Histopathological Response and Adhesion Formation After Omentectomy with Ultrasonic Energy, Bipolar Sealing, and Suture Ligation

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Abstract This study was designed to evaluate the histopathological response and intra-abdominal adhesion formation after an omentectomy in rats using the bipolar vessel-sealing device, ultrasonic coagulator, and suture ligation techniques. Forty Wistar albino rats were used, divided into four random groups. The rats underwent a midline laparotomy, and a partial omentectomy was performed using a 3–0 silk suture with suture ligation in group 1, bipolar device in group 2, and ultrasonic coagulator in group 3; only a laparotomy was performed on the control group. Lateral thermal damage was examined the same day, and a piece of the omentum was left in the animals to be examined on post-operative day 15. A relaparotomy was performed to assess adhesion formation and histopathological response. In pairwise comparisons, there was no statistically significant difference among the ultrasonic device, bipolar device, and suture ligation groups in terms of microscopic adhesion scoring; however, the scores of the bipolar device and suture ligation groups were significantly higher compared with those of the control group ($p < 0.01$). Furthermore, the macroscopic adhesion scores were significantly lower for the ultrasonic device group when compared with those of the bipolar device and suture ligation groups ($p < 0.05$ and

$p < 0.01$). The ultrasonic device seems to be superior to the bipolar device and suture ligation in terms of macroscopic adhesion formation, but no significant difference was found in terms of the histopathological response in rats following an omentectomy. Further research may be required.

Keywords Adhesion · Electrosurgery · Omentum · Peritoneum

Introduction

Adhesion formation and prevention strategies remain one of the major challenges of surgery. The first adhesion was described in a patient with peritoneal tuberculosis after a post-mortem examination in 1836 [1]. Following this report, Bryant reported a fatality due to an adhesion-related bowel obstruction after an ovary tumor resection in a patient in 1872 [2]. This case was a curious example for surgeons in terms of understanding the clinical importance of adhesion formation. Over 100 years have passed since this report, but postoperative adhesion formation is still a serious clinical problem in every surgical specialty.

Energy-based devices such as the Ultracision Harmonic Scalpel (Ethicon Endo-Surgery, Inc., Cincinnati, OH; ultrasonic coagulator (UC)) and the LigaSure (Covidien Valleylab, Boulder, CO; bipolar vessel-sealing device (BP)) have increasingly been used in both conventional and laparoscopic surgeries [3–5]. BP is an electrothermal vessel-sealing system presented as an alternative to sutures, hemoclips, staplers, and ultrasonic coagulators for ligating vessels. It is a feedback-controlled device that uses high current (4 A) and low voltage (< 200 V) and that includes a tissue-sensing technology. With BP, the coagulation effect is obtained by denaturing collagen and elastin within the vessel wall and in the surrounding connective tissues [6]. UC is another device for hemostasis

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that has been used in open and laparoscopic procedures. It is based on a high-frequency ultrasound energy that supplies the coagulation and cutting of tissues at low temperatures [7]. UC includes a piezoelectric ceramic device that converts electrical impulses to mechanical energy, resulting in a high-frequency vibration (55,500 times per second). The hydrogen bonds in the protein structure are broken by this vibratory effect, and coaptive coagulation occurs [8].

The aim of this study is to investigate the histopathological response and the adhesion formation in rats that may occur after an omentectomy is performed either through BP, UC, or suture ligation (SL).

Materials and Methods

The study was initiated after approval was received from the Animal Care Ethics Committee and was performed in accordance with institutional guidelines. A total of 40 female Wistar albino rats weighing 200–260 g were used. The animals were randomly divided into four groups of ten animals each, and all rats were housed under controlled temperature and humidity.

Surgical Procedure

The animals were anesthetized by an intramuscular injection of 40 mg/kg ketamine hydrochloride and 5 mg/kg xylazine. The abdomen was shaved, and the skin was cleaned with povidone iodine. The rats underwent a midline laparotomy; after the identification of the omentum (Fig. 1), a partial omentectomy was performed using 3–0 silk suture with SL in group 1, BP in group 2, and UC in group 3. In the control group (group 4), only a laparotomy was performed, without the manipulation of any tissue. Lateral thermal damage was examined the same day, and a piece of the omentum was left in the animals to be examined on postoperative day 15. The

Fig. 1 An image of rat omentum separated from other tissues

laparotomy was closed with a 3–0 silk suture. On postoperative day 15, animals underwent another operation, and part of the omentum was taken from the most damaged area and fixed in 10% buffered neutral formalin for day 15 evaluation. Rats were then sacrificed by intracardiac potassium infusion.

