COMPARATIVE ANALYSIS OF IN VITRO ANTIMICROBIAL ACTIVITY OF 5-FLUOROURACIL AND 5-BROMOURACIL

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ABSTRACT

In the leptospiral culture medium, along with basic nutrients, supplements including bovine serum albumin and vitamin complexes, the selective agent 5-Fluorouracil are also added to avoid the other microbial contaminations. The leptospiral culture medium is highly sensitive to contamination due to its high substantial nutrients. Thus, a study to determine the in vitro antimicrobial activity of 5FU was done compared with 5-Bromouracil. The standard microbiological procedures were followed to analyze the antimicrobial effect using a battery of bacterial and fungal pathogens. Results revealed the wide antimicrobial properties of 5FU whereas 5BU showed poor and mostly negative interpretations. This study concluded that the usage of 5FU in leptospiral culture medium is mandatory in order to avoid the contamination.

KEYWORDS: 5-Flourouracil, 5-Bromouracil, antimicrobial activity.

INTRODUCTION

In general, the bioactive molecules are having soft and hard donor sites of nitrogen, oxygen and sulphur atoms which are abundantly found in nature. Among them, 5-Fluorouracil (5-FU) is an analog of pyrimidine which exhibit antineoplastic and antimetabolite activity that are having the high active bioactive molecular potential.[1]

Metabolites of 5-Fluorouracil also possess a different mechanism of action mainly the synthesis of DNA is interfered by 5-FU through blocking the thymidylate synthetase conversion of deoxyuridylic acid to thymidylic acid.[2]

5FU is a white crystalline powder, almost soluble in 97% ethanol and lightly soluble in water. Especially, 1g of 5-FU dissolves in 80ml of water, 170ml of ethanol and 55ml of methanol, compound solubility in water increase with an increasing pH due to salt formation.[3] It melts at 282-286°C and produce highly toxic fluorides and nitrogen oxides vapors with decomposition. Breakdown of 5-FU done in two ways including thermal and photochemical decomposition which creates the opening of the pyrimidine ring between N1 and C4 and N1 and C3 it produce urea.[4,5]

Production of barbituric acid and uracil by alkaline hydrolysis then further it degrades urea. At the rate pH 9.0, alkaline hydrolysis is increase.[6] Generally the antimetabolite role is to inhibit the essential biosynthetic process or sometime being incorporate into the macromolecules such as DNA and RNA thus inhibit their normal functions but 5-FU does both the above mentioned functions, 5-FU highly used in the cancer treatment which includes colorectal, breast cancer and cancer in aerodigestive tract. Sometimes, 5-FU combines with some chemotherapeutic agents which improve the rate of response.[7]

A major side effect caused by the 5-FU treatment is oral mucositis, oral cavity ulceration and inflammation.[8,9,10] It is a major impact that affects the patient life span, which includes problems with eating, speaking and drinking and concentration only focus on pain relief and oral hygiene.[11] Clinical application of 5-FU is purely limited because of the drug resistance, it could be the result from various causes, which including alteration of drug influx and efflux development of drug inactivation and the drug target mutations. Still many activities of 5-FU not fully demonstrated, but in nearby future using some smart technologies the mechanism of anti-tumor action and drug target of 5-FU will be concluded.[10] 5-FU acts as a
pyrimidine analogue, which exhibit a marked inhibitory effect on a number of heterotrophic bacteria.\textsuperscript{[11,12]}

New combination of antimicrobial agent is developed for selective isolation of \textit{Leptospira}.\textsuperscript{[13]} an aerobic, spiral, gram negative bacteria, occur in soil and water. It is slow growing organisms have a generation time of nearly 24-28 hours at 30°C and it require a rich medium for their growth at the neutral pH.\textsuperscript{[14]} Saprophytic and pathogenic leptospires have a same morphological structure, but they vary in some biological characters, thus they differentiate by using a selective media.\textsuperscript{[15]} The selective media contain 5FU which inhibit the growth of other microbial loads in the leptospiral culture media.

