Evaluation of Antibacterial Activity of Famotidine, Ranitidine and Cimetidine: In Vitro Study

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Abstract

Background: Selective antihistamine receptor type 2 blockers like famotidine, ranitidine and cimetidine are widely used for treatment of peptic ulcer, and because many nonantibiotics agents have antibacterial activity.
Aims:This study aimed to evaluate the antibacterial effects of famotidine, ranitidine and cimetidine.
Material and methods: Tewnty two of bacterial isolates were selected and obtained from National Chemical Laboratory (NCL), from conjunctiva: 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 measurment of zone of inhibition and minimal inhibitory concentration(MIC).
Results: Results showed that cimetidine and ranitidine have non significant and negligible antibacterial effects, while famotidine have significant antibacterial effects ($P \leq 0.01$) against *Escherichia coli* (MIC8mg/ml and inhibition zone 25mm) and moderately against *Staphylococcus aureus*.
Conclusions: famotidine have significant antibacterial activity especially against *Escherichia coli.*

Keyword: antibacterial, cimetidine, famotidine and ranitidine

تقييم التأثير المضاد للبكتريا لدواء الفاموتدين و الرانتدين والسمتيدين: دراسة خارج الجسم

الخلاصة

الخلفية: مضادات الهستامين الخاصة بالنوع الثاني من المستقبلات مثل الفاموتدين و الرانتدين والسمتيدين تستعمل كثيرا لعلاج قرحة المعدة.
االهدف: هذه الدراسة تهدف الى تقيم التأثير المضاد للبكتريا لدواء الفاموتدين و الرانتدين والسمتيدين لان هناك الكثير من المضادات الغير حيوية المعروفة وجد بان لها دور مضاد للبكتريا.
المواد وطريقة العمل: ٢٢ مسحة بكتيرية اختيرت ٥ منها لبكتريا Staphylococcus aureus ،و ٥ E.coli ، و ٦ Pseudomonas aeruginosa و ٦ Klebsilla pneumoniae . تأثير هذه الأدوية حدد بواسطة الأوساط البكتيرية المناسبة وتم تقيم المنطقة المتأثرة واقل تركيز محدث للتأثير.
النتائج: وجد إن دواء الرانتدين والسمتيدين ليس لهما تأثير ملحوظ على البكتريا في حين إن دواء الفاموتدين كان له تاثير فعال ($P \leq 0.01$) ضد بكتريا E.coli اقل تركيز محدث للتأثير ٨ ملغم لكل مل ومنطقة متأثرة حوالي ٢٥ ملم و تأثير متوسط ($p<0.05$) ضد staphylococcus aureus.
الاستنتاج: من هذا نستنتج أن دواء الفاموتدين له تأثير مضاد للبكتريا وخاصة ضد E.coli

مفاتيح الكلمات : مضادات البكتريا، الفاموتدين ، الرانتدين ، السمتيدين

Introduction

There is an 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 or as lead compounds for new and effective antibacterial agents [6,7].

Selective antihistamine type antagonists 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.

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 committee of Department of Pharmacology and Microbiology and licensed by conncil of medical college.

The microbial isolates investigated in this study were identified isolates and were obtained from National Chemical Laboratory (NCL, Baghdad), from conjunctiva. The studied bacterial strains comprise [5] *Staphylococcus aureus*, [5] *Escherichia coli*, [6] *Pseudomonas aeruginosa* and (6)*Klebsiella pneumoniae* microorganisms [that confirmed according to collee et al procedure [9]were maintained at 4 °C on nutrient agar slants.

Materials and Methods

The antibacterial assay was performed by 2 methods: Agar disc diffusion method [10] and Agar well diffusion method [11]. The media (Mueller Hinton Agar) along with the inoculums (10^8 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 200mg, ranitidine 150mg and famotidine 20mg) 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 over night at 37 °C. Microbial growth was determined by measuring the diameter of the zone of inhibition.

For each bacterial isolates, 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 isolates 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 determine 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 antibiotic.

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

	Zone of Inhibition (mm)			
Antibiotics	***Staphylococcus aureus***	***Escherichia coli***	***pseudomonas aeruginosa***	***Klebsiella pneumoniae***
• ***Chloramphenicol (30 mcg/disc)***	***16***	***14***	***7***	***0***
• ***Ciprofloxacin (5 mcg/disc)***	***16***	***4***	***25***	***1***
• ***Gentamicin (10 mcg/disc)***	***0***	***18***	***16***	***0***
• ***Piperacillin (100 mcg/disc)***	***5***	***1***	***0***	***1***

Regarding the antibacterial effects of selective H2 antagonist both cimetidine and ranitidine showed minimal antibacterial activity in comparison with control ,also famotidine produced minimal antibacterial activity against *Pseudomonas aeruginosa and Klebsiella pneumoniae and moderate* ($p<0.05$) antibacterial activity (against *Staphylococcus aureus* but the most significant effect ($P\leq 0.01$) appeared on *Escherichia coli* when famotidine produced 25 mm zone of inhibition table [2].

