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Dietary Supplementation with Calcium in Artemisinin-Based Combination Drug Administered Rat and Risk of Cardiovascular Disease

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Abstract: Reports on the role of calcium on predisposition to cardiovascular disease have been rather inconsistent while studies on its interaction with other medications are ongoing. Researchers therefore, investigated the effect of separate and combine administration of calcium supplement with artemisinin-based combination drug on hepatic and serum lipid profile. A total of 32 male wistar rats were randomly assigned into 4 groups of 8 rats each. The control (Group A) received water. Group B and D were placed on 10 mg kg^{-1} calcium twice daily for 4 weeks. On the 30th day, therapeutic dose of artemisinin-based combination was simultaneously administered to group C and D twice daily for 3 days. All the rats were then sacrificed after 12 h fasting, blood was withdrawn and the liver removed and homogenized in an appropriate buffer. Biochemical analysis showed no significant ($p>0.05$) variation in hepatic triaacylglycerol in all the treated groups whereas calcium supplementation was observed to induce a significant ($p<0.05$) reduction in hepatic cholesterol. Significant elevations due to calcium supplementation were also observed in serum total cholesterol, LDL cholesterol level and atherogenic risk index with a concomitant reduction in serum HDL cholesterol. No significant change was observed in serum total cholesterol, triacylglycerol and serum lipoproteins in all other treatment groups. This study suggests that calcium supplementation may predispose to cardiovascular disease and that its co administration with ACT may not aggravate nor reduced the predisposition risk.

Key words: Lipid profile, cardiovascular disease, calcium, ACT, malaria, rat, Nigeria

INTRODUCTION

Calcium is the fifth most abundant element in the human body with ~1000 g present in adults. It plays a key role in skeletal mineralization as well as a wide range of biologic functions. Calcium is an essential element that is only available to the body through dietary sources. Current dietary calcium recommendations range from 1000-1500 mg day^{-1}, depending on age (Institute of Medicine (IOM), 1997). It has been noted (Mccabe *et al*., 2004) that some individuals, particularly the elderly may require calcium supplements in order to achieve the recommended dietary calcium intake. An individual requirement for calcium is dependent on the state of calcium metabolism. This in turn is regulated by three main mechanisms: Intestinal absorption, renal reabsorption and bone turnover. These in turn are regulated by a set of interacting hormones, including Parathyroid Hormone (PTH), 1,25-dihydroxy Vitamin D [1,25$(OH)_2$D], ionized calcium itself and their corresponding receptors in the gut, kidney and bone.

Malaria is one of the worst health problems in this decade. Globally, the disease takes the heavy toll of >1 million lives every year. The worsening problems of drug resistance in many parts of the world and the limited number of antimalarial drugs available have led to increasing difficulties for adequate disease management. Artemisinin and its derivatives are among the most effective antimalarial drugs known today. They rapidly cure even drug-resistant falciparum infections. Artemisinin-based Combination Therapies (ACTs) are being used for treatment, instead of mono-therapies to delay the emergence of resistant strains of *Plasmodium falciparum* to this vital class of drugs. ACTs are increasingly being adopted as first-line treatments in malaria-endemic regions of the world that are afflicted with *P. falciparum* strains resistant to conventional antimalarial drugs.

Many researchers have reported on the metabolic effects of artemisinin and on the pharmacological effects of its interaction with some other medications

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(Ekong *et al*., 2008; Obianime and Aprioku, 2009; Tijani *et al*., 2010). It has been suggested that high doses of artemisinin drugs can produce neurotoxicity, prolongation of the QT interval, bone marrow depression with the possibility of long term toxicity in human beings (De Vries and Dien, 1996). However, no report on the pharmacological effects of its interaction with calcium has been cited. A study reported that calcium and calcium containing products interact with drug such as rosephine and cause dangerous deposits in the heart, lungs and kidneys (Ekong *et al*., 2008). Another study reported that calcium can decrease the absorption of other drugs such as tetracycline antibiotics (e.g., doxycycline, minocycline), bisphosphonates (e.g., alendronate), estramustine, levothyroxine and quinolone antibiotics (e.g., ciprofloxacin, levofloxacin) (Peacock, 2010).

