Trimebutine as a potential antimicrobial agent: a preliminary in vitro approach

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Abstract
Aim: The aim of this preliminary study was to investigate the in vitro effect of “non-antibiotic” trimebutine against reference strains Staphylococcus aureus ATCC 29213, ATCC 25923, Escherichia coli ATCC 25922, ATCC 35218, Pseudomonas aeruginosa ATCC 27853 and Enterococcus faecalis ATCC 29212; microbiota that are potentially involved in the pathophysiology of post-infectious functional gastrointestinal disorders.

Methods: Trimebutine activity was assessed by the broth microdilution method according to Clinical and Laboratory Standards Institute recommendations against reference strains S. aureus ATCC 29213 and ATCC 25923, E. coli ATCC 25922 and ATCC 35218, P. aeruginosa ATCC 27853 and E. faecalis ATCC 29212. Bactericidal activity of the compound was determined by spreading a 10 μL aliquot on Mueller-Hinton agar from each dilution showing non-visible growth. All tests were carried out in triplicate.

Results: Trimebutine was active against all strains tested presenting with MIC ranging from 1024 to 4000 mg/L. MIC and MBC were similar for E. coli ATCC 25922 and P. aeruginosa ATCC 27853 whereas for Gram-positive isolates and E. coli ATCC 35218 the MBC was higher.

Conclusions: We demonstrated the in vitro bacteriostatic/bactericidal activity of trimebutine against bacteria frequently involved in the gastrointestinal tract and potentially involved in human gastrointestinal infections that might trigger post-infectious functional gastrointestinal disorders. Hippokratia 2012, 16, 4: 347-349

Key words: trimebutine, antimicrobial effect, post-infectious irritable bowel syndrome, functional dyspepsia, gastrointestinal reflux disease

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Introduction
Trimebutine [3,4,5-trimethoxybenzoic acid 2-(dimethylamino)-2-phenylbutyl ester], a prokinetic drug, has been found to be effective in controlling symptoms in some patients with irritable bowel syndrome (IBS), gastroesophageal reflux disease (GERD) and functional dyspepsia (FD); overlaps among GERD, FD and IBS are frequent having negative impacts on patients’ quality of life. Specifically, apart from GERD, recent data indicate that post-infectious IBS and FD appear to be common disorders, the pathophysiological role of the gut microbiota has been recently elucidated, the risk of developing IBS increases six-fold after gastrointestinal infection, and remains elevated for at least 2-3 years post-infection. In this regard, a variety of pharmaceutical agents termed “non-antibiotics”, applied in the therapy of non-infectious diseases, possibly including trimebutine, have shown in vitro some antimicrobial activity.

In view of the fact that post-infectious functional gastrointestinal disorders constitute a large medical burden in society in terms of consultations, drug consumption and even surgery, the aim of this pilot study was to investigate the in vitro effect of trimebutine on microbiota frequently involved in the pathophysiology of post-infectious functional gastrointestinal disorders.

Material and Methods
Trimebutine activity was assessed by the broth microdilution method according to Clinical and Laboratory Standards Institute (CLSI) recommendations against reference strains Staphylococcus aureus ATCC 29213 and ATCC 25923, Escherichia coli ATCC 25922 and ATCC 35218, Pseudomonas aeruginosa ATCC 27853 and Enterococcus faecalis ATCC 29212. Trimebutine powder was provided by Galenica, Greece and the concentrations used were 0.25, 0.5, 1.2, 4, 8, 16, 32, 64, 128, 256, 512, 1024, 2000, 4000, 8000 and 16000 mg/L. After incubation at 37°C for 16-18 hours, minimum inhibitory concentration (MIC) was the highest dilution that showed non-visible growth. Bactericidal activity of the compound was determined by spreading a 10 μL aliquot on Mueller-Hinton agar from each dilution showing non-visible growth. The plates were incubated at 37°C for 18-24 hours and minimum bactericidal concentration (MBC) was considered the dilution which produced >99.9% killing of the inoculum. All tests were carried out in triplicate.
in order to confirm the reliability of results.

