The American Journal of Surgery (2016) 211, 189-196

The American Journal of Surgery®

Research

Thermal tissue change induced by a microwave surgical instrument in a rat hepatectomy model

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KEYWORDS:
Microwave coagulation surgical instrument;
Lateral thermal injury;
Tissue heating;
Acid phosphatase enzyme staining

Abstract

BACKGROUND: Microwaves exhibit great potential in tissue heating, which causes effective coagulation. Using this energy, we have developed the microwave coagulation surgical instrument (MWCX) for clinical application. Here, we characterized the impact of MWCX on tissues including heating property, tissue change, and spread of thermal injury.

METHODS: Hepatectomy was performed with MWCX using a rat model. The resections were completed using various energy levels and powers. Tissue temperature during radiation was recorded. Tissue change and lateral thermal injury (LTI) was assessed immediately, 7 days, 3 months, and 6 months after resection.

RESULTS: All cutting and hemostasis procedures were successfully accomplished. Major histologic findings consisted of deformation or destruction of hepatocytes, tissue edema, and peripheral hemorrhage. At various energy levels, 200 to 1000 J, the tissue was heated up to approximately 80°C to 140°C causing 2.7- to 6.5-mm LTI on the 7th day. LTI was then decreased gradually in the following term. At certain energy levels, the application of neither 20 W nor 40 W induced significant difference in both heating and LTI.

CONCLUSIONS: MWCX achieved effective tissue coagulation with relevant tissue injury, and it should be a good candidate for clinical application.

Energy-based surgical devices have been widely used in modern operations, especially in the field of laparoscopic surgery. Technological advancements have facilitated the development of energy-based surgical devices that use various energy sources, such as ultrasound, radiofrequency, cryogenics, light, and hydromechanics, to achieve effective hemostasis, less tissue injury, and safe and quick application.[1,2] Microwaves are a part of the electromagnetic spectrum and have a frequency that ranges from 300 MHz to 300 GHz. During radiation, water molecules and other polar molecules in the tissue are forced to oscillate at microwave frequencies, a process known as dielectric hysteresis. The increase in thermal agitation causes tissue heating and cell desiccation. This heat has been thought to result in protein denaturation, which induces cellular death via coagulation necrosis.[3–5] Microwave coagulation has been applied in medicine for decades for purposes including the ablation

The authors declare no conflicts of interest.

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Manuscript received January 23, 2015; revised manuscript July 17, 2015

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http://dx.doi.org/10.1016/j.amjsurg.2015.07.008

of tumors in the liver,[6–8] kidney,[9,10] lung,[11,12] and prostate,[13,14] the treatment of cardiac arrhythmias,[15] and hyperthermia therapy.[16] Using microwave energy, we have developed microwave energy-based surgical devices for clinical operation. The scissor-type microwave coagulation surgical instrument (MWCX) demonstrated a great capability in sealing vessels.[17] With specific tissue heating properties, microwaves allow more efficient and uniform transmission of heat throughout the irradiated tissue, possibly improve tissue sealing ability, and allow quick application compared with externally applied heat.[17]

Acid phosphatase enzyme (AcP) histochemistry staining has been reported to distinguish the difference between the heating effects of microwaves from induction heating.[4] The aim of this study was to characterize further properties of this instrument, such as heating property, histologic changes using AcP and Hematoxylin and Eosin (HE) staining, and spread of tissue thermal injury to establish evidence for clinically relevant application. The experiment was based on a rat model of liver resection with the use of MWCX. Resection procedures were performed with different modes of output power in watt (W) and total energy for one bite in joules (J).

Methods

Microwave surgical device system

The microwave surgical device system comprised a microwave generator connected to MWCX (Fig. 1A) by a flexible low-loss coaxial cable. The generator radiates microwave energy at a frequency of 2,450 MHz from a magnetron (Magnetron; Alfresa Ltd., Osaka, Japan) and is capable of a power output from 10 W to 100 W.

Liver resection with microwave coagulation surgical instrument

Experiments were performed under the approval of the Committee on Animal Care of Shiga University of Medical Science, Shiga, Japan.

A total of 150 female Wistar rats, 10- to 12-week old, weighing from 250 to 300 g were used for the experiment. To examine tissue changes, spread of tissue damage, and heat generation in the lateral sides of MWCX blades, we performed liver resection using a single MWCX device (Fig. 1B). Each rat was anesthetized under general anesthesia with isoflurane inhalation. A 3-cm-midline incision of the upper abdomen was then performed to expose the liver. The resection method is described in Fig. 1B. The transection plane was approximately 2 to 3 cm in length and .5 to 1 cm in width. The rats were categorized into 3 groups with 50 rats in each group (group I, II, and III). Each group was subsequently divided into 5 randomly equal subgroups (10 rats in each subgroup) to be resected with an energy level of 200, 400, 600, 800, or 1,000 J. In each rat, 20 W of power was applied for resecting the right median lobe, and 40 W of power was applied for resecting the left lateral lobe. We immediately collected the resected liver specimens (day 0 samples). The abdominal incision was then closed, and the rats were returned to the animal facility. Rats in group I, II, and III were killed 7 days, 3 months, and 6 months, respectively, after the operation for sample collection.

Temperature recording

During liver resection, we used a high-sensitivity infrared thermography system (AIR 64 Professional MT) to record the tissue temperature of contiguous MWCX blades. After each application, MWCX blades were cooled down to 30°C before the next radiation. The peak temperature was recorded, and the mean temperature values were summarized for each resection procedure. The mean range of error was 0°C.

Histology evaluation

The day 0 samples were stained for AcP activity, an enzyme highly expressed in healthy hepatocytes, to evaluate enzyme activity that indicates cellular viability. To prepare an AcP enzyme histochemistry stain, a series of 5-μm frozen sections were made using a cryostat (Leica CM1900; Leica Microsystems Nussloch GmbH, Nussloch, Germany). We used the diazonium salt coupling method with naphthol AS series of phosphate esters as previously described.[4] The incubation solution was 40 mL of buffer (.2-M CH_3COOH) containing 12 mg of naphthol AS-BI phosphate, 30 mg of Fast red salt, 250 μL of N,N-dimethylformaldehyde, and 2 drops of 10% $MgCl_2$. The sections were incubated in this solution at 37°C for 30 min.

All samples collected at 4 time points (day 0, day 7, month 3, month 6, n = 50 in each) were stained with HE to evaluate morphologic change and with fluorescein by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay to detect cell death regions. The TUNEL assay was performed according to the manufacturer's protocol (Promega DeadEND Fluorometric TUNEL system; Promega Corporation, Fitchburg, WI). Briefly, the 3-μm paraffin-embedded sections were pretreated by deparaffinization, rehydration, fixation, and permeabilization of cell membranes. Then, the sections were equilibrated in equilibration buffer, incubated with recombinant terminal deoxynucleotidyl transferase buffer, and terminated by immersing in saline sodium citrate solution. Nuclei were counterstained with 4′,6-diamidino-2-phenylindole (Vector Laboratories, Burlingame, CA).

The HE-stained and AcP-stained sections were observed under a light microscope (Nikon ECLIPSE 90i; Nikon Corp., Tokyo, Japan) equipped with Image-Pro Plus software (version 7.0J; Media Cybernetics, Inc., Rockville, MD). The TUNEL-stained sections were observed under a fluorescence microscope (Olympus BX61TRF; Olympus optical Co. Ltd., Shinjuku, Tokyo, Japan) at 520 nm to view the green fluorescence of fluorescein and 40 nm to view the blue 4′,6-diamidino-2-phenylindole. The lateral thermal