In Vitro Antibacterial Effects Of Selective Histaminic Receptor Type 2 Blockers: A Novel Study

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In Vitro Antibacterial Effects Of Selective Histaminic Receptor Type 2 Blockers: A Novel Study

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Abstract

Selective antihistamine receptor type 2 blockers are widely used for treatment of peptic ulcer, these drugs are famotidine, cimetidine, nizatidine and ranitidine and because many nonantibiotics agents have antibacterial activity so this study aimed to evaluate the antibacterial effects of selective H2 blockers. Twenty two of bacterial strains were selected 5 Staphylococcus aureus, 5 Escherichia coli, 6 Pseudomonas aeruginosa and (6) Klebsiella pneumoniae. The drug’s effects determined by Agar disc diffusion and Agar well diffusion methods, then evaluation done through measurement of zone of inhibition and minimal inhibitory concentration (MIC).

Results showed that cimetidine and ranitidine have insignificant and negligible antibacterial effects, while famotidine have significant antibacterial effects against Escherichia coli (MIC 8mg/ml and inhibition zone 25mm) and moderately against Staphylococcus aureus. In conclusion famotidine have significant antibacterial activity specially against Escherichia coli.

Introduction

There is a unremitting and critical need to discover novel antimicrobial compounds with miscellaneous chemical structures and novel mechanisms of action because there has been an startling increase in the occurrence of new and reemerging antibiotic resistances. Another large distress is the progress of resistance to the antibiotics in current clinical use. (1). In brightness of the new emergence of the bacteria that are resistant to multiple antimicrobial drugs posing a challenge for the treatment of infections, the need to ascertain new antimicrobial substances for use in combating resistant bacteria becomes relevant, resistant bacteria representing a confront in the treatments of various well-known infections necessitated the need to find new-fangled substances with antimicrobial properties to be used in the combat against these microorganism (2). The non-antibiotics reported so far, more than a few have been found to improve the activity of certain antibiotics and even non-antibiotics against specific bacteria, e.g. methdilazine in combination with streptomycin (STR), kanamycin and gentamicin showed augmentation of their antibacterial effects, resulting in synergism. (3) Synergism has also been noted between two non-antibiotic drugs, for example between methdilazine and bromodiphenhydramine, diphenhydramine and m-DOPA (4) and between chlorpromazine and thioridazine (5). These studies have collectively led to the recognition that, as a group, nonantibiotics such as phenothiazines are agents that must be considered either for immediate use when the management of the bacterial infection is challenging (6) or as lead compounds for new and effective antibacterial agents (7). H2 blockers are a class of drugs that work on the cells that line the stomach, reducing the production of acid. They include: cimetidine, famotidine, nizatidine and ranitidine, and come in various different brand names; they have also been used as one part of a treatment to get rid of Helicobacter pylori, a bacterium found in the stomach which can cause ulcers (8). Because of many nonantibiotics agents have antibacterial effects therefore, in the present study, H2-blockers were screened for their antimicrobial potential against selected members of bacteria.

Methods

This study was carried out in Department of Pharmacology, College of Medicine, Al-mustansiriya University, Baghdad – Iraq, from October to December 2010. It is approved by scientific jury of Department of Pharmacology and microbiology and licensed by board of medical college.

The microbial strains investigated in this study are identified strains and were obtained from National Chemical Laboratory (NCL). The studied bacterial strains comprise (5) Staphylococcus aureus, (5) Escherichia coli, (6) Pseudomonas aeruginosa and (6) Klebsiella pneumoniae microorganisms were maintained at 4 °C on nutrient agar slants. The antibacterial assay was performed by 2 methods: Agar disc diffusion method (9) and Agar well diffusion method (10). The media (Mueller Hinton Agar) along with the inoculums (108 cfu/ml) was poured into the petri plate. For agar disc diffusion method, the disc (0.7cm, Hi-Media) was saturated with 100 µl of the test compound (cimetidine, ranitidine and famotidine)
allowed to dry, and introduced on the upper layer of
the seeded agar plate. Into the well, 100 µl of the test
compound was introduced. The plates were incubated
overnight at 37 °C. Microbial growth was determined
by measuring the diameter of the zone of inhibition.
For each bacterial strain, negative controls were
maintained where pure solvents were used instead of
the extract. For positive control, 4 antibiotics, namely
Chloramphenicol (30 mcg/disc), Gentamicin
(10mcg/disc), Ciprofloxacin (5 mcg/disc), , and
Imipenem (10 mcg/disc) were used. The experiment
was performed 2 times and the mean values are
presented.
Two bacterial strains sensitive to famotidine were
chosen, Staphylococcus aureus and Escherichia coli.
Each strain was grown in 4 ml nutrient broth (NB) for
18 h; 2 ml of this culture was then added to 4 ml of
fresh NB and incubated at 37°C for 2 h to help the
strain attain logarithmic growth phase. At this stage,
the CFU count was determined, and famotidine was
added at a concentration higher than the respective
MIC level. CFU counts from the cultures were
individually taken after 2, 4, 6 and 18 h of adding the
drug this used to determined the kinetic effects of
famotidine on bacterial growth.
Serial dilutions of the H2-blockers were made in Muller
Hinton broth which was inoculated with a standardized
number of organisms and incubated for 24 hours. The
lowest concentration of drug preventing of turbidity is
considered to be the minimal inhibitory concentration
(MIC).
Drugs Were Obtained From Private Pharmaceutical
Company Ltd; Cimetidine 200mg (Cimedine Dar Al
Daw), Ranitidine 150mg (Histac Ranbaxy Laboratories
Limited At:Kother,Mahaboob Nagar Dist.A.P-509228)
And Famotidine 20mg
(UlceranMedochemie Ltd Limassol Cyprus).

