

Effects of some anti-histamine on erythrocyte aspartate amino transferase and alanine amino transferase activities in Wistar albino rats

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Abstract

The present study was conducted on the rat population of animal house in Madonna University, Elele Campus, Nigeria to evaluate the status of some biochemical parameters of rats administered with varied concentrations of chlorpheniramine maleate (piriton), cyproheptadine hydrochloride (cypron), and clemastine fumarate (tavagyl). Blood samples and liver organ were collected from 51 male and female albino rats, 48 were given different concentrations of each of the antihistamine drugs for 30 days, while 3 rats were used as control. Alanine amino transferase (ALT) activity and Aspartate amino transferase (AST) were lower in the control group compare to the groups administered with piriton, Cypron, and Tavagyl at 0.04mg/ml, 0.02mg/ml, 0.01mg/ml, and 0.005mg/ml. From the above results it is concluded that piriton, cypron, and Tavagyl increased the activities of AST and ALT enzymes.

Keyword: Alanine amino transferase, Aspartate amino transferase, piriton, cypron, and Tavagyl, Rat.

Introduction

Anti-histamines are drugs that inhibit the action of histamine in the body by binding onto the histamine receptors (Kemp, 1992). Anti-histamines act upon the H1, H2, H3, and H4 histamine receptor. While first generation H1- receptor antagonists are responsible for vast majority of poisoning, all antihistamine classes have been associated with serious toxicity.

All H1 histamine antagonists are reversible competitive inhibitors of histamine receptors. First generation H1 receptors blockers also are potent competitive inhibitors of muscarinic receptors and may cause anti-cholinergic syndrome. In addition, antihistamine disrupt cortical neurotransmission and block fast sodium channels (Leurs *et al.*, 2000). Some non-sedating anti-histamines are known to inhibit the potassium rectifier currents which show repolarization.

H2- receptor antagonists are primarily regulators of gastric acid secretion in the CNS. Histamine (H1, H2) modulates activities such as arousal, thermoregulation, neuroendocrine and vegetative functions. H2 receptor antagonist is considered relatively benign in overdose. H2-receptor antagonist also inhibits hepatic oxidative metabolism by most cytochrome P450 enzymes and, thus the metabolism of a variety of drugs.

H3- receptors are presynaptic regulators of synthesis and release of histamine into the synapses (Benett *et al.*, 2003). Some side effects of antihistamine include sedation, drowsiness, headache, difficult in passing urine, dry mouth, blurred vision, feeling sick or vomiting, constipation or diarrhea, irritation, arrhythmia, High blood pressure, allergic reactions, dizziness, confusion, depression, disturbed sleep etc (Baker *et al.*, 2003).

Chlorpheniramine maleate commonly called piriton is a first generation alkylamine antihistamine used in the prevention of the symptoms of allergic conditions (Katzung, 2007). It prevents histamine from reaching its site of action and it also crosses the blood-brain barrier of effected H1 receptors in the brain thereby inducing sedation (Mustapha, 2008).

Chlorpheniramine works as a serotonin-norepinephrine reuptake inhibitor (Rieveschl & Macchi, 2000). Chlorpheniramine is often combined with phenylpropanolamine to form allergy medication with both antihistamine and decongestant properties (Wilkinson *et al.*, 1999).

Cyproheptadine hydrochloride commonly called cypron is an anti-histamine/anti-chlonergic and anti-serotonergic agent (Katzung, 2007). Cyproheptadine has showed effectiveness in the treatment of nightmares, management of moderate to severe cases of serotonin syndrome and in the disease carcinoid in which serotonin is over produced by tumour cells. It blocks the sensitizing action of serotonin on body cells and hence reduces the allergic reaction like itching (Mustapha, 2008).

Cyproheptadine when used in conjunction with oral potassium salt can cause lowing of GIT transit and increase the local exposure to high potassium concentration (Ashton *et al.*, 2003).

