The Inhibitory Effect of Some Benzoic Acid Derivatives on Human Erythrocyte Catalase Activity

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Abstract

In order to study the kinetic of human erythrocytes catalase a well –known enzyme uses H_2O_2 as substrate as well as hydrogen acceptors, in non smokers and smoker individuals.
Anthranilic acid and p-Amino Benzoic Acid ($_p$ABA) were used to study their effect on the enzyme.
The kinetic study confirmed that anthranilic is a non-competitive inhibitor with Km values of 0.95 and 1.0 for non smokers and smokers respectively ($_p$ABA) was found to be a competitive inhibitor with Vmax values of 8.0 and 8.9 for nonsmoker and smoker respectively

Introduction

The univalent reduction of oxygen results in a series of cytotoxic oxygen species.
These highly reactive species can cause cell damage including lipid peroxidation, inactivation of enzymes, alteration of intra-cellular oxidation-reduction state and damage to DNA (1) (2).
Catalase (human erythrocyte catalase E.C:1.11.1.6). Its functions include catalyzing the decomposition of hydrogen peroxide to water and oxygen. ,also oxidizes different toxins, such as formaldehyde, formic acid and alcohol. In doing so, it uses hydrogen peroxide according to the following reaction (3):

$$H_2O_2 + H2R \longrightarrow 2H_2O + R$$

Catalase has one of the highest turnover rates for all enzymes; one molecule of catalase can convert millions of molecules of hydrogen peroxide to water and oxygen per second (4).
Catalase is atetramer of four polypeptide chains. It contains four porphyrin heme (iron) groups which allow the enzyme to react with the hydrogen peroxide (5)
Anthranilic acid (ortho amino benzoic acid) and its derivatives were used as a drug for pain, temperature (pyrexia) and inflammatory processes, among non steroidal anti inflammatory drug agents (6) (7).
Para amino benzoic acid (PABA) is a co-enzyme associated with the vitamin B-complex forms part of the structure of folic acid and it thought as a vitamin within a vitamin.
Para amino benzoic acid stimulates the intestinal bacteria enabling them to produce folic acid, which in turn aids in the production of pantothenic acid .PABA, also helps to maintain healthy intestinal micro-flora. As a coenzyme PABA functions in the breakdown and utilization of proteins and in the formation of red blood cells (8) (9).

The objectives of the present study are to evaluate the lipid peroxidation marker Malondialdehyde (MDA) in sera of non smokers and smoker's healthy individuals and to indicate the effect of anthranilic acid and Para amino benzoic acid on erythrocyte catalase activities, also to evaluate Km and Vmax in the presence of the above compounds.

Experimental part

All reagents were of highly analytical grade.

Blood samples were collected from healthy individuals; the erythrocytes were obtained by centrifugation of blood after coagulation at 2500rpm for 30 min at room temperature. Hemolysate of RBC s was obtained by washing the erythrocytes three times with normal saline solution (0.9%NaCl).

Hemolysate was prepared by adding four parts volume of distilled water to one sediment volume of erythrocyte. A chloroform-ethanol extract was prepared by adding (0.5ml) hemolysate to (3.5ml) ice cold DW , followed by (1ml) ethanol and (0.6ml) chloroform. After centrifugation, 1:500 A dilution of this concentrated hemolysate was prepared with phosphate buffer immediately before the assay.

Determination of serum Malondialdehyde (MDA) levels

The determination is based on the formation of colored complex upon the reaction of MDA with thiobarbituric acid (TBA) according to the method described by Rehnerona et al .The absorbance of complex was measured at 532 nm (10).

Determination of erythrocyte catalase activity

The assay of catalase was performed on the ability of the enzyme to decompose H_2O_2 to give water and oxygen. This assay was based on the reduction in the absorbance of hydrogen peroxide at 240nm. The difference in absorption (ΔA_{240}) per unit time is a measure of catalase activity(11).

-Preparation of stock solution of anthranilic acid and Para amino benzoic acid with concentration of 10^{-2} mM of each:-

Four different dilutions from organic compound were prepared by the following steps:

From the stock (10^{-2}mM),one ml was transferred to 100ml volumetric flask and completed with ethanol to the mark to get 10^{-3} mM concentration,also 10^{-4} , 10^{-5} and 10^{-6} dilutions were prepared .

