A Comparison Study of the Inhibitory Effect Between Tin and Ame'tycine on the Adenosine Deaminase (ADA) Activity in Sera of Anemia, Rheumatiod Arthritis and in both .

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Abstract:
In this work a comparsion study was carried out of the effect of 0.025M of Tin and Ame'tycine on Adenosine deaminase (ADA) activity in patient's sera of Anemia, Rhematiod Arthritis andin both diseases.The activity percentage after inhibition by Tin were 12.4%, 22.7% and 61.4% while by Ame'tycine were 22.5%, 44.9% and 74.6% in the cases of Anemia, RheumatiodArithritis and in both, recepectively, indicating that Ame'tycine was more potent inhibitor than Tin in all cases.These results may have important theorpatic implications.
Key words: Adenosine Deaminase (ADA), ADA inhibitors, Ame' tycine, Tin as inhibitor, Coformcin.

Introduction:

Adenosine deaminase (ADA) EC 3.5.4.4, is a crucial enzyme in purine metabolism that irreversibly deaminates adenosine and 2'–deoxyadenosine to inosine and 2'–deoxyinosine, respectively [1-2] . It is ubiquitous in human tissues, but the highest level are found in the lymphoid system such as lymph nodes, spleen and thymus[3-4] .

The enzyme is a glycoprotine consisting of a single polypeptide chaine of 311 amino acids.

The primary amino acid sequence of ADA is highly concerved across species[5] .

Studies on the crystal structure of mouse ADA showed that the protine is composed of an eight-stranded α /β motif with five additional α-helices, and the activity sites is located at the β- barrel carboxy terminal end[6] .The crystal structure has also revealed that ADA is a metalloenzyme that complexes one mole of Zn^{+2} per mole of proteine.

The zinc ion is located deep within the substrate binding ɜ cleft and coordinated in a tetrahedral geometry to three Nε2 atoms of His-17 and His-214, and the Oσ2 of Asp-295. A water molecules which shares the ligand coordination site with Asp-295, is plarized by the metal giving rise to a hydroxylate ion that replaces the amino group at the C-6 position of adenosine through a sterospecific additional-elimination mechanism. Experiment confirm that in the active enzyme, zinc play a critical role in catalysis[7] .

The function of ADA is critical in controlling the effects of adenosine in many system. Adenosine is an endogenous antihypoxic and anticonvulsant, as well as a molecular of platelet aggregation, lipolysis, glycolysis, blood flow and neurotransmission, therefore, modulation of ADA with the use of highly specific inhibitors might modify the action of endogenous adenosine under various physiological and pathological condition[8-9] .

There are several studies on ADA inhibitors which are analogues of adenosine that been used in treatment drugs[10-16] .Coformycin and its derivaatines exhibited potent effect as ADA inhibitors [17-22] .

Materials and Methods

Materials:-

The study population : Nineteen serum specimens of three groups patients with Anemia, Rheumatiod Arthritis and in both diseases,were used in this study, based on clinical manifestation and labortary results in Tikrit General hospital as summarized in table(1) .Patients with other diseases that may interfer were excluded. A 4th group of (13) samples of normal individuals was used as control.

Table(1): The host information of patients included in this study.

Group	Number	Type of disease	Age range (years)	Gender	
				F	M
I	6	Anemia	10-47	2	4
II	8	RhematiodArithitis	42-55	1	7
III	5	Both	37-50	2	3
IV	13	Non	19-60	6	7

Blood sampling: blood samples(3-5 mL) were collected from individuals, the whole blood sample was left for 20 min. at room temperature, after coagulation, the serume were separated by centrifugation, aspirated carefully.

Methods:

1 .Activity measurment of ADA: The activity of ADA was carried out according to Galanti&Giusti method[23] .

2 .Effect of Sn^{+2} activity on ADA: The same method in(1) was cattied out, 0.1N of Sn^{+2} was prepared, 1mL of the solution prepared were added to all tubes in the detemination of ADA activity.

