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ACTIVATION OF HUMAN/RAT SERUM AMYLASE BY CHLOROQUINE AND FANSIDAR

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ABSTRACT

The effects of two antimalarials - fansidar (Sulphadoxine-pyrimethamine) and chloroquine on rat and human serum α-amylase (E.C 3.2.1.1) activity were investigated in vivo and in vitro, at an optimum temperature of 37°C and an optimum pH of 7.0. In vivo, fansidar significantly ($P<0.001$) increased serum α-amylase activity in a concentration dependent manner. At a maximum fansidar dose of 7.20 mg/g body weight α-amylase activity was increased by 28.83 ± 1.60% within 96 hours of the drug administration. In vitro, at fansidar concentration of 26.24mg per 100ml, serum α- amylase activity increased significantly by 5.90×10^2%.

Similarly, in vivo, chloroquine was found to increase α-amylase activity. At an optimal chloroquine dose of 2.88mg per 100g body weight, the activity of the enzyme increased by 6.88 +1.21% within 96 hours of the drug administration. In vitro at optimal chloroquine concentration of 41.65mg per 100ml, α-amylase activity increased by 7.51×10^2%.

These findings could hold some significance in relation to the biological function of the enzyme as well as clinical diagnosis involving the enzyme especially in subjects/ patients who are placed on these drugs therapeutically or otherwise.

KEY WORDS: Chloroquine, Fansidar, Serum α-amylase, Rat, Humans.

INTRODUCTION

Amylases are a group of hydrolases that split complex carbohydrates (such as starch) constituted of α-D-glucose units linked through carbon atoms 1 and 4 located on the adjacent glucose residues. They hydrolyse both straight-chain polyglucans, such as amylopectin and glycogen, though at different rates (Datta and Ottaway, 1979). The α-1, 6- linkages at the branch points are not attacked by the enzymes (Ekeke *et al* 1999).

Two types of amylases are known: Beta -amylase ,and alpha-amylase. β-amylase is found in plants and bacteria where it acts only at the terminal reducing ends of polyglucan chain and split off two glucose units (maltose) at a time. Alpha amylases are human amylases. They act rapidly on large polysaccharide molecules and break them down into small units. They are calcium metaloenzymes. Thus calcium is absolutely required for the functional integrity of the enzyme. (Varley et al 1980).

The enzyme displays full activity only in the presence of anions such as Cl , Br , No_3 and HPO_4, Cl and Br being the most effective activators (Whitby et al 1980). Alpha amylase is present in a number of organs and tissues, but has its greatest concentration in the pancreas where it is synthesized by the acinar cells.

Amylase activity assays are useful in diagnosis of disease of the pancreas and in the investigation of pancreatic function. In acute pancreatitis, there is a transient rise in serum amylase activity within 2-12 hours of onset of pain, with a peak at 12-48 hours. Amylase assays have also been found useful in detecting the development of complications such as

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pseudocyst, ascites and pleural effusion following acute pancreatitis (Tietz, 1987).

Drugs, even when taken in the right doses, elicit a number of pharmacologic effects at the site of action. This may include deleterious effects in addition to the desired clinical effects. (Katzung 1982).

In the world today, malaria is a common disease, especially in tropical Africa sequel to the mosquito infested environment. Fansidar and chloroquine are among the many drugs in common use for the treatment of the disease (WHO,1973). Like any other drug, they have some side effects which, however, may be unknown to the users. This is moreso when they are used in the treatment of other diseases. For instance, chloroquine is used in the treatment of rheumatoid arthritis or discoid lupus erythematosus (Olatunde, 1970). One of the most notable serious consequences of this is damage to the retina (Meyer, 1964 and Peters 1968). Some antimalarials also show their side effects on the gastrointestinal tract, cardiovascular system and skeletal muscles (Olatunde, 1970). High doses of chloroquine could lead to hyper excitability and convulsion within two hours (Katzung, 1982). Death may result due to cardiovascular collapse from extreme vasodilation and hypotension (Olatunde, 1970). There is a reported finding that the pyrimethamine component of fansidar when given in a single dose of 25mg daily for seven weeks caused the development of megaloblastic anaemia which readily disappeared after stopping the drug (Olatunde, 1970).

Chloroquine exerts its antimalarial action by blocking the enzymatic synthesis of DNA and RNA in the plasmodium parasite (Pearlman and Hall, 1975). This leads to the formation of DNA complex which cannot perform its natural role as a template for its own replication or transcription to RNA.

