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ANTI-BACTERIAL EFFECTS OF BISMUTH COMPOUNDS AND ITS SYNERGY WITH TETRACYCLINE AND METRONIDAZOLE ON *HELICOBACTER PYLORI*

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ABSTRACT

A bacteriological investigation performed on the biopsies of 130 patients with gastrointestinal disorders at the GI section of Imam Khomeini hospital concluded in the isolation of 100 cases of *Helicobacter pylori*. The antibacterial effects of bismuth salts alone and in synergy with tetracycline and metronidazole were evaluated on the basis of disc diffusion method on 5 randomly selected pure cultures of *H. pylori* - the main target - and 4 standard cultures of other bacteria including *Staphylococcus aureus* (ATCC 25923), *Brucella abortus* (CS BPI - A 103) S99, *Salmonella typhi* (CS BPI - 196) 0-901 and *Escherichia coli* (ATCC 25922) in order to control the accuracy of procedures.

In this research, growth inhibition zones by bismuth subcitrate (128 µg/disc potency) were determined as 15 ± 2.4 mm diameter against *H. pylori* (mean ± SD), and 15 ± 2.6, 20±3.1 and 14 ± 2.5 mm against the given standard cultures, respectively, except for *E. coli*. Tetracycline (30 µg potency) produced inhibition zones of 30 ± 2.6 mm against *H. pylori* and 30 ± 2.4, 40 ± 2.9, 25 ± 2.3 and 21 ± 2.8 mm against the standard strains, respectively. Diameters of inhibition zones by metronidazole were 4 ± 1 against *H. pylori*, and 3 ± 1.2, 8 ± 2.4 and 3 ± 1.3 mm against the standard strains, respectively, except for the resistant *E. coli*.

In another test in order to demonstrate the synergistic effects of bismuth subcitrate together with the two mentioned antibacterial agents, a composition of 4 parts of bismuth subcitrate, one part tetracycline and 6 parts metronidazole was used (359 µg potency). The obtained data were as follows: the growth inhibition zone against *H. pylori* was 40 ±3 mm and 42± 2.2, 45 ± 2.1, 35± 2.6 and 27 ± 2.3 mm against the control strains. Therefore, these results indicate that bismuth cubcitrate has a remarkable antibacterial effect against *H. pylori* among the bismuth salts which increases when used together with tetracycline and metronidazole in peptic ulcers.

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INTRODUCTION

Helicobacter pylori seems to be one of the etiologic factors of peptic ulcers,[1-4] still a serious problem in gastric ulcers which are cured for a limit of time by common remedies such as cimetidine and ranitidine.[1-4]

Therefore, specialists are focusing on the synergistic activity of antibiotics together with bismuth compounds, in particular bismuth subcitrate and subsalicylate, for the treatment of certain patients. Although the effectiveness of such regimens has been confirmed by many reports,[1, 3, 4, 7] diversity in opinions exists among scientists at the present time which has in fact been the motive for this research, with a main purpose of ruling out the equivocalities concerning the efficacy of bismuth alone and in synergy with antibacterial agents in the treatment of peptic ulcers.

MATERIAL AND METHODS

Sampling

Specimens obtained from biopsies of duodenal lesions were carried in transport media (thioglycolate and 5 antibiotics) and submitted to the laboratory for consequent bacteriological investigations.

Preparation of smears

Two thin smears were prepared from each sample by crushing between two slides and staining with methylene blue and fuschin for 10 min. *H. pylori* was recognized microscopically as short spiral bacteria in clusters.

Culture

Samples from biopsies were homogenized aseptically, inoculated on brucella agar containing 5 - 10 percent sheep whole blood, vancomycin 3-6 mg/L, amphotericin B 2-6 mg/L, trimethoprim 5 - 20 mg/L, colistin 25000 units/L or cefsulodin 5 mg/L and incubated at 36± 1°C for 4 --7 days under microaerophilic conditions. For final determination of positive cultures, biochemical tests were used. The effects of bismuth subcitrate on bacteria and it's probable synergistic action with tetracycline and metronidazole were then studied.

Preparation of dises

Filter paper lacking antibacterial activity in the form of 6 mm dises was macerated with determined amounts of the above-mentioned materials. They were air dried or dried in a jar containing P pentoxide.

In order to standardize the dises, concentrations of the solutions were changed and determined in a manner to produce zones equal to the control ones.

Standardizing of bacterial suspensions

Turbidity of microbial suspensions were selected as the turbidity of 0.5 ml of barium chloride "1. 175% (w/v)" and 99.5 ml of sulfuric acid 1%.

