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ANTIBACTERIAL ACTIVITY OF SOME NON-STEROIDAL ANTI-RHEUMATIC DRUGS

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Abstract: Based upon some reports indicating the role of bacterial pathogens in rheumatoid arthritis, this study was undertaken to determine the anti-bacterial activity of some non-steroidal anti-rheumatic drugs including some of their copper(II) complexes. Thus, D-penicillamine, levamisole, flurbiprofen, tiaprofenic acid, pirprofen, ketoprofen, sulphasalazine, phenylbutazone, aspirin, Cu(II)-aspirinate, Cu(II)-penicillamine and some Cu(II)-amino acid complexes (possible active metabolites of the copper complexes of the drugs) were tested for their anti-bacterial activity against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. All the compounds exhibited from moderate to high activity against one or more strains. These results substantiate the speculated role of bacterial pathogens in the causation and/or perpetuation of rheumatoid arthritis.

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INTRODUCTION

Rheumatoid arthritis has been thought to be an atypical form of tuberculosis. Some of the drugs now used for this disease have been initially developed as anti-bacterials. During the clinical studies of sulphasalazine[1,2] , it was observed that its activity was mainly due to the anti-bacterial component, sulphapyridine. Thus, it was reiterated that bacterial infection may have a role in the causation or perpetuation of rheumatoid arthritis. This role is not firmly established and requires further work. One way to investigate into this speculation could be to study the anti-bacterial activity of well-known anti-rheumatic drugs in use, which is the subject of the present paper. Complexes of non-steroidal anti-inflammatory drugs (NSAID's) with copper(II) are considered as potential anti-inflammatory drugs with enhanced activity[3,4]. The action of these complexes is thought to be, in part, due to *in vivo* formation of copper-amino acid complexes[5]. Therefore, some of the Cu(II)-amino acid complexes have also been included in this work to make the study more comprehensive.

MATERIALS AND METHODS

Materials

The drugs used were : D-penicillamine (Fluka), levamisole (ICI), flurbiprofen (Boots), tiaprofenic acid (Hoechst), pirprofen (Ciba Giegy), ketoprofen (Rhone-Poulene), sulphasalazine (Wilsons Pharmaceuticals), phenylbutazone (Ciba Giegy) and aspirin (Reckitt & Colman). The amino acids used were : L-alanine, L-arginine, L-histidine, L-lysine, L-proline and L-threonine (Sigma Chemical Company). Copper(II) complexes of aspirin[3], D-penicillamine[3] and amino acids[6] used were prepared according to the reported methods.

Blood agar Muller Hinton medium (Difco), penicillin G (10 μg) and ampicillin (10 μg) standard discs (bioMerieux) and Whatman susceptibility discs (Difco) were used for the sensitivity measurements. The bacterial strains used were : *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923) *and Pseudomonas aeruginosa* (ATCC 27853).

Methods

The solutions of the compounds under investigation were prepared by dissolving the appropriate amount in sterilized distilled water or petroleum ether or alcohol, as appropriate, to obtain 1μg/ml solutions. The standard strains were grown on blood agar medium and transferred on the slants. The slants were then incubated at 37 °C for 24 hours. The standard bacterial dilutions were prepared by transferring the growth in distilled water and adjusting against the MacFarlan's tube 1.

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The bacterial dilutions were then inoculated on the Muller Hinton medium plates in triplicate. The test solutions (10 µl, containing 10 µg of the compound) were loaded on the susceptibility discs and air-dried. The standard and the test discs were placed on the plates, pressed slightly against the medium and incubated at 37 °C for 24 hours. The susceptibility zones were measured in mm and recorded.

RESULTS AND DISCUSSION

The susceptibility zones observed for the compounds under study are recorded in Tables 1 and 2. All the NSAID's under investigation showed significant anti-bacterial activity against one or more strains (Table 1). The susceptibility zones represent the mean of triplicate measurements. D-Penicillamine did not show significant activity against *E.coli*, while it was highly active against *S.aureus* and *P.aeruginosa*. Previously, D-penicillamine has been reported to be effective against experimental tuberculosis[7]. The present study, supporting this, clearly establishes the anti-bacterial activity of the drug. Levamisole, similarly, showed high activity against *S.aureus* and *P.aeruginosa* but no activity against *E.coli*. Flurbiprofen exhibited medium activity against *E.coli* and *S.aureus* and no activity against *P.aeruginosa*. Tiaprofenic acid showed a similar behaviour but with enhanced activity. Pirprofen was active against all the strains tested. Ketoprofen and sulphasalazine exhibited moderate activity against these strains. Phenylbutazone was active against *P.aeruginosa*. This drug has frequently been used for rheumatoid arthritis in the past. Aspirin, the most widely and consistently used drug, has shown some activity against *E.coli* and *P.aeruginosa*. This is an interesting observation and may help in furthering the understanding of the mode of action of the drug in a variety of diseases for which it is being used.

