are those that act on the food vacuole, drugs that block metabolic synthesis and oxidative processes, and those that interfere with membrane processes[5]. Knowledge of these sites of action has enabled identification of new drugs with the most promising potential for development. Current antimalarial strategies prioritize combination therapies such as atovaquone/ proguanil or artemether/lumefantrine and prolonged treatments to limit the risk of inducing drug resistant *Plasmodium* [6].

An antimalarial drug containing arthemether and lumefantine. The availability of artemisinin-based compounds, derived from the Chinese plant *Artemisia annua*, during the past decade has introduced a new era in the treatment of malaria. Effective and well tolerated, these drugs appear to offer substantial benefits over traditional agents such as chloroquine or sulfadoxine-pyrimethamine. Artemisinin-based combination therapies (ACT) are recommended as first-line therapy for malaria by the World Health Organization (WHO) and have been accepted as a mainstay of treatment in more than 70 countries [7]. The mechanism by which artemether exerts their antimalarial activity remain contentious. Nevertheless, most studies concur that the activity of artemether and many if not all of its potent derivatives results from reductive scission of the peroxide bridge by reduced heme iron, which is produced inside the highly acidic digestive vacuole as it digests hemoglobin. In support of this, a recent study with fluorescent artemethertrioxone derivatives provided evidence for their rapid accumulation in the digestive vacuole and their activation by neutral lipid-associated heme. The selection of artemether and lumefantine as a combination is due to their reported synergistic effects against *P. falciparum in vitro*. Lumefantine is also chosen due to its large apparent volume of distribution [8].

Lipases (EC 3.1.1.3 triacylglycerol acylhydrolase) are a group of water soluble enzymes, which exhibit the ability of acting at the interface between aqueous and organic phases. They primarily catalyze the hydrolysis of ester bonds in water insoluble lipid substrates. However, some lipases are also able to catalyze the processes of esterification, interesterification, transesterification, acidolysis, aminolysis and may show enantio selective properties [9]. Serum lipase elevations have been reported to be positively associated with a correct diagnosis of acute pancreatitis, with diagnostic efficiencies of 94 per cent. A

close correlation between elevation of serum lipase has been observed in both extrapancreatic and pancreatic disease processes. Serum lipase is a better test than serum amylase either to exclude or to support a diagnosis of acute pancreatitis [10].

Administration of an antimalarial drugis sometimes done with caution due to some reported side effects, such as skin rashes, difficulty in sleeping, nausea, vomitting, diarrhea, and coughing. Administration of an antimalarial drugtablets in high doses can lead to toxicity of the hepatocytes [11]. Hence, the present study investigated the effect of oral administration of an antimalarial drugon lipase activity as an index of possible pacreatic toxicity.

Materials and Methods

Collection of Samples

Twenty (20) adult male albino rats weighing 87 – 105g were obtained from Zoology Department, University of Nigeria Nsukka, Enugu state, and transported to the animal house, Department of Biochemistry, Ebonyi State, University, Abakaliki. Two packets of An antimalarial drugwere purchased from Elegant drug store Abakaliki, Ebonyi State, Nigeria.

Animal (Rats) Handling and Treatment

Ethical approval for animal use in research was given by Ebonyi State University Ethics and Research Committee.

The 20 Albino rats were randomly placed in 4 cages, labeled (A, B, C and D), each containing 5 rats. All the animals were allowed free access to water and feed (growers mash), and were acclimatized for 7 days before administration of sample commenced. After acclimatization, doses of 8, 16, and 32mg/kg body weight of drugsolution were administered orally to rats in groups A, B, and C respectively for seven consecutive days. Group D served as control and received distilled water. After seven days of drug administration, the rats were starved overnight, sacrificed under a mild anesthesia using chloroform, and blood samples were collected from the albino rats by cardiac puncture.

Measurement of Parameters

Serum activity of lipase was measured according to the method of Yang and Biggs [12], while Lowry [13] method was adopted to determine total protein.

Statistical Analysis

Data generated were expressed as mean ± SD. Statistical significance of difference was determined using the program SPSS 12 (SPSS, USA) by performing one-way ANOVA with post-hoc comparisons between the control group and each of the treated groups by Ducan's multiple comparison test. A p-value less than 0.05 was considered statistical significant.

Results and Discussion

Treatment of the animals with a solution of the antimalarial drug resulted in a decrease in their physical activities and rate of feed and water intake relative to the control (data not shown). The actual biochemical mechanism behind this observation is still been investigated. However, it may be due to an upset in general metabolism caused by the drug. Some mild and uncommon side effects of artemether-lumefantrine combination include loss of appetite and weakness [14]. The finding is consistent with the report of Foley and Tilley [15] on

treatment of albino ratscoaterm (a combination of artemether-lumefantrine).

