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Sutureless end-to-end bowel anastomosis in rabbit using low-power CO_2 laser

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INTRODUCTION

The use of laser energy to weld biological tissues and produce sutureless anasto mosis has its advantages over conventional silk- sutured anastomosis since it was reported in small vessels[1] and fallopian tubes[2], in the la te 1970s. Since then, more investigators have welded a larger variety of tissues[3-13] and have expanded its application to welding trials of ent ertomies of rabbit and rat small intestine[14-17]. Sauer *et al*[18] reported results from Nd: YAG laser in reconstruction of end-to-end wel ding in rabbit small intestine. Recently, controlled temperature during YAG and argon laser-assisted welding of entertomies of rabbit and rat was implemented to eliminate exponential increases in the rate of denaturation associated with r apidly increasing temperature[19,20]. Yet there was no report of sutureless end-to-end bowel anastomosis using low-power CO_2 laser. This is a repor t of a circumferential end-to-end laser welding bowel anastomosis in rabbit by using 3 different CO_2 laser powers to explore the feasibility of CO_2 laser welding of a circumferential intestinal tissue and to determine the optimal la ser-welding parameter. Then the appropriate CO_2 laser power was chosen to weld bowels in rabbit and its long-term healing effect was evaluated.

MATERIALS AND METHODS

Animals and equipment

Twenty-eight Japanese white rabbits weighing between 1.8 kg and 2.5 kg (6 for a cut e and 22 for chronic phase of experiment) regardless of sex were supplied by Exp erimental Animal Center of Wenzhou Medical College. A hand-held Model JZ-5, po wer 500 mW-5 W adjustable CO_2 laser made in Shanghai Optical Machinery Institute, which was modified to provide a 100 mW-1000 mW potentimeter, was used. The laser spot diameter was 0. 4 mm. The laser power output was calibrated by a Model SD2490 CO_2 laser power meter produced by Subei Electronic Equipment. The pres sure was measured by a PT-6B pressure transducer and PTM-6B physiological pre ssure meter, jointly produced by Shanghai Fudan University and Zhejiang Ouhai El ectronic Equipment Factory, with normal saline intraluminal infusion at an average rate. The flow was monitored by a computed infusion pump (DYB-1) made in Jia ngsu Haimeng Electronic Equipment Factory.

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Acute phase

A rabbit was fasted for 24 h prior to surgery and sodium pentobarbitol (30 m g/kg, im) was used for anesthesia. Using a 5-cm upper abdominal incision, the ileum was identified. At 10 cm proximal to the ileocecal junction, intestinal circ umference was measured. Ileum was cut apart and then reconstructed. Each subsequent test was moved approximately 10 cm and a bowel anastomosis was welded by using 3 different CO_2 laser powers of 250, 500 and 1000 mW respectively. At two circumferential cut-edges of ileum, 3 silk stay sutures were placed to hold tissue together and allow for accuratere-approximation and inversion. Before and a fter each welding, the laser power output was calibrated and modified. Tissue blanching and constriction, and fusing of both egdes of the anastomosis were marks of the completion of a tissue weld. The circumferential welding was completed in sequence.

One min after each laser welding, a 10-cm segment of bowel including the anas tomosis was isolated between occlusive bowel ligature, and the bursting pressure of anastomosis was tested by the intraluminal infusion of normal saline at a rate of 16 mL/min. The pressure was recorded until the anastomosis burst which was visible both as leakage of infusate from the anastomosis and as a sharp drop of pressure. Each rabbit was welded 3 to 5 times and all subjects were sacrificed after the experiment.

Chronic phase

A rabbit was fasted for 24 h prior to surgery and sodium pentobarbital (30 mg/kg, im) was used for anesthesia. Using upper midline abdominal incision, ileum was circumferentially cut apart for a laser-welded anastomosis and a conventio nal one-layer silk sutured anastomosis. It was randomly determined which anasto mosis was 10 cm or

35 cm proximal to ileocecal junction. Three silk stay sutures were placed to hold the cut intestinal edges in welded anastomosis, and 16-20 stiches of interrupted one-layer 0 silk suture were used in sutured anastomosis.