RESULTS

The drug sensitivity of 5 selected strains from 100 cultures of *H. pylori* was assessed (Table 1). Diameters of zones around bismuth subcitrate against *H. pylori* and the sensitive strains of *Staphylococcus aureus* (ATCC: 25923), *Brucella abortus* (CSBPI - A103) S-99 and *Salmonella typhi* (CSBPI - B196) 0-901 were measured as 15± 2.4 mm, 15 ± 2.6 mm, 20 ± 3.16 mm and 14 ± 2.1 mm, respectively. The resistant *E. coli* strain showed no inhibition zone. The growth inhibition zone of tetracycline with a potency of 30 µg/disc was 30 ± 2.6 and 30 ± 2.4, 40 ± 2.5, 25 ± 2.3 and 21±2.8 against the standard cultures, respectively. The inhibition zone diameter of metronidazole (with a potency of 200 µg/disk) against *H. pylori* was 4 ± 1 and 3 ±1.2, 8 ±

Table I. Potency of dises and growth inhibition zone diameters.

Dises	Antibacterial substances (µg/disc)	Inhibition zone against *H. pylori* (mm)	Inhibition zones against control sensitive strains (mm)			Inhibition zone against *E. coli* (mm)
			S. aureus	*B. abortus*	*S. typhi*	
Bismuth subcitrate	128	15 ± 2.6	15 ± 2.6	20 ± 3.1	14 ± 2.1	
Tetracycline	30	30 ± 2.6	30 ± 2.4	40 ± 2.9	25 ± 2.3	21 ± 2.8
Metronidazole	200	4 ± 1	3 ± 1.2	8 ± 2.4	3 ± 1.3	-
Triple drugs: Bismuth subcitrate Tetracycline Metronidazole	359	40 ± 3	42 ± 2.2	45 ± 2.1	35 ± 2.6	27 ± 2.2

Table II. Potency of doubled discs and inhibition zones.

Doubled Discs	Antibacterial substances (µg/disc)	Inhibition zone against *H. pylori* (mm)	Inhibition zones against control sensitive strains (mm)			Inhibition zone against *E. coli* (mm)
			S. aureus	*B. abortus*	*S. typhi*	
Tetracycline + Metronidazole	230	33 ± 3	32 ± 2.5	40 ± 4	27 ± 2.5	24 ± 2
Tetracycline + Bismuth	158	38 ± 3.5	40 ± 4	42 ± 3.6	30 ± 2.7	27 ± 2.4
Metronidazole + Bismuth	328	18 ± 2.1	16 ± 1.8	26 ± 2.6	15 ± 1.8	3 ± 0.5

2.4 and 3 ± 1.3 mm against the standard strains (excluding the resistant *E. coli* strain). In another test demonstrating the synergistic effects of bismuth subcitrate, tetracycline and metronidazole (4 parts bismuth subcitrate, one part tetracycline and 6 parts metronidazole with a potency of 359 µg/disc), the inhibition zones were 40 ± 3 for *H. pylori* and 42 ± 2.2, 45 ± 2, 35 ± 2.6 and 27 ± 2.2 for control cultures. The double disc antibacterial test and the results are shown in Table II.

DISCUSSION

Helicobacter pylori has been known to be one of the most important etiologic agents of gastroduodenal disorders in the recent years, and has been incriminated for the recrudescence of the illness and as possibly being the reason why common treatments are not successful in peptic ulcers.[27] Although temporary recovery occurs in most cases, recurrence of the disorder produces difficulties for the patients in long term. *H. pylori* was isolated from 100 of 130 patients at the endoscopy section of the hospital, thus confirming the role of *H. pylori* in the induction of gastroduodenal ulcers. Therefore, we believe that the most effective regimen for treatment and prevention of recurrence should be focused on eradication of *H. pylori*.

It has been shown that bismuth compounds and bismuth subcitrate in particular, in combination with other antibacterial drugs present better results in the treatment of ulcers.[7] The mechanism of action of bismuth is not clear; some believe that bismuth protects ulcers against the destructive actions of pepsin and hydrochloric acid by covering them. Others declare that bismuth acts as an antagonist of histamine receptors, thus preventing acid secretion, and finally some specialists compounds are antibacterial and prevent recurrence of illness by eradicating the responsible bacterium.[4,5,7,17] Therefore, in order to clarify this vagueness we decided to study the probable antibacterial effects of bismuth compounds and their relevent synergy with other drugs *in vitro*, by using the disc diffusion technique on solid media. As shown in Table I, bismuth subcitrate is definitely effective against *H. pylori* . Prohibition zones produced by triple discs (bismuth subcitrate, metronidazole and tetracycline) together are more extensive than those by each of the drugs separately, indicating the obvious synergistic effects of these materials. As can be seen, metronidazole is not effective alone, possibly due to the bacterial resistance against it.

An insignificant increase in antibacterial activity by two agents rather that one (bismuth and metronidazole, bismuth and tetracycline) can be observed in Table II. In this study, it was elucidated that bismuth is an antibacterial substance capable of inhibiting the growth of bacteria alone and in synergism with other antimicrobial agents such as metronidazole and tetracycline, these two drugs showing the highest level of synergy.

It is mentionable that calculations in this study have been based on comparison with standard sensitive bacteria such as staphylococci, brucella and salmonella.

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