Determination of percentage inhibition :-

Using different concentrations of anthranilic acid and Para amino benzoic acid , while the concentration of the substrate [S] was fixed to get the percentage of inhibition or activation, according to the equation:

%Inhibition=100-(activity with inhibitor / activity without inhibitor) x 100

The inhibitor concentration that gives the highest percentage of inhibition was used throughout the study to obtain the type of inhibition.

Determination of type of inhibition

It was carried out by using different concentrations of substrate, while there are fixed concentrations of anthranilic acid and Para amino benzoic acid. The same method of catalase activity was used by utilizing the same concentrations of substrates without using anthranilic acid and Para amino benzoic acid.

The effect of ethanol, which was used as a diluent , was determined by adding a quantity equivalent to the sample and all steps completed as in the method of determination of erythrocyte catalase activity (12).

Results and Discussion

Lipid peroxidation marker Malondialdehyde (MDA) in healthy non-smokers were found to be 10.3 mM/dl ,while it was found to be 18.5mM/dl in healthy smoker individuals.

The high levels of MDA in sera of smokers over non smoker could be due to the damage in the tissue caused by over generation of free radicals in the body of smoker compared to non smoker; also research has shown that many diseases are the direct results of free radicals on human body (13)(14)

Figure (1) and (2) showed Michaelis-Menten for erythrocyte catalase in non smokers and smokers respectively. The values for Vmax were (8 U/g) and (9.5 U/g) respectively.

The Km values were 0.5 and 0.58 for non smokers and smokers respectively

The catalase activity increased in erythrocyte of smoker compared to non smokers is due to the increase in free radical generation in smokers which was shown in the present study accompanied with the increase in MDA concentration to counter balance by the increase in catalase activity especially in erythrocytes which are rich in this enzyme (Chance . etal) (15) also Boon .etal reported that the elevated antioxidant enzymes defence systems in smokers erythroctes for protection against oxidative stress (16)

The effect of anthranilic acid on erythrocyte catalase activity of non smoker and smoker is shown in figure (3).

Anthranilic acid showed an inhibitory effect (34.2 ,40.9 ,62.7 ,60 %) and (41.6,40.3,37.1,53.7%) at concentrations (10^{-3},10^{-4},10^{-5} and 10^{-6} mM) of non smoker and smoker respectively.

The effect of Para amino benzoic acid on erythrocyte catalase activity of non smoker and smoker is shown in figure (4).

Para amino benzoic acid showed an inhibitory effect (58.2, 40.3, 38.2, and 46.1%) and (65.9, 32.8, 44.4, 39.4 %) at concentrations (10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} mM) of non smoker and smoker respectively.

The effect of the solvent ethanol showed a negligible inhibitory effect on erythrocyte catalase activity. The percentage of original activity is considered to be 100%.

Figure (5) and (6) showed that Anthranelic acid is a non-compatative inhibitor for the erythrocytes catalase of non smokers and smokers respectively according to linweaver-barke plot with Km values of 0.95 and 1.01 for non smokers and smokers respectively. From figure (5), the Vmax for the uninhibited enzyme is 9.1 and 3.9 for the inhibited enzyme in figure (6), the Vmax was found to be 8.0 for smokers and 2.5 for the inhibited enzymes in smokers.

Figures (7) and (8) showed the linweaver – burk plot for nonsmokers and smokers respectively which indicate a compatative inhibition of PABA on catalase activity with the same Vmax (8.0 for both the inhibited and non inhibited catalase in non smokers) the Km values for the uninhibited is 1.0- while for the inhibited enzyme is 2.5 in non smokers from figure (8) the V max is 8.9 for the erythrocyte smokers with Km values of 1.0 for the uninhibited enzyme and 2088 for the inhibited enzyme.

The complete Mechanism of catalase is not currently known, the reaction is believed to occur in two stages:

H_2O_2 + Fe (III)-E _____ H_2O +O=Fe (IV)-E

H_2O_2 + O=Fe (IV)-E ____ H_2O + Fe (III)-E + O_2

(Where O=Fe (IV)-E represents the iron center of the heme group attached to the enzyme (17).
A study conducted on the inhibitory effect of azide could be due to the removal of some catalase-Fe (III) from the reaction medium as the catalase – Fe (III)-azide complex (18).

