Evaluation of percutaneous microwave coagulation therapy for hepatic artery injury

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Abstract

Objectives: To evaluate the in vivo efficacy of 915 MHz percutaneous coagulation in the treatment of hepatic artery injury.

Methods: After inducing hepatic artery injury, 8 dogs in each group underwent 915 MHz microwave percutaneous coagulation therapy and 8 dogs were injected with batroxobin and α-cyanoacrylate.

Results: The hemostatic effects of 915 MHz microwave were better than drug injection, and the amount of bleeding was significantly lower (p < 0.05). Pathological examination showed that vessel wall necrosis were greater.

Conclusion: Contrast ultrasound guided 915 MHz microwave percutaneous coagulation treatment has potent hemostatic effects in the repair of in vivo hepatic artery injury.

Keywords: 915 MHz microwave, Vascular injury, Percutaneous treatment, Contrast ultrasound guidance, Drug injection
1. Introduction

Liver trauma is the most common abdominal injury associated with blunt multiple traumas affecting up to 25% of these patients [1] [2]. Although surgical repair is associated with poor prognosis, non-operative approaches have resulted in a considerable increase in survival [3] [4] [5]. Two non-operative methods are currently used to stop bleeding: embolization and cauterization. Embolotherapy consists of the administration of hemostatic agents, such as gelatin pledgets, alcohol foam, or fibrin sealant, to occlude the bleeding site [6]. These embolization agents have limitations because they may lead to immunologic reactions or may be washed away by blood leaving the wound uncovered [7] [8] [9] [10]. In contrast, cauterization by percutaneous radiofrequency ablation [11] [12] or microwave coagulation [13] [14] specifically targets the vascular injury. Microwave coagulation was recently shown to be more efficient than radiofrequency ablation because it covers a larger area more rapidly [15], a clear advantage in urgent scenarios like liver trauma.

Microwave ablation has utilized 2450 MHz and 915 MHz microwaves [15]. The latter is characterized by a longer wavelength and a deeper penetration depth than the former [16] [17] [18] [19]. In addition, 915 MHz microwave has greater ablation ability than 2450 MHz microwave, resulting in a larger ablation zone, sealing the tissue around the wound and preventing further bleeding. We have therefore compared the coagulation capacity of contrast-enhanced, ultrasound (CEUS)-guided 915 MHz microwave therapy and CEUS-guided hemostatic drug injection.

2. Materials and methods

2.1. Animals and ethics

Experiments were performed in dogs, weighing 16–22 kg (mean, 19.38 ± 2.57 kg), without injured hepatic arteries, obtained from and fed by the Experimental Animal Center of The General Hospital of Jinan Military District (Fig. 1A). The study was approved by the institutional animal care committee of The General Hospital of Jinan Military District.

2.2. Microwave system and ultrasound scanner

The 915 MHz microwave ablation system (KY2000-915, Nanjing Kangyou Biological Energy Co. Ltd) included a microwave generator, a flexible coaxial cable, and a cooled-shaft antenna. The generators could generate a power of 1–100 W. A 2 mm slit-radiating segment was placed 25 mm from the tip, a distance optimal for minimizing power feedback and maximizing energy to the tissue. The antenna shaft contained dual channels, through which distilled water
at 0 °C was circulated by a peristaltic pump to continuously cool the shaft. During the procedure, the temperature of each antenna was kept below 43 °C by adjusting the flow of the cold distilled water.

2.3. Animal model of liver trauma

The animals were fasted overnight, anesthetized with 10 mg/kg ketamine. During all procedures, they were constantly infused with 0.9% saline at a rate of 10 ml/kg/h and blood pressure was kept stable by adjusting the infusion rate. Systemic heparin sodium (5 mg/kg) was also administered to counteract self-hemostasis [20]. Each animal was injected via the femoral vein with 1.2–1.5 ml contrast agent (SonoVue; Bracco, Italy), followed by a bolus of

![Figure 1](http://dx.doi.org/10.1016/j.heliyon.2015.e00030)

