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Certain Antibiotics Having Inhibitory Effect on Xanthine Oxidase (E.C.1.2.3.2) Activity

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Abstract

The possible inhibitory effects of some antibiotics (sulphamerasine, penicillin-G, chlaritromycine, and ampiciline) on xanthine oxidase were investigated. Sulphamerasine and penicillin-G were found to have non-competitive, and the others un-competitive inhibitory effects on the enzyme. The assay was based on determination of uric acid at 294 nm formed from xanthine. Inhibition types were analysed and detemined by devising Lineveawer-Burk's double reciprocal plots.

Key words: Xanthine oxidase, antibiotics, sulphamerasine, penicillin-G, chlaritromycine, ampiciline

Introduction

Xanthine oxidase is a key enzyme of metallo-flavo type effective in controlling the purine pool in cytoplasm. This enzyme was inhibited by nebularine, a purine nucleoside antibiotic produced by *Streptomyces yokosukanensis*, *Clitocybe (Lepista) nebularis*, and a novel *Microbispora sp*, non-competitively (Ehrenberg *et al.*, 1946a-b, Löfgren *et al.*, 1954, Isono & Suzuki, 1960; Nakamura, 1961; Cooper *et al.*, 1986; Brown & Konuk, 1995) and this inhibition was later removed by the use of kinetin (Konuk *et al.*, 1996). Since antibiotics affect the biosynthesis of nucleic acids directly or indirectly (and nebularine has the same effect on the xanthine oxidase) we aimed to search any other possibly effects on the purine pool regulation system, as they break down the replication of DNA or transcription of mRNA.

The aim of this study was to investigate the possible nebularine type inhibitory effects of certain clinically used antibiotics on xanthine oxidase.

Material and Methods

The antibiotics (sulphamerasine, penicillin-G, ampicilline, and chlarithromycine) used in this study were supplied by the Konya branch of Roche pharmaceutical company; chemicals were purchased from Merck, and xanthine from Sigma (X 0125, Sigma Grade I, obtained from butter milk, suspension in 2.3 M $(NH_4)_2 SO_4$ containing 1 mM sodium salicylate). Enzyme activities were determined spectrophotometrically (ΔA_{294}, Shimadzu-1210 UV-Visible spectrophotometer) based on the appearance of uric acid at ambient temperature.

Phosphate buffer (0.1 M) containing EDTA (1 mM) was prepared by dissolving 22.52 g of $K_2HPO_4.3H_2O$, 1.157 g of KH_2PO_4 and 0.372 g EDTA in 1000 ml of water, and the pH was adjusted to 7.8.

Xanthine solution (9 mM) was prepared by dissolving 14 mg xanthine in 1 ml NaOH (0.1 M). All the antibiotics used in this study were dissolved in 0.1 M phosphate buffer containing EDTA (1 mM), at pH: 7.8.

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The essay employed in this study was the method of Kalckar's described in 1947 and devised by Heinz and Reckel in 1983.

Results and Discussion

Using a saturated concentration of the substrate (20 μM), the effect of antibiotics in the same concentrations were recorded (Table). The type of inhibitions were examined in further experiments using substrate (xanthine) concentrations of 5, 10, and 20 μM and inhibitors (separated) concentrations of 0, 5, and 10 μM, as final concentrations. The data were analysed using Perkin Elmer Biokins kinetics programme and Lineveawer-Burk plot obtained as shown in Figs. 1-4. It was observed that sulphamerazine and ampicilline were non-competitive; while penicillin-G and chlarithromycine were un-competitive inhibitors of xantine oxidase.
Kinetic measurements are useful for distinguishing between diferrent types of inhibition as well as providing quantitative information of the effectiveness of various inhibitors. This information is essential for an understanding of how cells regulate their enzymic activities. Comparison of the effects of a series of inhibitors also can help in mapping the structure of an enzyme's active site, and such studies are a key step in the rational design of therapeutic drugs.

Table. Inhibitory effects of antibiotics on xanthine oxidase.

Antibiotics	No antibiotics, xanthine conc. (20 μM)	With antibiotics (20 μM)	Inhibition %
Sulphamerazine	0.145	0.100	29.6
Penicillin-G	0.142	0.094	33.8
Ampicilline	0.143	0.126	11.3
Chlarithromycin	0.147	0.080	43.7

Data are the mean of triplicate determinations. S.D. ±0.08 .