The laser power output 500 mW was used in the laser welding. Tissue blanching and constriction, and fusing of both egdes of the anastomosis by naked-eye were marks of the completion of a tissue weld. The time used for laser welded anastomosis was about 5-7 min while sutured anastomosis needed 10-15 min.

Gentamycine (4 mg, im) was administered 12 h pre and post-operatively. Al l rabbits were sacrificed at d 3, wk 1, 3 and 5 and pathological study was done macroscopically and microscopically.

RESULTS

Acute phase

The intestinal circumference was 26-30 (28.2 ± 1.3) mm. The laser was delivered within 1 mm of both intestinal cut-edges. Based on this calculation, the laser delivery area of each anastomosis was (26-30) × 2 mm. The delivery time for 250 mW group, 500 mW group and 1000 mW group was 92 s-153 s (average 123 s), 59 s-84 s (average 70 s) and 25 s-45 s (average 40 s) respectively. According to the above pa ra meters, the laser power density and energy density of each anastomosis could be calculated. The number of bowel anastomosis using 3 different CO_2 laser powers and the bursting pressure are shown in Table 1.

Table 1 The bursting pressure of the welded and sutured anastomoses

Groups	Number of ananstomosis	Bursting pressure ($x \pm s$) kPa (mmHg)
Silk-sutured	5	6.1 ± 1.9 (46 ± 14)
250 mW-welded	7	1.1 ± 0.4 (8 ± 3)
500 mw-welded	6	2.7 ± 0.7 (20 ± 5)[a]
1000 mW-welded	6	1.7 ± 0.8 (13 ± 6)

[a]$P<0.05$ *vs* other three groups

Chronic phase

In chronic phase experiment, except that one animal died at 1 day and another at 3 days after operation from unknown causes without anastomotic disruption by au topsy, the remaining 20 rabbits,which were dissected at a different postoperative times, were divided into four groups, each consisting of 5 rabbits.

At d 3 after operation, both modes of anastomoses healed well without leaks. Loose fibrous adhesion, stiffness, and edema were found in the adjacent bowel including the anastomosis and mesentery. Suture was seen in the sutured anastomosis while the laser welded anastomosis was smooth.Microscopically, both anastomoses showed edema, acute inflammatory cell infiltration, consisting of mainly neutrop hils, lymphocytes, and macrophages, but comparatively less in the laser-welded a nastomosis. At 1 wk after operation, edema was markedly reduced but the cut-edge in the sutured anastomosis was still swollen. Adhesion was observed surroun ding both anastomoses, less fibrous adhesion was seen in all laser-welded anast omose except in one that had a small walled-off leak surrounded by fibrous form ation without pus or purulent fluid in the abdominal cavity. Some inflammatory cells infiltration, mainly lymphacytes and plasmacytes, was found in both groups. Broad gap of mucous due to excessive inversion was seen in the sutured anastomos is while the gap was much narrower in the laser-welded anastomosis.

At 3 wk after operation, anastomotic tissues were morphologically normal and soft. Linear scar along the cut-edge of sutured anastomosis was seen while lase r anastomosis showed less scar formation. Fibrous adhesion was remarkably decrea sed in the sutured anastomosis than before. No adhesion in 2 cases and very little adhesion in 3 cases was found in the laser-welded group. Microscopically, in flammatory cell infiltration and fibrous proliferation still existed in both ana stomoses. In the sutured anastomosis, there was more and thicker fibrous tissue than in the laser-welded anastomosis, especially at the sutured site. At 5 wk after operation,white linear seams were seen around the sutured anastomoses. Slightly sunken scar was found on one anastomosis and some fibrous adhesion in an other. At both cut-eges of anastomosis dotted line of scars resulted from the silk suture were vaguely observed, but no residual black suture remained. Two la ser-welded anastomoses were hardly distinguishable and the calibre of the other three anastomoses was identical to that of the normal bowel, though thread-like scarring was found at the cut-edges.