Results

Trimebutine was active against all strains tested presenting with MIC ranging from 1024 to 4000 mg/L. MIC and MBC were similar for *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 whereas for Gram-positive isolates and *E. coli* ATCC 35218 the MBC was higher. Results are reported in Table 1.

Discussion

This pilot study demonstrates for the first time the *in vitro* bacteriostatic/bactericidal activity of trimebutine against bacteria frequently colonizing the gastrointestinal tract (GIT) and are potentially involved in human gastrointestinal infections that might trigger post-infectious functional gastrointestinal disorders. Indeed, *S. aureus, E. coli, P. aeruginosa* and *E. faecalis* have the potential to pass from colonization to infection of the GIT depending on their virulence factors gene pool and their interaction with the host.

It is known that the actions of trimebutine on the gastrointestinal tract are mediated via: (i) an agonist effect on peripheral mu, kappa and delta opiate receptors and (ii) release of gastrointestinal peptides such as motilin and modulation of the release of other peptides, including vasoactive intestinal peptide, gastrin and glucagon. In particular, trimebutine acts mainly as an opioid agonist by triggering phase III of the migrating motor complex in the intestine and modulates the contractile activity of the colon and therefore differs from pure spasmylic drugs, including mebeverine, tiemonium, phloroglucinol and prokinetic agents such as cisapride, metoclopramide, and domperidone. There is no evidence to indicate that the compound acts at the level of the central nervous system, and it seems unlikely that the drug crosses the blood-brain barrier. Instead, trimebutine has been shown to interact with sensory neurons of the dorsal root ganglia and the drug has also been found to have local anesthetic activity which is 17 times more potent than that of lidocaine. Opioid peptides are implicated in the control of esophageal motility, both locally and at the level of the central nervous system. Leu- and met-enkephalin exert opposite effects on the lower esophageal sphincter pressure, but they appear to act on the nervous control of esophageal motility by modulating the adrenergic and excitatory cholinergic activity, whereas the inhibitory vagal pathway seems to be unaffected. In the stomach, trimebutine administration accelerates gastric emptying and shortens the lag period (i.e., period of time before the onset of constant gastric emptying). Clinical studies have demonstrated that gastric emptying in gastric ulcer patients receiving omeprazole plus trimebutine improved significantly post-treatment, while no significant changes were noticed in the patients that received omeprazole as a monotherapy for the same period of time. Moreover, clinical data indicate that trimebutine appears to be effective in patients with GERD and IBS. Specifically, trimebutine, which modulates the calcium and potassium channels, relieves abdominal pain in patients with IBS. Trimebutine has also been shown to be effective in reducing the colonic muscle hypercontractility of experimental postinfectious IBS and to decrease reflexes induced by distension of the gut lumen in animals and it may therefore modulate visceral sensitivity; clinically, trimebutine has proved to be effective in the treatment of functional bowel disorders, especially IBS, at doses ranging from 300 to 600 mg/day.

Summarizing these data, it is possible that trimebutine might exert viable effects on the colonization of gut microbiota via the aforementioned actions on the GIT.

Trimebutine is a mentioned “non-antibiotic” and the exact mechanism leading to its *in vitro* bacteriostatic/bactericidal effect observed in our study is not clear. Therefore, further research is needed to investigate any potential effect of trimebutine in bacterial growth and/or metabolism. Nevertheless, its novel antibacterial activity shown in the present preliminary study may entail potential additional therapeutic administration of this agent in aforementioned post-infectious functional and even organic gastrointestinal disorders. Changes in fecal and mucosa-associated microbiota, a link with small intestinal bacterial overgrowth and an up-regulation of the gastrointestinal mucosal immune system suggest a role for the gastrointestinal microbiota in the pathogenesis of IBS and other gastrointestinal disorders; post-infectious IBS is a frequent sequel of infectious intestinal disease, leading to a considerable disease burden compared with other outcomes. Given this evidence, rifaximin, a poorly absorbed broadband antibiotic, was approved for the treatment of traveler’s diarrhea caused by non-invasive enteropathogens; this pharmaceutical agent bears a high potential in many other indications currently under clinical investigations, including mainly post-infectious IBS.