Fletchers and EMJH media are enriched, semisolid medium used for the cultivation of \textit{Leptospira}, contain 5FU is a fluorinated pyrimidine analog that inhibits bacterial contaminants without affecting the growth.\textsuperscript{[16]} 5-FU interfere the fungal cell in the pyrimidine metabolism and protein synthesis which ultimately leads to cell apoptosis. 5-FU have a capacity to inhibit the \textit{Mycobacterium tuberculosis}, confirmed by trace level of metabolite labeling that affect the \textit{M. tuberculosis} cell wall synthesis\textsuperscript{[17]} and gives a high rate of antifungal and fungistatic activity.\textsuperscript{[18]}

Uracil, in general apart from 5-FU, 5-Bromouracil are the derivatives of benzylchlorides having wide antitumor, antibacterial properties. Most of the studies suggested that 5-BU have more antitumor properties than antimicrobial.\textsuperscript{[19]} Thus an attempt was made to determine the effect of antimicrobial activity of 5-FU with a battery of Clinical pathogens compared with 5-BU.

**MATERIALS AND METHODS**

**Preparation of chemicals and media**

The test drugs (chemicals) of both 5-FU and 5-BU in various concentration starts from 1 to 3\% were prepared using sterile double distilled water. The culture media (Mueller Hinton agar) was prepared and MHA plates were prepared aseptically. The chemical diluents were stored at -80°C until use. The prepared culture plates were checked for sterility overnight and stored in low temperature until use.

**Bacterial and fungal cultures**

The following bacterial and fungal cultures isolated and identified from the clinical specimens were included in this study. The bacterial cultures including \textit{Acinetobacter baumanii}, \textit{Escherichia coli}, \textit{Enterococcus faecalis}, \textit{Flavobacter sp}, \textit{Klebsiella pneumoniae}, \textit{K. oxytoca}, \textit{Pseudomonas aeruginosa}, \textit{Proteus mirabilis}, \textit{P. vulgaris}, \textit{Providentia sp}, \textit{Staphylococcus aureus}, \textit{Salmonella typhi}, \textit{S. paratyphi A}, \textit{S. paratyphi B}, \textit{Serratia marcescens}, \textit{Shigella dysentriae}, \textit{S. sonnei} and fungal culture – \textit{Candida albicans} were impregnated for antimicrobial analysis. All the cultures were sub-cultured in nutrient broth on the day of performing antimicrobial activity.

**In vitro antimicrobial activity**

By using various concentrations of 5-FU and 5-BU, the in vitro antimicrobial activity was performed by well cutting method. The test bacterial and fungal cultures were inoculated by surface plating method. The known sized wells were cut with appropriate distance aseptically. Further, various concentrations of diluted 5-FU and 5-BU were loaded in the pre-designated wells. These set up were incubated in un-inverted position at 37°C for 24 to 48 hours for determining the zone of inhibition. By using standard millimeter scale, the zone of inhibition was recorded, compared and interpreted.

**RESULTS**

A series of incorporation that analyzing the \textit{in vitro} antimicrobial properties of 5-FU was performed against the panel of bacterial and fungal pathogens by well diffusion method using Mueller Hinton agar. After appropriate incubation of the plates, the zone of inhibition were measured and interpreted in table 1. The measured zone of inhibition (in mm) against the growth of bacterial and fungal strains is shown in figure 1.

By observing the results, the bacterial pathogens that are inhibited in maximum are \textit{Staphylococcus aureus} followed by \textit{K. pneumoniae}, \textit{P. aeruginosa}, \textit{K. oxytoca}, \textit{E. faecalis} and \textit{Flavobacterium} sp. The fungal pathogen \textit{C. albicans} showed maximum inhibition at the concentration of 1, 2 and 3\% as 6, 12 and 23mm respectively. In contrast, \textit{A. baumanii} and \textit{S. dysentriae} were not responded even at the highest concentrations of 5-FU. Very less inhibition rate was observed at highest concentration of 5-FU among \textit{E. coli}, \textit{P. vulgaris}, \textit{S. typhi} and \textit{S. paratyphi A}.
Table 1: Observation of in vitro antimicrobial activity of 5-FU.