Results

The antibacterial susceptibility of various standard
antibiotics against the selected bacteria showed that
Klebsiella pneumonia was less susceptible toward
most selected antibiotics ,while pseudomonas
aeruginosa showed higher sensitivity for ciprofloxacin
but Escherichia coli is highly sensitive for gentamicin
and chloramphenicol and less sensitive for piperacillin
and ciprofloxacin respectively table (1).So most of the
selected bacterial strains regarded sensitive to the
standard antibiotics
Regarding the antibacterial effects of selective H2
–blockers both cimetidine and ranitidineshowed
minimal antibacterial activity in comparison with
control ,also famotidine produced minimal antibacterial
activity against Pseudomonas aeruginosa and
Klebsiella pneumoniae and moderated antibacterial
activity against Staphylococcus aureus but the most
significant effect appeared on Escherichia coli when
famotidine produced 25 mm zone of inhibition table[2.

Discussion

The histamine receptor antagonists are cimetidine,
rantididine, famotidine, these agents bind to the
H2-receptors on the cell membranes of parietal cells
and avert histamine induced stimulation of gastric acid
secretion. After protracted use, down-regulation of
receptor production occurs, resulting in tolerance to
these agents. H2-blockers are approved for the

treatment of gastro esophageal reflux disease, acute
ulcer healing, and post–ulcer healing maintenance
therapy (11).
The current study introduced a new utilize and
indications of H2-blockers that is the antibacterial
effects, in this study neither cimetidine nor rantidine
producing any considerable antibacterial effects but
famotidine inhibit bacterial growth of Escherichia coli
and to a petite extend on Staphylococcus aureus
growth.
The lack of susceptibility of Pseudomonas aeruginosa
to those agents could be accredited to the reality that
this bacteria are naturally resistant to many antibiotics
due to the permeability barrier afforded by its outer
membrane. Moreover; Pseudomonas aeruginosa tend
to colonize in a biofilm form which makes these
bacteria unreceptive to therapeutic concentrations of
most antibiotics. Inside view of the fact that its natural
habitat is the soil, living in association with bacilli,
actinomycetes and molds, it has developed resistance
to a variety of their naturally occurring antibiotics (12).
Furthermore; daily famotidine monotherapy resulted in
a significant decrease (P≤ 0.01) in the mean count of
epithelium-adherent Helicobacter pylori bacteria
relative to that of the positive control group after 2
weeks of treatment with complete eradication from the
epithelium in one of six mice at that time. This
suggests that Helicobacter pylori bacteria adhering to
surfaces of epithelial cells deep within the ion (acidic)
channels of gastric glands were disrupted via blockage
of histamine H2 receptors (13)
Icatlo1994 study showed that the direct antibacterial
activity of famotidine on Helicobacter pylori, is inert in
vitro at a concentration of 2 mg/ml (14). In this study
minimal inhibitory concentration of famotidine was
8mg against Escherichia coli which coincide with this
Moreover, famotidine and cimetidine salts showed significant antibacterial effects; they possess valuable pharmacological activities; namely, they show gastric acid secretion inhibitory, gastrectroprotective effect and antibacterial effect against the bacterial strain Helicobacter pylori.(15)

Furthermore, famotidine enhanced the induction of non-reparable DNA damage in Escherichia coli strains that lacking the post-replication repair pathways (16). The nitrosation products of all h-2 drugs mainly induced base-pair substitutions in Salmonella DNA, to a greater extent at sites containing G.C base pairs (strain TA100) in the case of famotidine and cimetidine, and at sites containing A.T base pairs (TA102) in the case of ranitidinealso famotidine cause differential toxicity in Escherichia coli, and reverse mutation in Salmonella typhimurium, (17;18;19).

