Effects of Radiofrequency Induced Local Hyperthermia on Normal Canine Liver

Chang Ok Suh, M.D., John J.K.Loh, M.D., Jin Sil Seong, M.D., Sun Rock Moon, M.D.
Hyung Sik Lee, M.D., Hyun Soo Shin, M.D., Sung Sil Chu, Ph.D.
Gwi Eon Kim, M.D., Chan Il Park, M.D*. and Eun Kyung Han, M.D.*

Department of Radiation Oncology and Pathology, Yonsei University, College of Medicine, Seoul, Korea*

In order to assess the effects of radiofrequency-induced local hyperthermia on the normal liver, histopathologic findings and biochemical changes after localized hyperthermia in canine liver were studied. Hyperthermia was externally adminsitered using the Thermotron RF-8 (Yamamoto Vinyter Co., Japan; Capacitive type heating machine) with parallel opposed electrodes.

Thirteen dogs were used and allocated into one control group (N=3) and two treatment groups according to the treatment temperature. Group I (N=5) was heated with 42.5±0.5°C for 30 minutes, and Group II (N=5) was heated with 45±0.5°C for 15-30 minutes. Samples of liver tissue were obtained through a needle biopsy immediately after hyperthermia and 7, 14, and 28 days after treatment. Blood samples were obtained before treatment and 1, 3, 5, 7, 14 and 28 days after treatment and examined for SGOT, SGPT and alkaline phosphatase. Although SGOT and SGPT were elevated after hyperthermia in both groups (three of five in each group), there was no liver cell necrosis or hyperthermia related mortality in Group I. A hydropic swelling of hepatocytes was prominent histologic finding. Hyperthermia with 45°C for 30 minutes was fatal and showed extensive liver cell necrosis. In conclusion, liver damage dy heat of 42.5±0.5°C for 30 minutes is reversible, and liver damage by heat of 45±0.5°C for 30 minutes can be fatal or irreversible. However, these results cannot be applied directly to human trial. Therefore, in order to apply hyperthermic treatment on human liver tumor safely, close observation of temperature with proper thermometry is mandatory.

Hyperthermic treatment should be confined to the tumor area while sparing a normal liver as much as possible.

Key Words: Hyperthermia, Liver

INTRODUCTION

It is generally accepted that heat kills cells, interacts with radiation in a synergistic way, and potentiates some chemotherapeutic agents. However, the role of hyperthermia in the managenent of different kinds of cancer is still under investigation. Designing and building equipment to heat designated tumor volumes accurately and precisely is unsatisfactory. Difficulty in measurement of temperature during hyperthermia limits the development of clinical hyperthermia. Heating and thermometry of superficial tumor is relatively successful; however, heating a deep-seated tumor is hard to achieve.

Therefore, interstitial hyperthermia of intraoperative hyperthermia were attempted in deep seated tumors. Recently, with development of a hyperthermia machine, delivery of a heat dose lethal to the tumor mass became possible, but effective exclusion of normal tissue from heating is difficult. Although the theory that heat is more toxic to tumor cells than normal cells justifies the clinical application of hyperthermia, we should identify normal tissue tolerance to heat before hyperthermic treatment. Effects on liver tissue by heat is well document by victims of heat stroke and in animal studies with isolated perfusion of liver or interstitial hyperthermia. The change to the liver would differ according to the methods of heating.

We studied biochemical and histopathological changes after hyperthermia in normal canine liver using 8 MHz capacitive type heating machine, in order to assess the effects of radiofrequency induced local hyperthermia on the normal liver and to

*연세대학교 의과대학 교수연구비에 의하여 이루어 졌음(1988).

clarify the tolerance of the liver to heat.

MATERIALS AND METHOD

Thirteen normal, healthy dogs with a weight range of 13~15 Kg were used for this study. Ten dogs received external hyperthermia to the liver using.

Thermotron RF-8 (8 MHZ capacitive type heating machine, manufactured by Yamamoto Vinyter CO.). Of those, five were heated with a liver temperature of 42.5±0.4°C for 30 minutes (group I), and the other five were heated with 45±0.5°C for 15~30 minutes. Three dogs were used for the control group. After overnight NPO, venous blood sampling was done before anesthesia. Anesthesia was induced with intravenous injection of pentobarbital sodium (50 mg/kg). After shaving the upper abdomen and back, the dogs were fixed in a supine position on a wooden board which had a 20 cm diameter sized hole in the center to apply electrodes. On fluoroscopic guidance, a 18 Gauge Vinca needle (Angiocath) was inserted into the liver, and a 4 channel thermometer (Copper-constantan thermocouple) was introduced through inner channel of vinca needle to monitor temperature (Fig. 1).

