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Original Article

Comparison of dry and moist cotton gauze for hemostasis in a rodent liver injury model

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Abstract: Massive bleeding in hepatic resection is one of the major factors for intraoperative and postoperative morbidity and mortality. Surgical cotton gauze is the most popular hemostatic dressing for surgeons to use in surgery and wet gauze is the modality has been widely applied. By comparison of two gauze modalities, moist and dry, we attempt to provide better choice for surgeons as hemostatic dressing. Thus, we investigated the hemostatic efficiency and postoperative adhesion in a liver injury rodent model. Forty male Sprague-Dawley rats were subjected to present experiment, and divided into two main groups with compression pressure of 10 g weight (Control group) and 20 g weight (Experimental group). Each group divided into another two subgroups based on dry and moist gauze treatment, with ten rats in each group. The coagulation time and blood loss were documented as measurement of hemostatic efficacy. At 14th day after operation, animals were euthanized for evaluation of adhesion score. All the results were corrected by dividing the weight of the resected liver during the operation. Rats treated with dry gauze exposed to shorter hemostatic time compared to moist gauze group with pressure of 20 g weight ($P < 0.05$). However, in evaluation of blood loss and adhesion score, there is no significant difference ($P > 0.05$). In control groups, there were no remarkable differences in hemostatic time ($P=0.548$), blood loss ($P=0.201$) and adhesion score ($P=0.501$). In conclusion, dry gauze exhibited the better hemostatic efficiency with shorter hemostatic time under pressure of 20 g weight. Our results suggest that dry gauze might be a better option for reducing the blood loss in short amount of time.

Keywords: Moist/dry gauze, hemostatic efficiency, adhesion scores

Introduction

Hepatic resection remains a major challenge to surgical procedure that involves in massive bleeding caused by vascular anatomy of the liver [1]. High mortality rate, which is mostly caused by increased blood loss, has been considerably reduced as the introduction of modern transection techniques and becoming much more acceptable. However, since the major blood loss during the surgery or after the surgery requires excessive blood transfusion and biliary complications, liver resection surgery accompanied with blood loss is till associated with considerable morbidity [2, 3]. Thus, reducing unexpected blood loss has been taken into account as first priority in medical surgery. For decades, there have been several conventional hemostatic methods applied to perform hepatic resection, such as manual compression, cauterization and ligation, leading to vasoconstriction [4]. However, yet gauze compression has been the most popular procedure modality for surgeons and widely used in hepatic surgery, considering the cost of expense and proficiency at using gauze. And this consideration was based on the common fact that the skilled operation of surgeons is another major factor in hemostasis, since the surgeon's preference can potentially influence the intraoperative and postoperative morbidity with the choice of surgical technique for reducing bleeding [5].

Hemostatic dressing with chemical components have been widely introduced to liver surgery with a hope to improve intraoperative hemostasis, bleeding complications and minimization of postoperative pain [6-8]. And yet,

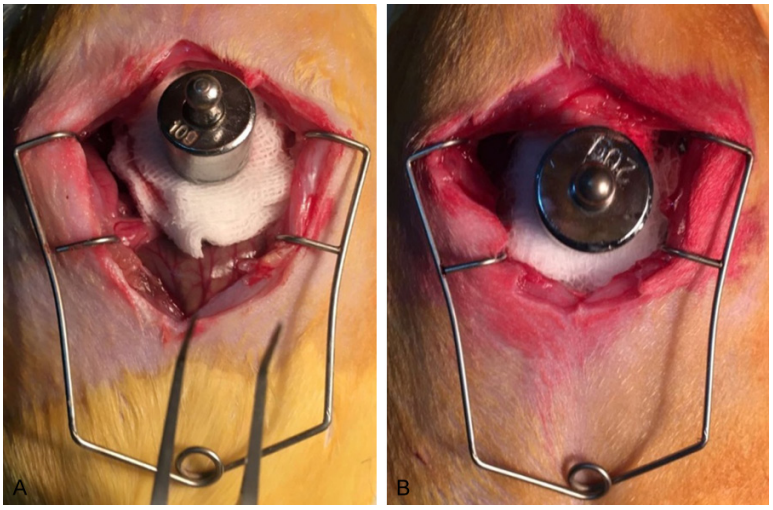

Figure 1. Manual compression simulation by pressing the bleeding site with either 10 g weight (A) or 20 g weight (B).