The potential effects of inadequate or excessive calcium supply on Cardiovascular Disease (CVD) are receiving growing attention a number of prospective epidemiologic studies have examined the relationship between dietary calcium intake and CVD incidence or mortality in middle-aged and older adults, however the results were inconsistent (Pasi *et al*., 2003; Skerret, 2010; Wang *et al*., 2012). A report by Larrtey-Rowser (2009) noted that the relationships between calcium intake and body mass index, total cholesterol, high-density lipoprotein and low-density lipoprotein in African American adults vary between genders (From being a positive relationship to a negative relationship depending on the factor). What researchers do not know is whether this process is influenced by the amount of calcium a person takes in each day from supplements.

In the present study, researchers have investigated the modulating effect of calcium supplementation and the effect of co-administration of calcium with artemisinin-based combination drug on lipid profile of both the plasma and the liver. This was done as an attempt to understand the role of separate and combined administration of these medications in predisposition to cardiovascular disease.

MATERIALS AND METHODS

Drug: Leonart® (Bliss GVS Pharma Ltd., India) composed of 80 mg Artemether and 480 mg lumefantrine and calcium compound (GNLD Int. Spartan, South Africa and Lagos, Nigeria) composed of 300 mg calcium glycinate) was obtained from Boorepo Chemist, Osogbo, Nigeria.

Experimental subjects: Thirty two male Wistar rats weighing 100-150 g were obtained from the animal facility centre, University of Ibadan, Nigeria. All animals were kept in individual cages in an environmentally controlled room with a 12 h light/12 h dark cycle. The animals were placed on standard rat feed (Ladokun feed, Ibadan, Nigeria) and clean drinking water *ad libitum*. They were all acclimatised for 1 week. The care of the animals was in accordance with the US Public Health Service Guidelines (National Research Council, 1999) and approved by the Ethics Committee, Kwara state University, Ilorin.

Grouping of animals: The rats were randomized into four groups (each containing 8 rats), labeled and treated as indicated:

- Group A is normal control
- Group B is calcium supplement
- Group C is artemisinin-based combination drug
- Group D is calcium supplement+Artemisin-based combination drug

Experimental procedure and administration of drugs: The calcium supplement was administered (to rat in group B and D) at a therapeutic dose of 10 mg kg^{-1} body weight twice daily while artemisinin-based combination was administered at a therapeutic dose of 2.67 mg artemether/16 mg lumefantrine kg^{-1} body weight twice daily (to rats in group C and D). All drugs were administered in an aqueous suspension by oral galvage. The drug suspensions were continuously agitated in order to deliver the drug homogenously to the animals. The calcium was first administered to the rats for 4 weeks. On the 30th day, artemisinin-based combination drug was simultaneously administered (Group C and D) for another 3 days. After the last administration, the rats were fasted for 12 h and were then sacrificed. Blood was drawn from their eye vein and delivered into lithium heparinised tube by inserting a capillary tube. Plasma was then prepared by centrifugation at 3000 rpm for 10 min. The liver was also removed, clean of blood, weighed and homogenised.

Analytical procedure: Plasma triacylglycerol, total cholesterol and HDL-c were determined by colorimetric methods after enzymatic reaction with peroxidase (Microlab 300, Leatherland, Dieren, UK). Low-density lipoprotein cholesterol was estimated by the Friedewald *et al*. (1972) formula which is reliable when triacylglycerol levels are <400 mg dL^{-1}. Hepatic triacylglycerol and total cholesterol were measured using established methods (Biggs *et al*., 1975; Folch *et al*., 1957; Zlatkis *et al*., 1953). Hepatic lipid was extracted using the method of Folch *et al*. (1957) and was gravimetrically determined. Liver triacylglycerol was measured using the method described by Biggs *et al*. (1975) and total cholesterol, using the method described by Zlatkis *et al*. (1953). Very low Density Lipoprotein (VLDL) was estimated by mathematical computation using friendship relationship VLDL = TG ÷ 2.2. Atherogenic Index (AI) was

calculated using the formula of Abot *et al.* (1988) and Coronary Rrisk Index (CRI) was obtained by the method of Allard *et al.* (1994).

Statistical analysis: Data analyses were performed using SPSS software (SPSS 10.0 for Windows, SPSS Inc, Chicago, IL). All data are expressed as mean±SEM. Analysis of variance was used to test for differences between the groups. Duncan's multiple range test was used to determine the significance of differences among the mean values at the level of $p<0.05$.

