Comparative In Vitro Antimicrobial Activity of Pantoprazole, Tetracycline and a Fixed Dose Combination in Helicobacter pylori Infection

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Abstract: Fixed dose combinations of Pantoprazole and Tetracycline (PT) have a wider range of susceptibility than any one of these two drugs individually. The present study was carried out to determine the Minimum Inhibitory Concentration (MIC) and Time kill curve of Pantoprazole, Tetracycline and a fixed dose combination of these two drugs in four groups (HP-B1, HP-B2, HP-B3, HP-B4) of Helicobacter pylori (H. pylori) bacteria. The MIC were found to be 0.03125 mg/l, 0.03125 mg/l, 0.0625 mg/l and 0.0625 mg/l for PT, 32mg/l, 64mg/l, 32mg/l, 64mg/l, respectively, for Pantoprazole and 0.125 mg/l, 0.125mg/l, 0.125mg/l, 0.25mg/l for tetracycline, respectively, in the different groups.

In all H. pylori groups studied, the time-kill curve analysis demonstrated maximum bacterial killing at 4 hours with PT having the most pronounced effect. From the present findings it can be concluded that a fixed dose combination of pantoprazole and tetracycline has better efficacy and more bacterial inhibiting properties than Pantoprazole or Tetracycline alone.

Key Words: Minimum inhibitory concentration, time kill curve, fixed dose combination, pantoprazole, tetracycline.

INTRODUCTION

Helicobacter pylori (H. pylori) is a small, gram-negative bacillus, curved, microaerophilic and motile organism with multiple polar flagella. It resides in the stomach of man and other primates, lining the gastric mucosa secreting cell. More than 50% of the world population is colonized with H. pylori [1,2]. H. pylori commonly causes peptic ulcers, a chronic inflammatory condition of the stomach and duodenum, in which patients undergo with recurrent abdominal pain. It is a major cause of morbidity in infected patients as it is associated with 90% of duodenal ulcers and 80% of gastric ulcers [3]. The disease has a low mortality, but it results in substantial human suffering, and hence, loss of manpower. H. pylori is also associated with gastric mucosa associated lymphoid tissue (MALT) lymphomas and gastric adenocarcinoma. Before the scientists Warren and Marshall isolated H. pylori from mucosa specimens of patients with chronic active gastritis and peptic ulcer in 1983, the disease was attributed to stress, dietary factors and injurious effects of digestive secretions such as gastric acid. Humans appear to be the only reservoir of H. pylori infection, and therefore, human contacts remain the major mode for its transmission. Iatrogenic spread through contaminated gastrointestinal equipment has also been documented [2], while water has been shown to be a source for H. pylori infection too [4].

H. pylori infection probably occurs when an individual ingests the bacteria via food, fluid or perhaps from contaminated utensils [5]. This infection is one of the most common infections worldwide. The rate of infection increases with age, so it occurs more often in old people. It also frequently presents in younger people from developing countries since the infection tends to be more common where sanitation is poor or, and living quarters are cramped. In many cases, the infection is not symptomatic, and therefore, the infection can go unnoticed. The infection remains localized to the gastric area and that is probably the main reason for ulcers [5, 6]. A peptic ulcer is a sore on the lining of the stomach or, duodenum, which is a part of the small intestine. These peptic ulcers are caused by H. pylori bacterium [6,7]. Pantoprazole sodium is a proton pump inhibitor (PPI), viz. 5-Fluorouracil benzimidazole-2-yl-tetra-methyl sulfoxide. It is extensively metabolized, mainly via the hepatic cytochrome P450 (CYP) 2C19 isoenzyme [8].

Pantoprazole is a substituted benzimidazole, which accumulates in acidic environments of patient's cells after absorption. It binds to the H+/K+ ATPase transporter, thus, inhibiting the proton pump, and thereby, causing potent long lasting suppression of basal and stimulat gastric acid secretion (acetilcholine, histamine, gastrine) [8,9]. Pantoprazole becomes active in higher acidic condition and gets inactive in alkaline environment i.e. in higher pH.

Tetracycline is a cheap and effective antibiotic with better broad spectrum activity against H. pylori infections, [10,11]. Tetracycline prevents bacteria from growing by interfering with their protein synthesis and it binds to the subunit 30S of the bacterial ribosome, and thus, inhibits the translation of peptides. Tetracycline is more effective against certain gram-negative bacteria H. pylori [12].

Treatment with a combination of the antibiotic Tetracycline plus the PPI Pantoprazole has shown increased effi-
Treatment of Helicobacter pylori infected mice with potent fixed dose combination of Pantoprazole and Antibiotics by histological attribute.

