LABORATORY INVESTIGATION

Potential Mechanisms of Vascular Thrombosis after Microwave Ablation in an in Vivo Liver

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ABSTRACT

Purpose: To evaluate potential biologic and thermal mechanisms of the observed differences in thrombosis rates between hepatic vessels during microwave (MW) ablation procedures.

Materials and Methods: MW ablation antennae were placed in single liver lobes of 2 in vivo porcine liver models (n = 3 in each animal; N = 6 total) in the proximity of a large (> 5 mm) portal vein (PV) and hepatic veins (HVs). Each ablation was performed with 100 W for 5 minutes. Conventional ultrasound imaging and intravascular temperature probes were used to evaluate vessel patency and temperature changes during the ablation procedure. Vascular endothelium was harvested 1 hour after ablation and used to characterize genes and proteins associated with thrombosis in PVs and HVs.

Results: Targeted PVs within the MW ablation zone exhibited thrombosis at a significantly higher rate than HVs (54.5% vs 0.0%; $P = .0046$). There was a negligible change in intravascular temperature in PVs and HVs during the ablation procedure (0.2°C ± 0.4 vs 0.6°C ± 0.9; $P = .46$). PVs exhibited significantly higher gene expression than HVs in terms of fold differences in thrombomodulin (2.9 ± 2.0; $P = .0001$), von Willebrand factor (vWF; 7.6 ± 1.5; $P = .0001$), endothelial protein C receptor (3.50 ± 0.49; $P = .0011$), and plasminogen activator inhibitor (1.46 ± 0.05; $P = .0014$). Western blot analysis showed significantly higher expression of vWF (2.32 ± 0.92; $P = .031$) in PVs compared with HVs.

Conclusions: Large PVs exhibit thrombosis more frequently than HVs during MW ablation procedures. Biologic differences in thrombogenicity, rather than heat transfer, between PVs and HVs may contribute to their different rates of thrombosis.

ABBREVIATIONS

GAPDH = glyceraldehyde 3-phosphate dehydrogenase, HV = hepatic vein, MW = microwave, PCR = polymerase chain reaction, PLAT = Tissue Plasminogen Activator, PROCR = Endothelial Protein C Receptor, PV = portal vein, qRT-PCR = quantitative real-time polymerase chain reaction, SERPINE1 = Serpin Peptidase Inhibitor 1, THBD = Thrombomodulin, vWF = von Willebrand Factor

Thermal ablation is an accepted alternative to surgical resection in the treatment of liver cancer as a result of its association with less bleeding, quicker recovery, and fewer comorbidities compared with surgical resection (1). The underlying goal of a thermal ablation is to coagulate the entire tumor volume plus a circumferential boundary of at least 5 mm (2–4). One limitation of thermal ablation treatments is that they are less effective in the presence of nearby vasculature, which draws heat away from the ablation zone (5). This "heat-sink" effect can lead to incomplete tumor ablation and an increased incidence of local tumor progression (4,6,7).

High-powered microwave (MW) ablation systems have been used to overcome this heat-sink effect by using an electric field to heat through a variety of tissue conditions, including desiccated and charred states (8). The quick and efficient energy-delivery mechanism leads to larger ablation zones, even in well-perfused tissue (9). However, there is a lack of information regarding how individual vessels respond to the high temperatures associated with MW energy. Current literature documenting the incidence of

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thrombosis during ablation is found mostly in the form of case reports and small retrospective studies (4,10–13). Damage of small hepatic vessels is common but unlikely to have clinical consequence; thrombosis of larger vessels appears more rare but could cause more serious complications in patients with cirrhosis or compromised liver function (14). In the absence of established clinical studies characterizing rates of thrombosis in large vessels, physicians may avoid aggressive treatment of perivascular tumors, leading to higher rates of local tumor progression (7). Further insight into intravascular thrombosis in a controlled, in vivo setting can shed insight into this phenomenon and may help physicians maximize the efficacy of the ablation treatment while minimizing the risk of clinically important vascular damage.

Early in vivo ablation studies demonstrated that vessels smaller than 3 mm in size are more likely to show thrombosis (8,9,15). More recent studies have identified greater thrombosis rates in smaller portal veins (PVs) compared with hepatic veins (HVs) in porcine and human livers (7,16). The purpose of the present study was to evaluate potential mechanisms for the observed differences in thrombosis rates by quantifying thrombosis-related gene and protein expression in normal vessels as well as differences in heat transfer during the ablation procedure.