Clemastine fumarate commonly called Tavagyl belongs to the benzhydryl ether group of anti-histamine compounds. Clemastine is an anti-histamine with anti-cholinergic and sedative side effects. It antagonizes the increase of capillary permeability that occurs in histamine release and it also inhibits the constrictor effect of histamine on respiratory muscle (Mustapha, 2008).

Clemastine are indicated for the relief of symptoms associated with allergic rhinitis such as sneezing, pruritus and also indicated for the relief of mild, uncomplicated allergic skin manifestation of urticaria (Mustapha, 2008).

Materials and methods

For this study a total of 51 Wister albino rats (*Rattus rattus*) were used and were obtained and housed at the animal house of Madonna University Elele Nigeria during year 2010. The feed used for the rats was the normal rat chow processed by Vital Feed Limited and purchased freshly from Owerri town market in Imo State, Nigeria during the year 2010. The rats were housed in 25 cages labeled P1, P2, P3, P4, C1, C2, C3, C4, T1, T2, T3, T4 and control. Each cage contains 4 rats, while the control cage contains 3 rats. Rats in cage P, C, and T were administered with varied concentrations of piriton, cypron and tavagyl respectively for a period of 30 days. Four different concentrations of piriton, cypron and Tavagyl were made from a stock of 8mg/100ml. Rats in cage P1, P2, P3, and P4 were administered with 0.04 mg/ml, 0.02 mg/ml, 0.01 mg/ml, and 0.005 mg/ml piriton respectively for 30 days. Rats in cage C1, C2, C3, and C4 were also administered 0.04 mg/ml, 0.02 mg/ml, 0.01 mg/ml, and 0.005 mg/ml cypron respectively for 30 days. Rats in cage T1, T2, T3, and T4 were administered 0.04mg/ml, 0.02mg/ml, 0.01mg/ml, and 0.005mg/ml tavagyl respectively for 30 days.

At the end of 30 days of drug administration, the rats were sacrificed and blood collected by commercially available 2ml disposable syringes. The blood was stored in lithium heparin anticoagulant bottles. Then the samples were transported to the laboratory for further investigations. Samples were washed several times using 0.9g/l sodium chloride and hemolysate was collected and stand at -2°C before test. Erythrocyte Alanine amino transferase and aspartate amino transferase activities were determined by Reitman, and Frankel method (1957) using enzyme kit obtained from Randox laboratories United Kingdom. The test was performed according to the manufacturer's instructions. Optical density (OD) value was recorded in a digital UV Spectrophotometer at 540nm.

Results

The present study was carried out on Wistor albino rats administered with varied concentrations of piriton, cypron, and tavagyl to study the status of erythrocyte alanine amino transferase and aspartate amino transferase. The erythrocyte alanine amino transferase activity was found to be lower in the control group (122.40 ± 0.06 u/l) than in the group administered piriton at 0.005mg/ml (148.80 ± 0.06 u/l), 0.01mg/ml (139.00 ± 0.01 u/ml), 0.02mg/ml (128.35 ± 0.03 u/l), and 0.04mg/ml (124.95 ± 0.03 u/l). The aspartate amino transferase was also found to be lower in the control group (199.80 ± 0.03 u/l) than in the group administered piriton at 0.005mg/ml (224.40 ± 0.03 u/l), 0.01mg/ml ($231.60 \pm$

0.06u/l), 0.02mg/ml (248.80 ± 0.06 u/l), and 0.04mg/ml (252.00 ± 0.01 u/l).

The erythrocyte alanine amino transferase activity was found to be lower in the control group (122.40 ± 0.06 u/l) than in the group administered cypron at 0.005mg/ml (127.50 ± 0.03 u/l), 0.01mg/ml (137.70 ± 0.06 u/l), 0.02mg/ml (178.20 ± 0.06 u/l), and 0.04mg/ml (196.05 ± 0.03 u/l).

The Aspartate amino transferase was also found to be lower in the control group (199.80 ± 0.03 u/l), than in the group administered cypron at 0.005mg/ml (248.40 ± 0.06 u/l), 0.01mg/ml (262.80 ± 0.06 u/l), 0.02mg/ml (298.00 ± 0.01), and 0.04mg/ml (252.00 ± 0.01 u/l).

