Effects of He-Ne laser irradiation on chronic atrophic gastritis in rats

Xue-Hui Shao, Yue-Ping Yang, Jie Dai, Jing-Fang Wu, Ai-Hua Bo

AIM: To study the effects of He-Ne laser irradiation on experimental chronic atrophic gastritis (CAG) in rats.

METHODS: Sixty-three male adult Wistar rats were randomly divided into five groups including normal control group, model control group and three different dosages He-Ne laser groups. The chronic atrophic gastritis (CAG) model in rats was made by pouring medicine which was a kind of mixed liquor including 2% sodium salicylate and 30% alcohol down the throat for 8 wk to stimulate rat gastric mucosa, combining with irregular fasting and compulsive sporting as pathogenic factors; 3.36, 4.80, and 6.24 J/cm² doses of He-Ne laser were used, respectively for three different treatment groups, once a day for 20 d. The pH value of diluted gastric acid was determined by acidimeter, comparing among different treatment groups. Morphology, structure and volume of the parietal cells all recuperated or were closed to normal.

RESULTS: In model control group, the secretion of gastric acid was little, pathologic morphological changes in gastric mucosa, the morphology and structure of parietal cells were observed, and the thickness of mucosa was significantly thicker than that in model control group (<0.01), the gastric sinus secret cells reduce, function is weakened, gastric acid secretion reduces, especially pathological changeable epithelium often contains intestine epithelium metaplasia and atypical hyperplasia, which are often seen as precancerous lesions of gastric carcinoma[5-7]. To the author's knowledge, clinics still have no perfect and effective treatment projects[8]. So we successfully established an animal model with CAG in rats[9], used different radiation intensity of He-Ne laser to irradiate at the gastric projective area of rat with CAG, to study the therapeutic effects by examining changes of gastric acid and observing changes of pathologic histology in gastric mucosa.

CONCLUSION: 3.36 J/cm² dose of He-Ne laser has a significant effect on CAG in rats.

Key words: Chronic atrophic gastritis; Laser; He-Ne; Rat

© 2005 The WJG Press and Elsevier Inc. All rights reserved.
Small dose He-Ne laser treatment: irradiated 7 min each time, 3.36 J/cm² of dosage;
Inside dose He-Ne laser treatment: irradiated 10 min each time, 4.80 J/cm² of dosage;
Big dose He-Ne laser irradiation: irradiated 13 min each time, 6.24 J/cm² of dosage.

**Process**
The rats in each group were killed at the desired time-point. The animals were deprived of food but were offered enough water before being killed at 24 h. The rats were anesthetized with ether, the belly was opened immediately, cadre was ligature, the whole stomach was taken off, and the surface blood stains were washed by normal saline water, sucked dry with filter paper, then the gastric cavity was opened along greater curvature of stomach, flushed with distilled water, and the dilution gastric liquid (provided to measure the gastric acid pH value) was collected, then front and back gastric sinus and parts of stomach organized roughly into 3 mm×10 mm were taken in parallel with lesser curvature of stomach, fixed in neutral formalin, embedded in paraffin wax, 6 µm sections, stained with hematoxylin-eosin (HE) for pathological examination.

**Determination of gastric acid**
The rat's gastric acid (pH value) was determined five times for each example by acidimeter (PHS-3C) from Shanghai Instrument Factory, and took the average value as the pH value of this animal.

**Pathology examinations**
Take three sliced pieces for each example, and select three different visual fields for each slice such as the body of stomach, gastric sinus and the area of the body of stomach marked with gastric sinus. The whole gastric mucosa was observed under light microscope, which included the following aspects: (1) the immunity degree of the gastric mucosal ingluvitis cellular was divided into two grades as negative (-) (no ingluvitis cells or some ingluvitis that jot spread at the gastric mucus) and positive (+) (more ingluvitis cell at the gastric mucosa or series of ingluvitis cells aggregated inside the mucus), counted the positive rate respectively by each visual field; (2) measured the thickness of the gastric mucosa with microscope, regarded µm as the unit, adopted the average of the mucosa thickness to show the reflection hyperplasia circumstance; (3) observed the parietal cell including the appearance, construction and arrangement.

**Statistical analysis**
Software SPSS 10.0 was used in the statistical analysis, parameters were expressed as mean±SD, and compared using One-way ANOVA analysis of variance, followed by χ² tests and differences were considered significant at P<0.05.