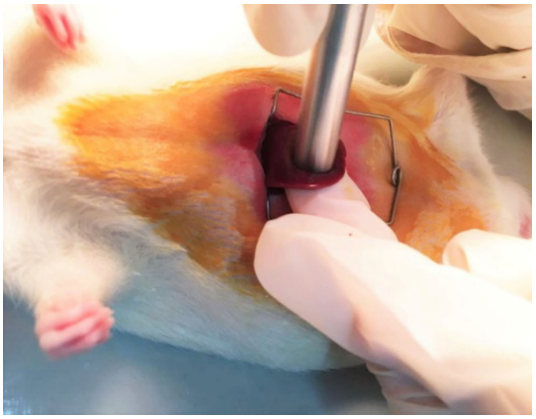

Figure 2. Resection procedure was conducted by pressing a round tube with the diameter of 1 cm on the left bottom of the left lateral lobe and the depth was about 1 mm.

no technique has been claimed successful than other in reducing the hemorrhage rate as well as in postoperative pain and employment of these techniques usually results in adverse effects [9]. This might be a reason why do so many surgeons look to cotton gauze treatment modality as their first choice [10]. Thus, improvement of basic and common hemostatic technique to prevent unexpected secondary injury is as urgent as discovering novel technique.

Much of this literature emphasized to use moist gauze (usually saturated with saline solution) in order to provide moist wound healing environment that prevents tissue dehydration and cell death, accelerates angiogenesis, increases the breakdown of dead tissue and fibrin, and potentiates the interaction of growth factors and their target cells. Same as in practical procedure, these dressings that maintain the moist environment are superior treatment modalities, assuming that dry gauze might extract too much fluid from tissues and organs which may increase the fragility of vessels and lead to secondary hemorrhage [11]. However, there is no experimental evidence could support this hypothesis. Thereby, in this study we attempt to compare hemostatic efficiency of moist and dry cotton gauze via Sprague-Dawley rat model.

Methods

Animals

Forty male Sprague-Dawley rats, weighted 400 ± 50 g, were recruited to this study and randomly divided into four different groups with 10 rats in each group; Group A (dry) and B (moist) serve as controls and the use of direct pressure was implemented by putting the 10 g weight of objects at the bleeding site. Group C (dry) and D (moist) were experimental groups with addition of pressure (20 g), which is apply to simulate manual compression in a practical operation (**Figure 1**). Each rat was weighted and marked with ear tag. The animal study protocol was approved by the animal Welfare Committee of Tongji University, School of medicine.

Surgical procedure

The 32 layers of cotton gauze was cut into pieces (2 cm × 2 cm) and weighted before the surgery, assuring all the cotton gauzes applied to study have equal weight. Rats were anesthetized by intraperitoneal injection of 3% pentobarbital sodium (3 ml/kg). A longitudinal midline incision around 3 cm was made below the xiphoid. The left lateral lobe is account for about 30% of the whole liver of the rats, and it has an arrow pedicle containing portal as well

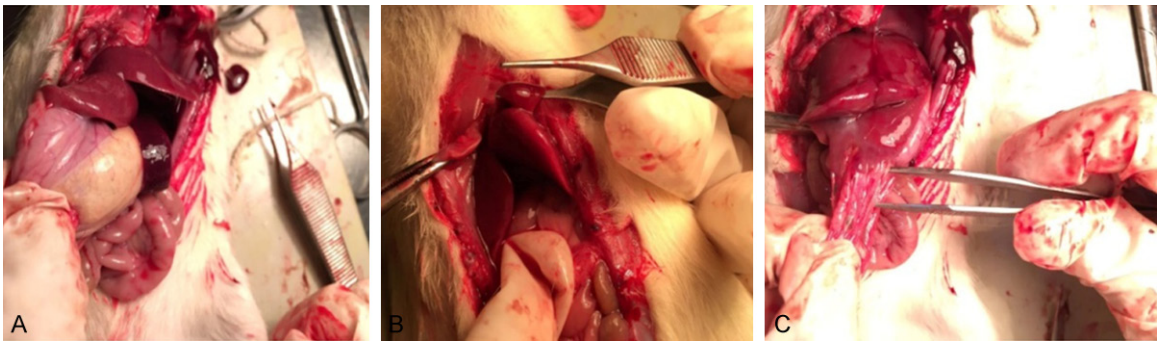

Figure 3. Adhesion scoring at Day 14: A. The adhesion between the liver and stomach; B. The second picture in the middle showed the adhesion between liver lobes; C. The third picture on the right showed the adhesion between the liver and mesentery.

