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Photoeradication of *Helicobacter pylori* Using 5-Aminolevulinic Acid: Preliminary Human Studies

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Background and Objectives: *Helicobacter pylori* (HP) is an endemic pathogenic bacterium causing gastritits and gastroduodenal ulceration in humans and is linked to the development of gastric malignancies. These first human in vivo studies investigated the photoeradication of HP using laser and white light.
Study Design/Materials and Methods: In 13 HP-positive volunteers, a zone of gastric antrum was irradiated with laser (410 nm, 50 J/cm^2) or endoscopic white light (10 J/cm^2) 45 minutes after oral 5-aminolevulinic acid (5-ALA) 20 mg/kg. HP-eradication was assessed by biopsy urease test and HP-culture from irradiated and control zones 5 minutes, 4 and 48 hours post-irradiation.
Results: A maximum eradication effect was achieved at 4 hours post-irradiation when 85% of biopsies in the monochromatic and 66% in the white light exposed zones, and 58 and 33% in the respective control zones were HP-negative.
Conclusions: HP numbers were greatly reduced following exposure to 5-ALA and either laser or white light in vivo. Photoeradication appears feasible, but further light dosimetry and the development of convenient application methods is required. Lasers Surg. Med. 31:18–22, 2002. © 2002 Wiley-Liss, Inc.

Key words: aminolevulinic acid (ALA); antibacterial therapy; gastritis; *Helicobacter pylori*; human; laser; photodynamic therapy; photosensitizer

INTRODUCTION

Helicobacter pylori (HP) is a common Gram negative bacterium infecting the stomach, which causes chronic gastritis and is implicated in peptic ulceration and gastric neoplasms [1–3]. Eradication with a combination of antibiotics and acid inhibitors is generally effective, but must be balanced against the disadvantages of increasing antibiotic resistance, toxicity, reduced compliance due to complicated dosing regimens, and cost. Photodynamic antibacterial chemotherapy could be an effective treatment alternative, as various bacteria exhibit very low bactericidal light thresholds after incubation with photosensitizers [4,5]. Preliminary in vitro studies with different photosensitizers demonstrated good photosensitivity of HP [6–8]. 5-Aminolevulinic acid (5-ALA) is a commonly used precursor of the photosensitizer protoporphyrin IX, itself a precursor in the biosynthesis of haem. In vitro studies with 5-ALA demonstrated potent photosensitization of HP, with kill rates of > 95% at ALA concentrations of < 0.01 μmol/L at a pH between 4 and 5 and 20–40 minutes irradiation with laser light of wavelength of 407 and 630 nm [8]. Light doses between 10–300 J/cm^2 showed similar efficacy. HP kill rates with only irradiation or only 5-ALA were < 10%. Based on these results two similar open human in vivo pilot studies of focal gastric HP photoeradication were designed.

MATERIALS AND METHODS

Two successive endoscopic eradication studies were performed. In the first study, seven (five males, two females, age 26–46 years) and in the second study six (three males, three females, age 30–47 years) asymptomatic HP-positive volunteers were recruited. Before the start of the study, HP infection was confirmed by histology using Giemsa and immunohistochemical staining (Novocastra Laboratories Ltd, Newcastle, England), the rapid urease biopsy test (HUT®; AstraZeneca, Zug, Switzerland), culture of gastric antrum and corpus biopsies, and the C^{13} urea breath test [9]. Exclusion criteria were a history

of gastrointestinal, hepatic or dermatological disease, coagulation disorders, pregnancy, lactation, and porphyria. University Ethics Committee approval and written informed consent were obtained.

