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GLUCOSE HOMEOSTASIS IN CHLOROQUINE-TREATED NORMAL RATS

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ABSTRACT

Glucose tolerance (GT), peripheral insulin activity (A) and beta cell function (CIR) were improved on prolonged chloroquine administration to normal male rats, while the acute effects comprised hyperinsulinaemia with unaltered blood glucose levels. This study explains some of the seemingly conflicting results due to chloroquine treatment and concludes that the drug is not damaging to glucose homeostasis in long term treatment.

INTRODUCTION

Besides its extensive use as a prophylactic and therapeutic antimalarial agent, chloroquine is used in rheumatoid arthritis therapy (Asamoah *et al.*, 1989) and in the long-term treatment of collagen diseases (Andersson *et al.*, 1980). Despite its wide spread use, there is a number of contradictory reports regarding its effect on glucose homeostasis.

Chloroquine was reported to have an inhibitory effect on the hepatic degradation of insulin (Pease *et al.*, 1985) through its lysosomotropic

action (Dennis and Aronson, 1981) thereby causing retention of hepatic insulin (Terris *et al.*, 1979).

Furthermore, chloroquine was shown to increase the accumulation of insulin in the Golgi-lysosomal area of the adipocytes (Hammons and Jarett, 1982), hepatocytes (Varandani *et al.*, 1982) and pancreatic acini, as well as blocking the action of insulin on glucose uptake (Iwamoto *et al.*, 1983). It was also reported that it inhibits glycogen synthesis (Hofmann *et al.*, 1980 and Varandani *et al.*, 1982) and promotes insulin release (Dencker *et al.*, 1976 and Tjälve *et al.*, 1980) thereby theoretically hyperinsulinaemia together with hyperglycaemia might be anticipated. Experimentally, hypoglycemia was shown to occur in both man (Smith *et al.*, 1987) and animals (Okitolonda *et al.*, 1984) with unaltered plasma insulin levels.

On the other hand, other studies were unable to show any effect of chloroquine on blood glucose and insulin levels in man (Ericsson *et al.*, 1981) while others reported beneficial effects on glucose homeostasis (Smith *et al.*, 1987 and Quatraro *et al.*, 1988). Thus, it was found of interest to study glucose homeostasis in extended *in vivo* studies in normal rats, in search of a better understanding of the mechanism (s) underlying the effect of chloroquine.

MATERIAL AND METHODS

Sprague Dawly male rats weighing 150-200 g, were used in this study. The animals were housed in separate cages and allowed free access to standard diet and water.

For the chronic effects of chloroquine twenty rats were injected intramascularly three times a week by chloroquine diphosphate (20mg/kg body weight; Sigma chemical company. St Louis, Mo, USA) dissolved in 0.2ml saline (150 mM NaCl) over a period of 4 months. Control rats were administered saline only.

Blood was obtained from the retro-orbital venous

plexus of the eye using a heparinized capillary tube (Madway *et al.*, 1969) under light ether anesthesia. Oral glucose tolerance test (GTT) was undertaken at the start and after 2 and 4 months of treatment. The tests were performed after an overnight fasting. Glucose (40% solution in water) was introduced directly into the stomach using a stomach tube, at a dose of 2g/kg body weight.

The acute effects of chloroquine ten minutes after its administration, were studied on 18 rats divided to three groups. A blood sample was obtained prior to the start of the experiment and another was collected 10 minutes after. Only glucose was injected intraperitoneally to the 1st group, glucose and chloroquine (20mg/kg body wt) to the second group, while the third group was injected with chloroquine only.

Blood glucose was measured on the day of the experiment by Bio-Merieux glucose oxidase kit, (Trinder, 1969). The serum was then frozen at - 20°C until insulin was measured by coat-A count radio-immuno-assay kit (Berntorp *et al.*, 1983).

The areas under glucose and insulin curves during the oral glucose tolerance test were computed as described by Haffner *et al.* (1986), the beta-cell response (CIR) was deduced according to Sluiter *et al.* (1976a) while a parameter of peripheral insulin activity (A) and glucose tolerance (GT) was calculated according to Sluiter *et al.* (1976b).

For the statistical calculations the students t-test for paired observations were used.

RESULTS

Acute effects of chloroquine

The studies on the acute effects of chloroquine on insulin secretion showed that the level of serum insulin was higher after the injection of chloroquine, even more than when chloroquine was administered with glucose solution. No statistically significant

effects on glucose levels were recorded for chloroquine (Table 1).

Long-Term effects of chloroquine.

There was a significant decrease in the basal levels of glucose, while basal insulin levels remained unchanged during the 4 months of treatment. In response to oral GTT there was a decrease in the areas under glucose and insulin curves (Table 2 and Fig.1). The glucose tolerance (GT_p) was improved primarily due to improvement of peripheral insulin activity (A) and increased pancreatic function (CIR_p) (Table 3).

