

Antibacterial Activity of Some Antineoplastic Drugs Against *Staphylococcus aureus* Isolated for UTI

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Summary

Antibacterial activity of methotrexate , 6-mercaptopurine, thioguanine and 5-fluorouracil against twenty isolates of *Staphylococcus aureus* isolated from UTI in children were studied .Ten of these isolates were isolated from urine samples of children suffering from leukemia in addition to UTI and showed completely resistance to all of these drugs, comparing with the other isolates were showed sensitivity to the drugs above except methotrexate. A phenomenon synergism between methotrexate and each of 5-fluorouracil and 6-mercaptopurine to inhibit the isolates from non-leukemic children were detected .Synergism also occurred between each of methotrexate, 6-mercaptopurine and 5-fluorouracil and the antibiotics :ciprofloxacin, amoxicillin, co-trimoxazole and ceftazidime to inhibit these isolates. Hemolysin production by *Staph. aureus* were inhibited in presence of methotrexate in the culture.

Introduction

One of the most common complications involved in treating patients with hematologic cancer is infections. In many cases there are multiple factors that predispose these patients to infections such as neutropenia induced by therapy or bone marrow involvement, hypogammaglobulinemia, T-cell dysfunction, and mucosal damage. The spectrum of infections has changed with the use of purine analogs and the advent of monoclonal antibodies [1].

Gram-positive organisms account for 60% to 70% of microbiologically documented infections in most reports [2]. *Staphylococcus aureus* is one of the most frequently pathogens cause infections especially in hematological and oncological patients with febrile neutropenia [3].

Antineoplastic drugs used for treatment of malignant diseases and affect all cells with rapid turnover. Antimetabolites is one of antineoplastic drug groups, includes methotrexate , 6-mercaptopurine , thioguanine , 5-fluorouracil , fludarabine and cytarabine.Their mechanism of action by inhibiting the production of DNA and

RNA by inhibiting formation of purine and pyrimidine [4 , 5]. Several antineoplastic drugs are known to have an antibacterial effects[6 -8]. The previous studies revealed bacteriocidal effect of antineoplastic drugs which used for treatment of leukemia on intestinal and oral flora [8,9] . 5-Fluorouracil was found to inhibit strains of *Staphylococcus aureus* and *Staph. epidermidis* in low concentration [10] .In addition to that *Staph aureus* were sensitive to the antineoplastic drug etoposide [11].

Bacteriocidal effect (synergisms & antagonisms) of combinations of antibiotics and antineoplastic drugs commonly used in clinical practice to certain bacteria were detected in some studies [8 , 12,13] .

The aim of this study is to reveal the antibacterial effect of some antineoplastic drugs used for treatment of leukemia on clinical isolates of *Staphylococcus aureus* isolated from UTI cases in leukemic and non-leukemic children and to detect the occurrence of synergism and antagonism between antibiotics and these drugs.

Materials and Methods

Bacteria : twenty isolates of *Staphylococcus aureus* were isolated from samples of urinary tract infections in children(7-15 years males&females).Ten of these isolates from children suffering from leukemia (in addition to UTI) under chemotherapy .The isolates were identified according to Diagnostic microbiology[14] .

Antineoplastic drugs : Methotrexate , 6-Mercaptopurine , Thioguanine , 5-Fluorouracile supplied by Wellcome Co. , England.

Antibiotics : vancomycin , amoxicillin , cephalothin , ceftazidime , cefotaxim , ciprofloxacin , co-trimoxazole , gentamycin , amikacin were supplied by Bioanalyse Co., India.

Antibacterial activity of each of antineoplastic drugs and antibiotics against *Staph. aureus* were tested on Mueller Hinton agar by disc diffusion method. Antineoplastic disc prepared in a concentration 25 & 50 µg [8].

The activity of combinations of antibiotics and antineoplastic drugs (synergism & antagonism) were initially screened by disc diffusion method. A big disc (30 mm diameter) saturated with sub-MIC of antineoplastic drugs placed in the dish center and antibiotic discs were placed around it. Appearance of clear zone between the central disc and any of antibiotic disc recorded synergism. [8]

Minimal inhibitory concentration (MIC) were done by microtitration method [14], two ranges of two fold serial dilution were prepared. Dilutions were made from 18 hr. cultures of *Staph. aureus* to results approximately 10^6 CFU/ml .The wells were inoculated with 0.1 ml of bacterial suspension .

The effect of antineoplastic drugs on hemolysin production(activation or inhibition) was done on blood agar by adding antineoplastic disc on lines of bacterial growth[15].

Results:

Results of antibacterial activity test of antineoplastic drugs showed completely resistance of *Staph. aureus* which was isolated from leukemic children to all of these drugs, while the isolates from children suffering from UTI only showed sensitivity to each of 5-fluorouracile , 6-mercaptopurine and thioguanine with inhibition zone more than 18 mm diameter, and resistance to methotrexate.

Minimal inhibitory concentration values of antineoplastic drugs for isolates from leukemic children were higher than that for other isolates from non leukemic children (table 1).

Table 1: MICs values($\mu\text{g/ml}$) of antineoplastic drugs for *Staph aureus* isolates.