<table>
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<tr>
<th>ATCC strain</th>
<th>MIC (mg/L)</th>
<th>MBC (mg/L)</th>
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<tr>
<td><em>Staphylococcus aureus</em> ATCC 29213</td>
<td>1024</td>
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<tr>
<td><em>Staphylococcus aureus</em> ATCC 25923</td>
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<tr>
<td><em>Escherichia coli</em> ATCC 25922</td>
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<td><em>Escherichia coli</em> ATCC 35218</td>
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<td><em>Pseudomonas aeruginosa</em> ATCC 27853</td>
<td>4000</td>
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<tr>
<td><em>Enterococcus faecalis</em> ATCC 29212</td>
<td>1024</td>
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but also *Clostridium difficile*-associated diarrhea and pseudomembranous colitis, small bowel intestinal bacterial overgrowth, symptomatic uncomplicated diverticular disease, and hepatic encephalopathy. Of note, the GIT is a vulnerable area through which pathogens may be also involved in the pathogenesis of systemic diseases; they may influence the brain and induce multiple sclerosis pathologies.

Importantly, post-infectious IBS is typically of the diarrhea-predominant type. In this respect, in treating patients with functional dyspepsia coexisting with diarrhea-dominant IBS, trimebutine has the advantage of high efficacy, low cost and few adverse reactions. These and the aforementioned experimental data, might provide evidence for trimebutine to treat postinfectious IBS patients effectively.

Viewing all the aforementioned data, we can speculate that trimebutine combined with rifaximin might exert a synergistic effect mainly against post-infectious gastrointestinal functional disorders and even systemic pathologies. It is important to note that the aforementioned consideration is essential because in this study only high trimebutine dosage appears to be bactericidal, whereas combined therapy with rifaximin or other antibiotics plus trimebutine’s low dosage used in clinical practice might be equally effective. However, further relative studies are needed to elucidate this field.

A variety of additional microorganisms have been involved in the pathophysiology of post-infectious gastrointestinal functional disorders including *Campylobacter jejuni*, *Helicobacter pylori* (H. pylori) and the non-invasive protozoan *Giardia lamblia*. Specifically, *H. pylori* appears to play a role in the pathogenesis of FD and possibly GERD disease; both FD symptoms and GERD are highly prevalent in the population representing a major burden for healthcare systems and the individuals. In this regard, trimebutine appears to be effective in patients with GERD and IBS associated with FD symptoms. Because rifaximin-based regimens for *H. pylori* eradication showed optimal compliance though a limited eradication rate compared with standard first-line treatment, the addition of trimebutine might increase the success of rifaximin-based eradication regimens. Again, further relative studies are needed to elucidate this speculation.

Our series has further certain limitations. We did not measure the trimebutine effect against all the aforementioned pathogens involved in the pathogenesis of functional gastrointestinal disorders. Moreover, we did not consider the *in vivo* effect of trimebutine with or without combined relative agents (i.e., rifaximin) against gut pathogenic microbiota; however, these were beyond the aim of this pilot study. In vitro analysis aimed to identify a potential antibacterial effect of the drug.

In conclusion, this pilot study shows that trimebutine appears to be active against certain bacterial strains possibly involved in the pathophysiology of common functional gastrointestinal disorders, affecting substantially both the individuals and the society. However, further large-scale studies are needed to confirm its relative efficacy.

**Conflict of Interest**

There is no conflict of interest.

**References**