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Single Adult-human Equivalent Dose of Intramuscular Chloroquine did not Lower Blood Glucose Level in Fasted Wistar Rats

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Abstract

Objectives: The 4-Aminoquinoline, Chloroquine is still indicated for treatment of malaria fever due to sensitive Plasmodium species. The hypoglycemic effects of Chloroquine have been sparsely reported in human and animal models of type 2 diabetes and a widely held perception among health caregivers in this part of the world is that administering Chloroquine intramuscularly to fasting malaria patients could be complicated by syncope, directly caused by hypoglycemia. This study investigated the effects of a single adult human intramuscular bolus of Chloroquine on the blood glucose level in fasting non-diabetic Wistar rats.

Methods: Fifteen acclimatized adult male rats were randomly divided into three groups of 5 per group. Group I and II rats were fasted overnight and received 4.17 mg/kg of intramuscular Chloroquine in the morning. Group III rats were also fasted but received 1 ml of sterile water injection. Fasting blood sugar level was determined immediately after injection and at 2 h and 4 h intervals using Accucheck glucometer.

Results: The results were recorded as Mean ± SEM. The mean sugar levels of the groups were compared with each other within a given time point as well as between the different time intervals within a given group, using the Student's t test of significance. There were no observable statistical significant differences between the Mean blood glucose levels of the groups.

Conclusion: The results of this study suggested that intramuscular Chloroquine injection in fasted non-diabetic rats did not significantly lower their blood glucose level and syncope in fasting adult humans immediately following treatment by this route is unlikely to be related to a Chloroquine-induced hypoglycemic effect.

Keywords: Adult-human equivalent dose, Intramuscular, Chloroquine, Blood glucose, Fasted rats

Introduction

The 4-Aminoquinoline, Chloroquine is still effective for treating malaria fever due to sensitive Plasmodium species in many developing countries [1,2]. It is readily available, affordable and has very good oral bioavailability in healthy subjects and children with uncomplicated falciparum malaria [3,4]. In seriously ill-patients, this route of administration may not be effective because they are either vomiting or unconscious [5]. Chloroquine injection is commonly used in these situations, intramuscular more often compared to either the intravenous or subcutaneous routes [6]. Occasional deaths have been recorded following intramuscular Chloroquine, with the principal toxicities of intravenous 4-Aminoquinolines, Chloroquine inclusive, reported to include hypotension and dysarrythmias [7]. The choice of using the intramuscular route is often made in the setting of vomiting or diarrhea, but there are instances where malaria patients volunteer it as the only effective treatment-route for them, an observation that could be adduced to the fivefold higher peak plasma concentration achievable following either intramuscular or subcutaneous Chloroquine injections, compared to the oral route [6]. Parenteral administration of Chloroquine could become complicated with syncope and sudden death [8,9]. This is considered by a few health caregivers to be the consequence of an induced hypoglycemia following the intramuscular administration of the drug to fasting anorexic patients. This fear of hypoglycemia rather than hypotension have sometimes resulted in caregivers delaying the commencement of prescribed Chloroquine injections in fasting patients, insisting that these patients must eat good meals before any commencement of prescribed treatment, even with intravenous dextrose infusion already set up and running. Both hypoglycemic and hyperglycemic effects of Chloroquine have been suggested in two reported *in-vivo* studies and changes in insulin release and clearance rates have been proposed as possible mechanisms of action, but the doses used in both of them did not simulate the dosing pattern in adult humans [10,11]. This study investigated the effect of a single bolus of intra-muscular Chloroquine, within the anti-malarial therapeutic dose range of adult humans, on the blood glucose levels of fasted Wistar rats to either validate or refute this mythical rapid-onset intramuscular Chloroquine-induced fatal hypoglycemic syncope.

Methods

Animal husbandry

Fifteen adult male Wistar rats weighing between 151-184 g were procured from the breeding colony of the University of Benin animal facility. They were acclimatized for 2 weeks under standard hygienic conditions at the animal house of the Faculty of Basic Medical Sciences, Delta State University Abraka before the experiment commenced. They had free access to clean drinking water and Food (Top Feeds Nigeria) as well as 12 h light/dark exposure cycles. The room temperature of the animal macro-environment was 29-32°C during the study period. The work was approved by the Research Ethics Committee of the College of Health Sciences, Delta State University, Abraka, Nigeria. The care and handling of the animals were humane throughout the project [12].

Grouping of the animals

The animals were randomly allotted to 3 groups of 5 rats per group (n=5). Group I rats were fasted overnight, given intramuscular Chloroquine injection (Yanzou Xier-Kangtai Pharmaceuticals, China) in the morning and had their blood glucose level determined immediately post injection and at two hourly intervals until the 4th h. They remained fasted throughout the study period.

