J. Exp. Biomed. Sci. 2011, 17(4): 363~366

Brief Communication

Antibacterial Effect of Photodynamic Therapy using Photogem and a 632 nm Diode Laser on *Helicobacter pylori*

Jin-Chul Ahn[2], Jang-In Shin[2], So-Young Chang[2], Hong-Ja Kim[3] and Seong-No Hong[1,†]

[1]*Department of Clinical Laboratory Science, Dongnam Health College, Suwon 440-714, Korea,*
[2]*Medical Laser Research Center, Dankook University, Cheonan 330-751, Korea*
[3]*Department of Internal Medicine, Dankook University, Cheonan 330-751, Korea*

Eradication of *H. pylori*, usually carried out by using antimicrobial agents, is essential for preventing gastric ulcers and cancers. The *H. pylori* isolates, however, have continuously grown antimicrobial resistance, which have caused difficulty in treating the bacteria and in turn, photodynamic therapy (PDT) has been found to be effective in inducing deaths of variety of bacteria. After PDT treatment, the number of colony forming units (CFU), the morphologic changes, and flow cytometry were observed. In the PDT group containing 100 and 200 μg /ml photogem, no live *H. pylori* was observed, while 10 and 50 μg/ml photogem were only partially effective. *H. pylori* of the PDT group also displayed distortion and shrinkage in morphology. This study demonstrated that photogem-mediated PDT effectively induces deaths of *H. pylori*.

Key Words: Photodynamic therapy, Photogem, *Helicobacter pylori*

Helicobacter pylori is a gram-negative, microaerophilic bacterium which selectively colonizes in the mucus layer of the human stomach and duodenum (Dunn et al., 1997; Muller et al., 2007). More than 70% of Koreans aged 40 years or older are infected with *H. pylori*, which is closely associated with upper gastrointestinal tract diseases such as acute gastritis, chronic gastritis, peptic ulcer and gastric cancer (Choi et al., 2010). One of the most currently accepted modality for treating *H. pylori* infection is a triple therapy, which combines the antibiotic clarithromycin and amoxicillin with a proton pump inhibitor such as omeprazole. This chemotherapeutic regimen, however, has been found to have side effects and it was observed to fail in eliminating the infection in 10 to 30% of the patients (Ching et al., 2008). Also, the increasing development of antibiotic resistance among *H. pylori* isolates suggests that alternative strategies for *H. pylori* eradication are needed.

Photodynamic therapy (PDT) is a potential therapy against cancerous tumors or localized infectious diseases (Trushina et al., 2008; Stukavec et al., 2009). It involves a light-sensitive compound (photosensitizer), light, and molecular oxygen. Reactive oxygen species generated by the photodynamic reaction induce damage to multiple cellular structures including the cell membrane, cell wall and nucleic acids, which leads to bactericidal and anti-cancer effects (Wainwright et al., 1998). As an effective modality in inducing unwanted-cell deaths, PDT has recently been studied against a wide range of bacteria, fungi, yeasts, and viruses that cause serious problems in contemporary medicine (de Souza et al., 2006; Chabrier-Rosello et al., 2005; Hamblin et al., 2005; Peloi et al., 2008; Donnelly et al., 2008). The purpose of this study is to contribute to the search for alternative therapies in treating *H. pylori* infections, evaluating the effects of PDT using photogem and 632 nm diode laser *in vitro*.

H. pylori was provided by JH Lee M.D. (Asan Medical Center, Korea) and was grown on horse serum agar plates for 4 days in a microaerophilic environment. The horse

*Received: 21 June, 2011 / Revised: 12 December, 2011
Accepted: 13 December, 2011
†Corresponding author: Seong-No Hong, Department of Clinical Laboratory Scicence, Dongnam Health College, 937 Jeongja-dong Jangan-gu Suwon-city Gyeonggi-do, 440-714, Korea.
Tel: +82-31-249-6411, Fax: +82-31-249-6410
e-mail: snhong@dongnam.ac.kr

Table 1. Colony forming units (CFU) for the following studied groups; control group not treated with laser or with photosensitizer; laser group treated only with laser; ps group treated only with photosensitizer; and PDT group irradiated with laser in the presence of photosensitizer

Test conditions	Photogem conc. (μg/ml)	Viable count ($\times 10^6$ CFU)	
		No irradiation	Irradiation
PIT = 3 h ED = 4 J/cm^2	0	>3.0	>3.0
	10	>3.0	1.46
	50	>3.0	0.18
	100	>3.0	0
	200	0.98	0