3. Effect of Ame'tycine on ADA activity: the same procedure in (1) was carried out, in which 1mL of

0.025M of Ame'tycine was added to each tube which used in ADA determination experiment.

Results:

The studay included a total of 19 patients having Anemia, Rheumatiod Arthritis and both diseases with age from (10-55) years. These patients were diagnosed by doctors and clinical examination, and distributed into three groups:-

Figure (1) shows ADA activity in sera of all groups, while figure (2) shows the comparsion of the inhibitory effect of Tin and Ame'tycine on ADA activity in sera of all groups.

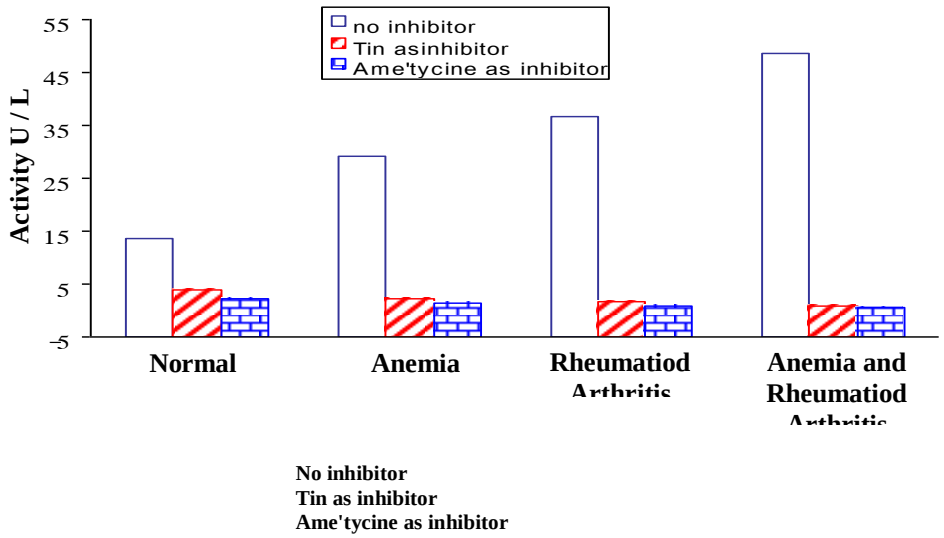

Figure (1): ADA Activity on sera of Normal, Anemia, Rheumatiod Arthritis and on both Anemia and Rheumatiod Arthritis.

Table (2) : Effect of Tin and Ame'tycine on ADA activity on sera of Normal, Anemia, Rheumatiod Arthritis and in both Anemia and Rheumatiod Arthritis.

Groups	Activity percentage % after inhibition	
	Tin	Ame'tycine
Normal	3.5	6.3
Anemia	12.4	22.5
Rheumatiod Arthritis	22.7	44.9
Anemia and Rheumatiod Arthritis	61.4	74.6

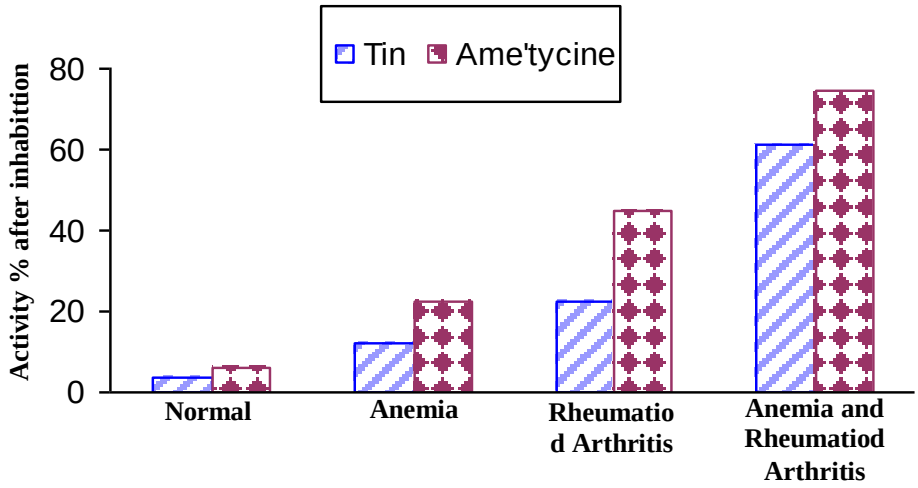

Figure (2): Compartion of the inhibition percentage between Tin and Ame'tycine in ADA