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التأثير التثبيطي لبعض مشتقات حامض البنزوك في فعالية انزيم الكاتليز في كريات الدم الحمراء البشريه

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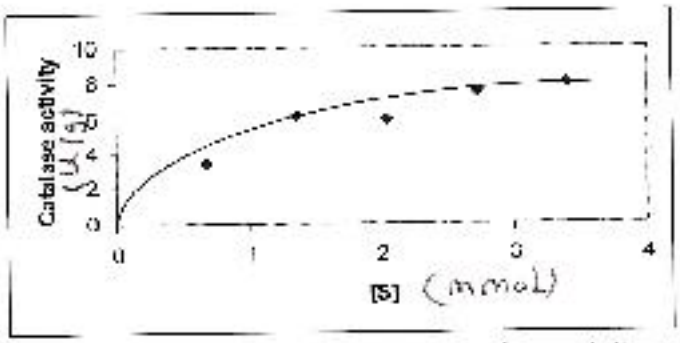
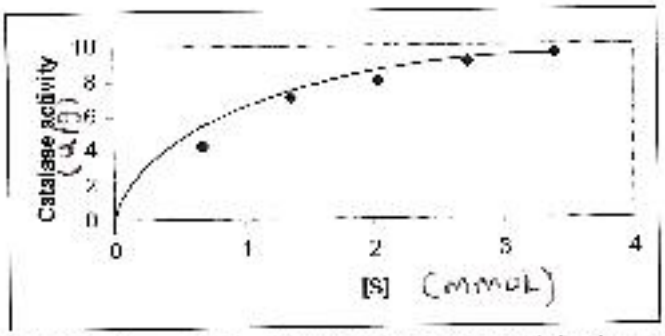
الخلاصة

لاجل دراسة حركية انزيم كتليز كريات حمراء الدم البشري بأ ستعمال H_2O_2 مادة اساس وكذلك مستقبلا للهيدروجين في الافراد غير المدخنين والمدخنين باسراف
حامض الانثرانيلك و بارا –امينو حامض البنزوك استعملت لدراسة تاثيرهما في هذا الانزيم .

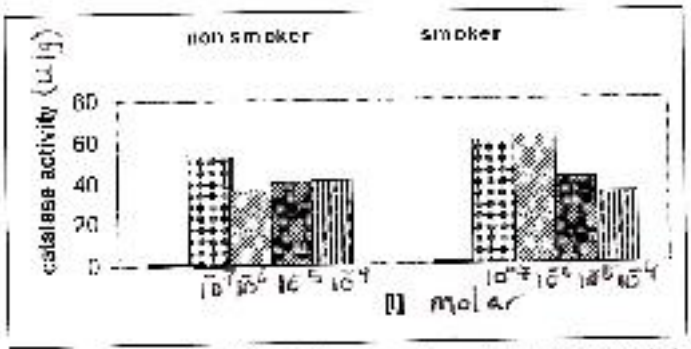
اكدت الدراسة الحركية ان الانثرانلك مثبط لا تنافسي بقيم ل Km 0.95 و 1.0لغير المدخنين والمدخنين على التوالي ، بارا امينو حامض البنزوك وجد لكي يكون مثبطا تنافسيا مع قيم ل Vmax 8.0 و 8.9 لغير المدخنين والمدخنين على التوالي



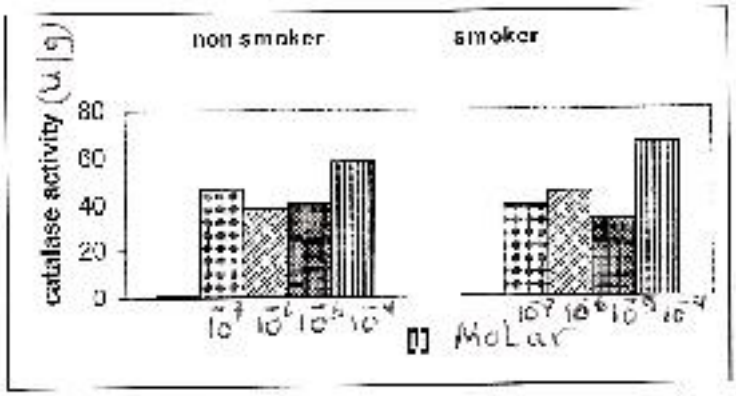
Figure(1): Michaelis Menten plot for erythrocyte catalase activity in non smoker individuals.