Group II rats were fasted overnight, given intramuscular Chloroquine in the morning and their blood sugar levels were determined immediately post injection after which they were allowed free access to their food and water. Their blood sugar levels were subsequently determined at 2 hourly intervals until the 4th hour.

Group III rats were fasted overnight and administered 1 ml of sterile water by intramuscular injection. Blood sugar level immediately post-injection and 2 hourly intervals were then determined until the 4th hour as above.

Determination and administration of chloroquine dose to the Rats

The injections were administered to the thigh muscles of each rat using disposable 25 G needles and 1 ml syringe. The dose of Chloroquine administered to each rat was calculated as one-sixth of 25 mg/kg of animal weight because this total Chloroquine injection dose is traditionally administered to humans as six equally spaced (eight hourly or twelve hourly intervals) boluses for 2 or 3 days in this part of the world, with excellent treatment outcomes [13].

Determination of the blood glucose level of rats

The blood glucose levels were determined using Accu-Check glucometer which uses a Glucose oxidase method, according to the manufacturer's instruction. Each blood sample collection was done using a sterile needle puncture of the Saphenous vein [14]. Hemostasis was secured with digital pressure for 1 minute after each sample collection before the animals were returned back to their cages.

Statistical analysis

The blood sugar levels of each group were presented as Mean ± SEM at zero hour, 2 h and 4 h. The Means were compared within and between groups for significant statistical difference using Student's t-test with the aid of Microsoft Excel 2003 computerized statistical software. Level of statistical significance was set at P-values less than 0.05.
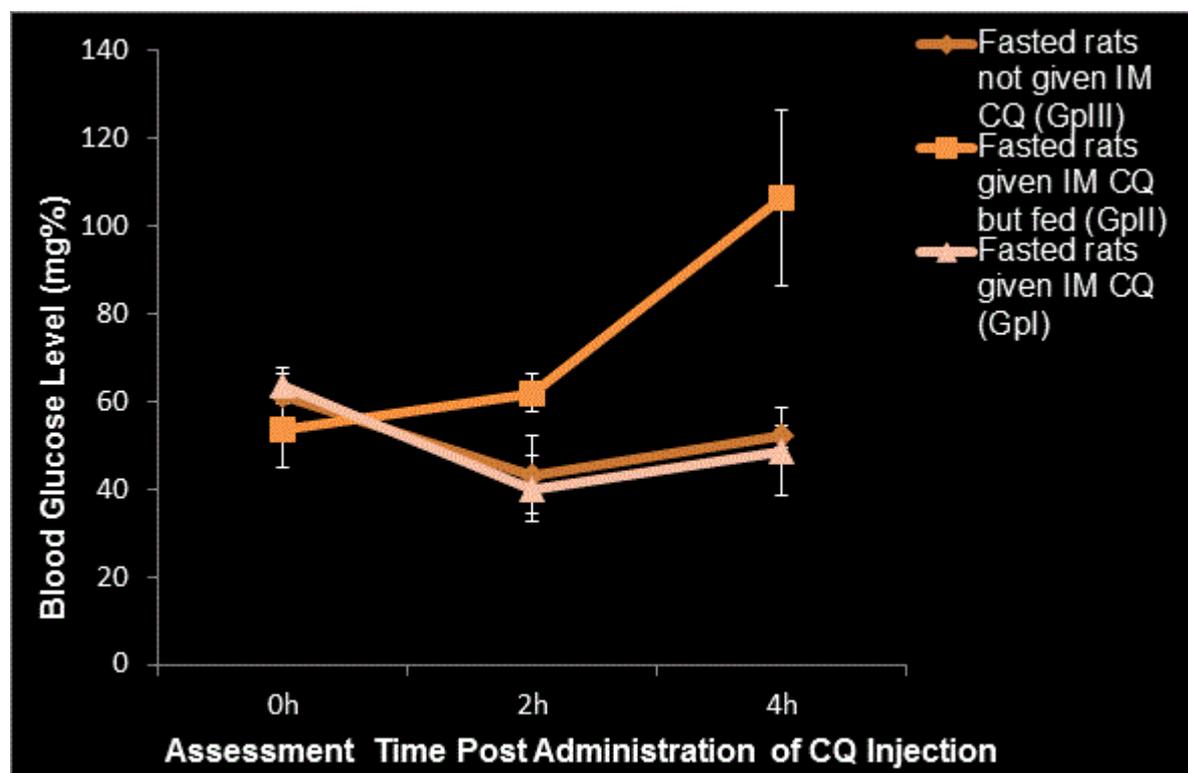
Results

