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A study on the combined administration of chloroquine and insulin effect on serum transferases and phosphatases activity during increased dietary fat and calcium consumption

Ajani E. O.*, Salau B. A., Ezima E. N. , Oyefuga O. H. and Osineye P. O

Department of Biochemistry, Faculty of Basic Medical Sciences, Olabisi Onabanjo University, Remo Campus, Ikenne, Ogun State, Nigeria.

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Previous studies reported on relationship between chloroquine administration, insulin and glucose homeostasis, but the role of dietary fat and the combined effect of the drugs on liver function have not been exploited. The present study was set up to investigate the effect of combined administration of insulin and chloroquine on serum transferase and phosphates activity during increased dietary fat and calcium consumption. Thirty (30) adult male albino rats randomly assigned into five (5) groups were used in the study. Group A was placed on normal diet (normal control); B (test control) C, D, and E were placed on an increased dietary fat and calcium. Drugs were administered as follows: group C, insulin (100 µg/kg body weight once daily); group D, chloroquine (Imarsel Chemical Co. Ltd., Chinon, Hungary, 15 mg/kg body weight) thrice weekly over a period of 12 weeks, and group E, chloroquine combined with insulin. All rats were sacrificed after 15 weeks treatment period. Blood was withdrawn and activities of the enzymes were determined. Combined administration of the drugs increased serum alanine amino transferase, aspartate transaminase and alkaline phosphate activity and decreased acid phosphatase activity. The result suggests that combined administration of insulin and chloroquine may result in hepatic injury particularly in individual whose dietary regimen includes increased fat and calcium.

Key words: Chloroquine, insulin, liver function, transaminases, phosphatases, dietary calcium, fat.

INTRODUCTION

Calcium is a key component in all cells for maintenance of proper structure of membranes and organelles. It is also a pivotal regulator for a wide variety of cellular functions as a major second messenger from plasma receptors (Newmark, 2000; Newmark et al., 1984). The ubiquitous role of calcium and its binding proteins in the regulation of cell function is an established principle of cellular physiology (Ramussen et al., 1984; Chung, 1980). Calcium is recognized to be a kind of universal second messenger within cells, transforming a variety of signals into appropriate cellular actions and involving a broad range of processes ranging from blood clotting, neuromuscular transmission, secretion and cell division (Robert, 1990).

*Corresponding author. E-mail: immanbisi@yahoo.com. Tel: 08055533192.

Calcium is regarded so powerful in this regard that the intracellular free calcium ion levels in a resting cell must be kept extremely low (four to six order of magnitude below the level in extracellular fluid). Because of this central importance of calcium for fundamental cell functions, most organisms have evolved a variety of mechanisms for dealing with excess or deficiencies of calcium (more often than not it is the excess) (Heaney et al., 1977). Calcium toxicity takes two forms: hypercalciuria and hypercalcemia.

Chloroquine is a drug that is widely used in West Africa for the treatment of malaria fever. It has been reported to be concentrated in the liver and many other tissues following its administration (Adelusi and Salako, 1982) . In toxic doses, it is known to cause appreciable cellular damage to liver, kidney and heart muscle (deGroot et al., 1981; Ngaha, 1982). It is an acidophilic weak drug that will accumulate in the acid environment of the endosome,

raising its pH. This may interfere with the dehydration of the internalized insulin receptor complex. It has a wide distribution in the body with a particular affinity for melanin-containing tissue and may affect the function of tissues such as the eye and the uterus (Anderson et al., 1980). A direct interaction of chloroquine with insulin receptor, resulting in a reduced rate of dissociating insulin from the receptor has been reported (Adelusi and Salako, 1982). This may increase the biological half-life of the activated receptor and may prolong the action of the insulin (Smith et al., 1987).

The release of insulin is calcium dependent and appears to be associated with changes in the plasma membrane potential. It is believed that the mechanism of insulin release is that an increase in blood glucose increases the influx of calcium ions into the B cells leading to membrane depolarization and subsequent release of contents of the granules (Bevan et al., 1997). Although it is usual to associate insulin with regulation of blood sugar, it is well known that insulin affects fat and protein metabolism almost as much as it does for carbohydrate metabolism. Indeed in diabetic patients, abnormalities of fat metabolism result in such complications as acidosis and atherosclerosis which could be the cause of death (Frank et al., 1994).

Of particular interest to this study are reports linking chloroquine to insulin and glucose homeostasis. In an earlier study, we reported that administration of insulin and chloroquine resulted in reduction of serum glucose level and an improved kidney function (Ajani et al., 2004). The liver plays essential role in drug metabolism and this possibly explains the wide variety of drug-induced liver lesions that are produced and their relative frequency. The measurement of the activities of various enzymes in tissues and body fluids provided a significant and well known aid in disease investigation, diagnosis, assault on the organs/tissues and to a reasonable extent the toxicity of the drug (Yakubu et al., 2003).