Table 1. Postoperative deaths and inefficient hemostasis

	Group A (n=10)	Group B (n=10)	Group C (n=10)	Group D (n=10)
Postoperative death	0	0	0	1
Inefficient hemostasis	0	1	2	2

Inefficient hemostasis is defined as the cessation time of bleeding over 10 minutes; group A, 10 g, dry gauze pads group (n=10); group B, 10 g, wet gauze pads group (n=10); group C, 20 g, dry gauze pads group (n=10); group D, 20 g, wet gauze pads group (n=10).

as hepatic veins and is not connected to the paracaval liver [12]. We excised a piece of liver with diameter of 1 cm at the bottom margin of the left lateral lobe and the depth was about 1 mm (**Figure 2**) and recorded the weight of each piece of dissected liver.

In experimental groups, time was recorded after the gauze was applied with 20 g weight pressure (**Figure 2**). The gauze was lifted at 3 minutes, then with the interval of 1 minute for 2 times, after that, the interval was 30 seconds till the cessation of bleeding. Hemostasis was observed and verified by two researchers. In control groups, nothing was administrated in the initial 3 minutes after the bleeding model was completed, then either dry gauze in group A or moist gauze in group B was applied to verify coagulation and soaked the extra within 30 seconds. After cessation of hemorrhage, the liver was observed for at least 1 minute to verify whether the coagulation was durable. If coagulation was not observed in 10 mins, it will be defined as hemostatic failure [13]. Rats with experienced hemostatic failure were excluded from the study. The gauze was weighted before and after surgery to calculate blood loss.

14 days after the primary surgery, all rats were euthanized and the abdominal adhesions were evaluated.

Macroscopic evaluation and adhesion scoring

Abdominal adhesions were assessed by a surgeon based on a scale described by *Leach et al*. [14]. This scoring system took into consideration abdominal adhesion extent, severity and degree. We counted the adhesion sites instead of evaluating the adhesion area which requiring higher skill to make it accurate. Degree and severity of each adhesion site was measure, after that, an average figure of degree and severity for one rat was calculated. Adhesions were characterized on gross examination for severity as follows: 0= no adhesions; 1= Filmy avascular; 2= Vascular or opaque; 3= Cohesive attachment of liver lobes to each other or other abdominal structure. Adhesions for severity were as follows: 0= No adhesion; 1= Separated from tissue with gentle traction; 2= requiring moderate traction; 3= requiring sharp dissection. The total score was the average figure of degree and severity plus the number of adhesion sites. Examples of different types of adhesions were showed in following pictures (**Figure 3**). Because we made the bleeding liver model manually, the weight of resected liver tissues was not exactly the same and all the adhesion scores were fixed by dividing the resected liver tissue's weight.

Statistical analysis

Statistical analyses were performed using the SPSS 21.0 software. Values were reported as

Table 2. Weight of resected liver

	Group A (n=10)	Group B (n=10)	Group C (n=10)	Group D (n=10)
Resected liver (g)	0.091±0.019	0.079±0.012	0.082±0.019	0.078±0.011

Group A, 10 g, dry gauze pads group (n=10); group B, 10 g, wet gauze pads group (n=10); group C, 20 g, dry gauze pads group (n=10); group D, 20 g, wet gauze pads group (n=10).

means ± SD. Student's t-tests and Mann-Whitney U tests were performed to identify significant differences between two independent groups. Probability values < 0.05 were considered to be statistically significant.

Results

Rats in dry gauze group have better hemostatic response to the postoperative survival

Hemostatic failure was defined as the cessation time of bleeding was over 10 minutes. All rats in group A survived after the surgery, and the hemostatic time was within 10 minutes. All of the rats in group B also survived after the surgery, but one rat was exposed to hemostatic failure.

One out of ten rats in group C was defined to be hemostatic failure and no rat died after surgeries in their recovering days. Two out of ten rats in group D were defined to be hemostatic failure and two out of 10 rats died after the surgery.

When under the same pressure, there was no difference in the death rate between group A and Group B, but there were more rats died in group D than group C. Taken together, there were more rats died under the pressure of 20 g than under the pressure of 10 g (**Table 1**).

When under the same pressure, more rats failed to hemostasis within 10 minutes in group B than in group A, but the inefficient hemostatic rate was the same between group C and group D. As a conclusion, more rats failed to stop bleeding within 10 minutes with 20 g pressure than 10 g pressure.