MATERIALS AND METHODS

In Vivo Study

All studies were performed with approval from the institutional animal care and use committee and complied with National Research Council Guidelines (17). Female domestic swine (n = 2; 50 kg; Arlington Farms, Arlington, Wisconsin) were sedated with intramuscular tiletamine hydrochloride/zolazepam hydrochloride (7 mg/kg; Telazol; Fort Dodge Animal Health, Fort Dodge, Iowa) and xylazine hydrochloride (2.2 mg/kg; Xyla-Ject; Phoenix, St. Joseph, Missouri). Anesthesia was maintained with inhaled 1.0%–2.0% isoflurane (Halocarbon, River Edge, New Jersey). An ear vein was cannulated with a 20-gauge angiocatheter for administration of intravenous fluids.

Individual MW antennae (Neuwave Medical, Madison, Wisconsin) were inserted to create a single MW ablation in each individual liver lobe (n = 3 for each animal, for a total of N = 6) to within 20 mm of larger PVs and HVs (> 5-mm diameter) under ultrasound (US) guidance (Antares; Siemens, Issaquah, Washington). Each MW antenna was strategically placed to target multiple PVs and HVs simultaneously. Preablation Doppler US imaging was performed to measure the peak blood flow velocity in each vessel. Fiberoptic temperature probes (MTG-170; Opsens, Quebec, Canada) were then inserted under US guidance into the nearest PV or HV. Temperature probe tips were identified to guide their placement onto the same imaging plane as the MW ablation antenna heating ceramic (**Fig 1a**). Ablation zones were then created by applying 100 W for 5 minutes, with the fiberoptic temperature probes recording temperatures every 1 second. Postablation US imaging was performed to confirm the placement of the fiberoptic temperature probe and evaluate vascular damage such as vessel stricture or thrombus formation (**Fig 1b**).

After the ablation procedure, animals were euthanized with an intravenous injection of Beuthanasia-D (390 mg/mL pentobarbital sodium and 50 mg/mL phenytoin sodium at 0.2 mL/kg; Schering-Plough, Kenilworth, New Jersey). The liver was then removed en bloc and sectioned along the axis of each antenna to expose the transverse cross-section of the nearest vessels and confirm the location of the temperature probe (**Fig 1c**). Normal sections of the PV and HVs, identified outside of the ablation zone, were excised by using sterile technique and frozen for quantitative polymerase chain reaction (PCR) and Western blot analysis.

Gene Analysis

Endothelial cells were detached by dissection of the inner lining of the vessel from PVs (n = 3) and HVs (n = 3).

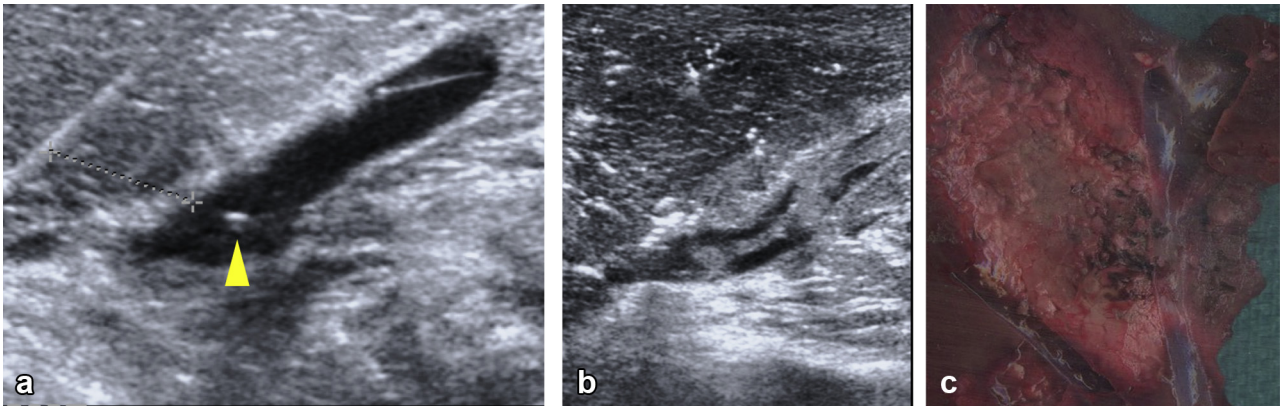

Figure 1. MW ablation antenna being placed next to a large PV. **(a)** The tip of the temperature probe (arrowhead) appears hyperechoic under US imaging and was used to guide placement within the vessel. **(b)** Immediate postablation imaging of the PV shows an echogenic mass within the vessel. The ablation zone next to the vessel appears hypoechoic, reflecting the increased tissue density within the ablation zone. **(c)** Postablation gross histologic image confirms the location of the ablation zone next to the PV and thrombus formation.