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Abstract:
The present study clearly indicates that the combination of Pantoprazole-Tetracycline, Pantoprazole-Clarithromycin and Pantoprazole-Amoxicillin are effective in treatment of infections caused by H. pylori (ATCC43504) in laboratory condition. There have been gradual recovery in treatment of infection induced in mice Mus musculus, with Pantoprazole-Tetracycline (A), Pantoprazole-Clarithromycin (B), Pantoprazole-Amoxicillin (C). After 60 days of treatment there was completely recovery in all groups. Best in vivo results was found in the case of combination of Pantoprazole-Tetracycline, as compared with Pantoprazole-Clarithromycin and Pantoprazole-Amoxicillin. The histological recovery in case of stomach ulcer was almost similar in all combination our of antibiotics with Pantoprazole. Pantoprazole mediated successful treatments of H. pylori infections were done in laboratory animals in combination with Tetracycline, Clarithromycin and Amoxicillin. There was maximum efficacy reported of Pantoprazole-Tetracycline combination. It may be concluded that any of these three combinations can be used for the treatment of H. pylori infections in human being after successful clinical evaluation.

Key Words:
Histopathological analysis, Helicobacter pylori infection, Fixed dose combination, Pantoprazole and Antibiotics.

Introduction:
Helicobacter pylori is a gram-negative bacillus, curved, microaerophilic and motile organism with multiple polar flagella (Mendall, 1995). H. pylori is a fragile bacteria that has found an ideal home in the protective mucous layer of the stomach. These bacteria have long threads protruding from them that attach to the underlying stomach cells. The mucous layer that protects the stomach cells from acid, also protects from H. pylori. Infection-fighting white blood cells move into the area, and the body even develops H. pylori antibodies in the blood (Megaard, 1995). Ulceration is caused by an imbalance between damaging factors (gastric acid and peptic enzymes) and protective factors (gastric mucus secretion, local secretion of alkali). H. pylori appears to cause direct damage to the epithelial cells as well as produce enzymes that break down the surface mucus. This allows access of acid to mucosal tissues leading to the formation of an acute ulcer. If the process proceeds unchecked, then the ulcer can erode through the full thickness of the stomach or duodenal wall, leading to perforation and escape of gut contents into the peritoneal cavity. Generally, the destructive process is arrested by an acute inflammatory response. Tissue repair then begins with the formation of

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Comparative in vitro Microbial Efficacy of a Fixed Dose Combination of Pantoprazole and Metronidazole with Pantoprazole and Metronidazole alone for *Helicobacter pylori*

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The present investigation was carried out to study the Minimum inhibitory Concentration (MIC) and Time kill curve of Pantoprazole (P), a proton pump inhibitor, a substituted benzimidazole: viz. 5-Difluoromethoxy benzimidazole-2-y1-3,4-dimethoxy-2-pyridylmethyl sulphoxide. It is used for inhibition of gastric acid secretion. Metronidazole [M] is a antibiotic with better broad spectrum activity against *H. pylori* infections, a gram negative organism. Pantoprazole and Metronidazole (PM) a fixed dose combination (FDC) has a wide range of susceptibility to any of these drugs individually. Chemical vector Mediated compatibility(CMVC) was obtained at the R& D center for PM and FDC of P and M in Combination of 40mg: 500mg ratio. The MIC was determined by broth micro dilution method as per guidelines of National Committee for Clinical Laboratory Standards (NCCLS). This study was aimed at evaluating microbial efficacy of PM in comparison with Pantoprazole and Metronidazole alone. Efficacy was evaluated on the basis of MIC and time kill curve analysis in *H. pylori* (HP-B, HP-B1, HP-Bp, and HP-Ba). In case of HP-B, HP-B1, HP-Ba, and HP-Bp MIC were found to be 0.5 mg/l, 0.5 mg/l, 0.25 mg/l, and 0.5 mg/l for PM respectively. In pantoprazole alone the MIC was found to be 32 mg/l, 32 mg/l, 32 mg/l and 64 mg/l respectively. For Metronidazole alone, these values were 2 mg/l, 2 mg/l, 1 mg/l, and 2 mg/l respectively. In all organisms under study, time kill curve analysis demonstrated bacterial maximum killing at 4 hours. In conclusion, under in vitro analysis PM was found to have more bacterial inhibiting properties than pantoprazole and Metronidazole alone in vitro analysis.

Key words: Minimum inhibitory concentration, Time kill curve, PM Combination, pantoprazole and Metronidazole.

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*H. pylori* is a predominantly extracellular, Gram negative, short, S-shaped, flagellated and motile bacterium Colonization by this Gram negative, microaerophilic bacterium is characterized by acute inflammatory reaction. Nowadays, there is a general consensus that *H. pylori* infection is the main etiological factor of primary gastritis in children and adults. Significant correlation between *H. pylori*-associated gastritis and peptic ulcer has been found, especially with duodenal ulcer. Gastric cancer and lymphoproliferative
COMPARATIVE MICROBIAL EFFICACY OF A FIXED DOSE COMBINATION OF PANTOPRAZOLE AND CLARITHROMYCIN WITH PANTOPRAZOLE AND CLARITHROMYCIN ALONE FOR HELICOBACTER PYLORI