RESULTS AND DISCUSSION

Table 1 presents the result of hepatic lipids determined after treatment. Separate administration of calcium supplement was observed to decrease liver total cholesterol from the nirmal control value of 4.58±0.10 to 2.61±0.12 mmol L^{-1} but does not alter the triacylglycerol level significantly ($p>0.05$). Neither separate administration of ACT nor its combined administration with calcium significantly alters the liver total cholesterol and triacylglycerol level.

Table 2 shows the result of the effect of treatment on plasma lipids. Calcium supplementation was observed to have significantly increased the plasma total cholesterol from the normal control value of 6.02±0.10 to 7.94±0.22 mmol L^{-1}. Separate administration of ACT and combined administration of ACT with calcium, however did not significantly affect plasma total cholesterol. The observed plasma cholesterol of 6.28±0.18 mmol L^{-1} following separate administration of ACT and the observed value of 7.47±0.20 mmol L^{-1} when co-administered with calcium were not different from each other. A similar trend in triacylglycerol level was observed with calcium administration, however the observed triglyceride level following separate administration of ACT and when co administered with calcium were not different from that of the normal control value.

The observed trend in HDL-c shown in Table 2 indicates that whereas separate administration of calcium significantly lowered plasma HDL-c from the normal control value of 3.30±0.39 to 1.94±0.31 mmol L^{-1}, separate administration of ACT and its combined administration with calcium does not significantly altered plasma HDL-c value. A raised plasma LDL-c was observed with calcium treatment, ACT administration, however was not observed to affect plasma LDL-c value. The observed value of 2.49±0.07 mmol L^{-1} was not different from the observed value of 2.13±0.2 mmol L^{-1} when ACT and calcium were co administered. Neither separate administration of calcium and ACT nor their combined administration significantly affects plasma VLDL-c value.

Table 3 indicates that calcium supplementation increased artherogenic risk index but does not significantly altered the coronary risk index. Separate administration of ACT and its combined administration with calcium however do not caused any variation in both artherogenic and coronary risk indexes.

High levels of total cholesterol and LDL-c are major risk factors for coronary diseases whereas increased HDL-c is associated with a decrease in coronary disease risk (Wilson, 1990). Another previous study also noted that elevated triacylglycerols could increase the incidence of coronary heart disease (Bainton *et al.*, 1992). Data generated from the study showed that calcium supplementation increased levels of serum total cholesterol, triacylglyserol and LDL-c. These results suggest that calcium supplementation may predispose to cardiovascular disease risk. The result is further supported by the increased artherogenic risk observed in the study when the subjects were placed on calcium supplements. It is well known that HDL-c plays an important role in the transport of cholesterol and cholesterol ester from the periphery (away from the tissues) to the liver by the reverse cholesterol transport pathway where they are further converted to bile acids. Therefore, the observed decrease in serum HDL-c in the

Table 1: Effect of treatment on hepatic cholesterol and triglyceride

Group/treatment	Total cholesterol (mmol L^{-1})	Triglyceride (mmol L^{-1})
A (Normal control)	4.58±0.10^a	1.69±0.07^a
B (Calcium)	2.61±0.12^b	1.58±0.06^a
C (Artemisinin combination)	4.51±0.18^a	2.11±0.21^a
D (Artemisinin+calcium)	5.12±0.56^a	1.71±0.05^a

Results are mean±standard deviation of mean of five samples; all means in the same column with similar superscripts are not different from each other; the significant difference was at $p<0.05$

Table 2: Effect of treatment on plasma lipid

	Plasma lipid (mmol L^{-1})				
Group/treatment	Total cholesterol	Triglyceride	HDL-c	LDL-c	VLDL-c
A (Normal control)	6.02±0.10^a	1.37±0.26^a	3.30±0.39^a	2.58±0.26^a	0.63±0.21^a
B (Calcium)	7.94±0.22^b	2.78±0.23^b	1.94±0.31^b	3.29±0.12^b	0.81±0.20^a
C (ACT)	6.28±0.18^a	1.60±0.09^a	2.59±0.20^a	2.49±0.07^a	0.57±0.04^a
D (Calcium+ACT)	7.47±0.20^b	1.13±0.42^a	2.82±0.19^a	2.33±0.21^b	0.52±0.20^a

Results are mean±standard deviation of mean of five samples; all means in the same column with similar superscripts are not different from each other; the significant difference was at $p<0.05$

