Attenuation of erythrocytic acetyl cholinesterase by paracetamol and chloroquine: Evidence in an *in vitro* study

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Abstract: The present study deals with the erythrocytic acetylcholinesterase inhibitory activity of paracetamol and chloroquine in an *in vitro* protocol using Michaelis Menten parameters (Apparent Michaelis Constant (aKm) and Apparent Maximum Velocity (aVm). Paracetamol showed marked inhibition of the erythrocytic acetylcholinesterase. The inhibitory values for aKm and aVm were 65.6% 51.36% respectively, which reduced with respect to control and therefore, proposed an un-competitive type of antagonism. When chloroquine was tested, it showed 45.14% inhibition for aKm which increased while 69.21% for aVm decreased with respect to control; proposed a mixed type of antagonism. In conclusion, the cholinergic intervention by paracetamol in this study suggested a new mechanism for its analgesic activity as acetylcholinesterase inhibitors have already shown both peripheral and central analgesic activity, while the cholinergic activation by chloroquine provided explanation for some of its side effects.

Keywords: Acetylcholinesterase inhibition, paracetamol, chloroquine

INTRODUCTION

Acetylcholinesterase (AchE; EC 3.1.1.7) is a membrane bound enzyme which is frequently found in the erythrocyte. AchE induces the hydrolysis of acetylcholine into choline and acetic acid and thereby increasing the concentration of endogenous acetylcholine in the vicinity of cholinoreceptors (Khan *et al*., 2007; Khan *et al*., 2011). Alteration of the enzyme concentration has been recognized in several human disorders such as Alzheimer's diseases, diabetes, autoimmune heamolytic anemia, menstruation, pregnancy, protein malnutrition, spleenomagaly, addiction, and ureamia (Niazi *et al*., 2011). It is therefore, prevention of over expression of AchE is considered as an ideal therapeutic strategy to achieve effective management of such conditions.

The current study was designed to estimate the enzyme inhibitory profile of two commonly used drugs, paracetamol and chloroquine on acetylcholinesterase in an *in-vitro* experiment using Michaelis Menten parameters (Apparent Michaelis Constant (aKm) and Apparent Maximum Velocity (aVm).

MATEREAL AND METHODS

Blood sample preparation

Blood samples were taken from the healthy volunteers students of the Institute of Chemical Sciences, University of Peshawar. Blood was collected by sterile vein-puncture and added to acid citrate dextrose solution as anticoagulant 0.44% (w/v) citrate acid 1.3% (w/v) sodium citrate: 1.47% (w/v) glucose in the proportion of 5 part of blood to 1 part o acid citrate dextrose solution. This was centrifuged at 15^0 with 2000 rpm for 5 min. The plasma, and one-third upper portion of the packed cells were sucked off. The remaining packed cells were washed 3 times with 10 volume of ice-cold 0.9% (w/v) NaCl (Niazi *et al*., 2011).

Enzyme preparation

Haemolysate was prepared by adding 0.02 ml cells to 100 ml ice cold deionized water. After approximately 15 min, it was diluted, with an equal volume of ice-cold potassium phosphate buffer (0.2M, pH 7.4), whole cell suspension was prepared by suspending 0.2 ml cells in 100 ml of the same buffer (0.1M, pH 7.4). The enzyme activity was assayed in triplicate at 30^0 and pH 7.4, using acetylthiocholine iodide (ATChI) as substrate and 5,5-dithiobis (2-nitrobenzoic acid (DTNB) as colour re-agent. To 3 ml of the haemolysate was added 100 µl of 10 mmol/I DtNB (final concentration 160 µmol/I) and then after a 10 min pre-incubation period, 50µl of ATChI was added as 200 µmol/I concentration. The change in absorbance (ΔE) at 412 nm (Tariq *et al*., 2013), due to the formation of 5-thio-2 nitrobenzoate yellow coloured anion was recorded per min and the rate of reaction was determined by applying the Ellmman equation.

Enzyme parameters

The absolute activity was expressed as ΔEa/min per ΔEb where ΔEb represent the absorbance due to haemoglobin

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content of the haemolysate measured at 540 nm (Ullah *et al*., 1990).

All the assays were run by the same observer at concentrations of substrate, one was much lower (S_1=10 µ mol/l) and the other much higher (S_2=160 µ mol/l) and aVm were calculated by fitting the corresponding given linear regression equation, which were derived from S/V versus s plot (Ullah *et al*., 1993) to the date.

$aKm = (S_1/VS_1)\ (S_2\text{-}S_1)\text{-}(S_1/S_2)\text{-}(S_2/VS_2)\text{-}(S_1/VS_1)\text{-}S_1$

and

$aVm = [(S_2/VS_2) - (S_1/VS_1) - (S_2\text{-}S_1)]$

Where VS_1 and VS_2 represent absolute activities at S_1 and S_2, respectively

STATISTICAL ANALYSIS

Data are presented as mean ±SEM of three different readings.

RESULTS

The experimental result showed inhibition (%) as well as mechanism or type of antagonism using Michaelis Menten parameters. Regarding effect of paracetamol on the enzyme, marked inhibition was observed as shown in table 1 and fig 1. The inhibition of aKm and aVm were 65.6% and 51.36% respectively, suggested a non-competitive type of antagonism (table 3). As shown in table 2 and fig. 2, the chloroquine caused prominent inhibition of the enzyme and the inhibition value for aKm and aVm were 45.14% and 68.21%, respectively and thus mediated through mixed type of antagonism (table 3).

Table 1: Effects of paracetamol on aKm and aVm on enzyme activity

Experiment	aKm (µM)	aVm
Control	21.83±0.71	83.30±8.10
Test	11.44±0.16	78.90±9.60

Values represent mean ±SEM of three different readings.