DISCUSSION

Laser which is used to weld living tissue differs from high energy laser for other medical purposes in that the former requires specific low power energy. Which type of laser is to be chosen depends on the structure of the target tissue and welding requirement as well. Sauer *et al*[16] used CO_2 laser to weld a 0.5 cm longitudinal incision in rabbit bowel and acquired the bursting power of 5.4 kPa. In this experiment low-power CO_2 laser was used to weld circum ferential bowel anastomosis,which attempted to make laser-welding more applicable to clinical practice. The busting pressure of 500 mW group was 2.7 kPa. The bu rsting pressure was lower probably because the welded anastomoses in this group were circumferential instead of a small longitudinal incision and therefore greater tension was expected. Besides, the distance between each stay suture to be welded was as long as 1.0 cm, much longer than Sauer's 0.5 cm[16]. This study shows that the 500 mW group produced remarkably higher bursting pressure than the other two groups. Group 250 mW cannot produce a desired welding effect due to much too low power and prolonged delivery while charring tissue occurs in the 1000 mW group so that the tensile strength of the welded anastomosis is affected. Furthermore, Cilesiz *et al*[19,21] believed that temperature feedback control improved the quality and stability of laser- assisted enterotomy closures in surviving animals, temperature (90-95) ℃ was considered optimal.

Though no information is available about the maximum bursting pressure of bowel of the conscious rabbit, Abbott *et al* reported that the basic pressure of the distal bowel was 0.83-1.03 kPa, and Fink reported that the pressure of normal ileum was no more than 0. 92 kPa with the peak pressure less than 2.03 kPa in the hum an body. Therefore we infer that the bursting pressure produced in the anastomoses of the 500 mW group is adequate to avoid the anastomotic disruption caused by peak tensile strength.

The healing process of the welded bowel reveals that fibrous tissue or intestina l adhesion to various degree form around both kinds of anastomoses early after o peration. Later, the laser-welded anastomosis is hardly distinguishable from the normal intestine, while suture remnant and white linear scar are found in the sutured anastomoses. Microscopically, compared with the sutured anastomosis, the laser-welded anastomosis has less inflammatory cells early after operation and less fibrous proliferation, less scar formation, and no suture granulation later postoperatively. We therefore conclude that the laser-welding in intestinal an astomosis, as a new type of sutureless surgical technique, has its potential cli nical application value with the advantages including sutureless anastomosis, no foreign material, avoidance of needle trauma and suture remnant, minimal inflam matory response with reduction in stricture and infection. It is especially suit able for mini-bowel anastomosis in congenital intestinal atresia.

It is universally accepted that laser thermal effect is involved in the mechanism of laser-welding of biological tissue. It is also shown that adequate tissue seals occur in laser-welded wounds only when the tissue edges are directly oppo sed,whereas any blood in the interface selectively absorbs the laser energy and forms a fibrin seal which is too weak to tolerate increased intraluminal pressure[14,22]. According to Jain[1] in his study of YAG laser welding of the microvessels, it is the physical coherence of fibrous collagen that weld ed successfully the vascular walls, meanwhile laser does not result in degen era tion, coagulation, and necrosis. In contrast, it is held by most scholars that, based on the observation that coagulation and necrosis exists at the welded site , strong weld is not the result of physical coherence of collagen, but rather th e result of chemical bonding caused by laser thermal effect which can lead to collagenic degeneration and tissue necrosis[23-26]. In our welding experim ents, ideal bonds formed when the opposed cut edges of the bowel were directly and strongly held together and tissue blanching was seen during laser welding. Microscopically, slight tissue degeneration and necrosis could also be seen near the laser-welded anastomosis. However, recent studies show that solid protein used as solder can improve vascular welding effect[27,28]. Meanw hile, it is reported that collagen synthesis is stimulated during the healing process after laser welding[29,30], therefore, further study on mechanism of laser-welding is needed.

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