Subjects were gastroscoped four times. During the first endoscopy two zones in the gastric antrum of 2-cm diameter were marked using hot biopsy forceps. Mucosal pinch biopsies were taken for the HP tests as described above. Within 7 days, a second endoscopy was performed 45 minutes after ingestion of 20 mg/kg 5-ALA (ALAT AG, Zug, Switzerland). Subjects rotated onto their left and right sides to ensure an even distribution of the sensitizer. During the gastroscopy one of the antral zones was directly irradiated with laser (Study 1) or endoscopic white light (Study 2). The second antral zone (control) was exposed to the minimum dose of direct white light by keeping the endoscope tip at least 1 cm distant from the mucosa. In the first study, irradiation was with a light dose rate of 100 mW/cm^2 (light dose 50 J/cm^2) for 500 seconds using a Krypton laser of 410-nm wavelength (Model 171; Spectraphysics, CA). Microlens (FDI, Medlight, Ecublens, Switzerland) (n = 1) or cylindrical balloon (LB20, Medlight) (n = 6) diffusers were passed through the Olympus XQ 20 endoscope's biopsy channel and placed in contact with the mucosa for light application. In the second study, the antral zone was irradiated for 400 seconds with white light from an Olympus GIF 100 endoscope and the standard CLV U120 light source manually set to maximum output. The endoscope tip was positioned 1 cm from the mucosa. Based on pre-study spectrometric measurements with the same endoscope and power calculations, an incident light dose of 10 J/cm^2 was delivered to the irradiated zone in addition to an approximate light dose of 3–5 J/cm^2 due to the normal endoscopic procedure. The latter estima-
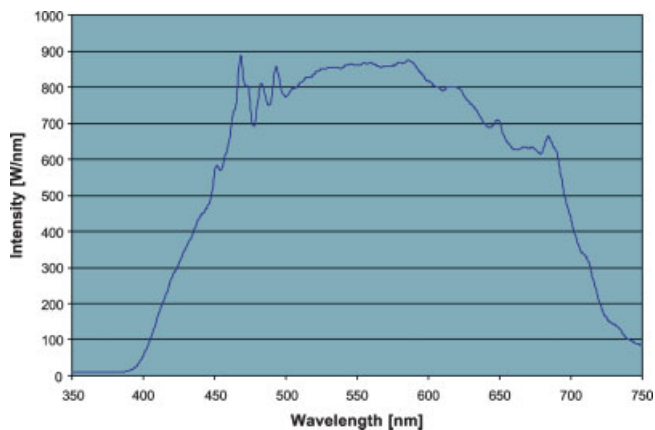

Fig. 1. Corrected spectrum of the endoscopic white light source (Olympus CLV-U20) used for irradiation in Study 2. The intensity is shown in Watt per nanometre.

tion was based on a gastric endoscopy time of 600 seconds, an average light dose rate of 4.5 mW/cm^2 due to illumination and an approximative correction for reflectance and scattering [10,11]. Therefore, it is estimated that the control zone received a light dose of approximately 3–5 J/cm^2. The corrected spectrum of the endoscopic white light used for irradiation is shown in Figure 1. The efficacy of the white light was calculated to be approximately 15 times smaller than light at 410 nm for a given fluence rate.

Subjects received midazolam 2–5 mg and scopalamin butylbromide 20 mg before intubation. Lansoprazole 30 mg was taken on the evening and morning before irradiation for optimal gastric pH for photoeradication.

TABLE 1. Zones (Numbers/Percentages) of Gastric Antrum *Helicobacter pylori* Negative by Culture or Biopsy Urease Test After Photosensitisation With 5-ALA 20 mg/kg and Focal Irradiation With Either Laser Light at 410 nm and 50 J/cm^2 (n = 7) or White Endoscopic Light at 10 J/cm^2 (n = 6)

	Culture		Biopsy Urease test	
	Irradiated zone	Control zone	Irradiated zone	Control zone
Test time	Laser irradiation (n = 7/100%)			
Pre-irradiation	0%	0%	0%	0%
5 minutes post	4/58%	3/42%	4/58%	3/42%
4 hours post	6/86%	3/50%[a]	6/85%	4/58%
48 hours post	5/72%	4/58%	7/100%	4/58%
	Endoscopic white light irradiation (n = 6/100%)			
Pre-irradiation	0%	0%	0%	0%
5 minutes post	0%	0%	3/50%	2/33%
4 hours post	4/67%	4/67%	5/83%	2/33%
48 hours post	0%	0%	5/83%	3/50%