Fifteen adult Wistar rats were fasted overnight and divided randomly into three groups n=5. Group I and II had 4.17 mg/kg of intramuscular Chloroquine (IM, CQ) injection while Group III rats had 1 ml of sterile water injected by the same route. Their blood glucose levels were determined immediately after they had the injections and Group II rats were allowed free access to food and water afterwards. Blood was again collected from the animals at the 2nd and 4th hour following their injections to monitor the trend in the blood sugar levels. Chloroquine injection did not significantly lower the Mean blood glucose level in fasting rats compared to untreated controls (Mean ± SEM, P>0.05, Student t Test). Access to food following injection Chloroquine allowed the blood glucose level to increase from the fasting level to much more safer ranges at the 2nd and 4th hours.

Discussion

The result of this study show that the zero hour sugar level (0 h) representing the immediate post-treatment fasting blood glucose level was higher in group III rats than in Groups I and II (Figure 1) but there were no significant differences between the observed Means of the groups (Table 1). This was suggestive that intramuscular Chloroquine injection had no significant immediate effect on the fasting blood sugar level. The 2 h and 4 h blood sugar levels of fasted rats, given intramuscular Chloroquine, closely paralleled those of fasted untreated rats, with slightly higher Means recorded in the former (Figure 1). This observation would be unexpected had the Chloroquine injection resulted in any immediate or sustained hypoglycemic effect in the fasted experimental subjects. The non-observance of any hypoglycemic effect is further supported by the observed lack of statistical significant differences in the observed Means within and between the groups at these two assessments time points (Table 1). The second hour (2 h) and fourth hour (4 h) postprandial glucose levels of group II rats that had free access to drinking water and food after receiving the Chloroquine bolus, progressively increased to higher levels than those of group I and III rats that remained fasted during the study period. Again there were no statistical significant differences (all P values >0.05) between the blood glucose levels for Gp II vs. Gp I and Gp II vs. Gp III at the different assessment times (Table 1).

Hypoglycemia is not often listed among the adverse effects of Chloroquine in popular and standard pharmacology textbooks and there are not many reported animal or clinical research studies supporting this side effect in the literature. A cited work reported hypoglycemic effects of Chloroquine in diabetic Wistar rabbits but used a supra-therapeutic dose level of 25 mg/kg twice daily for three days [11]. The unusually high dose used for the study negates the use of such finding as evidence linking Chloroquine-induced hypoglycemia as a possible cause of syncope observed following an intramuscular injection with the usual therapeutic anti-malarial dose, in fasted non-diabetic humans, An earlier study had also linked Chloroquine and Fansidar* with hypoglycemia [15].

Figure 1: Effect of Single Adult Human Equivalent Intramuscular Chloroquine Bolus on the Blood Glucose Level of Fasted Wistar Rats. Fifteen adult Wistar rats were fasted overnight and divided randomly into three groups n=5. Groups (Gp) I and II had 4.17mg/kg of intramuscular Chloroquine (IM, CQ) injection while Group III rats had 1ml of sterile water injected by the same route. Their blood glucose levels were determined immediately after they had the injections and Group II rats were allowed free access to food and water afterwards. Blood was again collected from the animals at the 2nd and 4th hour following their injections to monitor the trend in the blood sugar levels. Chloroquine injection did not significantly lower the Mean blood glucose level in fasting rats compared to untreated controls (Mean ± SEM, P> 0.05, Student t Test). Access to food following injection Chloroquine allowed the blood glucose level to increase from the fasting level to much more safer ranges at the 2nd and 4th hours.

The perception that intramuscular Chloroquine could cause post injection syncope if received without eating a meal could have originated from caregivers mistaking Chloroquine for Quinine which is known to produce hypoglycemia by stimulating insulin secretion by beta cells in the pancreas [16]. Although hypoglycemia causes syncope, collapse is more commonly associated with cardiovascular dysfunctions including hypovolemia and arrhythmias [17]. Chloroquine has been reported to produce these cardiovascular effects when administered as intravenous bolus [6], which could occur during a misdirected intramuscular injection [18-20]. A higher risk for cardiovascular toxicity of Chloroquine has been reported in volume-depleted and electrolyte deranged patients with gastroenteritis, and more so if the dose administered is inappropriately high for the patient's weight [21]. Chloroquine is safely administered as a slow intravenous infusion given over 30 minutes, but an inadvertent intravenous bolus could sometimes be given following failure to adhere to proper techniques of administering intramuscular injections [6,22-24]. Any confirmed hypoglycemia in a patient with malarial fever that collapsed immediately after an intramuscular injection of appropriate dose of Chloroquine could well have been a consequence of reduced appetite and food intake as well as consumption of blood glucose by the malaria parasite [25].