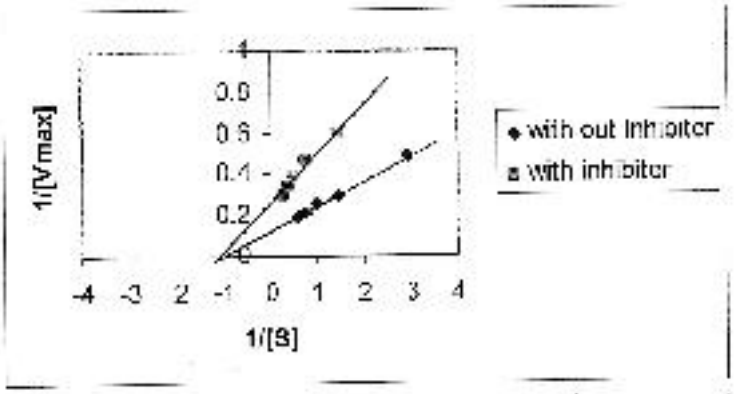
Figure(2): Michaelis Menten plot for erythrocyte catalase activity in smoker individuals.



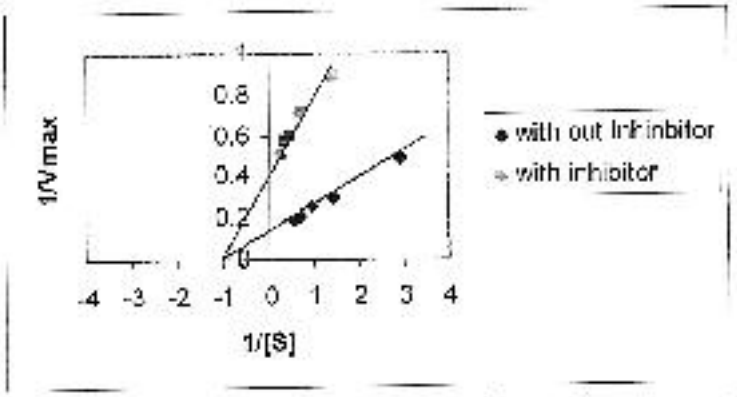
Figure(3): The effect of anthranilic acid on erythrocyte catalase activity of non smoker and smoker individuals.



Figure(4): The effect of Para amino benzoic acid on erythrocyte catalase activity of non smoker and smoker individuals.



Figure(5): Lineweaver-Burk plots for anthranilic acid on erythrocyte catalase activity of non smoker.



Figure(6): Lineweaver-Burk plots for anthranilic acid on erythrocyte catalase activity of smoker.

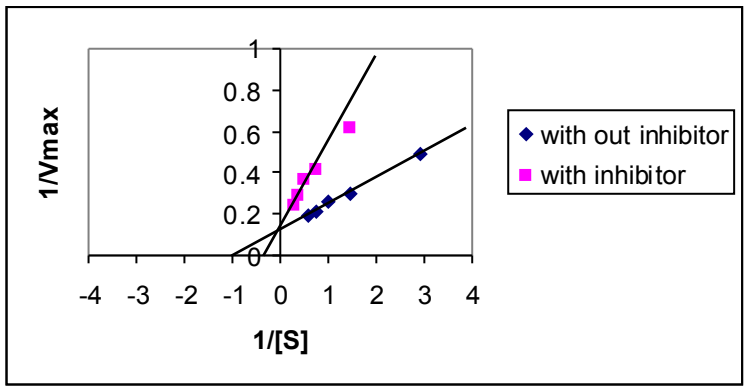


Fig.(7): Lineweaver-Burk plots for Para amino benzoic acid on erythrocyte catalase activity of non smoker.

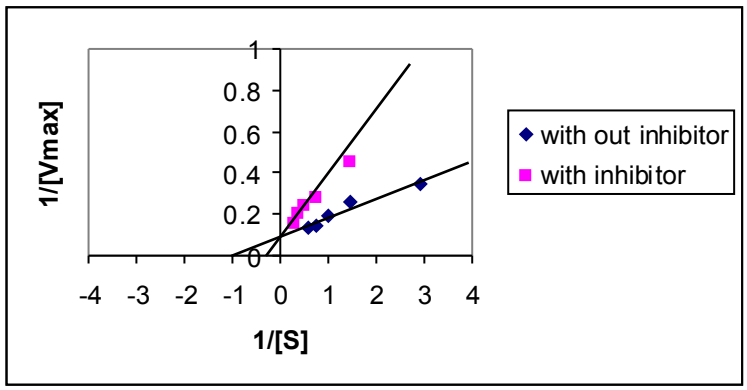


Fig.(8): Lineweaver-Burk plots for Para amino benzoic acid on erythrocyte catalase activity of smoker.