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Zone of inhibition (in mm) verses concentration of 5-FU</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>1%</td>
</tr>
<tr>
<td>Acinetobacter baumanii</td>
<td>-</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>4</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>6</td>
</tr>
<tr>
<td>Flavobacterium sp</td>
<td>6</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>7</td>
</tr>
<tr>
<td>K. oxytoca</td>
<td>6</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>7</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>4</td>
</tr>
<tr>
<td>P. vulgaris</td>
<td>3</td>
</tr>
<tr>
<td>Providentia sp</td>
<td>5</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>6</td>
</tr>
<tr>
<td>Salmonella typhi</td>
<td>-</td>
</tr>
<tr>
<td>S. paratyphi A</td>
<td>-</td>
</tr>
<tr>
<td>S. paratyphi B</td>
<td>5</td>
</tr>
<tr>
<td>Serratia marcescens</td>
<td>6</td>
</tr>
<tr>
<td>Shigella dysentriae</td>
<td>-</td>
</tr>
<tr>
<td>S. sonnei</td>
<td>6</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>6</td>
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</tbody>
</table>

While observing the antimicrobial activity of 5-FU at various concentrations, it showed negative results by providing no inhibition of any microbial pathogens listed in this study. Thus this study highlighted the importance of the usage of 5-FU in EMJH medium for leptospiral growth by avoiding the growth of contaminants. Even at very less concentrations of 5-FU, the growth inhibition rate of various bacterial and fungal pathogens were well recorded in this study.

[Antimicrobial properties of 5-FU: Fig. 1: A – Proteus mirabilis & Klebsiella oxytoca; B - Shigella sonnei & Klebsiella pneumoniae; Fig. 2: C – Pseudomonas aeruginosa & Flavobacterium sp; D – Enterobacter faecalis & Staphylococcus aureus; Fig 3: E – Salmonella paratyphi B & Providentia sp; F – Serratia marcescens & Candida albicans; Antimicrobial properties of 5-BU: Fig 4: G – Salmonella typhi & Escherichia coli; H – Serratia marcescens & Candida albicans; I – Pseudomonas aeruginosa & Flavobacterium sp.]
DISCUSSION
The in vitro activity and mechanism of action and catabolic nature and antimitagenic power of 5-FU have been the newer subjects of intense investigation. No previous report found of determining the antimicrobial activity of 5-FU alone in detail. But other studies suggested that the synergistic effect of antibiotics with 5-FU found significantly better bactericidal effect. A study suggested the presence of antitumour agents in blood samples for culturing may delay in detection of bacteria or sometimes even no growth because of inhibition of organisms during culture would also seem to be an infrequent concern based upon the data already published. No recent report found about the antibacterial effect of antineoplastic agents including 5-FU.

The mechanism of action of 5-FU against Mycobacterium tuberculosis was well studied and it was proved that association of mutational nature and conversion of FUMP leads to the maximum inhibition. Some studies suggested that investigation related to the determination of antimicrobial properties of 5-FU should be explored. Thus this study may provide some roadmap to use the 5-FU as antimicrobial in selective situations. However the effect on viability can be seen to be quite different in usage of FU and FUR (flourouracil riboside). 5-FU have bacteriostatic effect and occasionally kills bacteria while FUR is markedly bactericidal. This study determined the bactericidal effect of 5-FU by suing broad bacterial and fungal pathogens.

The magnitude of the global health problem of infectious diseases has underscored urgent need for new antimicrobials to treat drug resistant microorganisms. A study of determining risk of microbial contamination with multiple usages of 5-FU vials suggested that Staphylococcus, Pseudomonas, Klebsiella and Proteus are growing. But in this study these organisms are remarkably inhibited by various concentrations of 5-FU. In the mucosal region, there is a triggered alteration in the microbial environment upon chemotherapy of using 5-FU.

In conclusion, our study clearly described that 5-FU have wide bactericidal and anticandidal properties compared to 5-BU. A good rate of inhibition was observed against gallery of test bacterial pathogens showed it may be the future promise of using 5-FU as classical drug for various infections especially in immunocompromised state including cancer, transplantation etc. So, it is proved that 5-FU is mandatory in leptospiral culture media preparation for avoiding the contaminations. To get a complete picture of the mechanism and broad spectrum of antimicrobial properties, 5-FU has to be subjected further for research by determining the effect of 5-FU as anti-infective external coatings for central venous catheters and other catheters and cannulas.

REFERENCES