DISCUSSION

In vivo studies of the acute effects of chloroquine treatment showed that increased insulin levels (Andersson *et al*, 1980 and Asamoah *et al*, 1984) are not concomitant with hypoglycaemia which is in accord with the present study. The enhanced insulin release in the acute study might be attributed to be due to a direct membrane effect by virtue of chloroquine's marked ability to bind to cellular constituents (Seydel and Wasserman, 1976) and cell surface (DiDonato *et al.*, 1977) as well as to its inhibition of insulin degradation in target tissue (Kobayashi *et al.*, 1980).

On the other hand, the results of the long-term effects of chloroquine in normal rats showed a tendency towards hypoglycaemia unaccompanied with hyperinsulinaemia. Previous reports have referred to lower fasting glucose levels and unaltered plasma insulin levels (Okitolonda *et al.*, 1984 and Smith *et al.*, 1987), while others reported an increase in plasma insulin levels (Asamoah *et al*, 1989). Furthermore, when the curves were computed it was found that both the glucose and insulin areas were significantly decreased as was recorded in man (Smith *et al.*, 1987). A possible reason for the decrease in the circulating insulin during the oral GTT after prolonged chloroquine treatment would be the in-

hibition of proinsulin biosynthesis (Andersson *et al.*, 1980) and the decrease of insulin concentration in the pancreas (Okitolonda *et al.*, 1984). Healthy β-cells normally have a large overcapacity to synthesize and secrete insulin. The increased pancreatic function and improved peripheral insulin activity would thus, overshadow the slight inhibition of proinsulin synthesis by therapeutic doses of chloroquine. This supports what has been reported on cultured mouse islets (Okitolonda *et al.*, 1984) where insulin biosynthesis was found to be the most sensitive islet cell function to chloroquine. Moreover, chloroquine was reported to decrease insulin resistance dramatically (Blazer *et al*, 1984) thereby rendering the tissue more sensitive to insulin.

The present study showed that glucose tolerance was improved in rat by long-term administration of therapeutic dose of chloroquine. Although several other studies (Andersson *et al.*, 1980; Ericsson *et al*, 1981 and Okitolonda *et al*, 1984) reported that chloroquine does not alter glucose tolerance, Smith *et al.* (1987) stated that glucose tolerance improved significantly on glucose challenge. The classic criteria of glucose tolerance is the glucose levels at fixed intervals after an oral glucose load. While the formulae deduced by Sluiter *et al.* (1976b) and used in this study for evaluating glucose tolerance does not regard an empirically fixed time interval but applies to the glucose peak irrespective of the moment it is reached as the moment may be different in individuals.

In conclusion, the effect of chloroquine is time- dependent. Therefore although there appears to be some negative effects of the drug in short-term or culture tissue experiments, the long-term administration of the drug appears to have an overall beneficial effects on the glucose homeostasis, by improving glucose tolerance, and increasing pancreatic function and tissue insulin sensitivity. Furthermore,

it suggests the possibility that chloroquine might be used as a new drug in some aspects of insulin insufficiency or resistance.

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Table 1: Effect of chloroquine injection i.v. alone and together with glucose on serum glucose and insulin.

Agents	glucose	insulin
None	71.4	2.9246
Glucose (2g/kg)	98.75**	4.83**
Chloroquine (20 mg/kg)	76.625	9.694**
Glucose (2g/kg)+ Chloroquine (20mg/kg)	112.00*	5.875**

Serum glucose (mmole/l) and serum insulin (ng/ml) values are expressed as means ± S.E.M. Blood samples were taken before and 10 min after injection. Statistical significance of difference from untreated controls:
* P<0.01.
** P<0.05.

Table 2: Fasting glucose and insulin levels and glucose and insulin areas in response to chronic chloroquine treatment.

	Control	2 months	4 months
Fasting glucose level (mmol/l)	78.00	66.667**	70.333**
Glucose area	205.08	170**.00	166.8**
Fasting insulin level (ng/ml)	4.6963	5.8963	5.728
Insulin area	17.8256	14.6598**	14.738**

Values are presented as mean ± SEM for 8 control rats and 20 rats treated with chloroquine.
Statistical significance of difference from controls:
* P < 0.01
** P < 0.05

Table 3: Mean values for GTT parameters; pancreatic function (CIR), insulin activity (A) and glucose tolerance (GT) of rats before and after chloroquine treatment.

parameters / duration of treatment	G_p	I_p	CIR	A	GT
Before	123.428	12.2883	0.1870	6.6	1.2336
Two months	96.6667**	10.666	0.4302	9.9347	4.4064**
Four months	96.333*	4.7216**	0.372**	11.1894**	4.1783**

G_p refers to glucose value (mmole/p) at glucose peak,
I_p refers to insulin value (ng/ml) at glucose peak.
Statistical significance of difference from before treatment.
* $P < 0.01$.
** $P < 0.05$.