The erythrocyte alanine amino transferase activity was found to be lower in the control group (122.40 ± 0.06 u/l), than in the group administered tavagyl at 0.005mg/ml (146.20 ± 0.06 u/l), 0.01mg/ml (133.10 ± 0.06 u/l), 0.02mg/ml (127.70 ± 0.06 u/l), and 0.04mg/ml (124.95 ± 0.03 u/l). The Aspartate amino transferase was also found to be lower in control group (199.80 ± 0.03 u/l) than in the group administered tavagyl at 0.005mg/ml (250.20 ± 0.06 u/l), 0.01mg/ml (253.80 ± 0.06 u/l), 0.02mg/ml (264.60 ± 0.06 u/l), and 0.04mg/ml (246.60 ± 0.06 u/l).

The technique used for the *in vitro* assay is based on the original method of Reitman and Frankel (1957) and reagent 1 containing phosphate buffer, l-aspartate, and α -oxoglutarate was used as substrate for AST while reagent 1 containing phosphate buffer, l-alanine, and α -oxoglutarate was used as substrate for ALT. Four serial dilutions of each of the drugs were prepared (piriton: 0.00, 0.04, 0.02, 0.01, and 0.005mg/100ml. cypron: 0.00, 0.04, 0.02, 0.01, and 0.005 mg/100ml. Tavagyl: 0.00, 0.04, 0.02, 0.01, and 0.005mg/100ml). Exactly 0.5ml of buffer substrate pH 9.8 and 0.1ml erythrocyte was introduced into each of labeled four tubes including the control tube. The blank tube contains only 0.5ml buffer substrate. Both tubes were incubated at 37°C for exactly 30minutes. 0.2ml of each antihistamine drug preparation was introduced into the buffer except the control tube in which case is added 0.2ml of water. The tubes were mixed and left in the incubator for 15 minutes. 0.5ml of reagent 2 (2, 4-dinitrophenylhydrazine) was added into each tube and further incubated for 10 minutes at 37°C . 5ml of 0.4 mmol/l sodium hydroxide was finally added into each tube. The absorbance of test and control was read against the blank within 5 minutes with UV Spectrophotometer.

Discussion

Various blood biochemical parameters were studied to evaluate the effect of chlorpheniramine maleate (piriton), cyproheptadine hydrochloride (cypron), and Clemastine fumarate (Tavagyl) on amino rats.

The *in vivo* and *in vitro* studies showed that the activities of erythrocyte AST and ALT increased in presence of the three anti-histamine drugs after 30 days. In one of the reactions of amino acid metabolism (Transamination), AST transfers amino group from α -amino acid (glutamate) to α - keto acid (oxaloacetate) to



Table 1. In vivo Erythrocyte ALT and AST in control group and group administered different concentrations of piriton

Antihistamine	concentrations (mg/ml)	Mean \pm SD	
		ALT u/l	AST u/l
Piriton	0.00	122.40 \pm 0.06	199.80 \pm 0.03
	0.04	148.80 \pm 0.06	224.40 \pm 0.06
	0.02	139.00 \pm 0.01	231.80 \pm 0.06
	0.01	128.35 \pm 0.03	248.80 \pm 0.06
	0.005	124.95 \pm 0.03	252.00 \pm 0.01

Table 4. In vitro Erythrocyte ALT and AST in control group and group administered different concentrations of Piriton

Antihistamine	concentrations (mg/ml)	mean \pm SD	
		ALT u/l	AST u/l
Piriton	0.00	23.02 \pm 31	47.00 \pm 0.55
	0.04	23.90 \pm 0.50	49.50 \pm 0.22
	0.02	23.90 \pm 0.47	49.80 \pm 0.38
	0.01	32.90 \pm 0.28	55.50 \pm 0.55
	0.005	43.40 \pm 0.41	67.10 \pm 0.42