The antimalarial effect of fansidar is due to its ability to block folic acid synthesis in the protozoa (Katzung, 1982)

The manner in which these drugs are used , especially when malaria is not yet confirmed, necessitates a thorough biochemical research to elucidate any possible toxic effect this could have on the body of the users. The present study is to examine and/or elucidate the effects of fansidar and chloroquine on the activity of serum amylase, an enzyme which plays a vital role in the physiological integrity of the body system.

MATERIALS AND METHODS.

Fansidar and chloroquine were bought from ROCHE pharmaceuticals, Nigeria. Other chemicals used for the *in vitro* test were from BDH and M&B, London. For the *in vivo,* a total of 136 rats *(Rattus rattus)* of average weight: 100.15 ± 12.2g were used. All the rats were obtained from the Animal house of the Department of Biochemistry, University of Port Harcourt. The rats were divided into two groups, each of 68 rats. Eight rats served as control in each group. Five doses of each drug (fansidar: 0.60, 1.20,2.40,4.80 and 7.20 mg per 100g body weight and chloroquine: 0.24,0.48,0,96 1.92 and 2.88 mg per 100g body weight) were used. Each drug dose was administered intramuscularly to 3 rats from each group. The rats were on their normal diet (standard commercial feed) before the injection and were continued on same feed after the injection. The animals were monitored for a total of 144 hours which were subdivided into 0hr, 48 hr, 96hr and 144 hr intervals. Fifteen rats from each drug groups were sacrificed within each time interval. Blood of the rats were collected (by cardiac puncture) into heparinized anticoagulant bottles for analysis.

In vitro tests were conducted with human blood serum collected by vene-puncture from human healthy volunteers. The blood serum from the rats were separated and used immediately for the assay. A spectrophotometer (Reflotron) was used for the optical density reading. The enzyme activity was displayed in U/Lwithin 135 seconds as described by Varley *et al* (1980).

The technique used for the *in vitro* test was based on the saccharogenic method (Varley et al 1980). Starch was used as the substrate.

Five serial dilutions of each drug were prepared from initial concentration: 26.24 mg per 100ml for fansidar and 41.65 mg per 100ml for chloroquine. Each drug dilution was put into labeled test tubes and a sixth tube served as a control. 1.0ml of serum was put into each tube. 0.1ml of the respective serially diluted drugs was added into each of the labeled tubes, except the control tube, into which was added 0.1ml of normal saline. The tubes were incubated for 8 minutes at 37°C. 1.0ml of substrate (starch) was added to each tube and allowed to stand for 15 minutes. This was followed by the addition of 1.0ml of 0.1M NaOH, 1.0ml of 0.5M Ferrocyanide and 12.0ml of distilled water. The tubes were boiled for 20 minutes and then cooled to room temperature. 2.0ml of 50% acetic acid was then added followed by 10ml of iodide reagent (Varley et al 1980).

After 3 mins duration, the test tube contents were transferred into 250ml volumetric flasks and titrated with 0.025M sodium thiosulphate solution and the volume of the thiocyanate used in each case was recorded. The difference between two titrations for the test and control were related to the amylase activity in Norby unit/ml.

1 Norby unit = 1.8 U/L (Harold et al, 1980).

Statistical analysis: this was done using the student's t-test of statistical significance (Pearson and Hartley 1966).

RESULTS AND DISCUSSION

The effects of chloroquine on serum α-amylase activity, *in vivo* is shown in Table 1 and *in vitro,* Table 2, while that of fansidar is shown in Table 3 (*in vivo)* and Table 4 (*in vitro*). *In vivo*, α-amylase was significantly ($P \geq 0.05$) activated in the presence of increasing doses of chloroquine drug. For instance, at chloroquine dose of 2.88mg per 100g body weight of rats, serum α-amylase activity increased by 51.72 ± 10.6% within 48 hours of the drug administration. Also, it was observed to

TABLE 1–*IN VIVO* EFFECTS OF CHLOROQUINE ON RAT SERUM α-AMYLASE ACTIVITY

Drug dose mg/100g body weight	% Increase in Serum α-amylase Activity			
	0HR	48HR	96HR	144HR
0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
0.24	0.00 ± 0.00	41. 70 ± 8.90	28. 42 ± 3.80	13.2 ± 1.60
0.48	0.00 ± 0.00	47.87 ± 11.60	37.82 ± 8.60	5.43 ± 1.10
0.96	0.00 ± 0.00	53.06 ± 17.20	17.63 ± 5.20	8.43 ± 01.90
1.92	0.00 ± 0.00	54.01 ± 8.65	10.44 ± 1.60	9.28 ± 1.21
2.88	0.00 ± 0.00	51.72 ± 10.60	10.44 ± 1.60	3.97 ± 1.13