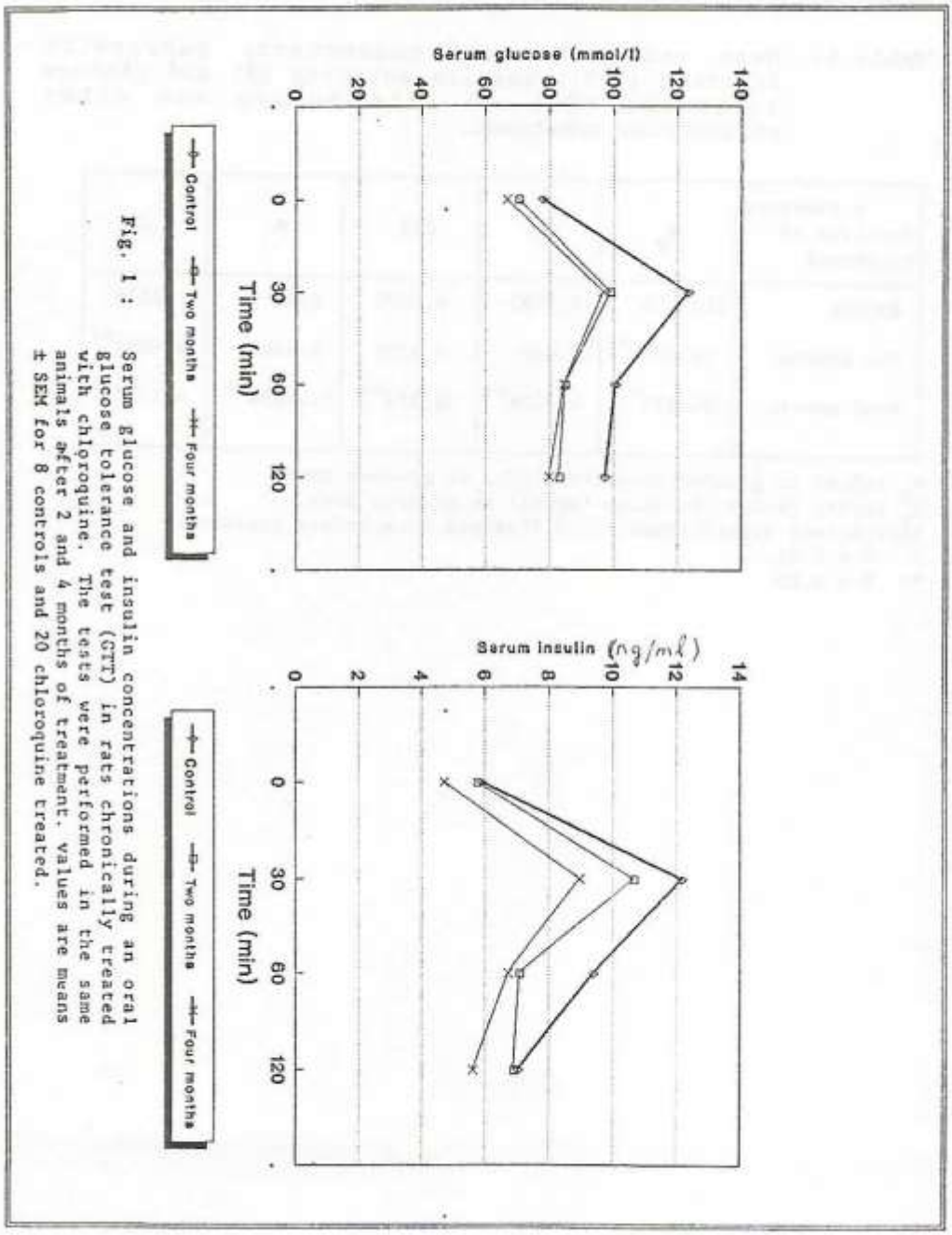

Fig. 1 : Serum glucose and insulin concentrations during an oral glucose tolerance test (GTT) in rats chronically treated with chloroquine. The tests were performed in the same animals after 2 and 4 months of treatment. Values are means ± SEM for 8 controls and 20 chloroquine treated.

الجمعية المصرية – الألمانية

لعلم الحيوان

العدد الرابع عشر (A) –مارس ١٩٩٤

الاتزان المتجانس للجلوكوز فى فئران طبيعية معالجة بالكلوروكين

خديجة محمد جعفر

قسم علم الحيوان ، كلية العلوم – جامعة القاهرة

مقاومة الجلوكوز ، النشاط الجانبى للأنسولين ووظيفة خلايا بيتا تحسنت بالتعاطى طويل المدى للكلوروكين لذكور الفئران الطبيعية بينما التأثير الحاد نتج عنه زيادة فى الأنسولين مع عدم تغيير فى مستويات السكر فى الدم.

هذه الدراسة أوضحت بعض النتائج المتضاربة ظاهريا للكلوروكين وتنتهى إلى أن العقار لايضر الإتزان المتجانس للجلوكوز فى العلاج طويل المدى.