Rats in the dry gauze group exhibited the shorter hemostatic time than moist gauze group

The rats that stopped bleeding exceeded 10 minutes (hemostatic failure) were excluded. There were 36 rats left: group A, n=10; group B, n=9; group C, n=9; group D, n=8. In order to be more accurate, the results of hemostatic time were normalized by dividing the weight of the resected liver (**Table 2**). In the experimental groups, the normalized hemostatic time in dry gauze group was shorter than moist gauze group (P=0.023), 56.74±21.36 (min/g) versus 89.68±24.57 (min/g). In control groups, the hemostatic time showed no remarkable differences (P=0.548), 59.83±21.41 (min/g) in group A versus 66.03±22.11 (min/g) in group B. In comparison of between control group and experimental group, we found that the probability value between group B and group D was 0.067 by performing Student's t-test (**Table 3**).

The amount of blood loss was not different between dry gauze and moist gauze groups

Among the 36 rats, all the weight of blood loss was corrected by dividing the weight of resected liver. There were no remarkable differences in experimental groups (P=0.1.32), 17.56 ± 9.08 in group C versus 20.16 ± 4.86 in group D. In control groups, there also no significant differences shown between dry gauze and moist gauze (P=0.201), 15.36 ± 5.26 in group A versus 14.12 ± 5.91 in group B. By comparing control groups with experimental groups, the probability value between group B and D was 0.069, no significant difference was shown (**Table 4**).

No significant difference was shown in tissue adhesion between dry and moist gauze groups

The rats that died during 14 days after the surgery were excluded. There were 35 rats left for adhesion scoring, namely, group A, n=10; group B, n=9; group C, n=9; group D, n=7. In experimental groups, hemostatic time in group C (dry gauze group) showed shorter normalized hemostatic time than group D (moist gauze group), there were no remarkable differences in normalized adhesion scores (P=0.078), namely 87.65 ± 17.28/g in group C versus 77.16 ± 37.46/g in group D. In control groups, there were also no remarkable differences between group A and B (P=0.501), namely 49.86 ± 8.42/g in group A and 63.52 ± 35.87/g in group

Table 3. Hemostatic Time

	Group A (n=10)	Group B (n=9)	Group C (n=9)	Group D (n=8)
Hemostatic time (min)	4.97.32±1.79	5.33±1.78	4.61±1.58	6.93±2.12
Fixed figure	59.83±21.41	66.03±22.11	56.74±21.36	89.68±24.57

Group A, 10 g, dry gauze group (n=10); group B, 10 g, wet gauze group (n=9); group C, 20 g, dry gauze group (n=9); group D, 20 g, wet gauze group (n=8); P > 0.05, group A compared with group B; P < 0.05, group C compared with group D; P > 0.05, group A compared with group C; P > 0.05, group B compared with group D; via Student's t-test.

Table 4. Blood Loss

	Group A (n=10)	Group B (n=9)	Group C (n=9)	Group D (n=8)
Blood loss (g)	1.19±0.38	1.23±0.51	1.48±0.71	1.37±0.35
Fixed figure	15.36±5.26	14.12±5.91	17.56±9.08	20.16±4.86

Group A, 10 g, dry gauze group (n=10); group B, 10 g, wet gauze group (n=9); group C, 20 g, dry gauze group (n=9); group D, 20 g, wet gauze group (n=8); P > 0.05, group A compared with group B, via Mann-Whitney test; P=0.201, group C compared with group D, via Student's t-test; P=0.132, group A compared with group C, via Student's t-test; P=0.069, group B compared with group D, via Mann-Whitney test.

Table 5. Adhesion Score

Group	N	Adhesion score	
		Total score	Fixed total score
A	9	5.08±2.01	49.86±8.42
B	9	4.96±2.87	63.52±35.87
C	9	7.00±2.58	87.65±17.28
D	7	5.47±2.32	77.16±37.46

Group A, 10 g, dry gauze group (n=9); group B, 10 g, wet gauze group (n=9); group C, 20 g, dry gauze group (n=9); group D, 20 g, wet gauze group (n=7); P > 0.05, group A compared with group B, via Mann-Whitney test; P > 0.05, group C compared with group D, via Student's t-test; P=0.078, group A compared with group C, via Mann-Whitney test; P > 0.05, group B compared with group D, via Student's t-test; All the comparisons were about fixed figure.