ED, energy density; PIT, pre-irradiation time; conc., sensitizer concentration.

serum medium consisted of Brucella broth (BD Biosciences, San Jose, CA, USA) supplemented with 10% horse serum (PAA laboratories, Morningside, QLD, Australia) and antibiotic mixture of 10 μg/ml vancomycin (Sigma, St. Louis, MO, USA), 5 μg/ml trimethoprim (Sigma), 2,500 IU/ml polymyxin B (Sigma), 2.5 μg/ml of amphotericin (Sigma) and 1.5% bacto agar (BD Biosciences). The liquid medium consisted of the same ingredients except for the 1.5% bacto agar.

The control group had not been exposed to neither the photosensitizer nor the laser, the photosensitizer only group was treated with various concentrations (0, 50, 100, and 200 μg/ml) of photogem, a hematoporphyrin derivative (Lomonosov Institute of Fine Chemicals, Moscow, Russia), the laser group was irradiated using a 632 nm diode laser (Biolitec AG, Jena, Germany) at a power density of 4 J/cm^2 and the PDT group was irradiated with laser in the presence of photogem.

H. pylori was cultured for 120 h on the horse serum agar plates and then the colonies were transferred into brucella broth and were incubated overnight at 37℃. The bacterial suspension was diluted to 1.5×10^8 CFU/ml with broth and were incubated with different concentrations of the photogem (0, 50, 100, and 200 μg/ml) for 3 h at 37℃ in the PS only and PDT groups. The suspensions of the PDT group were irradiated afterwards along with those of the laser group. After each treatment, 50 μl of the broth was plated in duplicate on agar and the number of CFU/ml was obtained after 5 days of incubation at 37℃.

Cell suspensions of the untreated control and PDT groups treated with 10 μg/ml of photogem were centrifuged and resuspended in phosphate-buffered saline (Dulbecco's phosphate buffered saline [PBS]; Hyclone, Logan, Utah, USA) to a concentration of 10^6 cells/ml. For flow cytometry analysis, the cells were incubated with 5 μl thiazol orange (TO) and propidium iodide (PI) from the BD cell viability kit (BD Biosciences, San Jose, CA, USA) for 15 min in the dark at room temperature. The stained cells were analyzed by using a FACSCalibur with the CellQuest program (Becton Dickinson). At least 20,000 events were collected for each sample. The observations were performed at 24 hours after the PDT.

To check the morphological changes in *H. pylori* after PDT, the samples were prepared for scanning electron microscope (SEM) and transmission electron microscope (TEM) observations. For the PDT group samples, cells treated with 10 μg/ml of photogem were obtained for each microscopy.

The number of CFU of *H. pylori* was recorded to be over 3.0×10^6 in the non-treated control, laser and PS groups except at the highest concentration of the photogem (200 μg/ml), which means that there was photocytotoxicity in highest concentration only. The PDT group presented the lowest CFU/ml value in relation to the other groups and the CFU of the PDT group was decreased in a dose-dependent manner, indicating that irradiation in the presence of the photogem was effective in reducing the viability of *H. pylori* (Table 1).

The analyzed, TO and PI stained *H. Pylori* also produced data which complied with the patterns of CFU for each groups. Based on its FL1 and FL3 fluorescence, it was observed that the number of live cells had decreased and dead cells had increased in the PDT group (Fig. 1). Compared to the mean value of 21.81 in control group, the values for PI and TO stained PDT groups were recorded to be 29.36 and 8.78, respectively.

Fig. 2 shows the scanning electron micrographs (SEM) and transmission electron micrographs (TEM) illustrating the ultrastructural damage of *H. pylori* after the PDT. The untreated *H. pylori* revealed coccoid, spiral, and slightly curved bacillary forms in which the cellular membranes