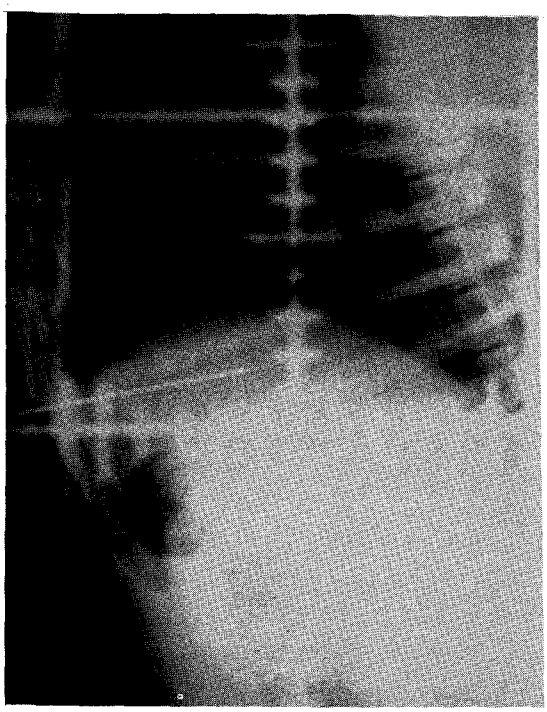


Fig. 1. In order to measure a temperature, a 18 G Vinca needle was inserted into the liver under the fluoroscopic guidance.

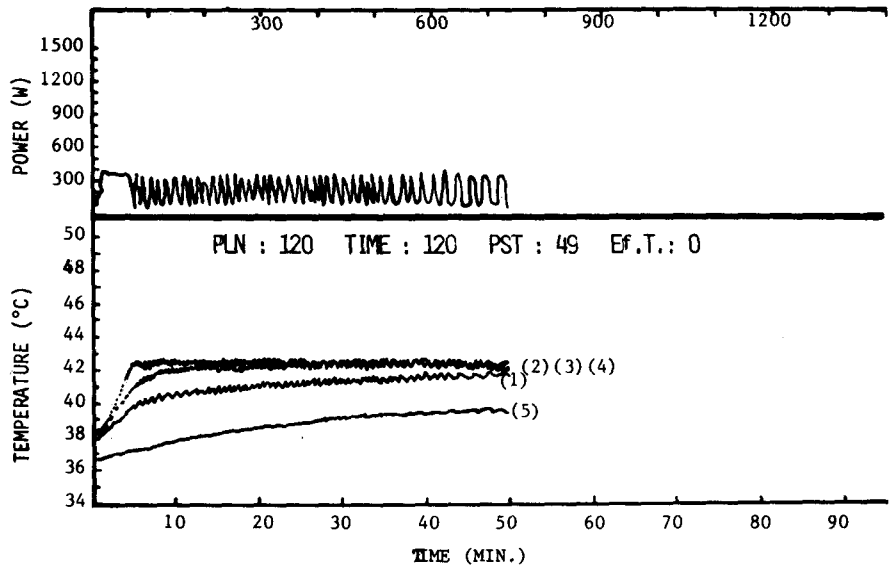
The Four channel thermometer had 1.5 cm spaced 4 sensors. To cover the entire liver, 14 cm diameter sized electrodes were attached in an anterior and posterior parallel opposed array. Hyperthermic treatment was done at a set temperature and time. Surface cooling was not done. Rectal temperature was monitored with thermocouple in the inserted Nelaton catheter. Immediately after hyperthermia, venous blood sampling for biochemical study and liver biopsy with Menguini needle were done. Blood sampling was repeated at 1, 3, 5, 7, 14 and 28 days after hyperthermia for SGOT, SGPT and alkaline phosphatase. Liver tissue was obtained by liver biopsy on 7 and 14 days after hyperthermia and 28 days after hyperthermia by euthanasia. Tissues were fixed in paraffin and stained with hematoxylin and eosin (H&E) for histological examination.