Histopathological Evaluation and Scoring

After scoring, tissue samples were collected and fixed in 10% neutral-buffered formalin for 24 h. All of the specimens were examined histopathologically. Tissues were processed, embedded in paraffin, sectioned at 3 μ m, and stained with hematoxylin–eosin; following this, histological scoring was performed. Histopathologically, polymorphonuclear leukocytes (PMNLs), microabscess formation, lymphocyte infiltration, granulation tissue formation, and fibrosis formation were all evaluated. The lateral thermal injuries were measured. All samples were evaluated by the same pathologist, who was unaware of the resection method. The abovementioned histological parameters were scored on a four-point scale as follows: 0, negative; 1, low; 2, moderate; and 3, high. According to the parameters mentioned above, a scoring model was formed that was mainly dependent on fibrosis scoring by the pathologist, since this was an adhesion study.

Evaluation of Macroscopic Adhesions

The scoring system defined by Rodgers et al. [9] was used for adhesion scoring. The abdominal cavity of each rat was opened with an inverted-“U” incision and explored completely. The grade and severity of the adhesions were evaluated by an investigator who was blinded to the groups. The tenacity of the adhesions was scored on a scale of 0=no adhesions; 1=mild, easily dissectible adhesions; 2=moderate adhesions, nondissectible, do not tear the organ; and 3=dense adhesion, nondissectible, tears organ when removed.

Energy Sources

The energy sources evaluated were the LigaSure with LigaSure V 5-mm forceps Lap System (Covidien Valleylab, Boulder, CO) and the Ultracision Harmonic Scalpel Generator with 5-mm 23p Harmonic ACE forceps (Ethicon Endo-Surgery, Inc., Cincinnati, OH).

Statistical Analysis

Adhesions and histopathological scores were graded, and the Kruskal–Wallis test was performed for multiple nonparametric variables. Pairwise comparisons were carried out using Dunn's *post hoc* comparison procedure for every two nonparametric variables. A value of $p < 0.05$ was considered

statistically significant. The Mann–Whitney U test was used to compare the spread of lateral thermal damage.

Results

Histopathological Evaluation

Polymorphonuclear Leukocytes

PMNL scores were 1.7 ± 0.95 in the UC group, 1.3 ± 0.82 in the BP group, 2.1 ± 0.99 in the SL group, and 0.8 ± 0.92 in the control group. A pairwise comparison revealed that PMNL scores were significantly higher in the SL group compared with those in the control group ($p < 0.05$).

Microabscess Formation

Microabscess scores were 0.6 ± 0.97 in the UC group, 0.0 ± 0.00 in the BP group, 2.1 ± 1.20 in the SL group, and 0.4 ± 0.84 in the control group. Pairwise comparisons revealed that microabscess formation was significantly higher in the SL group than in the UC group ($p < 0.05$), and it was statistically significantly higher in the SL group than in the control group ($p < 0.01$). Furthermore, there was a statistically significant difference between the BP and SL groups ($p < 0.001$).

Lymphocyte Infiltration

Lymphocyte scores were 2.0 ± 0.47 in the UC group, 1.7 ± 0.48 in the BP group, 2.0 ± 0.47 in the SL group, and 1.3 ± 0.48 in the control group. There were statistically higher lymphocyte scores in the UC and SL groups than in the control group in pairwise comparisons ($p < 0.05$).

Fibrosis (Fibroblast Scoring)

Fibroblast scoring was 0.7 ± 1.06 in the UC group, 1.8 ± 1.55 in the BP group, 1.6 ± 0.84 in the SL group, and 0.4 ± 0.84 in the control group. In multiple group comparisons, statistical analysis revealed a difference among groups ($p = 0.024$). Fibroblast scoring was higher in the BP group than in the UC group; however, the difference was not found to be statistically significant in pairwise comparisons.

Granulation

Granulation scores were 1.3 ± 0.67 in the UC group, 1.5 ± 0.85 in the BP group, 1.9 ± 0.32 in the SL group, and 0.4 ± 0.84 in the control group. There was a statistically significant difference between the SL and control groups in pairwise comparisons ($p < 0.01$). The multiple and pairwise comparison results are presented in Table 1.