Table 2 In vitro antibacterial activity of selective H2 blockerson bacterial strains.

	Zone of Inhibition (mm)			
Bacterial type	**cimetidine**	**ranitidine**	**famotidine**	**Distell water**
• ***Staphylococcus aureus***	**1±0.01**	**1.2±0.02**	**6.64±2.05†**	**2.2±0.16**
• ***Escherichia coli***	**0**	**0**	**25.2±2.32††**	**2.12±0.13**
• ***Pseudomonas aeruginosa***	**0**	**0**	**0**	**0**
• ***Klebsiella pneumoniae***	**0**	**0**	**0**	**1.1±0.12**

†p<0.05 †† p<0.01

Table 3 The minimal inhibitory concentration (MIC) of famotidine.

Bacteria	MIC
Klebsiella pneumoniae	**>64**
Staphylococcus aureus	**32 mg/ml**
Pseudomonas aeruginosa	**>64**
Escherichia coli	**8mg/ml**

Kinetic action of famotidine

The MIC of famotidine against *Escherichia coli* was found to be 8mg/ml. At the logarithmic growth phase of the cultures, when the CFU counts of the strains was $3.0 \times 10^{8;}$ 200 µg/ml of famotidine was added to each;subsequently, the CFU of the cultures were determined the CFU were 2.0×10^5 after 2 h, 3.0×10^4 after 4 h, 2.5×10^4 after 6 h and 1.2×10^4 at the end of 18 h [Fig. 1].

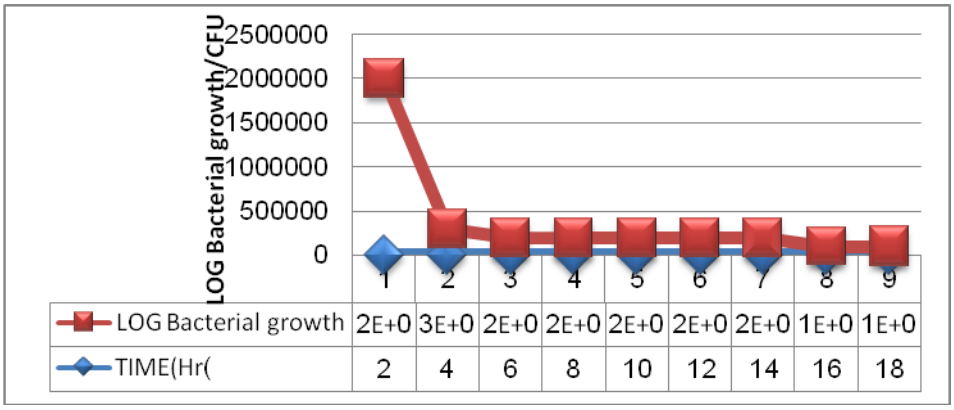

Figure 1 the action of famotidine on kinetic growth of *Escherichia coli.*

Discussion

The histamine receptor antagonists are cimetidine, ranitidine, 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 [12].

The current study introduced a new utilize and indications of common selective H2 antagonist that is the antibacterial effects, in this study neither cimetidine nor ranitidine producing any considerable antibacterial effects but famotidine inhibit bacterial growth of *Escherichia coli* and *Staphylococcus aureus* .

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 [13].

Furthermore; daily famotidine monotherapy resulted in a significant decrease ($P \leq 0.01$) 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 [14].

Icatlo1994 study showed that the direct antibacterial activity of famotidine on *Helicobacter pylori*, is inert in vitro at a concentration of 2 mg/mlm [15]. In this study minimal inhibitory concentration of famotidine was 8mg against *Escherichia coli* which coincide with this study.

Moreover; famotidine and cimetidine salts showed significant antibacterial effects; they possess valuable pharmacological activities; namely, they show gastric acid secretion inhibitory, gastrocytoprotective effect and antibacterial effect against the bacterial strain *Helicobacter pylori* [16].

Furthermore, famotidine enhanced the induction of non-reparable DNA damage in *Eschrichia coli* strains that lacking the post-replication repair pathways [17]. 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* [18-20].

Add to this famotidine, in having abenzene ring attached to another one, may be conceived to mimic a phenothiazine construction, per see explain the antibacterial activity because most drugs and agents have one or more benzene ring in their chemical structure possess antimicrobial effects [21].

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 [22].

Cimetidine predisposes critically ill patients to developing a diversity of infectious diseases [23]. 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 [24-26].

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 [27]. This also supports our opportunity of the use of antihistamine drugs for bacterial infection.

Accordingly, it was expected that histamine would enhance phagocytic recruitment and delay the clearance of *Escherichia coli* in the presence of histamine [28,29].

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 adhesin Expression [30].

Therefore; famotidine regarded as anti-infective 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 action.

From this study we conclude that Famotidine as the prototype of selective H-2 blockers possess significant antibacterial activity especially against *Escherichia coli* and *Staphylococcus aureus*

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