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Research Article

Drinking Ozonated Tri-Distilled Water Increases Helicobacter Pylori Eradication and Promotes Healing of Duodenal Ulcer Lesions

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Received: February 19, 2016; **Published:** April 07, 2016

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Abstract

Objective: Ozone is a physical bactericide that has been applied to disinfect drinking water. The study aimed to determine whether ozonated tri-distilled water in combination with the standard esomeprazole-based triple therapy could increase *helicobacter pylori (Hp)* eradication and promote healing of duodenal ulcer lesions.

Methods: In total, 132 patients were confirmed to be *Hp* infection positive using a Carbon 14 (14C) urea breath test, rapid urea enzyme test and pathological examination. The patients were subsequently divided into 4 groups with 33 patients per group. Groups 1 and 3 included the patients with chronic gastritis, whereas groups 2 and 4 included the patients with duodenal ulcers. The patients from groups 1 and 3 were administered standard esomeprazole-based triple therapy, including amoxicillin 1.0 two times per day, clarithromycin 0.5 two times per day, esomeprazole 20 mg one time per day, and hydrotalcite chewable tablets 1.0 three times per day. The patients from groups 2 and 4 drank ozonated tri-distilled water in combination with standard esomeprazole-based triple therapy: 300 ml ozonated tri-distilled water twice daily, amoxicillin 1.0 twice daily, clarithromycin 0.5 twice daily, esomeprazole 20 mg once daily, and hydrotalcite chewable tablets 1.0 thrice daily. The anti-*Hp* treatment period lasted 2 weeks, which was followed by esomeprazole and hydrotalcite for 4 weeks for all patient groups. Gastroscopy was performed for the patients in groups 3 and 4 after 4 weeks of anti-*Hp* therapy, and a 14C urea breath test was conducted for each patient after 4 weeks of drug withdrawal.

Results: Gastroscopy indicated that 21 patients in group 3 with duodenal ulcers were in the healing stage, and 29 patients in group 4 were in the healing stage, with a significant difference between these two groups ($p = 0.022$). The 14C urea breath tests were negative for 26 patients with a 79% rate of Hp elimination in group 1. The rates of Hp elimination were 97% in group 2 ($n = 32$), 73% in group 3 ($n = 24$), and 94% in group 4 ($n = 31$). The chi-square test demonstrated a significant difference between groups 1 and 2 ($p < 0.05$) and

between groups 3 and 4 ($p < 0.05$). The Hp eradication of drinking ozonated tri-distilled water in combination with standard esomeprazole-based triple therapy was significantly increased compared with the standard esomeprazole-based triple therapy

Conclusion: Drinking ozonated tri-distilled water in combination with the standard treatment substantially enhances Hp eradication and promotes healing of duodenal ulcer lesions.

Keywords: *Helicobacter pylori; Gastritis; Duodenal ulcer; Ozonated tri-distilled water*

Helicobacter pylori (Hp) is currently the only known stomach bacteria. *Hp* infection has an important role in the development of chronic gastritis and peptic ulcers, and it is a risk factor for gastric malignancies, adenocarcinoma and low grade gastric mucosa associated lymphoid tissue (MALT) lymphoma [1-4]. *Hp* is recognized as a carcinogenic microbial type and was the first confirmed carcinogenic to humans of prokaryotes. Anti-*Hp* treatment has become a routine treatment for *Hp*-associated chronic gastropathy. However, in recent years, antibiotic resistance has become the key factor for the eradication failure of Hp [4-7]. Discovering new treatment regimens to treat Hp appears to be a priority for both physicians and researchers. Research has demonstrated that ozone, such as chlorine, is applied in drinking water to treat E. coli and may eradicate *Hp* [8]. As a physical bactericide, ozone exhibits differential functions in the body compared with antibiotics. In the current study, we investigated the treatment effects of drinking ozonated tri-distilled water combined with the standard esomeprazole-based triple therapy in patients with Hp-associated chronic gastritis and duodenal ulcers and determined whether ozone promotes healing of duodenal ulcer lesions.

Materials and Methods

Patient recruitment

In total, 132 patients with chronic gastropathy disease were recruited for the study. The patients were ages from 21 to 65 years, and 72 patients were male, whereas 60 patients were female. All participants were clinical and hospitalized patients from the Shanghai Public Health Clinical Center who sought medical attention regarding symptoms, such as upper abdominal pain, acid reflux, eructation and abdominal distension. All patients provided informed consent.

