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Combined effect of chloroquine and insulin administration on some biochemical parameters in rats placed on high fat and calcium diet

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Summary

Effect of combined administration of Insulin and Chloroquine on fasting blood glucose, total protein, creatinine and uric acid concentration were investigated in rats placed on diets high in fat and calcium (SP diet). Thirty-six (36) rats (grouped into six) were placed on different treatment: Grams A and B were fed with NP (normal diet) and SP diet respectively; Group C was placed on NP diet and injected intramuscularly with 100µg insulin per day; Group D was placed on SP diet and also injected with 100µg insulin per day; Group E was placed on SP diet and injected with both insulin (100µg/day) and chloroquine (20mg/Kg thrice weekly); Group F was placed on SP diet and injected with chloroquine (20mg/Kg) thrice weekly. After 15 weeks of treatment, a significantly reduced concentration of glucose was observed in groups injected with insulin and those injected with insulin and chloroquine together (compared with the control groups, A and B). The serum total protein and uric acid level were however not significantly different in all the rats. Serum creatinine was also observed to be significantly lowered in the rats treated with insulin. The results of this study thus suggest that insulin and chloroquine administration may result in reduced blood glucose level (hypoglycemia). It also suggests that insulin and chloroquine administration may further effect an improved kidney function.

Key words: *Diet, calcium, high fat, insulin, chloroquine, creatinine and glucose.*

Résumé

L'effect de l'aministration combinée de l'insuline et la chloroquine sur le taux de glucose a jeune., des proteines totale, du taux de créatinine et la concentration d'acide urique étaient investigués aux rats sous régime lipide et de calcium. Trente six rats groupes en six étaient soumis a ces regimes obtaines des traitement different. Le grupe A et B nouris d 'un régime normale de NP et SP respectivement , le groupe C obtenait le NP plus 10 ug d insuline par jour intramusculaire, le grupe D du SP plus 100 ìg d insuline par jour , le groupe E du SP plus 100 ìg d'insuline parr jour et 20mg/kg trois fois par semaine et le groupe F du SP plus 20 mg/kg chlroquine trois fois par semaine. Apres 15 semaines de traitement on observait une réduction significative de la concentration du glucose aux groupes qui ont récu l'insuline et ceux qui récu l'insuline et la chloroquine. La quantite totale des protéines et le taux d acide urique étaient cependant pas significativement different chez les rats. La créatinine en sérum était aussi observée étant significativement faible chez les groupes qui récu l'insuline. Les résultats de cette étude montre que l'administration de l'insuline et la chloroquine pourrait résulter à la réduction du taux du glucose (hypoglycémie) et aussi pourrait améliorer la fonction rénale.

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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 [1,2]. The ubiquitous role of calcium and its binding proteins in the regulation of cell function is an established principle of cellular physiology [3, 4]. Dietary calcium has being noted to play a role in the promotion of mammary cancer by dietary fat [2]. Other factors that has being noted to play active roles include: (a) dietary amount of vitamin D, which facilitates the intestinal absorption of calcium, and (b) the level of dietary phosphate, which is readily absorbed leading to increased serum phosphate [5].

An increasing number of supportive scientific studies in humans and animals suggest a chemical interaction between components of digested fat and calcium [6] as one mechanism by which cell functions are regulated. In the arena of animal experimentation, most investigators have found support for the "calcium hypothesis". The hypothesis states that under certain conditions governed by colonic pH, the potential of dietary lipids to accelerate epithelial proliferation (a biological forerunner to cancer) can be largely modified [7, 8]. Calcium has being noted to have a capacity to bind ionized lipids forming complexes of calcium, soap and fatty acids [9]. As a rule, fatty acids

and bile acids soaps are thought to be far less damaging to tissue [10].