Most enzymes are sensitive to inhibition by spesific agents that interfere with binding of a substrate at the active site or with the conversion of the enzyme-substrate complex into products. Study of these effects can provide information about how an enzyme operates.
In many cases, an inhibitor is found to resemble the substrate strucrurally, and to bind reversibly at the same site on the enzyme. This effect is called *competitive inhibition.*, because the inhibitor and the substrate compete for binding.
Inhibitors of a different sort can bind at separate sites where they do not compete directly with the substrate. Instead, they act by interfering with reaction of the enzyme-substrate complex. An inhibitor that bind to en enzyme whether or not the active site is occupied by the substrate is termed *a non-competitive inhibitor.*
A third possibility is that the inhibitor binds only after formation of the enzyme-substrate complex. This effect called *un-competitive inhibition.* Un-competitive inhibition is most common in reactions involving more than one substrate.
After drawing the double resiprocal plots in accordance to the results obtained from this study, different types of lines have occured in the graphs. As a result of this lines antibiotics have both un-competitive (Figs.1-2) and non-competitive (Figs.3-4) inhibition on xanthine oxidase. In un-

competitive inhibition, plots of 1/υ versus 1/[S] at different values of [I] give a series of parallel lines (Figs. 1-2). In non-competitive inhibition, inhibitor decreases the maximum velocity but does not effect K_m,and plots of 1/υ versus 1/[S] in the presence of different concentrations of the inhibitor intersect at the same point on the abscissa, but pass through the ordinate at different points (Figs.3-4).

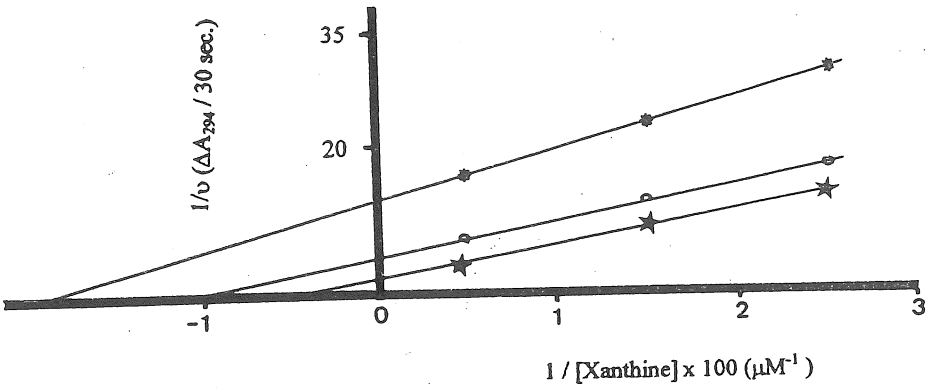

Fig. 1. Lineweaver-Burk plot of xanthine oxidase kinetics in the presence of chlarithromycin.

(—★—) Blank
(—●—) Chlarithromycin (5 μM)
(—●—) Chlarithromycin (10 μM)

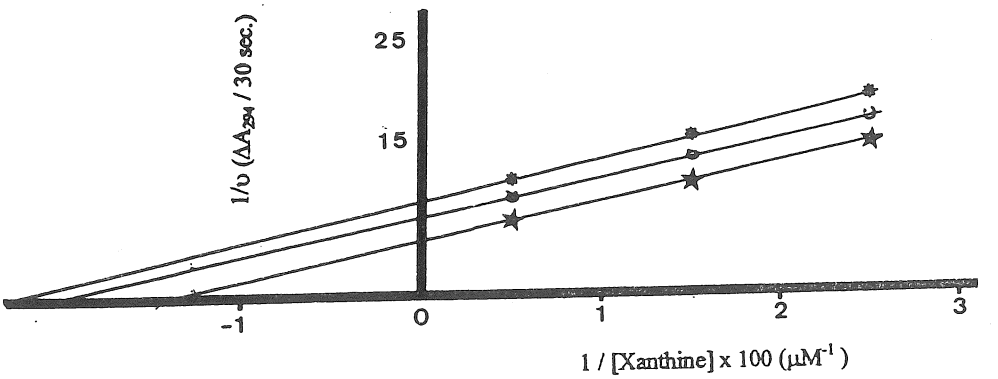

Fig. 2. Lineweaver-Burk plot of xanthine oxidase kinetics in the presence of penicillin-G.