**RESULTS**

**Gastric acid**
The determination results of gastric acid showed that the secret function of gastric mucosa in model control rats was more decreased than that in both normal control group and small dose He-Ne laser group (P<0.05, Table 1).

| Group                  | n  | pH value (mean±SD) |
|------------------------|----|--------------------|
| Normal control         | 6  | 3.72±1.02          |
| Model control          | 7  | 5.86±1.45          |
| Small dose He-Ne laser | 7  | 4.43±1.18          |
| Inside dose He-Ne laser| 6  | 4.66±1.00          |
| Big dose He-Ne laser   | 7  | 4.57±1.48          |

P<0.05 vs control group.

**Pathological findings**
The pathological histologies were observed under microscope. In normal control group, the gastric mucosa was thick, the epithelium was complete, only fell ingluvitis were spread, there was no obvious ingluvitis cellular immunity (Table 2). The parietal cells in normal control group were as follows: large volume, pyramid form, cell edge tactful, full, numerous, were arranged neatly and above 2/3 at a fundic gland (Figure 1A). In model control group, the gastric mucosa was thinner than that in normal control group (P<0.01), there were large quantities of immunity ingluvitis (P<0.01), most of the cells were lymphocytes and plasma cells. The parietal cells in model control group were as follows: volume sterigma, cell edge anomaly polygon, afterbirth syrup was wrinkled and arranged foul-up, intercellular space was enlarged, the ratio in syrup and nucleosidase increased the breadth of the gland antrum, and large quantity of cells were put through empty transformation (Figure 1B). In He-Ne laser groups, the gastric mucosa was thicker than that in model group (P<0.01), ingluvitis cells were less than that in model control group, particularly the small dose He-Ne laser group (P<0.05). The parietal cells in small dosage He-Ne laser

**Table 1 Detection of gastric results of pH value in Wistar rats**

| Group                  | n  | pH value (mean±SD) |
|------------------------|----|--------------------|
| Normal control         | 6  | 3.72±1.02          |
| Model control          | 7  | 5.86±1.45          |
| Small dose He-Ne laser | 7  | 4.43±1.18          |
| Inside dose He-Ne laser| 6  | 4.66±1.00          |
| Big dose He-Ne laser   | 7  | 4.57±1.48          |

**Table 2 Changes of gastric mucosa thickness and the observations of ingluvitis cellular immunity degree**

| Group                  | n  | The thickness of gastric mucosa (µm) (mean±SD) | Ingluvitis cellular immunity degree | Ingluvitis edema |
|------------------------|----|-----------------------------------------------|------------------------------------|-----------------|
|                         |    |                                               | -  +  Positive rate (%)           | Yes | No |
| Normal control         | 8  | 525.17±57.52                                  | 7  1  13                      | ✓   | ✓  |
| Model control          | 11 | 387.21±51.60                                  | 1  10  91                     | ✓   | ✓  |
| Small dose He-Ne laser | 11 | 499.06±57.25                                  | 6  5  45                      | ✓   | ✓  |
| Inside dose He-Ne laser| 11 | 456.77±47.56                                  | 1  10  91                     | ✓   | ✓  |
| Big dose He-Ne laser   | 11 | 475.62±53.79                                  | 2  9  82                      | ✓   | ✓  |

*P<0.05, **P<0.01 vs model control group.*
group were as follows: cell appearance, volume and construction were all near to normal, and arranged neatly (Figure 1C). Besides, there was no obvious ingluvitis edema in normal control group, model control group and small dosage He-Ne laser group, but it was obvious in inside and large dose He-Ne laser groups, and the cells were transformed in the two groups.

**DISCUSSION**

The cause of disease and pathogenesis of chronic atrophic gastritis (CAG) are not yet completely clean, generally speaking, it is a synthesis factor with more effects and actions such as immunity, gall back streaming, pyloric infection, food, wine, smoke, and drugs. Research expressed that sodium salicylate could hurt gastric mucosa and enzyme could inhibit gastric cell growth, alcohol could incite gastric mucus. In our experiment, we poured a mixture of 2% sodium salicylate and 30% alcohol down the rat’s throat for 8 wk, which could affect the natural cover and organize variety in gastric mucus, combined with fatigue from overwork, hunger and dissatisfaction in order to make the pathological changes of CAG appear on the gastric mucosa appear.

Since the parietal cells reduced and degenerated, there was disorder both in gastric acid secretion and digestion enzyme weakness, the performance was a series of weak symptoms of the function of digestion and absorption such as the reduction of gastric acid secretion, diminishing of the gland and atrophy, and metaplasia of the intestinal epithelium.