The present study aimed at investigating the activities of the phosphatases and transaminases enzymes, (which are important in diagnosing liver functions) when chloroquine and insulin are co- administered to subject whose dietary regimen consist of increased fat and calcium.

METHODOLOGY

Experimental subjects

The study was carried out with thirty (30) adult male albino rats of the Sprague- Dawley strain weighing 165 ± 18 g. The rats were randomly assigned into 5 groups and housed in plastic cages with wood-chip bedding in a humidity controlled room with 12 h light/dark cycle. The animals were kept in separate cages labeled A - E. They were all allowed free access to their respective diets and clean drinking water on which they were stabilized for three (3) weeks.

Dietary regime

Two (2) separate diets were used in the study. The feeds were composed according to Olowokere et al. (1994). The control diet consist of 57% carbohydrate; 28% protein; 7.0% fats; 1.5% vitamins and 5.95% salt mix (consisting of 21.7% calcium). Rats in group labeled A were placed on this diet. The test diet on the other hand consists of 46.7% carbohydrate; 24.5% protein; 21.3% fats; 1.5% vitamins and 5.95% salt mix (consisting of 51.97% calcium). Rats in groups labeled B, C, D and E were placed on this diet. Each group was maintained on their respective diet *ad libitum* for 15 weeks.

Administration of drugs

Insulin (100 µg/Kg body weight) was administered intramuscularly as a single dose daily into rats in groups C and E. Chloroquine phosphate (Imarsel Chemical Co. Ltd. Chinon, Hungary; 15 mg/Kg body weight) dissolved in 0.2 ml of sterile saline was administered subcutaneously into rats in groups D thrice weekly over a period of 12 weeks. All treatments were made to commenced 3 weeks after adaptation to respective diet. At the end of the treatment period, all rats were fasted overnight and anaesthetized by keeping in an enclosed container containing diethylether. Blood was then withdrawn by cardiac puncture.

Enzyme assays

Serum acid phosphatase (EC. 3.1.3.2) and alkaline phosphatase (EC. 3.1.3.1) were determined using carbonate acetate buffer by measuring the 4-nitrophenol liberated from 4-nitrophenyl phosphate at 400 nm (Wright et al., 1972a; Wright et al., 1972b). Protein concentration was measured by Biuret method (Wright et al., 1972b; Plummer, 1978). Serum alanine aminotransferase (GPT) was determined by monitoring the concentration of pyruvate hydrazone formed with 2,4-dinitrophenylhydrazine (King, 1978). Aspartate amino transferase (GOT) activity was measured by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4-dinitrophenyl hydrazine (Reitman and Frankel, 1957).

Statistical analysis

Results were expressed as mean ± SEM. n values were the same (5) for both control and test animals. The data were analyzed using paired Student's t-test, choosing a p value of < 0.05 as the level of significance.

RESULTS

Results for the serum phosphatase activities are presented in Table 1. Increased dietary fat and calcium signifycantly increased ($p < 0.05$) the serum acid phosphatase activity but has no significant ($p > 0.05$) effect on the serum alkaline phosphatase activity. Separate administration of chloroquine and insulin apparently does not alter the phosphatase activity. However, combined administration of insulin and chloroquine significantly reduced acid phosphatase activity. The observed activity for the acid phosphatase was not significantly different from the normal control value and the activity observed for the enzyme in other test groups, that is, groups D and E. The observed activity for the serum alkaline phosphatase, following combined administration of insulin and chloroquine was significantly higher than the activity of the enzyme in all other groups. Table 2 illustrates the effect

Table 1. Effect of treatment on phosphatase activity.

Group/ treatment	Enzyme activity (nM/min/mg protein)	
	Acid phosphatase	Alkaline phosphatase
A [Control diet]	0.17 ± 0.06^{a}	0.26 ± 0.03^{a}
B [Test diet, test control]	0.31 ± 0.04^{b}	0.25 ± 0.08^{a}
C [Test diet+ insulin]	0.19 ± 0.11^{ab}	0.29 ± 0.02^{a}
D [Test diet + chloroquine]	0.21 ± 0.08^{ab}	0.28 ± 0.06^{a}
E [Test diet + chloroquine + insulin]	0.19 ± 0.04^{a}	0.36 ± 0.03^{b}

Note:
- Values are mean ± SEM
- Number of subjects (n) = 5
- All values in the same column with different superscripts are significantly different from each other ($p < 0.05$).