The observation in this study that the trend in blood sugar levels of fasted rats after intramuscular Chloroquine injections was not significantly lower compared to distilled water treated rats contradicts the hypothesis that could directly induce hypoglycemia in fasted human subjects. The result of this study also showed that ingestion of food around the injection time, obviously helped to maintain the blood sugar level within safer ranges, reflected by the higher mean 2 h postprandial blood glucose level of group II rats compared to that of group I rats. Syncope or sudden death following intramuscular injection would more likely be a cardiovascular complications of an intravenous Chloroquine bolus [8,9] than a hypoglycemic action of the usual therapeutic intramuscular anti-malarial dose. Although a reduction in fasting plasma glucose level has been reported in rats following intravenous administration of 45 mg/kg Chloroquine in equal divided doses over nine days [26], the dose was by any standard higher than what is normally administered intramuscularly to adult humans. A similar study on the short term and long term effects of Chloroquine on glucose metabolism, reported lowering of the blood

glucose levels, but again the doses were higher than the 25 mg/kg recommended for treating malaria [27].

Blood Glucose Level Analysed	P Value
0 h vs. 2 h (GP1)	0.3485
0 h vs. 4 h (Gp1)	0.4162
2 h vs. 4 h (Gp 1)	0.4351
0 h vs. 2 h (Gp2)	0.1863
0 h vs. 4 h (Gp2)	0.2209
2 h vs. 4 h (Gp 2)	0.2846
0 h vs. 2 h (GP3)	0.1277
0 h vs. 4 h (Gp3)	0.170
2 h vs. 4 h (Gp 3)	0.2881
GP1 vs. GP2 (0 h)	0.2294
GP1 vs. Gp3 (0 h)	0.6302
GP2 vs. GP3 (0 h)	0.1724
GP1 vs. GP2 (2 h)	0.3197
GP1 vs. GP3 (2 h)	0.7788
GP2 vs. GP3 (2 h)	0.1233
GP1 vs. GP2 (4 h)	0.2403
GP1 vs. Gp3 (4 h)	0.4085
GP2 vs. GP3 (4 h)	0.2204

Table 1: Outcome of statistical analysis of blood sugar levels within and between the different treatment groups of Wistar rats. GP-group. Intergroup and intra-group comparisons of means were statistically insignificant (All P-values >0.05).

One recent study showed that L6 muscle cells treated with insulin and Chloroquine exhibited enhanced Akt phosphorylation, Glycogen synthase activity and glucose uptake compared to cells treated with insulin alone, citing this as one possible mechanism underlying the reported hypoglycemic effect on humans and animal models of diabetes [28]. Another isolated case report of hyperinsulinemic hypoglycemia was reported following the oral treatment of a patient with rheumatoid arthritis with hydroxylchloroquine [29], but there has been no similar report for Chloroquine even though both drugs belong to the same pharmacodynamic class. The observation that the Mean 2-hour blood glucose level of Group II rats that remained fasted during the study period was numerically higher than that of Group III which was subjected to same conditions except receiving the intramuscular Chloroquine, further negated the possibility of a fatal Chloroquine-induced hypoglycemia, that could culminate in syncope in fasted subjects treated with appropriate dose of intramuscular Chloroquine. The risk of immediate post-injection fatal hypoglycemia may be higher when abnormally high dose is administered to the subject or when the combined action of parasite depletion of blood glucose and anorexia, a common clinical symptom result in a fall in the blood glucose to symptomatic hypoglycemic levels, even before the administration of the injection. To avoid syncope complicating the intravenous administration of Chloroquine to patients in whom the intramuscular route is contraindicated, as in hemophiliacs, Chloroquine is usually given diluted in normal or dextrose saline, infused over several hours with a close watch over the blood pressure [21].

Conclusion

The commonly used divided dose of intramuscular Chloroquine in adult humans did not significantly lower the blood glucose level in fasted rats. And Chloroquine induced hypoglycemia was unlikely to be responsible for any syncope observed immediately after or around the time of an intramuscular injection provided the dose was appropriate for the subject. Further investigation using this same model can be undertaken in human subjects. This would help to either validate or refute the conclusion of this study. It might be more helpful for health care givers to pay more attention to calculation of the appropriate dose and use of the right technique during intramuscular administration of Chloroquine. Although inadvertent intravenous boluses rather than induced hypoglycemia are more likely to be the cause of complication of syncope and even sudden death of the subject, one study suggested that at very high doses, Chloroquine could induce hypoglycemia.

Conflict of Interest

The authors declare that there is no conflict of interest.

Acknowledgements

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