Table 2–*IN VITRO* EFFECTS OF CHLOROQUINE ON HUMAN SERUM α-AMYLASE ACTIVITY

Drug dose (mg/100ml)	% increase in α amylase activity($x10^2$)
0.00	0.00 ± 0.00
8.31	2.19 ± 0.15
10.40	3.96 ± 0.13
13.87	5.78 ± 2.20
20.65	7.11 ± 1.14
41.65	7.52 ± 0.99

Table 3-*IN VIVO* EFFECT OF FANSIDAR ON RAT SERUM α AMYLASE ACTIVITY

Drug dose mg/100g body weight	% Increase in Serum α-amylase Activity			
	0HR	48HR	96HR	144HR
0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
0.60	0.00 ± 0.00	42.13 ± 6.21	70.53 ± 15.20	13.75 ± 2.61
1.20	0.00 ± 0.00	77.84 ± 8.17	79.65 ± 3.10	19.12 ± 1.26
2.40	0.00 ± 0.00	138. 08 ± 9.86	89.01 ± 2.10	53.15 ± 3.12
4.80	0.00 ± 0.00	156.92 ± 10.61	97.34 ± 20.63	56.14 ± 7.12
7.20	0.00 ± 0.00	46.05 ± 16.31	28.83 ± 1.60	7.74 ± 2.11

Table 4 – *IN VITRO* EFFECT OF FANSIDAR ON HUMAN SERUM α AMYLASE ACTIVITY

Drug dose (mg/100ml)	% increase in α amylase activity ($x10^2$)
0.00	0.00 ± 0.00
5.24	0.38 ± 0.01
6.53	1.68 ± 0.22
8.74	3.26 ± 1.25
13.12	5.29 ± 0.96
26.24	5.91 ± 0.22

increase to a peak level of 54.01± 8.65% at 1.92 mg per 100 body weight within the same 48 hr duration, after which time, it decreased towards the normal (control). *In vitro*, at a chloroquine concentration of 41.65mg per 100ml, a percentage increase in α-amylase activity of 751.65 ± 98.7% was observed.

Similarly, fansidar increased percentage activity of serum α-amylase in a dose-dependent manner *in vivo* except at a fansidar dose of 7.20mg/100g body weight (Table 3). At a fansidar dose of 4.80 mg per 100g body weight, for instance, a percentage increase in activity of 156.92 ± 10.61% was observed within 48 hours of the drug administration. Beyond this time the activity started decreasing to normal. *In vitro*, at a fansidar concentration of 26.24mg per 100ml, for instance, a percentage increase of 590.74 ± 21.62% was observed. This decreased as the concentration of the drug decreased. For instance, at 5.24mg fansidar per 100ml, an increase of 38.22 ± 1.21% was observed.

The dose-dependent increase in serum α-amylase activity elicited by chloroquine could possibly be a result of electrostatic interactions of the chloride ion (Cl^-) of the chlorine atom at the active site of the enzyme. This is supported by the reported finding that serum α-amylase displays full activity in the presence of anions such as Cl^-, Br^-, No_2 and HPO_4^{2-}; Cl^- and Br^- being the most effective (Tietz 1987). It could also be that chloroquine induces the enzyme liberation from the pancrease either through biliary obstruction, or yet an unknown mechanism. The observed decrease of the enzyme activity back to normal (control) after 96 hours may possibly be a consequence of bio-transformation and/or excretion of drug (chloroquine).

Similarly, the observed rise in α-amylase activity, *in vivo* and *in vitro* in the presence of fansidar in a concentration- dependent manner and time could be attributed to a possible activatory effect of fansidar on the pancreas. This may have provoked a profuse secretion of the enzyme into the blood. The rise to a peak level within 48 hours is in conformity with the report of Tietz (1987) that in acute pancreatitis, there is a transient rise in serum α-amylase activity within 2-12 hours of the onset of pain, with a peak at 12-48 hours, and declining to normal level by the third day. The findings, in work of significant stimulatory effects of chloroquine and fansidar on serum α-amylase,

could hold some relevance in clinical diagnosis involving the enzyme. In routine laboratory diagnosis, elevated activity of the enzyme could either point to or confirm acute or sub acute pancreatitis (Tietz 1987). Considering the findings in this work, therefore, patients on these drugs, who are on test of pancreatic function, could be susceptible to wrong diagnosis especially in cases where other confirmatory tests are not performed. It is therefore pertinent to state that care should be taken in the use of these antimalarials especially, in the case of unconfirmed malaria disease, as any abuse may result in toxicity of the systems.

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