(—★—) Blank
(—●—) Penicillin-G (5 μM)
(—●—) Penicillin-G (10 μM)

While sulphamerazine and ampicilline have nebularine like inhibition (Brown & Konuk 1995), non-competitive inhibition, chlarithtromycin and penicllne-G showed another type of reversible (un-competitive) inhibition on xanthine oxidase.

This investigation showed a different biological property of the antibiotics which it should undoubtedly be considered as important effective factors in the catabolism of purines in living cells. In this point, the question of 'whether antibiotics can be used in the treatment of gout instead of allopurinol?' could be asked. Theoretically the answer might be 'yes'. But we still need to know a lot of things related the other effects of antibiotics in all over the cellular metabolism. The more we learn the more we go on the problems and the more overcome this sort of problems.

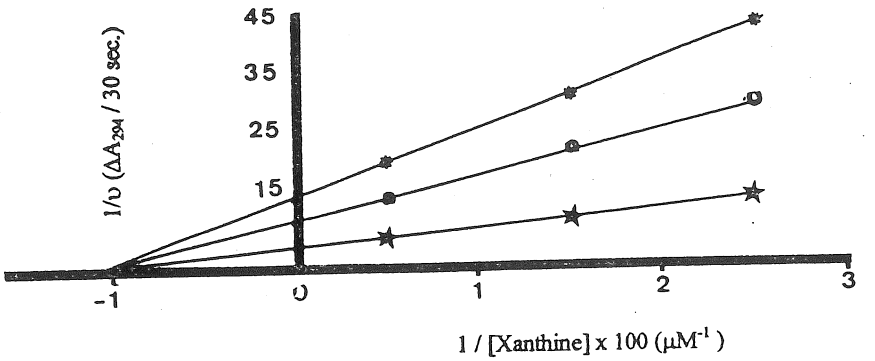

Fig. 3. Lineweaver-Burk plot of xanthine oxidase kinetics in the presence of sulphamerazine.

(—★—) Blank
(—o—) Sulphamerazine (5 μM)
(—•—) Sulphamerazine (10 μM)

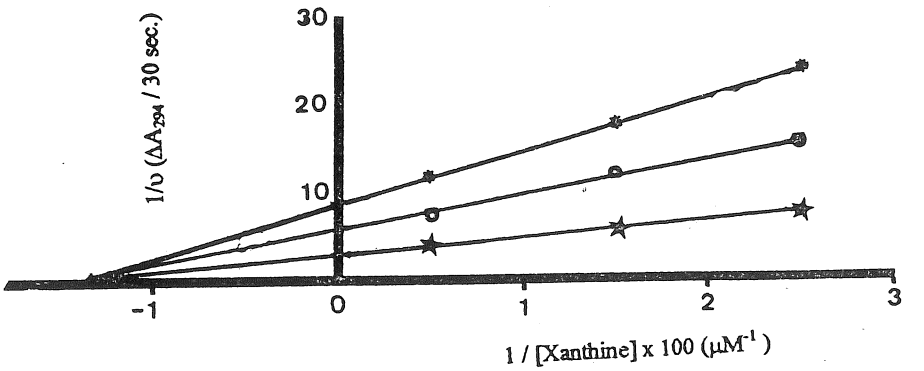

Fig. 4: Lineweaver-Burk plot of xanthine oxidase kinetics in the presence of ampicilline.

(—★—) Blank
(—o—) Ampicilline (5 μM)
(—•—) Ampicilline (10 μM)

Özet

Bu çalışmada, bilinen bazı antibiyotiklerin (sülfamerazin, penisilin-G, klaritromisin ve ampisilin) ksantin oksidaz üzerine olası inhibisyon etkileri araştırıldı. Sülfamerazin ve penisilin-G'nin non-kompetitif, diğer ikisinin (klaritromisin ve ampisilin) ise un-kompetitif şekilde ksantin oksidaz enzimini inhibe ettiği görüldü. Enzim aktivitesi 294 nm de ksantinden ürik asidin oluşumuna göre ölçüldü. İnhibisyon tipleri ise Lineveawer-Burk'un ikili ters grafiği çizilerek belirlendi.

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