Microscopic Final Adhesion Scoring

Mean microscopic adhesion scores were 1.4 ± 0.84 in the UC group, 2.2 ± 1.03 in the BP group, 2.0 ± 0.47 in the SL group, and 0.5 ± 0.85 in the control group (Table 2). In pairwise comparisons, there was no statistically significant difference among the UC, BP, and SL groups. Additionally, the microscopic adhesion scores of the BP and SL groups were significantly higher compared with those in the control group ($p < 0.01$).

Macroscopic Adhesion Scoring

For all groups, the macroscopic adhesion scores were evaluated. The mean adhesion scores were 2.5 ± 0.71 in the SL group, 2.4 ± 0.70 in the BP group (Fig. 2), 0.9 ± 0.57 in the UC (Fig. 3), and 0.6 ± 0.70 in the control group. In pairwise comparisons, the adhesion scores were significantly lower in

Table 1 Comparison of microscopic adhesion parameters

Groups	Microabscess score (mean $\pm$ SD)	PMNL score (mean $\pm$ SD)	Lymphocyte score (mean $\pm$ SD)	Fibroblast score (mean $\pm$ SD)	Granulation score (mean $\pm$ SD)
UC (1)	0.6 ± 0.97	1.7 ± 0.95	2.0 ± 0.47	0.7 ± 1.06	1.3 ± 0.67
BP (2)	0.0 ± 0.00	1.3 ± 0.82	1.7 ± 0.48	1.8 ± 1.55	1.5 ± 0.85
SL (3)	2.1 ± 1.20	2.1 ± 0.99	2.0 ± 0.47	1.6 ± 0.84	1.9 ± 0.32
Control (4)	0.4 ± 0.84	0.8 ± 0.92	1.3 ± 0.48	0.4 ± 0.84	0.4 ± 0.84
KW- χ^2	18.040	9.548	11.507	9.475	14.591
p	$p < 0.001$	0.023	0.009	0.024	0.002
Post hoc Dunn test	1 vs. 3, $p < 0.05$ 3 vs. 4, $p < 0.01$ 2 vs. 3, $p < 0.001$	3 vs. 4 $p < 0.05$	4 vs. 1, 3 $p < 0.05$	NS	3 vs. 4 $p < 0.01$

UC Ultracision, BP LigaSure, SL suture ligation, KW Kruskal–Wallis test, SD standard deviation

Table 2 Comparison of adhesion scores

Groups	Microscopic adhesion score (mean±SD)	Macroscopic adhesion score (mean±SD)
UC (1)	1.4±0.84	0.9±0.57
BP (2)	2.2±1.03	2.4±0.70
SL (3)	2.0±0.47	2.5±0.71
Control (4)	0.5±0.85	0.6±0.70
KW- χ^2	15.410	25.357
<i>p</i>	0.001	<i>p</i> <0.001
<i>Post hoc Dunn test</i>	4 vs. 2, 3, <i>p</i> <0.01	1 vs. 2, <i>p</i> <0.05
		1 vs. 3, <i>p</i> <0.01
		2 vs. 4, <i>p</i> <0.01
		3 vs. 4, <i>p</i> <0.001

UC Ultracision, BP LigaSure, SL Suture ligation, KW Kruskal–Wallis, SD standard deviation

the UC group than in the BP group (*p*<0.05). The adhesion scores of the UC group were significantly lower than those of the SL group (*p*<0.01). The adhesion scores of the SL and BP groups were significantly higher than those of the control group (*p*<0.01; Table 2).

Lateral Thermal Damage

The evaluated lateral injury was 2.29±1.11 mm in the UC group and 2.57±1.51 mm in the BP group. The mean lateral thermal damage was lower in the UC group, but there was no statistically significant difference between the two groups (*Z*=0.26, *p*=0.80).

Discussion

Beginning with Muller et al. [10] in 1886, surgeons have tried to find the most effective method to prevent adhesion formation. Despite the improvements in surgical techniques, intra-

Fig. 2 Severe adhesions in BP group involving the liver, spleen, stomach, and the omentum

Fig. 3 Adhesion between the omentum and abdominal wall in the UC group

abdominal adhesion formation still remains a common problem. The incidence of adhesion formation after an abdominopelvic operation is estimated to be as high as 50–100 % [11]. The severity of adhesion formation has a relationship with the frequency of surgeries performed [12].

Many complications may be seen after adhesion formation. Prolongation in the duration of surgery and corruption in the operation field are important complications related to adhesion formation. Additionally, with adhesion, a safe entry to the abdominal cavity becomes more difficult, and inadvertent organ injuries may complicate the operation [13]. Moreover, the adhesion may cause infertility problems and chronic pelvic pain [14].