Data for the irradiated and non-irradiated control antral zones are shown.
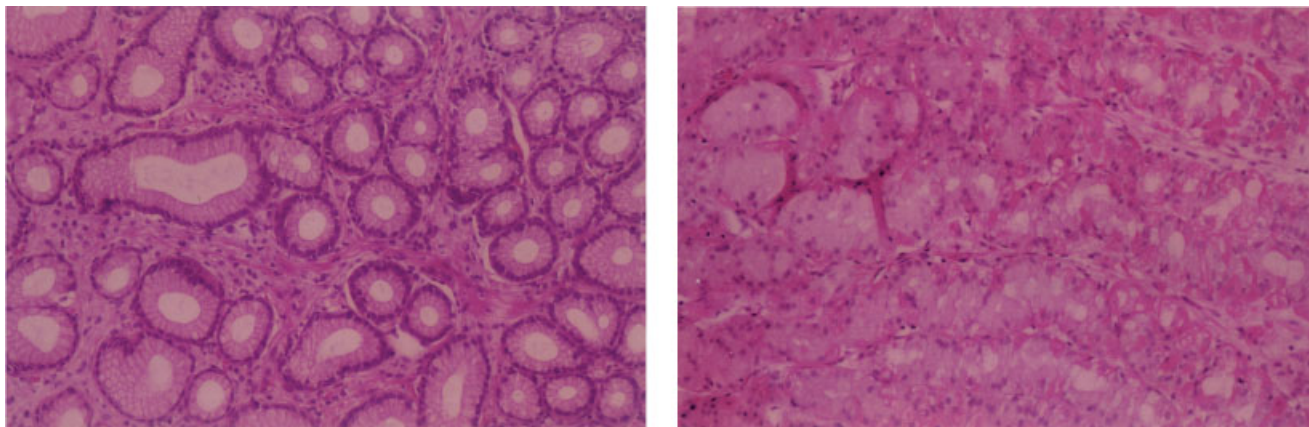[a]Data from one patient are missing.

Fig. 2. Histology of antral biopsy before (left panel: normal histology) and 4 hours after (right panel: edema of the lamina propria) laser irradiation. HE staining and a magnification of $\times 60$ were used.

In both studies, biopsies for HP-culture, histology, and urease testing were obtained with a different biopsy forceps from each zone. Endoscopies were repeated at 4 and 48 hours after irradiation for further biopsies. All biopsies were shielded from light until and during processing.

RESULTS

The irradiation protocol was completed in all subjects. Photoeradication rates, as determined by HP culture and the urease test, are shown in Table 1. Maximal eradication was achieved after 4 hours as assessed by culture and after 48 hours using the urease biopsy test.

Minor histological damage in the form of mild edema in the lamina propria and/or epithelial desquamation were described in two irradiated and one control zone in the laser irradiation group, and in one case in a white light

irradiated zone (Figs. 2 and 3). Slight cutaneous erythema after exposure to bright sunlight developed in the first 24 hours in three subjects with laser treatment.

DISCUSSION

These first human in vivo studies of focal photoeradication demonstrate considerable bactericidal effect on HP following 5-ALA ingestion, with a more profound effect of laser than white light irradiation. Endoscopically applied laser irradiation following 5-ALA ingestion eradicated HP successfully in all but one targeted zone in HP positive subjects. Both the laser energy and the 5-ALA dose applied were low compared to those used in tumour photodynamic therapy, where energies over 100 J/cm^2 and doses over 30 mg/kg are commonly used [12–16]. With normal white endoscopic light and the same dose of 5-ALA HP was
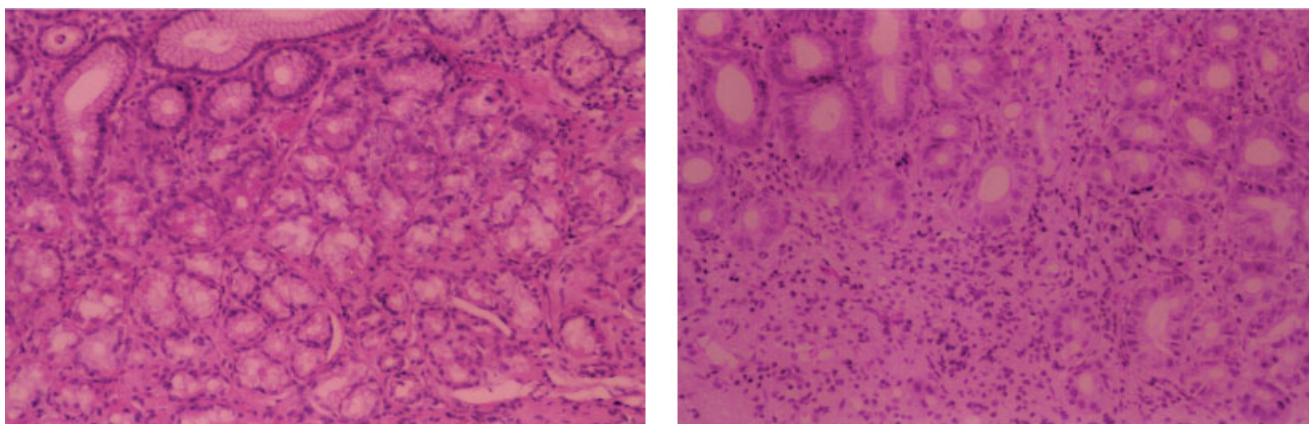