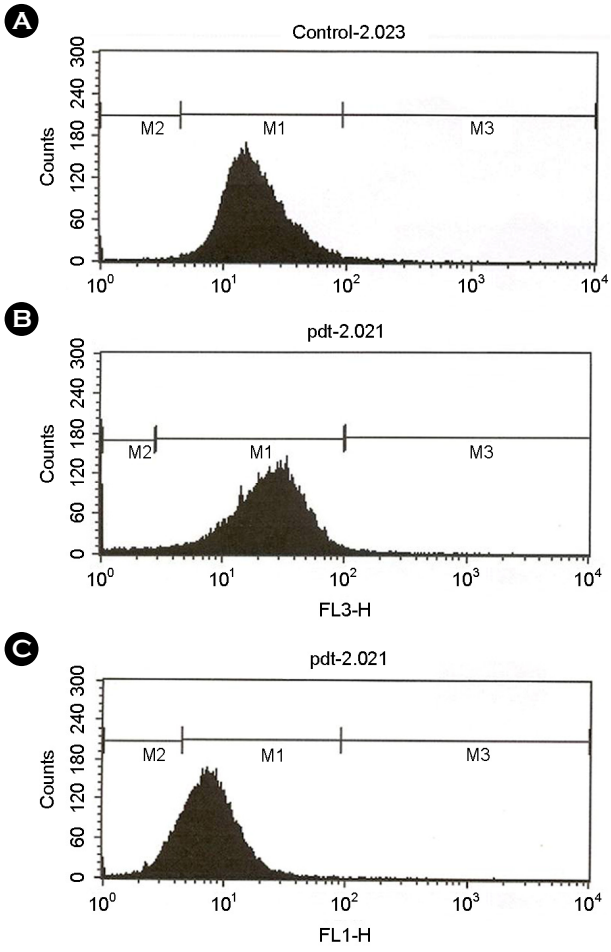

Fig. 1. Flow cytometry for *Helicobacter pylori* of control group and PI and TO stained cells of the PDT group. Compared to the control group (A), the values had increased for PI (B) and decreased for TO (C) in the PDT group.

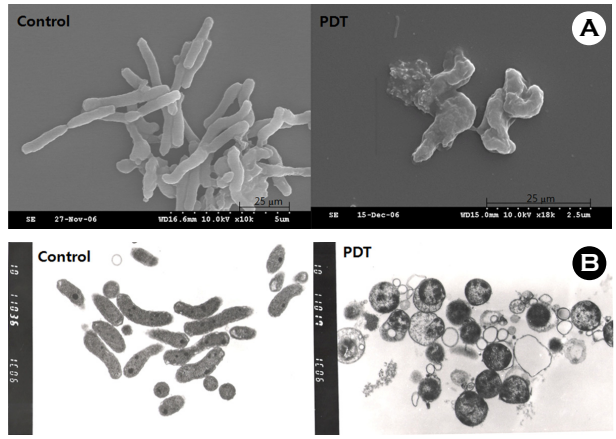

Fig. 2. Scanning electron microscopy (A, ×10,000 and × 18,000) and transmission electron microscopy (B, ×10,000) analysis of *H. pylori* irradiated with laser in the presence of photogem (10 μg/ml). The cell wall surface of *H. pylori* was severely damaged in PDT groups.

adhered closely to the smooth cell walls, as illustrated in Fig. 2A and B (left). Unlike the relatively homogeneous bacterial cytoplasm in the control group, *H. pylori* exposed to PDT was markedly swollen and distorted, with blebbing in the cellular membrane, as shown in Fig. 2B (right), indicating that the cell membrane was the main targets for the photogem.

PDT is capable of killing bacteria both *in vitro* and *in vivo* conditions. Although the use of PDT to treat infections is clearly in its infancy, PDT seems to be suitable as an alternative method of treating localized infections. Recently, some attempts to develop anti-bacterial PDT for the eradication of *H. pylori* were successful *in vitro* (Choi et al., 2010; Hamblin et al., 2005). Besides, a controlled, prospective trial of endoscopically delivered blue light to eradicate *H. pylori* in regions of the gastric antrum in 10 patients showed an overall 91% reduction in *H. pylori* colonies, between treated and control areas (Ganz et al., 2005). Hamblin et al. demonstrated that *H. pylori* accumulate quantities of endogenous coproporphyrin and protoprophyrin IX, which leads to bacteria killing by photodynamic action upon illumination.

In the current study, the antibacterial effect of PDT was confirmed by CFU, FACS and TEM analyses against *H. pylori*. The number of *H. pylori* CFU was significantly lower in PDT groups than the number in the untreated control, PS, and laser only groups. When the photosensitizer was administered without the laser no reduction in the number of CFU was observed, except at the highest concentration, suggesting that the photogem presented a cytotoxic effect on *H. pylori* only at the highest concentration. When observed microscopically, PDT group displayed disrupted cell walls, formation of dense cytoplasmic aggregates, cell wall blebbing and cell swelling. This morphologic progression correlates well with the viability data.