Table 2. In vivo Erythrocyte ALT and AST in control group and group administered different concentrations of cypron

Antihistamine	concentrations (mg/ml)	Mean \pm SD	
		ALT u/l	AST u/l
Cypron	0.00	122.40 \pm 0.06	199.80 \pm 0.03
	0.04	127.50 \pm 0.03	248.40 \pm 0.06
	0.02	137.70 \pm 0.06	262.80 \pm 0.06
	0.01	178.20 \pm 0.06	298.00 \pm 0.01
	0.005	196.05 \pm 0.03	252.00 \pm 0.01

Table 5. In vitro Erythrocyte ALT and AST in control group and group administered different concentrations of cypron

Antihistamine	concentrations (mg/ml)	mean \pm SD	
		ALT u/l	AST u/l
Cypron	0.00	23.02 \pm 0.31	47.00 \pm 0.55
	0.04	23.27 \pm 0.57	49.10 \pm 0.42
	0.02	26.47 \pm 0.64	49.40 \pm 0.55
	0.01	26.47 \pm 0.51	55.20 \pm 0.42
	0.005	36.83 \pm 0.38	59.50 \pm 0.42

Table 3. In vivo Erythrocyte ALT and AST in control group and group administered different concentrations of Tavagyl

Antihistamine	concentrations (mg/ml)	Mean \pm SD	
		ALT u/l	AST u/l
Tavagyl	0.00	122.40 \pm 0.06	199.80 \pm 0.03
	0.04	146.20 \pm 0.06	250.20 \pm 0.06
	0.02	133.20 \pm 0.06	253.80 \pm 0.06
	0.01	127.70 \pm 0.06	246.60 \pm 0.06
	0.005	124.95 \pm 0.03	246.60 \pm 0.06

Table 6. In vitro Erythrocyte ALT and AST in control group and group administered different concentrations of Tavagyl.

Antihistamine	concentrations (mg/ml)	mean \pm SD	
		ALT u/l	AST u/l
Tavagyl	0.00	23.02 \pm 0.31	47.00 \pm 0.55
	0.04	31.02 \pm 0.55	49.10 \pm 0.55
	0.02	30.02 \pm 0.62	51.50 \pm 0.55
	0.01	34.53 \pm 0.75	57.40 \pm 0.55
	0.005	46.04 \pm 0.25	69.20 \pm 0.42



form an α - amino acid (Aspartate) while ALT transfers amino group from α -amino acid (glutamate) to - keto acid (pyruvate) to form an α - amino acid (alanine). The increase in AST and ALT suggested the activatory ability of the drugs to the activities of the enzymes. It therefore suggested that the drugs have no binding effect on the active sites of the enzymes; rather they may have acted as a cofactor to the enzymes, speeding up the transamination reaction.

The dose dependent activatory effect of piriton, cypron and Tavagyl on erythrocyte AST and ALT activities could possibly result from electrostatic and hydrogen binding interactions between the drug and amino acid residue at the active site. High levels of AST and ALT induced by the drugs could be a consequence of hepatocellular and cardiac toxicity (Whitby *et al.*, 1980).

The activation of AST and ALT could promote transport of amino group across the cell membrane. AST and ALT are used as a marker for liver disease with ALT being more specific and myocardial infarction with AST being more specific (Tietz, 1986). The non concentration dependent increase in AST and ALT observed in some cases could be attributed to clinical limitations, technical limitations, disorders in the drug, absorption, bioavailability of the drug, biotransformation of the drug, and influence of drug metabolizing enzymes in the liver microsomes. AST and ALT are synthesized by the cells in response to any form of biliary obstruction and myocardium disorder. Some of the newly formed enzymes enter into the blood circulation and raise the level. This could be the possible cause of the observed rise in the level of AST and ALT activity. The rise in AST and ALT activities could also be as a result of the drugs causing high proliferation of liver and heart cells, low rate of enzymes clearance from circulation caused by the drugs and a high degree of induction of enzyme synthesis caused by the drugs.

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