He-Ne laser, its characteristics such as 632.8 nm of wavelength, good directivity, high intensity, good monochromaticity, is a low-level laser. The low-level laser has some biology effects such as cell vitality, phagocytosis, immune responses, but as far as its usage for the treatment of digestive disease, there were only few reports. On one hand, there was no difference between treating gastric ulcer through bark and by endoscope. On the other hand, external irradiation is easy, and patients can bear some pain with ease. We treated rats with CAG by external irradiation with He-Ne laser as amplified by a convex mirror, radiated at the gastric projective area. The results implied that the gastric acid secretion of the rats in CAG treated by He-Ne laser irradiation was increased, the best effect was on the animals treated by small dosage He-Ne laser. Some changes in gastric mucus such as thickness of gastric mucosa, alleviated ingluvitis, and parietal cell hyperplasia were found in this experiment. We think the mechanism of He-Ne laser treated CAG in rats as follows: (1) partial microcirculation was ameliorated; (2) body immunity was enhanced; (3) the organized growth of gastric mucosa was promoted; (4) inflammation was eliminated. Moreover, in our experiment we found that three different dosages of He-Ne laser preceding the incitement to the rat in CAG, resulted in different effects. The best effect was the small dosage of He-Ne laser, the inside dose and the large dose were not as well as the small dose of He-Ne laser, which showed the dependence on dosage. Perhaps it is because the biology effect of He-Ne laser closely related to the action time, power density that organized He-Ne laser. Under the same power density (8 mW/cm²), different action time (small dose for 7 min, inside dose for 10 min, large dose for 13 min), produced different effects. These results showed that small dose He-Ne laser was a positive action, but large dose was an inhibitory action to incite lombricine organization. Besides, some damaged cells, individual swelling, afterbirth syrup degeneration and ingluvitis edema in the inside dose He-Ne laser group and the big dose He-Ne laser group were found in gastric mucus of rats under microscope. This may indicate the light dynamic damage of He-Ne laser. So we draw a further conclusion that big doses of He-Ne laser were deformative. In a word, the experiment showed that 3.36 J/cm² dose of He-Ne laser is a valid dose of external irradiation to incite life, it could promote the secretion of gastric acid and recover the function of gastric mucus to the CAG in rats.

In summary, 3.36 J/cm²/dose of He-Ne laser irradiation is a well-tolerated, safe, and effective treatment in rats with CAG. The technique is easy, inexpensive, and of short duration. It is necessary to translate the outcome of this study into clinically relevant interventions by further studies which would develop a new way for the treatment of CAG.

**REFERENCES**

1. **He WB**, Gao GL, Hou S, Song G. Relationship between mucosal vascular lesion and gastric carcinoma in chronic atrophic gastritis of mice. *Shijie Huaren Xiaohua Zazhi* 1999; 7:130-132
2. **He RZ**. Pathology (fourth edition). *Renmin Weisheng Chubanshe* 2003: 128-129
3. **Asaka M**, Sugiyama T, Nobuta A, Kato M, Takeda H, Graham D. Atrophic gastritis and intestinal metaplasia in...
Rochkind S, Shao XH, Pogrel MH, Qin RJ. Nanjing pharmaceutical college. Medicament Chemistry.

Wang RJ, Du Q, Shao TY, Zhong TJ, Wu YL, Wang JH. Pathologic studies of Chinese drug Weiyanxiao on experimental chronic atrophic gastritis in rats. *Shijie Huaren Xiaohua Zazhi* 2000; 4: 382-285

Chen SY, Wang JY, Ji Y, Zhang XD, Zhu CW. Effects of *Helicobacter pylori* and protein kinase C on gene mutation in gastric cancer and precancerous lesions. *Shijie Huaren Xiaohua Zazhi* 2001; 9: 302-307

Wang XB, Wang X, Zhang NZ. Inhibition of somatostatin analog Octreotide on human gastric cancer cell MKN45 growth in vitro. *Shijie Huaren Xiaohua Zazhi* 2002; 10: 40-42

Yao XX, Yin L, Zhang JY, Bai WY, Li YM, Sun ZC. hTERT expression and cellular immunity in gastric cancer and precancerosis. *Shijie Huaren Xiaohua Zazhi* 2001; 9: 508-512

El-Zimaity HM, Ota H, Graham DY, Akamatsu T, Katsuyama K. Patterns of gastric atrophy in intestinal type gastric carcinoma. *Lasers Surg Med* 2002; 28: 212-218

Shao XH, Wang JG, Dai J. Establishment of chronic atrophic gastritis in a rat model. *Zhangjiaokou YiXueYuan Xuebao* 2002; 2: 11-13