Chloroquine-4-aminoquinoline is the most commonly used drug in the treatment of malaria worldwide and for rheumatoid arthritis therapy [11]. 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 tissue such as the eye and the uterus [12]. Of particular interest to this study are reports linking chloroquine to insulin and glucose homeostasis. A direct interaction of chloroquine with insulin receptor, resulting in a reduced rate of dissociating insulin from the receptor has been reported [13]. This may increase the biological half-life of the activated receptor and may prolong the action of the insulin [14]. Studies in *vitro* with pancreatic β-cells have shown that chloroquine inhibits insulin biosynthesis but promotes insulin release [15]. Chloroquine also inhibits insulin degradation by isolated rat adipocytes [16], mouse fibroblasts [17], rat hepatocytes [18], and rat kidney tubules [19]. Thus there is ample and consistent evidence *in vitro* that chloroquine profoundly inhibits the insulin degradative pathway resulting in the intracellular accumulation of intact ligand and a reduction in the release of degraded products.

In this present study, an attempt is made to investigate the changes in serum glucose, total protein, creatinine and uric acid level during a combined administration of insulin and chloroquine in rats that were placed on high fat and calcium diet.

Methodology

Experimental subjects

Thirty-six male (36) albino rats weighing between 150-180g obtained from the animal house, University of Ibadan were used for the study. The rats were divided into 6 groups labeled A, B, C, D, E and F and placed on separate diets on which they were stabilized for 3 weeks.

Formulation of feeds

Two (2) forms of feeds were used in the study: the SP diet which consists of carbohydrate, 17.40%; fats, 33.25%; protein, 39.25%; vitamin, 0.19% and calcium, 7.78% and the NP diet which on the other hand, consists of 22.27% carbohydrate, 54.44% protein, 16.04% fats, 0.22% vitamins and 4.55% calcium. The animals were maintained on their respective feeds *ad libitum* for 15 weeks as stated below:

Group A; normal control = NP diet.
Group B; test control = SP diet.
Group C; test = NP diet + insulin
Group D; test = SP diet + insulin
Group E; test = SP diet + insulin + chloroquine
Group F; test = SP diet + chloroquine.

Administration of drugs

Insulin (100 μg /kg) was injected intramuscularly as a single dose daily into rats in group C, D and E. Chloroquine diphosphate {(20mg/kg body weight) in 0.2ml of saline (150mmol/L NaCl)} was injected intramuscularly into rats in groups E and F thrice weekly over a period of 12 weeks. Group A was kept as normal control while group B was kept as test control. All treatments were made to commence 3 weeks after adaptation to respective feeds.

At the end of the treatment period, all rats were fasted overnight and then sacrificed by keeping in an enclosed container containing chloroform. Blood was then withdrawn by cardiac puncture.

Analytical methods

Serum glucose was determined by glucose oxidase method. In the determination, aminophenazone was used as oxygen acceptor. The hydrogen peroxide formed in the presence of glucose oxidase reacts under catalysis of peroxide with phenol and 4-aminophenazone to form a violet quinone that is estimated photometrically [20]. Total protein was determined by the biuret method. The test involves the reaction of plasma protein with cupric ions in alkaline medium to produce a violet colour whose intensity is estimated photometrically [21] Creatinine was estimated by the Jaffe reaction method. In the determination, creatinine reacts with picrate in alkaline solution and at room temperature. A red colour is produced within few minutes and this is estimated spectrophotometrically [22]. Estimation of uric acid was carried out by the Block and Geib method. The method relies on development of blue colour (tungsten blue) as phosphotungstic acid (PTA) is reduced by urate in alkaline medium. The colour is read spectrophotometrically [23].

Statistical analysis

Results are given as means SEM. n values are the same for both control and test groups. The data were analyzed using the paired Student's t-test, choosing a p value of 0.05 as the level of significance.