Table 3: Effect of treatment atherogenic and coronary risk index

Groups/treatment	Atherogenic index	Coronary risk index
A (Normal control)	2.49±0.32[a]	0.650±0.22[a]
B (Calcium)	3.54±0.26[b]	0.870±0.19[a]
C (Artemisinin combination)	2.15±0.13[a]	0.600±0.02[a]
D (Artemisinin+calcium)	2.89±0.37[a]	0.259±0.01[a]

Results are mean±standard deviation of mean of five samples; all means in the same column with similar super scripts are not different from each other; the significant difference was at $p<0.05$

rats administered with calcium may be related to the increase serum cholesterol and subsequently reduced hepatic cholesterol synthesis through depression of HMG-CoA reductase (Martynez-Gonzalez *et al*., 2004). This result corroborates previous laboratory studies (Li *et al*., 2012). Calcium may affect the risk of developing CVD through multiple mechanisms including blood cholesterol, insulin secretion and sensitivity, vasodilation, inflammatory profile, thrombosis, obesity and vascular calcification (Wang *et al*., 2005). In a previous study by Wang *et al*. (2005), it was reported that calcium may builds up in plaque, the cholesterol-filled pockets that grow inside arteries like tiny pimples. The consequent effect of narrowing arteries as a result of cholesterol deposition is that the plaque can choke off the supply of blood to heart muscle and other vital tissues. If a plaque bursts open, it can trigger a heart attack, stroke or sudden cardiac arrest. Based on data from the study therefore, researchers thus opined that over time, calcium can accumulate in arteries. This will makes them stiffer and less responsive to the demands of the body and thus contributing to high blood pressure, angina (chest pain with exertion or stress) and heart failure.

The LDL or harmful cholesterol transports free cholesterol and cholesterylester into the tissues where they accumulate and foster arteriosclerosis. The observed low level of hepatic cholesterol in rats placed on calcium supplement reported in the present study correlated with the decrease HDL cholesterol and increased LDL cholesterol observed in the same group of rats. Patients with arterial disease can have elevated VLDL or LDL or both (Murray *et al*., 1990). Although, the study with calcium supplements noted an increased LDL-cholesterol, the VLDL cholesterol was normal. Researchers also noted, that though separate administration of ACT does not contribute to alteration in these parameters, its co administration with calcium does not also prevent elevation of both total cholesterol of the plasma and plasma LDL cholesterol values elicited by calcium supplement. Data from this study therefore suggests that treatment of an individual with artemisinin-based combination drug when placed on calcium supplement may also predispose to cardiovascular diseases. No alteration was observed in the hepatic lipid with separate and combined administration of ACT with calcium. The findings in this study support the previous report of Georgewill and Ebong (2012) which noted in a toxicological study that artemisinin combinations causes no significant treatment related histopathological changes in the heart, liver and kidney of all treated rat groups as the tissues showed normal cytoarchitecture comparable to that of the control.

Body cells extract cholesterol from the blood by means of receptors on their surfaces. These receptors bind with the LDL particles (and their attached cholesterol) and draw them from the blood into the cell. When more LDL particles are captured by the receptors of the body cells, the formation of more receptors on that cell's surface is inhibited, thus lowering its future intake of cholesterol. Fewer receptors on the body cells mean that less cholesterol is ingested by the cells and that more remains in the blood stream. This thus increases the risk of cholesterol (LDL) accumulating in the internal walls of the blood vessels. The increase in LDL observed in the study with calcium supplementation researchers opined, may increase the rate of transportation of free cholesterol and cholesterylester into the tissue where they could accumulate and foster arteriosclerosis. Thus, the researchers suggest, it may be the mechanism by which increased calcium supplementation predispose to cardiovascular disease. Also, the decrease in HDL may have caused a reduction in the rate of transportation of free cholesterol and cholesterylester from tissues to the liver where it is converted to bile acid (George and Pamplona, 2005; Murray *et al*., 1990).

CONCLUSION

The result of this study further validate some of the initial studies which reported that increased calcium supplementation can predispose to cardiovascular diseases and disagree with studies that reported that calcium supplementation does not correlate with cardiovascular disease. Again, researchers are reporting based on the result of the present study that administration of therapeutic dose of artemisinin-based drug and its co administration with calcium may not increased the tendency of calcium supplement predispose to cardiovascular disease.

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