Gastroscopy

All patients recruited in the hospitals were examined and diagnosed via gastroscopy. In total, 66 cases comprised duodenal ulcers in the active stage, and 66 cases were chronic superficial gastritis.

Hp assessment in patients

All patients tested Hp positive with 14C urea breath tests. A gastric mucosa biopsy was performed utilizing an Olympus XQ260 gastrointestinal videoscopy. Further testing included 2 biopsies of the mucosa, 2-3 cm around the pylorus, were. Hp detection via a rapid urea enzyme test, histopathologic examination, and Giemsa stains of mucosa biopsies further confirmed Hp infections; pathological studies excluded cancerous ulcers. Serum carcinoembryonic antigen tests were performed with blood collected 4 weeks before and after treatment.

Generation of ozonated tri-distilled drinking water

A membrane electrolysis low tension voltage technique was used to generate ozone and produce ozonated tri-distilled water using a machine from Ningbo Tianyi Medical Instrument Limited Company. The ozone concentration in the tri-distilled water is 5 ppm per 300 ml. The ozonated tri-distilled water does not contain bromate

Experimental designs

The 132 patients were divided into 4 groups with 33 patients per group. Groups 1 and 2 included 33 cases of chronic gastritis, whereas groups 3 and 4 included 33 cases of duodenal ulcers. All other factors were randomized for grouping.

Anti-*Hp* therapy

The patients in groups 1 and 3 were administered standard esomeprazole-based triple therapy, including amoxicillin 1.0 two times per day, clarithromycin 0.5 two times per day, esomeprazole 20 mg one time per day, and hydrotalcite chewable tablets 1.0 three times per day. The patients in groups 2 and 4 were administered ozonated tri-distilled water combined with standard esomeprazole-based triple therapy: 300 ml ozonated tri-distilled water twice daily, amoxicillin 1.0 twice daily, clarithromycin 0.5 twice daily, esomeprazole 20 mg once daily, and hydrotalcite chewable tablets 1.0 thrice daily.

Determination of *Hp* eradication via 14C urea breath test and the healing stage of duodenal ulcers via gastroscopy

Anti-*Hp* treatment was administered for 2 weeks, which was followed by esomeprazole and hydrotalcite for 4 weeks for all patient groups. A second gastroscopy was performed for the patients in groups 3 and 4 after 4 weeks of anti-*Hp*, and a second 14C urea breath test was conducted for each patient for *Hp* detection after 4 weeks of drug withdrawal application.

Statistical analysis

All data are expressed as the eradication rate of Hp. Statistical analyses were conducted via chi-square tests. P values^{0}0.05 (two-tailed) were considered statistically significant, and p<0.01 (two-tailed) was considered an obviously significant difference.

Results

Standards for enrolling in the experiment

More than 100 dpm in the 14C urea breath test was used to diagnose Hp infection according to the certified kit manufacturer instructions. The rapid urea enzyme test, in which the chamber yellow color changes to red colour when the biopsied tissue placed in the chamber contains Hp (figure 1), indicated Hp was positive in the biopsied gastric mucosa. The pathological examination indicated the presence of a substantial amount of curved rod bacterial Hp in the lamina propria of the gastric gland from the patient biopsies examined via gastroscopy (figure 2).

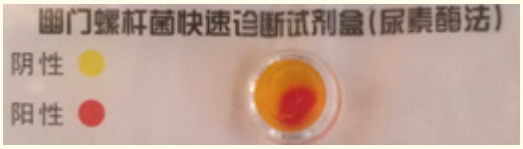

Figure 1. *Rapid urea enzyme test Hp kit.*

Yellow color indicates Hp negative when the biopsied sample is placed in the chamber for several minutes, and its round yellow colour changes to red colour when positive.

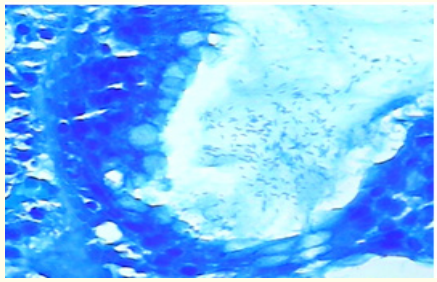

Figure 2: *Pathological examination confirmed substantial Hp in the gastric mucosa obtained from patient biopsies with gastroscopy. Giemsa stains × 400.*

Second gastroscopy for the patients with duodenal ulcers

Gastroscopy indicated there were 21 cases in the healing stage of duodenal ulcers in group 3, and 29 cases in the healing stage of duodenal ulcers in group 4, with healing rates of 63.63% and 87.88%, respectively. There was a significant difference between these two groups (p=0.022).