Table 2: Effects of chloroquine on aKm and aVm on enzyme activity

Experiment	aKm (µM)	aVm
Control	24.45±0.18	78.90±6.80
Test	29.71±0.15	35.10±5.55

Values represent mean ±SEM of three different readings.

DISCUSSION

The study revealed marked inhibition of paracetamol and chloroquine on erythrocytic acetylcholine esterase in an *in vitro* experimental model using Michaelis Menten parameters (Apparent Michaelis Constant (aKm) and Apparent Maximum Velocity (aVm).

Table 3: Showing the type of inhibition on the basis of aKm and aVm

S. No	aKm	aVm	Nature of inhibition
1	No change	Increased	Enzyme synthesis
2	Decreased	No change	High Enzyme Affinity
3	Increased	No change	Competitive Inhibition
4	No change	Decreased	Non-Competitive Inhibition
5	Decreased	Increased	Enzyme Activation
6	Increased	Decreased	Mixed Inhibition
7	Decreased	Decreased	Un-Competitive Inhibition

Extract from Mabood, 1981.

Pain is defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage (Khan *et al*., 2011). The nature of pain is highly subjective. Pain has both sensory (somatic) and psychological (affective) components (Boutaud *et al*., 2002). Paracetamol (fig. 3) is commonly used as an analgesic and antipyretic in clinical practice (Ouellet & Percival, 2001) and even as OTC (over-the-counter) product around the world. Researchers have been extensively investigated the mechanism of paracetamol and it is proposed that the paracetamol primarily act on the inhibition of prostaglandins. Additionally, the central analgesic effect of paracetamol could be attributed to activation of descending serotonergic pathways (Graham & Scott, 2005). The intrinsic cholinergic inhibitory pathways have been recognized as a crucial focusing side in pain management (Buerkle *et al*., 1999). Acetylcholinesterase inhibitors have shown both peripheral and central analgesic activity (Graham & Scott, 2005).

In our findings, paracetamol showed prominent attenuation of erythrocytic acetylcholinesterase in an *in-vitro* experiment and the type of inhibition was suggested as an un-competitive type of antagonism using Michaelis Menten parameters, aKm and aVm. Cholinergic activation of the paracetamol could be considered an additional mechanism for its analgesic activity.

Malaria is a challenging disease for more than 100 tropical and sub-tropical countries of the world and effect approximately 3.3 billion people every year including Pakistan (Khan *et al*., 2012). Chloroquine (fig. 3) is still used as a first line therapy for the management of malaria though facing problem of resistance and encounter several side effects such as gastrointestinal upset, pruritus, headaches, and blurring of vision (Pappaioanou *et al*., 1986; Horowitz & Carbonaro, 1992).

Chloroquine demonstrated marked inhibition of the erythrocytic acetylcholinesterase *in-vitro* thereby increasing acetylcholine concentration. Using Michaelis

Menten parameters, aKm and aVm, it exhibited mixed type of antagonism. This could explain the reason for the side effect of chloroquine such gastrointestinal upset due to over expression of cholinergic activity.

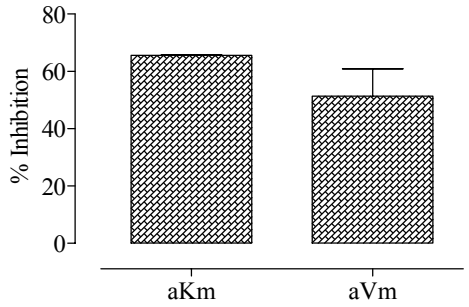

Fig 1: % inhibition (non-competitive antagonism) of acetylcholinesterase by paracetamol using Michaelis Mentin parameters Apparent Michaelis Constant (aKm) and Apparent Maximum Velocity (aVm) of the enzyme. Data are mean of S.E.M of five experiments.

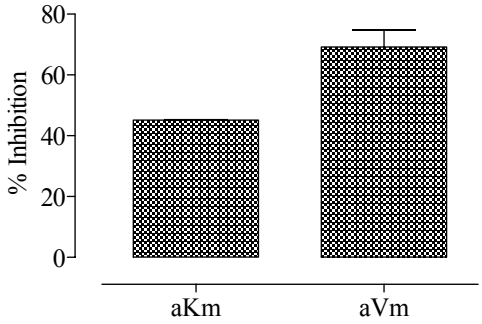

Fig 2: % inhibition (mixed type of antagonism) of acetylcholinesterase by chloroquine using Michaelis Mentin parameters Apparent Michaelis Constant (aKm) and Apparent Maximum Velocity (aVm) of the enzyme. Data are mean of S.E.M of five experiments.

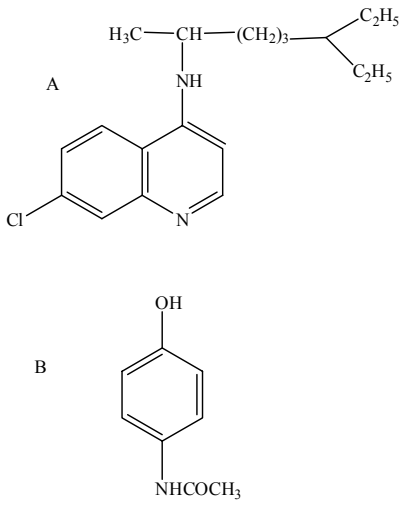

Fig 3: Structure of chloroquine (A) and paracetamol (B).

In conclusion, our finding suggested strong antagonism of paracetamol and chloroquine against acetylcholinesterase. The cholinergic activity of paracetamol provided a new mechanism for its analgesic activity while in case of chloroquine this suggested a mechanism for its side effect like dryness of mouth. However, further detail *in vivo* studies are required to ascertain these results.

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