Table 2. Effect of treatment on transaminase activity.

Group/ treatment	Enzyme activity (U/L)	
	Alanine amino transferase	Aspartate transaminase
A [Control diet]	10.21 ± 0.60^{a}	10.72 ± 0.03^{a}
B [Test diet, test control]	10.25 ± 0.30^{a}	10.69 ± 0.21^{a}
C [Test diet+ insulin]	30.65 ± 0.52^{b}	40.01 ± 0.08^{b}
D [Test diet + chloroquine]	30.45 ± 0.23^{b}	40.18 ± 0.14^{b}
E [Test diet + chloroquine + insulin]	43.61 ± 0.32^{c}	50.16 ± 1.01^{c}

Note:
- Values are mean ± SEM
- Number of subjects (n) = 5
- All values in the same column with different superscripts are significantly different from each other ($p < 0.05$).

of administration of the drugs on the serum transaminase activity. Increased dietary fat and calcium do not signify-cantly altered transaminase activity in the serum. Separate administration of chloroquine and insulin, both increase significantly alanine amino transferase and aspartate tranaminase activity ($p < 0.05$) when compared with either the normal control or the test control value. The activity obtained for the insulin administered group and the chloroquine administered group were however not different from each other. Combined administration of the drugs significantly raised ($p < 0.05$) the transaminase activity. The values obtained for the alanine amino transferase was significantly higher than that of all other groups, whereas the observed value for the aspartate amino transaminase though significantly higher ($p < 0.05$) than the control value was not significantly different from the values obtained for the separate administration of the drugs ($p > 0.05$).

DISCUSSION

Normally, enzymes will not be found in the serum except there is damage to one or more organs or tissues of the body. Therefore, enzymes from diseased tissues or organs and from drug assault and other xenobiotics may become manifested in the serum resulting in increased activity since the increased might be a consequence of leakage from damaged tissue. Calcium toxicity has been associated with calcium deposition in the kidney and to subsequent renal failure (Heaney, 1982). There has however, been no report sighted on its hepatotoxicity. The report from this study thus suggest that high calcium diet is largely tolerated and may not potentate liver damage. Calcium has been described as an extremely safe nutrient with a wide margin of safety between repletion and toxicity except for unusual combination circumstances (Johnston et al., 1950).

Alkaline phosphatase is a marker enzyme for the plasma membrane and endoplasmic reticulum (Akanji and Niumanse, 1987). It is often employed to access the integrity of plasma membrane (Khan et al., 1985). Similarly, a rise in serum alanine amino transferase and aspartate transaminase activities has been described as the most important determinant in liver diseases (King, 1978). The increased in alanine amino transferase, aspartate transaminase and alkaline phosphatase activity following combined administration of both chloroquine and insulin observed in this study could indicate a hepatocellular damage. In our opinion, the increase in the activity of these enzymes observed here could be attributable to the loss

of membrane components into the extracellular fluid. Chloroquine in toxic doses has been reported to cause appreciable cellular damage to liver, kidney and heart muscle (Kukoyi et al., 2000). Coupled with this is the fact that chloroquine interacts with insulin from the receptor (Kukoyi et al., 2000), hence prolonging the action of insulin. This interaction of chloroquine and insulin may be the predisposing factor in the hepatocellular damage suggested by the observed changes in the marker enzyme's activity. Though chloroquine administration independently increases the activity of these enzymes, the increase was more significant with combined administration. The values obtained for the serum alanine amino transferase and aspartate amino transaminase in the groups studied were within the normal range for rat (10-45U/L, for AST and 10-35U/L for ALT), (Karen, 2001) except in group E where the values obtained for the combined administration of the drugs were noticed to be above normal reference range (43.61 ± 0.32 U/L for alanine amino transferase and 50.16 ± 1.01 U/L for aspartate transaminase). In one study, Khan et al. (1985) noted that chloroquine injection caused a marked accu-mulation of both insulin and its receptor distribution.

The observed decrease in activity of acid phosphatase following combined administration of the drugs, may suggest the possibility of the drugs playing an important role in cancer chemotherapy (though not monitored here). An increase in plasma acid phosphatase activity has been observed in men with metastasis from prostate carcinoma (Bevan et al., 1997). Again, dietary calcium has been reported to play a role in the promotion of mammary cancer caused by dietary fat (Newmark et al., 1984).

Conclusion

The result of this study indicates that combined administration of chloroquine and insulin may result in hepatic injury. Based on the findings reported in this study, we thus opined that the role of combined administration of chloroquine and insulin in cancer particularly prostate cancer need to be further investigated.

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