	0 week	4 weeks after therapy
	Cases with active stage ulcer	Cases with healing stage ulcer
Group 3	33	21
Group 4	33	29

Table 1: *Effects of drinking ozonated tri-distilled water on duodenal ulcer healing.*
Notice: Group 3: Group 4, p=0.022

Figure 3: *Duodenal ulcer before and after treatment with drinking ozonated tri-distilled water combined with the standard esomeprazole-based triple therapy*

Figure 3 A shows a duodenal ulcer at the first endoscopy; Figure 3 B exhibits the ulcer in the healing stage when a second endoscopy was performed 4 weeks after anti-Hp, which comprised drinking ozonated tri-distilled water combined with standard esomeprazole-based triple therapy.

Second 14C urea breath test

Four weeks after esomeprazole withdrawal, the 14C urea breath test results of the 4 different treatment groups were evidently different (table 1). The 14C urea breath tests were negative for 26 patients with a 79% rate of Hp elimination in group 1. The rates of Hp elimination were 97% in group 2 (n = 32), 73% in group 2 (n = 24), and 94% in group 3 (n = 31). The chi-square test demonstrated significant differences between groups 1 and 2 ($p < 0.05$) and groups 3 and 4 ($p < 0.05$).

The results indicate that the Hp eradication of drinking ozonated tri-distilled water combined with standard esomeprazole-based triple therapy was increased compared with the standard esomeprazole-based triple therapy both in the patients with chronic gastritis and the patients with duodenal ulcers. No adverse side effect was identified during the treatment. The carcinoembryonic antigen levels were steady between 0-5 μg/ml before and after treatment.

	Before		After	
	Negative	Positive	Negative	Positive
Group 1	0	33	26	7
Group 2	0	33	32	1
Group 3	0	33	24	9
Group 4	0	33	31	2

Table 2: *14C urea breath test before and after treatment.*
Notice: Group 1: Group 2, p=0.02; Group 3: Group 4, p=0.02

Discussion

The combination of a Proton pump inhibitor and antibiotics for the treatment of Hp infection has become a routine approach for chronic gastritis and peptic ulcer therapy. Eradication of Hp also has a good effect on the improvement of functional dyspepsia epigastric pain and burning sensation symptoms. Usually, a standard triple therapy is consisting of two antibiotics and a proton-pump inhibitor proposed as the first-line treatment. The optimal Hp therapy should be eradication rate more 90% [9]. However, a successful Hp eradication therapy involves many determinants such as individual primary or secondary antibiotics resistance, mucosal drug concentration, patient compliance, side-effect profile and cost. The current standard triple therapy for Hp eradication rate is less than 80% in most parts of the world [9]. Our study shows that the triple therapy based on amoxicillin, clarithromycin and esomeprazole for chronic gastritis and duodenal ulcer patients with Hp infection eradication rates were 79% and 73% respectively. Hp resistance to antibiotics decreases the clinical treatment effect of chronic gastropathy and peptic ulcers. A previous randomized controlled trial [10] indicated that ozone nano-bubble water has the potential of a new antimicrobial agent. Fontes et al demonstrated that a single topical application via nebulization of a low ozone dose completely inhibited the growth of all potentially pathogenic bacterial strains with known resistance to antimicrobial agents [11]. In vitro, ozone exhibited strong bactericidal effects against multiple-drug-resistant bacteria and representative periodontopathic bacteria [10]. Thus, ozone may be used in medicine as an adjunct to primary treatment. Our study demonstrates that drinking ozonated tri-distilled water increases the eradication of the Hp infection in the gastric mucosa from patients with chronic gastritis and duodenal ulcers. These findings suggest that ozone not only may be used to eliminate microbial in water but also works on gastric Hp infections. Compared with traditional drug treatments, studies indicate ozone treatment quickens gastric mucosa repair, promotes Hp eradication, shortens the gastric ulcer recovery time, and reduces the recurrence chance [12], which is the same as our findings in which ozone promoted healing of duodenal ulcer lesions. Three methods may be applied in ozone therapy that comprises intravenous injections of ozonized physiologic treatments, which have been demonstrated to be effective in relation to improvements in clinical symptoms, regeneration processes, and Hp eradication; however, the combined regimen was optimal [13]. Our studies suggest that following the addition of ozonated tri-distilled water to the traditional treatment using antibiotics plus proton pump inhibitors, the eradication rate of Hp was further improved. These findings demonstrate that ozone increases the eradication rate of Hp of the traditional treatment using antibiotics plus proton pump inhibitor; thus, it will play a more important role in future Hp treatment. As antibiotic resistant bacteria increase [6], the unique physical anti-microbial properties of ozone may make treatment become more worthy and time efficient.