Results and discussion

After 12 weeks of treatment, the blood glucose levels of the control groups (A and B) were not significantly

different from each other. Significantly lowered serum glucose levels were however observed in all the test groups placed on insulin compared with the control groups. Insulin has been reported to affect transport of glucose and other substances across cell membrane and in many kind of cells in many different tissues [24] The blood glucose lowering of insulin has been hypothesized to be regulated by insulin-stimulated deployment of glucose transport protein into the cell surface [25]. The result also indicated that when combined, chloroquine and insulin reduces blood glucose significantly compared with when administered singly. Chloroquine has been reported to affect insulin metabolism in two ways: by reduction in insulin clearance from the circulation and by increasing its secretion during fasting and an hyper insulinemia [26]. A reduced fasting blood glucose level along with reduced weight gain has earlier being reported in rats administered with chloroquine [27]. In another report, a significant hypoglycaemic effect of chloroquine has been observed after 3 days in human subjects with non-insulin dependent diabetes mellitus, suggesting that it may be therapeutically useful in Type II diabetes [28]. In this study, it was also noted that the blood glucose level of rats treated with chloroquine alone (group F) was not significantly different from the control groups (group A and B). A possible reason may be that chloroquine, which is a cumulative drug may reach a threshold concentration in the tissue before its effects manifests themselves.

No significant alteration was observed in the serum total protein of all the test rats when compared with the control groups. The serum total protein did not also

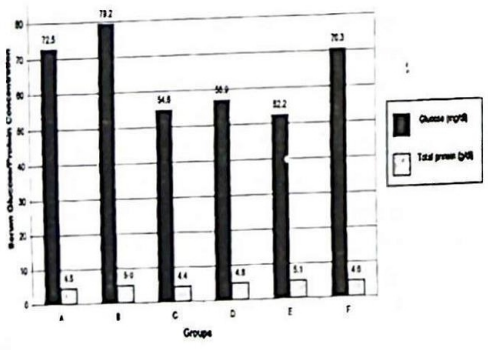

Fig. 1:

significantly differ between the test groups (Figure 1). Though data on serum total protein variation in rats during administration of insulin are scarce, many hormones and

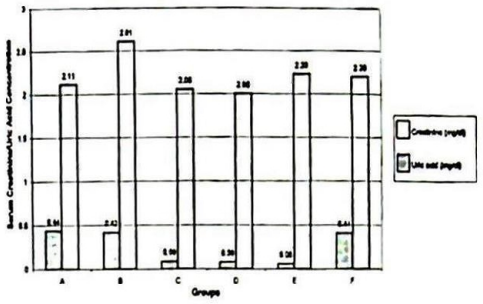

Fig. 2:

particularly insulin, when administered *in vivo* have been reported to markedly alter the rate of protein synthesis in a specific tissue and even more generally. It could be reported here that diet high in fat and calcium, does not significantly affect serum total protein level. This report is in close agreement with the earlier findings of Calloway and Margon [29]

The result for serum creatinine concentration is shown in Figure 2. No significant variation was observed in the serum creatinine level of the control groups. Administration of insulin alone or when combined with chloroquine is seen here to have significantly reduced the serum creatinine concentration. Creatinine is a protein produced by muscle and released into the blood. Since creatinine level in the serum is a measure of kidney function, if kidney function falls, the creatinine level will rise [30]. Impaired renal function may indicate obstructive nephropathy, although quite severe acidosis may occur before biochemical abnormalities develop. This result therefore suggests that administration of insulin may improve kidney function.

No significant variation in the serum uric acid of all the test animals was noticed when compared with the controls after the treatment period (Figure 2). This result suggests that neither the diet nor the administration of insulin and chloroquine significantly alter serum uric acid level. Uric acid results as a relatively insoluble product of purine metabolism. The concentration of uric acid in the plasma depends on dietary ingestion. Other factors such as increased renal tubular urinary uric acid secretion, decreased renal tubular uric acid reabsorption, decreased urine water content or increased hydrogen ion concentration may also affect the plasma uric acid concentration [29].

Conclusion

These results suggest that administration of insulin and chloroquine may subsequently results in hypoglycemia.

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