Table 1

Antibacterial activity of the anti-rheumatic drugs

Drugs	Susceptibility Zone (mm)		
	E.coli	*S.aureus*	*P.aeruginosa*
Penicillin G	20±0.82	36±0.82	32±0.00
D-Penicillamine	10±0.82	20±0.00	22±0.00
Levamisole	R	18±0.82	20±0.82
Flurbiprofen	12±0.00	14±0.00	R
Tiaprofenic acid	18±0.00	16±0.00	R
Pirprofen	16±0.82	16±0.00	12±0.82
Ketoprofen	10±0.00	12±0.82	12±0.82
Sulphasalazine	10±0.00	13±0.00	14±0.00
Phenylbutazone	R	R	20±1.63
Aspirin	10±0.00	R	12±0.00
Cu_2 (aspirinate)$_4$	10±0.00	12±0.00	12±0.00
Cu (penicillamine)$_2$	10±0.82	22±0.00	24±0.00

Values are means ± S.D. (n = 3) ; R = Resistant.

The copper complexes of aspirin and penicillamine, considered to be potential anti-inflammatory drugs, have shown significant antibacterial activity. A distinct behaviour of these complexes was noticed as compared to the parent drugs. Aspirin was resistant to *S.aureus*, whereas its copper(II) complex was active. Penicillamine exhibited enhanced activity on complexation against *S.aureus* and *P.aeruginosa*.

The copper-amino acid complexes, thought to be the active metabolites of the copper complexes of anti-inflammatory drugs, have also shown significant activity as compared to the amino acids and the copper salt (Table 2) used in preparation of the complexes.

Inspite of the fact that the amounts of the drugs and the copper complexes used in the test were equal to the amount of the established antibiotics (penicillin and ampicillin), all these compounds have shown significant activity against one or more strains of the microorganisms. In this respect, it may be noted that the minimum inhibitory concentrations of various drugs are usually different, therefore, an enhanced anti-bacterial effect could be achieved by increasing the dose of a particular compound taking due care of the toxic dose.

In the study about the mode of action of sulphasalazine in rheumatoid arthritis, it was suggested[2], without experimentally determining the anti-bacterial activity of the drug, that the anti-rheumatic effect of the drug could be due to an anti-bacterial effect of one of its components. In the present work, we have

Table 2

Antibacterial activity of the Cu(II)-amino acid complexes

Compounds	Susceptibility Zone (mm)		
	E.coli	*S.aureus*	*P.aeruginosa*
Ampicillin	22±0.00	30±0.00	30±0.82
Copper acetate	R	R	R
Alanine	R	R	R
Arginine	R	R	R
Histidine	R	R	R
Lysine	R	R	R
Proline	R	R	R
Threonine	R	R	R
Cu (alaninate)$_2$	15±0.00	15±0.82	17±1.63
Cu (argininate)$_2$	9±0.00	8±0.00	10±0.00
Cu (histidinate)$_2$	9±0.82	8±0.82	10±0.00
Cu (lysinate)$_2$	11±0.00	8±0.00	15±0.82
Cu (prolinate)$_2$	15±0.00	16±0.00	18±0.00
Cu (threoninate)$_2$	15±0.00	15±0.82	18±1.63
Cu (alaninate)$_2$+ Cu (prolinate)$_2$	15±0.00	15±0.00	18±0.82
Cu (alaninate)$_2$ + Cu (threoninate)$_2$	15±1.63	15±0.82	18±0.00
Cu (threoninate)$_2$ + Cu (prolinate)$_2$	15±0.82	15±0.00	18±0.00
Cu (alaninate)$_2$ + Cu (prolinate)$_2$ + Cu (threoninate)$_2$	15±0.00	15±0.00	18±0.82

Values are means ± S.D. (n = 3) ; R = Resistant.

provided an experimental evidence that most of the anti-rheumatic drugs including sulphasalazine, possess anti-bacterial activity. However, no obvious difference is apparent from the results between the NSAID's and second line or disease-modifying drugs studied.

The behaviour of the drugs is likely to be different *in vivo*, nevertheless the present *in vitro* study furthers our understanding about the mode of action of NSAID's, their copper complexes, and expected metabolites thereof. Thus, it indirectly substantiates the speculated role of bacterial infection in the causation or perpetuation of the rheumatic disease process.

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