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ABSTRACT: The present investigation was carried out to study the minimum inhibitory concentration (MIC) and time kill curve of pantoprazole (P), a proton pump inhibitor, a substituted benzimidazole: viz. 5-difluoromethoxybenzimidazole-2-yl-3,4-dimethoxy-2-pyridylmethyl sulfoxide. It is used for inhibition of gastric acid secretion. Clarithromycin (C) is a macrolide antibiotic with better broad spectrum activity against H. pylori infections, a gram negative organism. Pantoprazole and clarithromycin (PC) a fixed dose combination (FDC) has a wide range of susceptibility to any of these drugs individually. Chemical vector mediated compatibility (CMVC) was obtained at the R&D center for PC and FDC of P and C in combination of 40 mg : 500 mg ratio. The MIC was determined by broth micro dilution method as per guidelines of National committee for clinical laboratory standards (NCCLS). This study was aimed at evaluating microbial efficacy of PC in comparison with pantoprazole and clarithromycin alone. Efficacy was evaluated on the basis of MIC and time kill curve analysis in H. pylori (HP-B1, HP-B2, HP-B3, and HP-B4). In case of HP-B1, HP-B2, HP-B3 and HP-B4, MIC were found to be 0.0625 mg/l, 0.125 mg/l, 0.0625 mg/l and 0.0625 mg/l for PC respectively. In pantoprazole alone the MIC was found to be 32 mg/l, 64 mg/l, 32 mg/l, and 32 mg/l respectively. For clarithromycin alone, these values were 0.125 mg/l, 0.25 mg/l, 0.25 mg/l and 0.25 mg/l respectively. In all organisms under study, time kill curve analysis demonstrated bacterial maximum killing at 4 hours. In conclusion, under in vitro analysis PC was found to have more bacterial inhibiting properties than pantoprazole and clarithromycin alone in vitro analysis.

Key words: Minimum inhibitory concentration, Time kill curve, PC combination, Pantoprazole and clarithromycin.

INTRODUCTION

H. pylori is a predominantly extracellular, gram negative, short, S-shaped, flagellated and motile bacterium. Colonization by this gram negative, microaerophilic bacterium is characterized by acute inflammatory reaction, (Williams, 1997). Now a days, there is general consensus that H. pylori infection is the main etiological factor of primary gastritis in children (Bleck, 1996) and adults (Blaser, 1992). Significant correlation between H. pylori-associated gastritis and peptic ulcer has been found, especially with duodenal ulcer (Blaser, 1992 and Rautelin & Kosunen, 1991). Gastric cancer and lymphoproliferative gastric diseases also have been correlated with H. pylori infection (Wotherspoon et al., 1991). It is known that alcohol, aspirin and arthritis drugs such as ibuprofen can disturb the protective muscle layer (Lee, 1992). This allows the strong stomach acid to injure
IN VITRO MICROBIAL EFFICACY OF A FIXED DOSE COMBINATION OF PANTOPRAZOLE AND AMOXICILLIN IN COMPARISON WITH PANTOPRAZOLE AND AMOXICILLIN ALONE AGAINST HELICOBACTER PYLORI

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Abstract

The present investigation was carried to test microbial efficacy of Pantoprazole and Amoxicillin, alone and in fixed dose combination. Minimum inhibitory Concentration (MIC) and Time kill curve were used as method of investigation against these antibiotics. Pantoprazole (P) is a proton pump inhibitor, used for inhibition of gastric acid secretion. Amoxicillin (A) is a penicillin group antibiotic with better broad spectrum activity against Helicobacter pylori infections, a gram negative organism. Pantoprazole and Amoxicillin (PA) fixed dose combination (FDC) has a wide range of susceptibility to any of these drugs individually. FDC of PA was used in ratio of 1:12.5 of P:A. Efficacy was evaluated on the basis of MIC and time kill curve analysis in H. pylori groups HP-Ba, HP-Bc, HP-Bd, and HP-Be. In case of HP-Ba, HP-Bc, HP-Bd, and HP-Be, MIC were found to be 0.0625 mg/l, 0.03125 mg/l, 0.03125 mg/l, and 0.0625 mg/l for PA respectively. In pantoprazole alone the MIC was found to be 64mg/l, 32mg/l, 32mg/l and 32mg/l respectively. For Amoxicillin alone, these values were 0.25 mg/l, 0.125mg/l, 0.125mg/l, and 0.25mg/l respectively. In all organisms under study, time-kill curve analysis demonstrated bacterial maximum killing at 4 hours. In conclusion, in vitro analysis PA was found to have more bacterial inhibiting properties than Pantoprazole and Amoxicillin alone.

Key words: Minimum inhibitory concentration, Time kill curve, Pantoprazole and Amoxicillin.

Introduction

Helicobacter pylori is a gram-negative, curved, microaerophilic and motile organism with multiple polar flagella (Mendall, 1995). The Helicobacter pylori is 2.5-5 m long and 0.5-1.0 m wide. The bacterium is observed mainly in the spiral form, but atypical forms of H. pylori are seen. A common atypical