Fig. 1. A: Hepatic arterial blood flow images in 2 dogs, one from Group B (hepatic artery diameter, >1 to <2 mm; left), and one from group C (hepatic artery diameter, >2 to <3 mm; right). Blood flow speeds were about 40 and 85 cm/s, respectively. B: Cutting of the hepatic artery using a specially made puncture needle. The image at top above shows cutting of the hepatic artery using the specially made needle (arrow) under ultrasound guidance. The bottom panel shows two-dimensional ultrasound images, clearly showing that the artery had been severed.
5 ml normal saline. Using a Siemens 2000 ultrasound scanner (Siemens Medical Solution USA, Ino. Made in Korea), a convex array 4C1 probe, and a linear L9-3 probe, CEUS was performed by contrast pulse inversion harmonic imaging (cps), at a mechanical index range of 0.16–0.18 and with a puncture bracket and appropriate software. Ultrasound images clearly showed the hepatic artery was longitudinal. The internal diameters of the hepatic arteries were measured and the dogs were grouped according to the internal diameter of their hepatic arteries: <1 mm (Group A); 1–2 mm (Group B); and 2–3 mm (Group C) (Fig. 1A). Under ultrasound guidance, we chose as the target vessel, a hepatic artery that was as straight and with as little branching as possible to avoid interference from liver parenchymal hemorrhage. Using a specially made 14G needle with a sharpened edge, the liver parenchyma was pierced, and the selected blood vessel was severed (Fig. 1B). This procedure was repeated 5 times.

2.4. CEUS-guided percutaneous 915 MHz microwave coagulation therapy

Eight dogs each from Groups A, B, and C underwent 915 MHz microwave percutaneous coagulation therapy (3 min; 80W). Under CEUS guidance, a microwave electrode was inserted along the guide line in the trauma area and inserted into the gap at the active bleeding site. Our preliminary experiments showed that, below 80W, an increase in power was proportional to a more rapid hemostatic effect, but at 100W, the tissue around the electrode body became carbonized, preventing heat conduction. We also found that the hemostatic effect was proportional to ablation time. An ablation time of 3 minutes would result in the ablation zone covering the wound area, including the large vascular injury, but a longer ablation would increase damage to normal liver tissue. Microwave treatment was therefore applied at 80W for 3 min. At the end of treatment, the needle tract was burnt, and the generator was removed.

2.5. CEUS-guided hemostatic drug injection therapy

Eight dogs each from Groups A, B, and C received hemostatic drug injections. A 14G specially made puncture needle was placed into the active bleeding roots along the guide line. Each was first injected with 2.5 ml of 0.5 KU/mL batroxobin (SolcoBasle, Switzerland), followed by 1.5 ml α-cyanoacrylate [7] [8] [9] [10].

2.6. Assessment of hemostasis

Immediately after the end of the treatment, peritoneal effusion was measured at maximum depth in the upper outer, upper inner, lower outer, and lower inner quadrants by ultrasound. The abdomen of each animal was cut open, and hemostasis was assessed visually by covering the wound surface with clean
gauze for about 1 min. Because we only caused one injury to each dog, we used the gauze weighing method to calculate bleeding volume [8] [20]. Briefly, a piece of pre-weighed dry gauze was placed on each quadrant of the abdominal cavity and weighed, and the amount of bleeding was calculated.

2.7. Histopathological examination

The liver of each dog was removed, fixed in 10% formalin, embedded in paraffin, and sectioned. The sections were stained with hematoxylin and eosin (HE), and pathological changes were assessed by light microscopy. At low magnification, we observed global vascular changes, such as whether the vessel wall was necrotic, muscle fiber had degenerated, or an endovascular thrombus was present. At high magnification, we observed more subtle changes, such as small vessel occlusion and cell deformation.

2.8. Statistical analysis

All statistical analyses were performed using SPSS16.0 software. Fisher's exact test was used for univariate and multivariate analyses. Within-group differences were assessed by t tests and between-group differences by the $F$ and $q$ tests. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Ultrasound imaging of active bleeding after liver trauma

In Groups A (hepatic artery diameter $< 1$ mm) and B (diameter $> 1$ to $< 2$ mm), the trauma area showed a low echo and the contrast agent overflowed rapidly from the vascular stump showing linear enhancement along the needle tract. The liver lobe supplied by the hepatic artery was weakly enhanced (Fig. 2A). Ultrasound imaging of Group C (hepatic artery diameter $> 2$ to $< 3$ mm) showed contrast agent spraying from the vascular stump, subsequently showing clump-like accumulation. Because vascular amputation was successful in the absence of contrast agent, the liver lobe supplied by the hepatic artery was completely echoless (Fig. 2B, C).