Add to this famotidine, in having abenzene ring attached to another one, may be conceived to mimic a phenothiazine construction, per se explain the antibacterial activity (20).

Cimetidine and famotidine’s high lipidsolubility makes them expected to accumulate in lipid membranes, primary to an alteration of their physical properties. The consequential changes in conformation of the membrane-bound enzymes may be another mechanism of fundamental the inhibitory effect of famotidine on bacterial growth. In distinction, ranitidine, which is highly water-soluble (chloroform/water partition coefficient at pH 7 is 0.33), had no effects on membrane function. (21)

Cimetidine predisposes critically ill patients to developing a diversity of infectious diseases (22). In addition to facilitating bacterial overgrowth mediated by a decrease in gastric acidity, impairment of reactive oxygen species(ROS )production with cimetidine may have been accountable for these hazardous complications but famotidine also reduced the ROS production by neutrophils in a dose-dependent fashion. However, it is reasonable that this effect of famotidine has few clinical implications because clinically relevant concentrations of the drug abortive to impair the neutrophil function. However, the ROS produced extremely by neutrophils may play a essential role in pathogenesis of the host auto injury leading to multiple organ dysfunction syndrome associated with systemic inflammatory response (23;24;25).

Famotidine in this study mainly act on Escherichia coli and this is very important becausefamotidine antagonize the essential mediator of Escherichia coli-mediated pathogenesis which is histamine this supported by Hori 2002 which showed that two kinds of histamine receptor antagonists, famotidine and pyrilamine, promoted the clearance of Eschrichia coli in experimental peritonitis. The enhancement of recruitment of neutrophils was suppressed in the presence of the histamine agonists hepatanecarboxamide and dimaprit. Also histamine was first shown to be an important mediator in an Escherichia coli infectious peritonitis model, causing a delay in the elimination of bacteria.(26) This also support our opportunity of the use of antihistamine drugs for bacterial infection.

Accordingly, it was expected that histamine would enhance phagocytic recruitment, but our data definitely indicate that, on the contrary, the clearance of Escherichia coli was delayed in the presence of histamine.(27;28).

Likewise; Escherichia coli induce release of histamine from rat mast cells and human basophilic granulocytes by clinical Escherichia coli isolates and relation to hemolysin production and adherin Expression (29). Therefore; H2 blockers regarded as anti-infecctive and anti-inflammatory so decrease pathogen and or inflammatory induced tissue damage. Since this drug is in routine therapeutic usage, famotidine may, in course of time, be developed as the second or even the first line antimicrobial agent in many infections; such properties would further enhance its applicability in humans. Thus, the present study suggests that famotidine has a potential for being developed into a powerful antimicrobial agent. Further pharmacological studies are compulsory to confirm our findings on the possible utilize of this drug to treat bacterial infections. With suitable structural modifications, it may be possible to obtain compounds with greater antimicrobial actions.

Conclusions

Famotidine as the prototype of selective H-2 blockers possess significant antibacterial activity especially against Escherichia coli.

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Illustrations

Illustration 1

Table (1). Antibacterial susceptibility testing of various standard antibiotics against bacterial strains

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Staphylococcus aureus</th>
<th>Escherichia coli</th>
<th>Pseudomonas aeruginosa</th>
<th>Klebsiella pneumonia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloramphenicol (30 mcg/disc)</td>
<td>16</td>
<td>14</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Ciprofloxacin (5 mcg/disc)</td>
<td>16</td>
<td>4</td>
<td>25</td>
<td>1</td>
</tr>
<tr>
<td>Gentamicin (10 mcg/disc)</td>
<td>0</td>
<td>18</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>Piperacillin (100 mcg/disc)</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>
Illustration 2

Table (2): in vitro antibacterial activity of H2-blockers.

<table>
<thead>
<tr>
<th>Bacterial type</th>
<th>Zone of Inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cimetidine</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>1</td>
</tr>
<tr>
<td>Eschrichia coli</td>
<td>0</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>0</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>0</td>
</tr>
</tbody>
</table>
Illustration 3

Table (3): MIC of famotidine.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>MIC</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>&gt;64</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>32 mg/ml</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>&gt;64</td>
</tr>
<tr>
<td><em>Eschrichia coli</em></td>
<td>8mg/ml</td>
</tr>
</tbody>
</table>
Illustration 4

Figure (1): the action of famotidine on kinetic growth of Escherichia coli.
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