**Helicobacter pylori Eradication Using a Light-Emitting Diode and Methylene Blue**

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**Background and aims:** Eradication failures are increasing with the increasing antibiotic resistance of *Helicobacter pylori*. We examined the basic effect of methylene blue (MB) with sodium bicarbonate (NaHCO₃) on *H. pylori* eradication using antimicrobial chemotherapy activated by light.

**Material and methods:** When NaHCO₃ was added to MB, the pH became basic. We smeared *H. pylori* on a medium with basic MB and irradiated it using a red light-emitting diode with a wavelength of 660 nm. The applied energy fluencies were 10 J/cm² and 15 J/cm². After 4 days of culture, the effect of this intervention was determined according to the bacterial growth area.

**Results:** The basic effect of MB appeared between a pH of 8.6 and 9.0. The NaHCO₃ concentration was between 4% and 6%. The basic effect at 15 J/cm² was greater than that at 10 J/cm².

**Conclusions:** We concluded that antimicrobial chemotherapy activated by light with basic MB was effective in *H. pylori* eradication.

**Key words:** *H. pylori* • LED • antimicrobial chemotherapy activated by light • methylene blue • sodium bicarbonate • basic effect

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**Introduction**

*Helicobacter pylori* was first reported by Barry J Marshall and J. Robin Warren in 1984 [1]. *H. pylori* are spiral or curved bacilli that are gram-negative, flagellate, and microaerophilic [1]. These bacteria are present in almost all patients with active chronic gastritis, duodenal ulcers, or gastric ulcers [1]. *H. pylori* is considered as an important etiology of these diseases.

The combination of eradication therapy with proton pump inhibitors and antibiotics is used worldwide. However, because of an increase in antibiotic-resistant bacteria, it has become difficult to achieve complete eradication.

Therefore, a new eradication method that is independent of antibiotic resistance is required to destroy the resistant bacteria.

MB generates active oxygen in the presence of light energy [2]. We developed basic pH conditions by adding NaHCO₃ to MB and consequently devised a new photochemical eradication method using basic MB. We then performed antimicrobial chemotherapy activated by light and verified its bactericidal effect on *H. pylori*.

**Material and methods**

We used *H. pylori* strain JCM No.12093, which was obtained from National Research and Development Agency, Institute of Physical and Chemical Research (Japan). *H. pylori* was identified using the API Campy kit (SYSMEX bioMérieux Co., Ltd.).

We used 500 mL of stock solution containing 25 g of MB (Wako Pure Chemical Industries, Ltd.) and NaHCO₃, which was purchased from Nichi-Iko Pharmaceutical Co., Ltd.

We regulated the MB concentrations (0.01%, 0.05%, 0.1%, 0.2%, 0.5%, and 1%) in a sterile test tube with sterile water for injection. We also regulated the NaHCO₃ concentrations (2%, 3%, 4%, 5%, 6%, and 6.5%) similarly.

We adjusted the *H. pylori* count to $2.0 \times 10^8$ cells/ml in sterile physiological saline in a sterile test tube.

Using a sterile pipette tip, 100 µl of MB, 20 µl of *H. pylori*, and 10 µl of NaHCO₃ were taken out of the test...

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Received date: February 20th, 2017
Accepted date: January 22th, 2018

*Laser Therapy* 27.1: 21-25
tubes and smeared on a helicobacter agar medium (Nissui Pharmaceutical Co., Ltd.) using a sterile bacteria spreader.

The added NaHCO_3 was diluted 13-fold in the medium, and the final concentrations were 0.15%, 0.23%, 0.30%, 0.38%, 0.46%, and 0.50%.

After placing it in the dark for 5 min, the bacterial culture was irradiated from a distance of 5 cm using a red light-emitting diode (LED; CCS Inc.) with a wavelength of 660 nm. The energy fluencies were 10 J/cm^2 (at 2 min 40 s) and 15 J/cm^2 (at 4 min).