3.2. Hemostatic capacity of ultrasound-guided drug injection and 915 MHz microwave coagulation

Ultrasound-guided drug injection treatment successfully stopped bleeding in 17 of 24 (71%) dogs, with ultrasound contrast showing no active bleeding. After laparotomy, we found that, in all 17 dogs, the trauma surfaces were completely covered by film. In the remaining 7 dogs, however, treatment failed to stop the bleeding, with ultrasound contrast showed varying degrees of active bleeding. After laparotomy, we found that the hemostatic agents had failed to plug the
wound, the film failed to completely cover the trauma area, and blood clots were located in the surrounding area. Ultrasound-guided 915 MHz microwave coagulation therapy successfully stopped bleeding in all 24 dogs, with ultrasound contrast showing no effusion or overflow of contrast medium. After laparotomy, we found that the liver wound surfaces were corrugated, hardened, and dark brown. The wounds completely cured with no abnormal blood clots attached (Fig. 3A). In Group C dogs, microwave coagulation therapy was significantly superior to drug injection in

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**Fig. 2.** A: Ultrasound image of an area of liver parenchyma following trauma in a Group B dog. Damage to liver parenchyma was caused by devascularization, preventing contrast agents from entering, with or without enhancement (arrow). B: Two dimensional ultrasound images of a hepatic artery bleeding in a trauma area of a Group C dog. The left hepatic artery has ruptured with no echo area (yellow arrows). The wound area displays contrast agent clusters (yellow arrow), and the hepatic arterial blood supply to the area does not contain contrast agent (white arrow). C: Contrast-enhanced ultrasound images of a trauma zone of hepatic arterial bleeding in a Group C dog. The picture shows contrast agent spewing throughout the trauma area, similar to a “fountain” (arrow).
stopping bleeding, whereas, in Groups A and B, the difference between the two methods was not statistically significant (Table 1). Thus, microwave treatment was more effective for large, but not for small, blood vessels.

3.3. Bleeding amounts of ultrasound-guided drug injection and 915 MHz microwave coagulation

In each Group, the bleeding amount was significantly lower following microwave than following drug treatment ($p < 0.05$; Table 2), 12% in Group A,
14% in Group B, and 18% in Group C. Microwave-treated Group C dogs showed significantly higher bleeding than microwave-treated Groups A and B, and Group B bled more than Group A (p < 0.05 each; Table 2). Similar findings were observed in drug-treated animals. Thus, regardless of treatment, bleeding amount was proportional to the aggravation of trauma.

Examination of liver specimens after microwave ablation showed that their surfaces were brown and shrunken, with coagulative necrosis of the hepatic parenchyma. Cross-sectional examination showed an open ablation zone with visible wounds appearing carbonized and complete necrosis of the area of vascular rupture (Fig. 3B, C).

Pathologic examination of samples from drug-treated dogs showed no expansion of hepatic artery vessels, nearly complete vascular walls with no significant necrosis of the wall cell structure. Pathologic examination of samples from microwave-treated dogs showed the necrosis of the liver parenchyma, vascular

### Table 1. Successfully stop bleeding capacities of 915 MHz microwave coagulation and drug injection.

| Group | Drug injection (Number of dogs) | 915 MHz microwave (Number of dogs) | P     |
|-------|---------------------------------|-----------------------------------|-------|
| A     | 7                               | 8                                 | 1.000 |
| B     | 6                               | 8                                 | 0.2333|
| C     | 4                               | 8                                 | 0.0385*|
| Total | 17                              | 24                                | 0.0141*|

Dogs were assorted according to the internal diameter of the hepatic artery, as described in the Materials and methods section. Fisher's exact tests were used for univariate analysis. The hemostatic capacity of 915 MHz microwave coagulation therapy was superior to that of drug injection in dogs with large (>2 to <3 mm, p < 0.05), but not smaller hepatic arteries.

*Statistically significant (p < 0.05).