Discussion:

Adenosine deaminase (ADA) is a key enzyme in purine metabolsim, catalyzes the irreversible hydrolytic deamination of active adenosine (or 2'-deoxyadenosine) which yield the inactive metabolic inosine (or 2'-deoxyadenosine) and ammonia[25] .A deficiency of ADA activity is associated with a form of sever combined immunodeficiency disease also an increase of activity is associated with a form of chronic nonherocytic hemolytic disease, leukemia disease[22] and with inflammatory diseases[14,22-26] .

ADA inhibitors may have several clinical applications (i.e in the chemotherapy of lymphoproliferative disorders, in the immunosuppressive therapy, in adenosine levealmodulation[27-28] ,recently ADA inhibitors have been considered as anti-inflammatory drugs[29] also several reports have indicated common antirheumatiod drugs such as methotrexate, asprine and sulfasalazine might exert anti-inflammatory action by elevating the extracellular adenosine levels[30] .

In previous study, Al-Assi W[22] studied the effect of(Mn^{+2} ,Ca^{+2} ,Fe^{+2} ,Cu^{+2} ,Co^{+2} and Cd^{+2}) on the activity of ADA. The results showed that Mn^{+2},Ca^{+2} and Fe^{+2} have activation effect to ADA, while Cu^{+2} ,Co^{+2} and Cd^{+2} have inhibition effect to the enzyme in normal, Anemia, Rheomatiod Arthritis and in both.Also, Al-Obaidi A[17] stated that, Ame'tycine which is one of the new derivatives of Coformicin act as a good inhibitor of ADA in normal and abnormal C.S.F.

In this study, an attempte to compromise between Tin as inorganic inhibitor and Ame'tycine as an organic adenosine analoguse was accomplished.

Table (2) and figure (2) illustrated the effect of Tin and Ame'tycine on the activity of ADA in normal, Rheumatiod Arthritis and in both, which show that Ame'tycine is more potent inhibitor than Tin in all cases. These results might be explained by that, ADA molecule posseses secondary metal binding site(s) are avaliable that allow Tin to inhibit the holoenzyme[31] .Thus the inhibition is of non-competitive type while Ame'tycine has the same ring structure, so it's inhibitory effect is due to the competitive action with the substrate for binding to the enzyme active site. The nitrogen atom in the 3-position, is very important in the interaction of the azole ring with the inhibitory site on the enzyme[17] .

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دراسة مقارنة للتأثير التثبيطي بين القصدير والاميتايسين على فعالية أنزيم الادينوسين دي أمنيز ADA في مصل المرضى المصابين بالانيميا والتهاب المفاصل الرثوي وفي كليهما.

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الملخص :

تم دراسة تأثير كل من القصدير والاميتايسين على فعالية أنزيم الادينوسين دي أمنيز ADA في مصل المرضى المصابين بالانيميا، التهاب المفاصل الرثوي وفي كليهما حيث استخدمت تراكيز 0.025M من القصدير والاميتايسين وتم قياس تأثيرهما على فعالية انزيم ADAفي مصل المرضى. أظهرت النتائج ان الاميتايسين عامل مثبط اقوى من القصدير وفي جميع الحالات وهذا التأثير قد يكون له تطبيقات طبية مهمة.

الكلمات الدالة: أنزيم الادينوسين دي أمنيز ADA؛ مثبطاتADA؛ الاميتايسين؛ القصدير كمثبط انزيمي؛ الكوفورمايسين.