After irradiation, we placed the culture in an incubator at 37°C for 4 days. The bactericidal effect was evaluated according to the growth area of *H. pylori*, which was categorized into six grades.

The criteria for effectiveness were as follows:
The bactericidal effect in the absence of irradiation and MB was ineffective.
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The blank test included the bacteria and each MB concentration with no irradiation.

(-) indicated ineffectiveness.

(1+), (2+), (3+), and (4+) indicated a decrease in growth areas by 20%, 40%, 60%, and 80%, respectively, compared with the blank test using an ineffective concentration of 0.01% MB.

(5+) indicated no growth area.

We added 2000 µl of MB, 400 µl of sterile physiological saline, and 200 µl of NaHCO_3 to a test tube. We measured the pH using Seven Compact instruments (METTLER TOLEDO International Inc.).

**Results**

Table 1 shows the pH of MB (C_16 H_18 ClN_3 S) and sodium bicarbonate (NaHCO_3), and MB with the addition of NaHCO_3.

As the MB concentration increased from 0.01% to 1%, the pH decreased from 7.435 to 5.890.

| NaHCO_3 concentration (%) | MB concentration% |
|---------------------------|--------------------|
| 0                         | 0                  |
| 2                         | 8.141              |
| 3                         | 8.109              |
| 4                         | 8.082              |
| 5                         | 8.037              |
| 6                         | 8.03               |
| 6.5                       | 8.045              |
| blank test (no irradiation)|                     |

| NaHCO_3 concentration (%) | MB concentration% |
|---------------------------|--------------------|
| 0                         | —                  |
| 2                         | (4+)               |
| 3                         | (3+)               |
| 4                         | (3+)               |
| 5                         | (4+)               |
| 6                         | (3+)               |
| 6.5                       | (3+)               |
| blank test (no irradiation)|                     |

| NaHCO_3 concentration (%) | MB concentration% |
|---------------------------|--------------------|
| 0                         | (4+)               |
| 2                         | (4+)               |
| 3                         | (3+)               |
| 4                         | (3+)               |
| 5                         | (4+)               |
| 6                         | (3+)               |
| 6.5                       | (3+)               |
| blank test (no irradiation)|                     |

| NaHCO_3 concentration (%) | MB concentration% |
|---------------------------|--------------------|
| 0                         | —                  |
| 2                         | (4+)               |
| 3                         | (3+)               |
| 4                         | (3+)               |
| 5                         | (4+)               |
| 6                         | (3+)               |
| 6.5                       | (3+)               |
| blank test (no irradiation)|                     |

| NaHCO_3 concentration (%) | MB concentration% |
|---------------------------|--------------------|
| 0                         | (4+)               |
| 2                         | (4+)               |
| 3                         | (3+)               |
| 4                         | (3+)               |
| 5                         | (4+)               |
| 6                         | (3+)               |
| 6.5                       | (3+)               |
| blank test (no irradiation)|                     |
Figure 1: No irradiation (blank test)

Figure 2: The effect of MB irradiated at the fluence of 10 J/cm²

Figure 3: The basic effect of MB with the addition of 5% NaHCO₃ irradiated at the fluence of 10 J/cm²

Figure 4: No irradiation (blank test)

Figure 5: The effect of MB irradiated at the fluence of 15 J/cm²

Figure 6: The basic effect of MB with the addition of 5% NaHCO₃ irradiated at the fluence of 15 J/cm²
As the NaHCO₃ concentration increased from 2% to 6.5%, the pH decreased from 8.141 to 8.045.

When NaHCO₃ was added to MB, the pH increased to in the range of 8.550 - 9.0.

**Table 2** shows that, at an energy fluence of 10 J/cm², the MB concentration of > 0.2% was graded as 5+. With the addition of 2% and 3% NaHCO₃, the MB concentration of > 0.2% was graded as 5+. With the addition of 4% and 6% NaHCO₃, the MB concentration of > 0.1% was graded as 5+.