### Table 2. Bleeding amount following 915 MHz microwave and drug injection.

| Group | Bleeding amount on drug injection (g) | Bleeding amount on 915 MHz microwave treatment (g) | P     |
|-------|--------------------------------------|-----------------------------------------------|-------|
| A     | 66.7 ± 3.6 b,c                        | 58.9 ± 1.2 b,c                                 | 0.000 |
| B     | 109.6 ± 5.5 a,c                       | 94.5 ± 2.4 a,c                                 | 0.001 |
| C     | 267.3 ±8.4 a,b                        | 220.1 ± 5.3 a,b                                | 0.001 |

Student’s t test was used for intragroup comparisons, and the F and q tests were used for intergroup comparisons. *p < 0.05, vs. Group A; †p < 0.05, vs. Group B; ‡p < 0.05, vs. Group C. In all 3 groups, bleeding was reduced with 915 MHz microwave coagulation therapy than with drug injection (p < 0.05). The amount of bleeding was proportional to arterial diameters in all 3 groups, regardless of the treatment modality.
dilation of the hepatic artery in proportion to its original diameter, degeneration and necrosis of vascular wall endothelial cells and fibrous muscle cells, congestion within the lumen, discontinuous vessel walls, visible red blood cells, and fibrin effusion. A large intravascular thrombosis and small vessel occlusion were also present, with most vascular walls showing coagulative necrosis and hyalinization (Fig. 4A, B).

4. Discussion

Closed abdominal injury is often accompanied by internal bleeding and shock. Although bleeding is due mainly to the damage to the hepatic artery, previous studies did not simulate hepatic artery bleeding [21]. We therefore used ultrasound to precisely induce hepatic artery injury, allowing better diagnosis and treatment of active bleeding. Treatment with 2450 MHz microwave coagulation therapy was effective in treating liver trauma with active bleeding in pigs [22] and in rabbits [20]. However, coagulation hemostasis using 915 MHz is not reported. To our knowledge, this study is the first to evaluate percutaneous microwave coagulation therapy using 915 MHz microwave in the hemostasis of active bleeding of the liver.

**Fig. 4.** A: Histologic examination of the microwave ablation zone of hepatic arterioles under high magnification, showing hepatic arterial degeneration and necrosis, as well as lumen occlusion (arrow). B: Histologic examination of the microwave ablation zone of a hepatic artery under high magnification, showing incomplete degradation and necrosis of wall muscle fiber cells in the hepatic artery, and large thrombi visible in the lumen (arrow).
When we compared the ability of 915 MHz microwave coagulation and drug injection to stop bleeding, we found that both the methods had similar outcomes in Group A and B dogs, whereas, in Group C, microwave coagulation was more effective at stopping the flow of blood. Regardless, bleeding was significantly lower in the percutaneous microwave coagulation therapy group than in the injection group, and microwave treatment had a greater hemostatic effect than the injection.

Pathological examination showed that, in the drug injection group, there was no expansion of hepatic portal area vessels, the vascular wall was nearly complete, and there was no significant wall cell necrosis. Following microwave treatment, however, the liver parenchyma had areas of necrosis, the hepatic portal area had vascular dilation, there was congestion within the lumen, discontinuous vessel walls, and visible red blood cells and fibrin effusion. Most vascular walls had coagulative necrosis or hyalinization. Thus, the extent of necrosis is an important difference between the two methods.

Microwaves have a natural role of coagulation hemostasis, with the amount of microwave coagulation depending on the thermal effects on the organism. Microwave thermal effect refers to the heating of the large number of water and polar protein molecules. These polar molecules appear generally neutral. When these molecules are exposed to an alternating microwave field, their polarity orientation coincides with the electric field. As the polarity of microwave fields alternates, polar molecules rotate at a few miles per second, and the accompanying adjacent molecular friction generates heat, increasing the temperature of the molecular motor more rapidly [23] [24] [25]. This increase in temperature shows that the organization inside tissues can lead to the evaporation of water, protein denaturation and coagulation, as well as tissue and vascular wall coagulation; thus, microwaves play the role of a hemostatic agent.

Our method has several important advantages. Firstly, treatment duration was short. Secondly, therapy is a simple operation without laparotomy, and is feasible in critically ill patients. Furthermore, the instrument can be used repeatedly and is portable, so the cost of treatment is low. This study had several limitations, including a short experimental time and the short-term evaluation period, thus preventing the observation of long-term hemostatic effects in experimental animals. Although it is a long way from our experiment to clinical application, our study showed a promising and easy operational method to hemostasis therapy for hepatic artery injury.

**Declarations**

**Author contribution statement**

Lei Dong, Ping Liang: Conceived and designed the experiments.
Yuanyuan Sun: Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Guoming Zhang: Performed the experiments; Wrote the paper.

Jie Yu, Wei Liu: Analyzed and interpreted the data.

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**Competing interest statement**

The authors declare no conflict of interest.

**Additional information**

No additional information is available for this paper.

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