According to the results, *in vitro* PDT against *H. pylori* showed effective bactericidal activity. But, applying this new technique to real patient may has some limitation because gastric mucosa where *H. pylori* usually present will provide much more complex environment that might interfere with PDT. In addition, as the mechanism of action

of PDT involves the formation of ROS, it is necessary to know the toxic effects of this therapy on normal cells and adjacent healthy tissues, which are also susceptible to the damage generated by the free radicals. In fact, Ribeiro *et al*. Observed that association of Photogem and red LED caused severe toxic effects on normal cell cultures *in vitro*. Thus, it is essential to perform *in vivo* PDT using safe and effective conditions, in normal and infected tissue, respectively.

In conclusion, the results show that photogem-mediated PDT is effective in treating *H. pylori*. This theory encourages further *in vivo* studies, maybe by using an animal model, to explore the potential application of this protocol for eradication of *H. pylori* with antibiotic resistance.

Acknowledgements

This study has been worked by research fund at Dongnam Health College in 2010.

REFERENCES

Chabrier-Rosello Y, Foster TH, Perez-Nazario N, Mitra S, Haidaris CG. Sensitivity of Candida albicans germ tubes and biofilms to photofrin-mediated phototoxicity. Antimicrob Agents Chemother. 2005. 49: 4288-4295.

Choi SS, Lee HK, Chae HS. *In vitro* photodynamic antimicrobial activity of methylene blue and endoscopic white light against *Helicobacter pylori* 26695. J Photochem Photobiol B. 2010. 101: 206-209.

Ching SS, Sabanatha S, Jenkinson LR. Treatment of *Helicobacter pylori* in surgical practice: a randomised trial of triple versus quadruple therapy in a rural district general hospital. World J Gastroenterol. 2008. 14: 3855-3860.

De Souza SC, Junqueira JC, Balducci I, Koga-Ito CY, Munin E, Jorge AO. Photosensitization of different Candida species by low power laser light. J Photochem Photobiol B. 2006. 83: 34 -38.

Donnelly RF, McCarron PA, Tunney MM. Antifungal photodynamic therapy. Microbiol Res. 2008. 163: 1-12.

Dunn BE, Cohen H, Blaser MJ. *Helicobacter pylori*. Clin Microbiol Rev. 1997. 10: 720-741.

Ganz RA, Viveiros J, Ahmad A, Ahmadi A, Khalil A, Tolkoff MJ, Nishioka NS, Hamblin MR. *Helicobacter pylori* in patients can be killed by visible light. Lasers Surg Med. 2005. 36: 260-265.

Hamblin MR, Viveiros J, Yang C, Ahmadi A, Ganz RA, Tolkoff MJ. *Helicobacter pylori* accumulates photoactive porphyrins and is killed by visible light. Antimicrob Agents Chemother. 2005. 49: 2822-2827.

Muller S, Pflock M, Schar J, Kennard S, Beier D. Regulation of expression of atypical orphan response regulators of *Helicobacter pylori*. Microbiol Res. 2007. 162: 1-14.

Peloi LS, Soares RR, Biondo CE, Souza VR, Hioka N, Kimura E. Photodynamic effect of light-emitting diode light on cell growth inhibition induced by methylene blue. J Biosci. 2008. 33: 231-237.

Ribeiro AP, Pavarina AC, Trindade FZ, Inada NM, Bagnato VS, de Souza Costa CA. Photodynamic therapy associating Photogem and blue LED on L929 and MDPC-23 cell culture. Cell Biol Int. 2010. 34: 343-351.

Stukavec J, Duchac V, Horak L, Pouckova P. Photodynamic therapy of human colorectal carcinoma cell line. Photomed Laser Surg. 2009. 27: 107-110.

Trushina OI, Novikova EG, Sokolov VV, Filonenko EV, Chissov VI, Vorozhtsov GN. Photodynamic therapy of virus-associated precancer and early stages cancer of cervix uteri. Photodiagnosis Photodyn Ther. 2008. 5: 256-259.

Wainwright M. Photodynamic antimicrobial chemotherapy (PACT). J Antimicrob Chemother 1998. 42: 13-28.