B. By comparing group A with C, no significant difference was shown (**Table 5**).

Discussion

In present pilot study, we demonstrated that all the rats treated with dry gauze under 10 g pressure (group A) survived after the surgery and responded efficient hemostasis (within 10 minutes). Although, all the rats treated with wet gauze under pressure of 10 g (group B) survived after the surgery, but one rat was exposed to hemostatic failure (over 10 minutes). Rats subjected to 20 g pressure, two rats responded to hemostatic failure and no rat died after surgery with dry gauze treatment (group C), while two rats defined as hemostatic failure and two rats died after surgery with moist gauze treatment. No significant differences were shown in blood loss and adhesion between dry and moist cause treatment. Over all, more rats resulted in postoperative death under pressure of 20 g than 10 g pressure.

Liver is an organ with abundant blood vessel and constitute of extremely fragile tissues. In order to achieve better postoperative survival and hemostasis, two different light compression pressure, 10 g and 20 g, were apply to this study, considering the volume and resection surface of rat liver. Depending on the different subjected organs of different animals or human in surgery, manual compression pressure chosen differently, for example, pressure of 200 g material applied to hemostatic compression on rabbit's ear while 100 g pressure used on liver of same animal [15]. With employment of two different weighted materials, we are able to eliminate the unstableness of manual compression, which usually difficult to measure with operator's hand. Our result exhibited that 10 g pressure achieved better postoperative survival and less hemostatic failure.

Under the pressure of 20 g weight, dry gauze group exhibited higher hemostatic efficiency than wet gauze group ($P < 0.05$). Vessel-wall injury and the extravasation of blood from the circulation rapidly initiate events in the vessel wall and in blood that seal the breach. Circulating platelets are recruited to the site of injury, where they become a major component of the developing thrombus [16]. During platelet activation, platelets bear negatively charged phospholipids on their surface. If a positively charged dressing makes contacts with blood, it can lead to the rapid formation of a platelet plug [17]. Although the animal experiment showed no significant differences in hemostasis between moist and dry gauze with 10 g

pressure, further studies can be done to explore their physical or mechanical differences and observe their interaction with blood. It was also reported positively charged dressings interacted with negatively charged phospholipid on bacterial cell membranes, which may lead to the rupture of the bacteria [18]. We assumed that the difference of charge modality used in this study might be the reason for dry gauze had better hemostatic efficiency and less blood loss. Further study needs to be conducted to ensure this assumption. Our result also showed that there is no significant difference noted between two dry gauze groups under different compression pressure. The possible reason for this result might be that continuous compression with 20 g weight and frequent removal of gauze may disturb the hemostatic efficiency and blood loss. The liver lobes treated with dry gauze consistently had longer coagulation times. It was also reported in other studies. The documentation of efficacy requires removal of the pressure which is also associated with physical removal or disruption of the thrombus. Thus thrombus removal and continued hemorrhage is concomitant with observation and results are inherently skewed [13]. Experiments on oral surgery demonstrated that dry gauze is as efficient as gauze with tranexamic acid and fibrin sponge in controlling postoperative bleeding, suggesting that control of hemostasis is mainly dependent on platelet function, especially at the primary phase of coagulation, which is not directly influenced by the use of chemical agents such as warfarin [19-22]. However, there is no pathological explanation for this result claimed.

Postoperative adhesions are the pathologic fibrotic bands that develop between the peritoneal surfaces in the peritoneal cavity. The pathophysiology of adhesions originates from an inflammatory reaction stimulated by tissue trauma with increased vessel permeability, extravasation of immune cells, and deposition of fibrin [23]. In this experiment, we modified the adhesion scoring scale made by Leach et al. [14] to befit our experimental needs. In experimental groups, although dry gauze group (group C) showed higher hemostatic efficiency than moist gauze group (group D), the result of blood loss and adhesion score showed no remarkable differences. In a study that analyzed the effects of bismuth subgalate, *Arroyo et al.* found adhesions after using dry cautery in 80% of the sample [24]. Simões et al. conducted a study on mice that assessed hemostasis using dry electrocautery and found that adhesions occurred in 83.3% of those in which dry electrocautery was used [25]. However, these findings can't support and explain our result, since we applied dry and moist gauze manual compression method instead of dry electrocautery. Adhesion can be caused by several factors as it described earlier. Among all the factors, bleeding happened during and after surgeries was the most reasonable explanation for postoperative adhesions in the long term. Sterilized condition might be the reason that there is no difference in postoperative adhesion scores between two groups [26].

The sample capacity is one of the limitations in present experimental study. Further study needs to be conducted with enlargement of sample capacity to validate our results.

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Disclosure of conflict of interest

None.

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