Adhesion formation begins after trauma to the peritoneum and is caused by an inflammatory response to the damage. Fibroblasts begin to replace lost collagen by producing fibrous exudates. Under physiological conditions, the body responds to the damage by activating macrophages to clean the damaged area. Mechanical trauma, ischemia, and infection inhibit plasminogen activators. As a result, overaccumulation of fibrin exudates and impairment in the peritoneal fibrinolysis system result in adhesion formation [15]. In patients with a previous history of abdominal surgery, any complication related to adhesion formation may be seen at any time [16]. The 10th and 14th days after the peritoneal trauma were designated as a severe adhesion formation period after a tissue injury [17]. In this study, adhesion development after an omentectomy was evaluated in the postoperative day 15.

The omentum is a well-vascularized, adipose-rich tissue in the abdominal cavity. It consists of mesothelial cells, fibroblasts, histiocytes, monocytes, lymphocytes, and granulocytes. It contains inflammatory cells known as “milky spots” and has some tissue factors [18]. The cells in the structure of the omentum produce plasminogen activators and inhibitors, and in this way, the omentum plays a role in adhesion formation and prevention [19]. The greater omentum participates in adhesion formation in 80% of cases [11].

Furthermore, Cerci et al. [20] showed that an omentectomy reduced the peritoneal fibrinolytic activity in a rat model. For these reasons, the omentum resection was preferred for creating an adhesion formation in this experimental study.

The comparison of the healing processes following different ligation techniques has been discussed in some studies. Peterson et al. [21] reported the results of the healing processes obtained from vessels and tissue bundles ligated using a 3–0 silk suture and BP in dogs. Fourteen days after the initial surgery, the tissues ligated with BP demonstrated less inflammation and adhesions than the suture sites. Conversely, there was no significant difference between suture sites and sealed sites with BP in our study on the 15th day.

In a previous study, Phillips et al. [22] tested the tissue response to surgical energy devices in a porcine model. A full-thickness lateral energy spread caused by BP compared with that caused by UC was statistically higher in the arteries (4.5 vs. 0.6 mm) and the veins (5.5 vs. 1.5 mm) for each sample. Additionally, UC created less full-thickness damage in the bladder, peritoneum, stomach, and colonic tissues. In contrast, Diamantis et al. [23] compared the effects of UC and BP on rabbits' short gastric vessels. They reported greater thermal injury and inflammatory response after UC than after BP during the second postoperative week. However, in our study, we observed no significant difference between the two devices in terms of the thermal injury and histopathological response.

Some authors have evaluated the effects of these devices on different tissues. Goldstein et al. [24] compared the thermal damage on pig ureters after ligation with BP and laparoscopic shears. The mean length of thermal damage of the specimens using BP was 2.11 mm, whereas it was 1.92 mm in the ultrasonic energy group; no statistically significant difference between the two devices was found. These investigators' reports are congruent with our findings.

Person et al. [25] compared the duration of the sealing processes and thermal damage to the adventitial collagen caused by UC and BP in a porcine model. In their study, they found UC to be faster than BP (3.3 vs. 5.2 s). Additionally, they observed that UC created more thermal damage to the adventitial collagen than BP. In another study, Lamberton et al. [26] tested four laparoscopic 5-mm vessel ligation devices to seal 5-mm bovine arteries. According to their results, the BP was faster and produced more thermal spread than UC. These investigators' conflicting conclusions about the speed of sealing indicate that further studies are needed to elucidate the relationship between sealing time, tissue type, and thermal damage.

In our study, mean fibroblast scores were higher in the BP group than in the UC group (1.8 vs. 0.7). The accepted definition of adhesion is that it is a fibroproliferative and inflammatory response to injury. This description points to the effect of fibroblastic activity in adhesion formation [27]. Our study may serve as a clue in determining the correlation of

the histopathological and macroscopic scores of adhesion formation. In addition, we have seen that the microscopic scores of the BP and SL groups were significantly higher than those of the control group. This is an important finding in terms of supporting macroscopic adhesion scores, which represent a more subjective measure.

To the best of our knowledge, no report has compared two different energy modalities with suture ligation techniques in terms of adhesion formation along with histopathological and macroscopic evaluations of a rat omentectomy model. New studies designed with these devices to evaluate the results on postoperative days 7, 21, and 28 may be helpful in comparing the effects of these devices on adhesion formation.

Conflict of Interest The authors declare no conflict of interest and have no financial interest in the products presented in this work.

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