However, the effect of ozone on eliminating Hp has not been well published, and its mechanism remains unknown. Ozone is a powerful oxidant. It has been widely used as an antimicrobial agent for water and air purification and equipment cleansing, for example, the sterilization of perishables, disinfection of medical instruments, and cleaning or surface-conditioning processes in the semiconductor industry [8,10]. Ozone is an unstable gas; once generated, ozone in the gaseous state rapidly decomposes to oxygen (O2) and a signal oxygen atom (O). The gas is colourless, acrid in odour and explosive in liquid or solid form. It has a half-life of 40 min at 20°C and approximately 140 min at 0°C [14]; thus, the patients were required to drink the ozonized water generated by the generator in ten to twenty minutes in the study. The biological and chemical mechanisms of ozone elimination of microorganisms include three mechanisms. First, ozone may

directly react with bacteria and viruses and damage their organelles, DNA, and RNA [14-18]. Second, ozone may penetrate cell membranes, as well as react with lipoproteins and lipopolysaccharides on the outer membrane, which causes a permeability mutation and eventually cell lysis [14,15,18-20]. Dr. Bialoszewski., et al. demonstrated that Ozonated water is an effective bactericidal agent against biofilms after as little as 30 seconds of exposure; the bactericidal activity of the ozone-oxygen solution was substantially lower, in which prolongation of the duration of biofilm exposure to the gaseous disinfectant for 40 minutes led to a reduction in the viable cell count, which nevertheless remained high. The destruction of the bacteria biofilm of ozonated water is more effective than the ozone-oxygen mixture in vitro [19]. Third, ozone may stimulate the local or systemic cellular or humoral immune systems [21] of patients with Hp, which induces the release of interleukins, leukotrienes and prostaglandins [20], thus reducing inflammation and promoting wound healing.

During this entire study, no adverse side effect was identified. Dr. Kawara and his research team also demonstrated that ozone nanobubble water (NBW3) for the treatment of Helicobacter pylori in the stomach sustains its disinfective activity in a wide range of pH levels and did not exhibit cytotoxicity on mammalian cells or tissues; they suggested that pepsin inhibits NBW3 activity in a dose-dependent manner [22]. As a contamination free water disinfectant, because of its unstableness, ozone breaks down into oxygen and a single oxygen atom, which combines with oxygen quickly, and the ozone from the ozonated tri-distilled water does not leave behind toxic residues. The ozone dissolved in the tri-distilled water is released once it is passed through the stomach and intestines and works to eliminate Hp through the gastric mucosa. In medical use, the ozone produced from medical grade oxygen is administered in precise therapeutic doses, never via inhalation, and advocates that ozone has excellent health benefits in dental caries, decreases blood cholesterol and stimulation of antioxidative responses, modifies oxygenation in resting muscle and is used in the complementary treatment of hypoxic and ischemic syndromes [14]. Our study demonstrates the potential of ozone to eradicate Hp and, especially when combined with the standard treatment increases the Hp eradication rate. Moreover, the ozonated tri-distilled water has the advantages of simple manufacture, like our daily drinking mineral water, safety, convenience and economy. Thus, further research and discussion should be encouraged to identify the optimal and safe concentration for ozonated tri-distilled water that eliminates Hp. Ozone may potentially become a more widely applicable treatment method for fighting bacterial infections.

Conclusion

Drinking ozonated tri-distilled water in combination with the standard treatment substantially enhances Hp eradication and promotes healing of duodenal ulcer lesions.

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Volume 1 Issue 1 April 2016