With the addition of 5% NaHCO₃, the MB concentration of > 0.05% was graded as 5+.

With the addition of 6.5% NaHCO₃, the MB concentration of > 0.2% was graded as 5+. The effects on these media are shown in **Figures 1, 2, and 3**.

**Table 3** shows that, at an energy fluence of 15 J/cm², the MB concentration of > 0.05% was graded as 5+. With the addition of 2% and 3% NaHCO₃, the MB concentration of > 0.05% was also graded as 5+.

With the addition of 4% - 6% NaHCO₃, the MB concentration of > 0.01% was graded as 5+.

With the addition of 6.5% NaHCO₃, the MB concentration of > 0.1% was graded as 5+.

The bactericidal effect was attenuated compared with that of MB alone. The effects on these media are shown in **Figures 4, 5, and 6**.

**Discussion**

The chemical structure of MB³ is shown in **Figure 7**. In general, dyes are divided into acidic, basic, and amphoteric types⁵. Acidic dyes are negatively charged in an aqueous solution⁵, whereas basic dyes are positively charged⁵. Amphoteric dyes have both properties and maintain an equilibrium state⁵.

MB is a water-soluble and basic dye⁵. The positive charge of MB binds to a bacterium containing a negative charge⁵. Stainability is enhanced in the basic state⁵, 6.

We demonstrated the relationships between the minimal MB concentration for achieving a grade 5+ bactericidal effect, NaHCO₃ concentration, energy fluence, and pH (**Figures 8 and 9**). The pastel part of the figure is the region where a basic effect appeared and increased. We estimated that the basic effect of MB was present at 4% - 6% NaHCO₃ concentration and a pH of 8.6 - 9.0 at 10 J/cm² and 15 J/cm². The actual NaHCO₃ concentration was in the range of 0.30% - 0.46%.

**Figure 10** depicts the comparison of the basic effects of the two energy fluencies. The basic effect appears most strongly at pH 8.6. At the 4% - 6% NaHCO₃ concentration, the increasing energy decreases the MB’s effective minimal concentration to 0.01%. The basic effect is en-
enhanced by the increasing the energy.

The basic effect of MB is related to the following features:
1. NaHCO₃ concentration
2. basic pH
3. adjustment and balance

It is estimated that the basic effect appears and increases when the adjusted NaHCO₃ concentration and the basic pH are balanced.

Even when MB is sprayed in the stomach, the normal gastric mucosa is not stained. In cases of intestinal metaplasia, it is absorbed from the intestinal epithelium and is thus, useful for the diagnosis of intestinal metaplasia.

NaHCO₃, the proteolytic enzyme pronase, and stomach mucus antifoaming agents are orally administered in the form of aqueous solutions as a pretreatment during pigment endoscopy. In such cases, NaHCO₃ is used to increase the enzyme activity and decrease the mucus viscosity.

This is because the optimal pH of the proteolytic enzyme is between 7 and 10, and the enzyme activity is unstable under acidic conditions.

After sufficiently suctioning the dissolved mucus using endoscopic forceps, MB with NaHCO₃ is sprayed. Light is irradiated from the endoscopic light source after these procedures. NaHCO₃ is administered twice to achieve a basic pH because of the following reasons:
1. the optimal pH of pronase and
2. the enhancement of MB stainability.

The generation and maintenance of basic pH is particularly important for this new photochemical therapy.

**Conclusions**

Our eradication method is effective against antibiotic-resistant *H. pylori* and does not result in the production of resistant bacteria. This new photochemical method is a viable option for the eradication of *H. pylori*.

Endoscopic *H. pylori* eradication using basic MB can be applied. We propose that this method is termed as endoscopic antimicrobial chemotherapy activated by light.

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**Acknowledgments**

This paper was based on a lecture at the "Japan Society for Laser Surgery and Medicine" held on October 22, 2016.
This paper was based on contents published in “Optical Alliance” issued by JAPAN INDUSTRIAL PUBLISHING Co., Ltd. on November 1, 2016.