Fig. 3. Histology of antral biopsy before (left panel: normal histology) and 2 days after (right panel: edema of the lamina propria and epithelial necrosis) laser irradiation. HE staining and a magnification of $\times 60$ were used.

eradicated from the antral target zone in four of six subjects. HP regrowth in the irradiated areas occurred within 48 hours in both studies, probably due to reinfection from adjacent uneradicated areas, as HP culture excluded a bacteriostatic effect. Bacterial photoeradication was greatest at hours after light application. This delayed onset of photoeradication, observed in both laser and white light groups, indicates lysis or direct damage to the bacterium do not represent the major phototoxic effects. These would have resulted in rapid bacterial death due to the brief reproductive cycle of bacteria. Postulated bactericidal mechanisms with slow onset include complex host immune responses or progressive genetic damage resulting in accumulating disruption in the synthesis of cell walls or of the vital urease pump [14,17–20]. Phototoxic effects on bacterial reproduction and on the complex urease enzyme pump do not appear to be synchronous, as cell culture and rapid urease test results differed in magnitude and onset. A minor direct inhibitory effect of lansoprazole on urease activity is possible [21]. The HP urease test has also been shown to have a lower specificity and sensitivity (approximately 85 and over 90%, respectively) than HP culture (93–100 and 100%, respectively), which is considered the "gold standard." This discrepancy is further enhanced during concomitant antisecretory therapy [22,23].

The biopsy process itself is unlikely to explain the observed HP eradication effects, as only tiny pinch biopsies (< 2 mm in diameter) were taken, post-biopsy bleeding was minimal and any traces of blood were immediately rinsed off the mucosa during the endoscopic procedure. During routine endoscopy, over 95% of HP infections can be diagnosed with two pinch biopsies from the antral mucosa [22,23].

Although direct mucosal illumination of control zones was avoided, extensive scattering and reflectance of light sufficed to achieve good eradication rates. Based on pre-study dosimetry data light doses of at least 3–5 J/cm^2 were applied during normal endoscopy. Previous in vitro studies demonstrated HP eradication with light doses as low as 10 J/cm^2 with 5-ALA and other photosensitizers [6–8]. A synergistic effect with lansoprazole and other proton pump inhibitors is possible, as they have been shown to suppress HP virulence factors, as well as reduce the viability of HP [21,24]. However, the bactericidal effect in the control zones cannot be explained by lansoprazole alone, as no significant HP eradication occurs even when dosing lasts several weeks [25]. Because of the unexpected eradication effect in the control zones photoeradication rates cannot be calculated by comparison with the directly irradiated zones and a comparison to pre-irradiation baseline values must be performed instead. The observed bactericidal effects can however clearly be attributed to phototoxicity rather than spontaneous clearance or healing of HP infection, which has been shown to occur in less than 1% of infected individuals over an average follow-up period of 12 years [26].

Focal photoeradication of HP resulted in only minor toxicity. This can be explained by the favourable therapeutic window of 5-ALA uptake and conversion kinetics in gastric mucosa (t_{max} of gastric fluorescence approximately 3 hours after oral intake) versus HP (maximum uptake and kill rates 20–40 minutes after application), 5-ALA accumulation mainly in the superficial mucosa and rapid photobleaching, preventing deeper photodamage [27]. Moreover, the ALA concentrations required for photoeradication are 1/6–1/12 of those used in photodynamic tumour ablation.

Adequate irradiation of the entire stomach and duodenal bulb will have to be achieved before clinical application of HP photoeradication is feasible. In the current study, we switched from a microlens to a cylindrical balloon applicator to minimise motions artefacts during application. Light dosimetry using different light applicators and diffusers will be important to assess direct and indirect irradiation effects and side-effects.

ACKNOWLEDGMENTS

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