Nanjing pharmaceutical college. Medicament Chemistry. Beijing, *Renmin Weisheng Chubanshe* 1978: 172

Hu XM. Medical physics (fifth edition). Beijing: *Renmin Weisheng Chubanshe* 2001: 351-352

Qin RJ. Medical physics (third edition). Guilin: *GuangXi Shifan Daxue Chubanshe* 2002: 131-132

Stadler I, Lanzafame RJ, Oskoui P, Zhang RY, Coleman J, Whittaker M. Alteration of skin temperature during low-level laser irradiation at 830 nm in a mouse model. *Photomed Laser Surg* 2004; 22: 227-231

Rochkind S, Ouaknine GE. New trend in neuoscienee: low-power laser effect on peripheral and central nervous system. *Neural Res* 1992; 14: 2-11

Pogrel MH, Chen JW, Zhang K. Effects of low-energy gallium-aluminium-arsenide laser irradiation on cultured fibroblasts and keratinocytes. *Lasers Surg Med* 1997; 20: 426-432

Ben-Dov N, Shefer G, Irinitchev A, Oron U, Halevy O, Irinitchev A. Low energy laser irradiation affects satellite cell proliferation and differentiation in vitro. *Biochim Biophys Acta* 1999; 14: 372-380

Grossman N, Schneid N, Reuveni H, Halevy S, Lubart R. 780 nm low power diode laser irradiation stimulates proliferation of keratinocyte cultures: involvement of reactive oxygen species. *Lasers Surg Med* 1998; 22: 212-218

Rochkind S, Ouaknine GE. New trend in neuoscienee: low-power laser effect on peripheral and central nervous system. *Neural Res* 1992; 14: 2-11

Pessoa ES, Melhado RM, Theodoro LH, Garcia VG. A histologic assessment of the influence of low-intensity laser therapy on wound healing in steroid-treated animals. *Photomed Laser Surg* 2004; 22: 199-204

Brosseau L, Welch V, Wells G, DeBlie R, Gam A, Harman K, Morin M, Shea B, Tugwell P. Low level laser therapy (Classes I, II and III) for treating osteoarthritis. *Cochrane Database Syst Rev* 2004; 3: CD002046

Kans JS, Hutschenreiter T, Haina D, Waidelich W. Effect of low-power density laser radiation on healing of open skin wounds in rats. *Arch Surg* 1981; 116: 293-296

Hrnjak M, Kulijic-Kapulica N, Budisim A, Giser A. Stimulatory effect of low-power density He-Ne laser radiation on human fibroblasts in vitro. *Vojnosanit Pregl* 1995; 52: 539-546

Monteforte P, Baratto L, Molletta L, Rovetta G. Low-power laser in osteoarthritis of the cervical spine. *Int J Tissue React* 2003; 25: 131-136

Ohta A, Abergel RP, Uitto J. Laser modulation of human immune system, inhibition lymphocyte proliferation by a gallium-arsenide laser energy. *Lasers Surg Med* 1987; 7: 199-201

Karu T. Photobiology of low-power laser effects. *Health Phys* 1989; 56: 691-704

Fukutomi H, Kawakita I, Nakahara A. Endoscopic diagnosis and treatment of gastric cancer by laser beam. *Lasers Surg Med* 2002; 26: 199-204

Ouaknine GE. New trend in neuoscienee: low-power laser effect on peripheral and central nervous system. *Photomed Laser Surg* 2004; 22: 199-204

Rochkind S, Ouaknine GE. New trend in neuoscienee: low-power laser effect on peripheral and central nervous system. *Neural Res* 1992; 14: 2-11

Hrnjak M, Kulijic-Kapulica N, Budisim A, Giser A. Stimulatory effect of low-power density He-Ne laser radiation on human fibroblasts in vitro. *Vojnosanit Pregl* 1995; 52: 539-546

Monteforte P, Baratto L, Mollettta L, Rovetta G. Low-power laser in osteoarthritis of the cervical spine. *Int J Tissue React* 2003; 25: 131-136

Ohta A, Abergel RP, Uitto J. Laser modulation of human immune system, inhibition lymphocyte proliferation by a gallium-arsenide laser energy. *Lasers Surg Med* 1987; 7: 199-201

Karu T. Photobiology of low-power laser effects. *Health Phys* 1989; 56: 691-704

Fukutomi H, Kawakita I, Nakahara A. Endoscopic diagnosis and treatment of gastric cancer by laser beam. *Lasers Surg Med* 2002; 26: 199-204