Isolates source	5-fluorouracil	6-mercaptopurine	thioguanine	methotrexate
Leukemic children (10 isolates)	64	>64	>64	>100
Non leukemic children (10 isolates)	8	16	16	>64

The results revealed synergism between methotrexate and each of 5-fluorouracile and 6-mercaptopurine to inhibit the isolates and no antagonism were recorded.

A phenomenon of synergism occurred between each of methotrexate , 6-mercaptopurine and 5-fluorouracile and the following antibiotics : ciprofloxacin , amoxicillin , co-trimoxazole and ceftazidime. Activity of the other antibiotics, vancomycin, cephalothin, cefotaxim, gentamycin, amikacin were unaffected by the presence of antineoplastic drugs in the medium.

Antibiotic susceptibility test revealed high resistance percentages to the antibiotics by the isolates from leukemic children comparing with the other isolates ,and three isolates were resistant to vancomycin. (Table 2).

Table 2: Numbers of resistant isolates of *Staph aureus* to the antibiotics.

Isolates sources	No	Van co myc in	A mo x yci lli n	Cephal othin	Cefta zidim e	Cefo taxi m	Cipr oflox acin	Cotri m oxazo le	Gen ta myci n	Am ika cin
Leukemic children	10	3	10	10	6	6	3	10	8	6
non-leukemic children	10	0	5	6	3	2	0	5	4	2

The effect of sub-MIC of antineoplastic drugs on hemolysin production from both groups of *Staph. aureus* isolates were studied. No effects for these drugs on the

production of hemolysin were detected except methotrexate which inhibited the production .

Discussion :

The present study revealed bacteriocidal effects of 6-mercaptopurine, thioguanine and 5-fluorouracil except methotrexate on *Staph. aureus* isolated from non-leukemic children. Many previous studies reported occurrence of antibacterial action of some antineoplastic drugs on certain bacteria [7,8,16,17]. Other studies showed that 5-fluorouracil inhibit *Staph. aureus & epidermidis* while no effect found for methotrexate [10] .Selective resistance of pathogens to antineoplastic agents has been cited as an explanation for the prevalence of infections caused by bacteria in leukemic patients[6,8]. This explains the presence of vancomycin resistant *Staph. aureus* between isolates from leukemic patients in the present study. There are some ways enable the cells to be resistant to antineoplastic drugs : mutations, change in drug uptake path and enzyme changes in the cell [18] .Antineoplastic resistance in *Staph. aureus* and high MICs values as in the present study may explain their occurrence as opportunistic pathogens in compromised hosts[6].

Treatment of leukemia with combination of two or more of antineoplastic drugs is widely used[5]. Occurrence of synergisms between methotrexate and each of 6-mercaptopurine and thioguanine to inhibit growth of the isolates increase the risk of reduce number of normal flora and lead to change in the ecosystem and occurrence of colonization by resistant bacteria which cause diseases [8].

Bacteriocidal effect of combinations of antibiotics and antineoplastic drugs commonly used in clinical practice was investigated to analysed whether the combinations act synergistically have different or antagonistic antibacterial effect compared to the effect of the antibiotics alone.Previous studies reported an occurrence of synergism between some antineoplastic drugs and antibiotics and agree with the present study in the ability of 5-fluorouracil to synergize with antibiotics[12,13].It was concluded from the present study, that there is a variable degrees of synergisms between antibiotics and antineoplastic drugs depending on the type of drug and bacterial species ,and this may explain why only four of nine types of antibiotics synergize with the antineoplastic drugs in use.

Hemolysin production controlled by group of genes setting on a space called Pathogenicity island on chromosome or plasmid [19].The results revealed inhibition of hemolysin production by methotrexate only and no effect for the other drugs.This agreed with the last study[8] which reported inhibitory effect for methotrexate on hemolysin production in *E.coli* . Other study reported inhibitory effect on hemolysin production in presence of antibiotics [15] .

الخلاصة

درست الفعالية ضد ميكروبية لبعض أدوية الأمراض السرطانية على عشرين عزلة من المكورات العنقودية الذهبية عزلت من حالات التهاب المجاري البولية عند الأطفال. عشرة من هذه العزلات عزلت من عينات إدرار أطفال مصابين باللويميا ويعانون من التهاب المجاري البولية وقد أظهرت مقاومة تامة لكل أدوية الأمراض السرطانية المستخدمة في الدراسة مقارنة مع العزلات العشرة الأخرى والتي أظهرت حساسية تجاه الأدوية أعلاه عدا الميثوتريكسيت. سجلت ظاهرة التآزر بين الميثوتريكسيت وكل من فلورويوراسيل و ميركابتوبوريين لتثبيط عزلات الأطفال غير المصابين باللويميا. كذلك سجل التآزر بين كل من الميثوتريكسيت و فلورويوراسيل و ميركابتوبوريين والمضادات الحيوية سبروفلوكساسين، اموكسيلين، ترايموكسازول وسيفتازيديم لتثبيط العزلات. ظهر أن للميثوتريكسيت تأثيرا مثبتا على إنتاج الهيمولايسين من عزلات المكورات العنقودية الذهبية عند وجوده في الوسط الزراعي .

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