The average body weight of the animals in the groups administered the drug sample decreased, while that of the control increased (table 1). This result may be attributed to the reported decreased in feed and water consumption. As shown in table 2, the difference between total protein concentrations recorded in the serum of the rats treated with drug solution and the control was not significant (P>0.05). This indicates that the chemical constituents of the drug may play no significant role in regulation of protein synthesis and regulation.

Table.1 Average body weight of the rats during seven days of drug administration

Day of administration		BODY WEIGHT (g)		
	A	B	C	D
1	96.54±2.91	87.12 ± 2.40	105.60 ± 5.18	89.23 ± 5.78
2	97.65±4.66	84.12 ± 6.49	98.60 ± 3.30	90.33 ± 7.37
3	95.45±6.73	82.98 ± 3.17	95.67 ± 4.95	95.35 ± 6.45
4	92.50±4.90	78.32 ± 3.47	88.67 ± 7.89	98.32 ± 3.95
5	91.80±6.84	75.54 ± 7.89	79.54 ± 6.73	104.00 ± 6.94
6	88.98±5.18	71.45 ± 2.28	74.10 ± 7.89	107.50 ± 2.70
7	85.75±3.49	65.50 ± 7.89	68.76 ± 6.28	112.67 ± 2.89

All values are mean ± standard deviation; N = 5

Key: Group A = 8.0mg/kg body weight
Group B = 16.0mg/kg body weight
Group C = 32.0mg/kg body weight
Group D = distilled water

Table.2 Average serum lipase activity and protein concentration after seven days of drug administration

GROUP	ENZYME ACTIVITY (U/l)	TOTAL PROTEIN (mg/ml)
A	221.46±8.74^{a}	0.68 ± 0.034^{a}
B	288.04±3.68^{b}	0.62 ± 0.006^{a}
C	392.43±9.60^{c}	0.52 ± 0.016^{a}
D	209.70±5.48^{a}	0.77 ± 0.033^{a}

All values are mean ± standard deviation; N = 5. Values in the same column bearing different superscripts differ significantly (P<0.05)

Key: Group A = 8.0mg/kg body weight
Group B = 16.0mg/kg body weight
Group C = 32.0mg/kg body weight
Group D = distilled water

Serum lipase activity of the animals after seven days of treatment is presented in table 2. The activity of the enzyme obtained in groups administered the drug was significantly higher ($P<0.05$) than in the control. The exact mechanism responsible for this elevation of serum lipase activity is subject to further studies. However, it may be as a result of damage to pancreatic cells which causes the enzyme to leak into circulation. Lipase assay as a biomarker of pancreatic damage has a sensitivity and specificity of 80% and 60%, respectively [16][17]. The serum concentration of lipase increases within 3–6 hours of onset of damage and peaks within 24 hours [18]. The increased serum level stays for around 7–14 days before it comes down to the normal level [19]. In contrast to amylase, lipase is reabsorbed in renal tubules and stays for long at higher concentration, thereby giving greater sensitivity in patients with delayed presentation. Pancreatic lipase is four times more active than amylase and it is less affected by exocrine pancreatic deficiency occurring in patients of chronic pancreatitis [20]. Hypertriglyceridemia does not influence the serum lipase assay as happens in the case of serum amylase. Increased serum level of lipase can also be seen in many intra-abdominal pathologies including acute cholecystitis, appendicitits, inflammatory bowel disease, intestinal ischemia, obstruction, perforation, and renal insufficiency. According to recent guidelines from UK, serum lipase should be preferred for diagnosis of pancreatic damage over serum amylase wherever available [21].

The artemether-lumefantrine combination is generally well tolerated. The most frequently reported adverse effects in pediatric clinical trials have been fever (29%), cough (23%), vomiting (18%), headache (13%), and anorexia (13%). Dizziness, nausea, abdominal pain, diarrhea, rashes, arthralgia and myalgia, asthenia, fatigue, and elevations in serum transaminases have been reported in 1-10% of children [22].

These observed effects of oral consumption of an antimalarial drugon body weight and serum lipase activity were found to be dose-dependent.

Conclusion

From the findings of this research, it can be suggested that oral administration of an antimalarial drugmay be toxic to the pancreas. It may also affect appetite and physical health. However, studies are in progress in our laboratory to establish these findings, and identify the possible mechanisms responsible.

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