RESULT

Hyperthermia with a temperature of 42.5°C was well maintained with 100~300 Watt. Relatively homogenous heating verified by a 4 channel thermometer was achieved. Generally, however, mean temperature of entire liver was somewhat lower than planned temperature because the machine power automatically turned off if any one of the four temperature elevated above the planned maximum temperature (Fig. 2). Rectal temperature gradually increased up to 40.8°C. Four of the five group I dogs were alive until the planned sacrifice day, but one dog died on the 7th day after hyperthermia. An autopsy revealed hemoperitoneum as the cause of death. We thought the hemoperitoneum identified by examiners from an developed aorta puncture during the liver biopsy on day O, immediately after hyperthermia. In group II, only one dog lived until the planned sacrifice day, the first and second dogs died several hours after hyperthermia with 45°C for 30 minutes. In the remaining dogs, heating time was reduced to 15 minutes. The third dog died on the night of the hyperthermic treatment from hemoperitoneum after multiple traumatic liver biopsy. The liver was not changed grossly. The fourth dog died 2 days after treatment, and an autopsy revealed a congested liver.

1. Histologic Findings

In the three control dogs, there were no specific



no	temp.	max	probe location
(1)	41.8	42.6	7cm depth from skin surface
(2)	42.5	42.6	5.5cm depth from skin surface
(3)	42.4	42.6	4.0cm depth from skin surface
(4)	42.1	42.6	2.5cm depth from skin surface
(5)	39.6	42.6	rectal

Fig. 2. Temperature monitoring with 4 channel thermometer during hyperthermia demonstrated successful maintaining of desired temperature, 42.5 C.

pathologic findings in liver tissue. Histopathologic finding of heated liver tissue are summarized in Tables 1 & 2. In the group I, hydropic swelling of hepatocytes was the prominent finding throughout the observation period (Fig. 3). Inflammatory cell infiltration in the sinusoids and portal tract, dilatation of central or portal veins, and congestion of sinusiods were also noted (Fig. 4). Mild cholestasis was shown in the specimens on the 28th day after hyperthermia. Necrosis of the liver cell was not demonstrated. In the group II, the specimens obtained from the first and second dogs which died after hyperthermia with 45°C for 30 minutes revealed severe coagulative type necrosis which was thought to be cause of death (Fig. 5). Necrosis was more severe around the central veins while sparing the area around portal veins. In the cases heated with 45°C for 15 minutes, there was no demonstrable extensive liver cell necrosis. Only congestion of sinusoids and hydropic swelling of hepatocytes were noted.

2. Biochemical Changes

Change of SGOT and SGPT in each animal according to the time after hyperthermia are shown in Fig. 6 & 7. In six cases among ten, SGOT and SGPT were elevated immediately after heating, reaching a maximum values on one to three days after, and returning to normal values within one or two weeks. In contrast to SGOT, SGPT maintained a high level until third day after treatment. Values of alkaline phosphatase did not change after heating or throughout the observation period.

DISCUSSION

Pathologic effects of hyperthermia in normal tissue have been best described in the necropsies of fatal cases of heatstroke or patients treated by hyperpyrexia[1~6] and experimental heatstroke in animal models[7,8]. In these case of systemic hyperthermia, the central nervous system, liver, kidney, heart, adrenal, and bone marrow were mainly affected. They described histopathologic changes of the liver, such as congestion of centrilobular sinusoids, inflammatory cell infiltration, vacuolization of hepatocytes, centrilobular degeneration or necrosis of hepatocytes. Cholestasis was also seen in rather severe cases. And they suggested that the

Table 1. Histologic Findings in Group I (Hyperthermia, 42.5 ± 0.5°C)

No. of animal	Days of sampling	Histopathologic Findings					
		Congestion of sinusoids	Dilation of central & portal veins	Inflammatory cell infiltration	Hydropic change of hepatocytes	Necrosis of liver cells	Cholestasis
1	0	–	–	+	–	–	–
	7	++	+	+	–	–	–
2	0	insufficient material (skeletal muscle)					
	7	–	–	–	+	–	–
	14	–	–	–	+	–	–
	28	++	+	–	+	–	+
3	0	–	–	+	+	–	–
	7	–	–	–	+	–	–
	14	–	–	–	+	–	–
	28	–	+	–	+	–	+
4	0	insufficient material (skeletal muscle)					
	7	–	–	+	+	–	–
	14	–	–	+	+	–	–
	28	+	–	–	+	–	–
5	0	–	+	+	–	–	–
	7	–	+	+	+	–	–
	14	+	+	+	+	–	–
	28	–	+	+	+	–	+

+ Focal involvement ++ Extensive involvement

Table 2. Histopathologic Findings in Group II (Hyperthermia, 45 ± 0.5°C)

No. of animal	Days of sampling	Histopathologic Findings					
		Congestion of sinusoids	Dilation of central & portal veins	Inflammatory cell infiltration	Hydropic change of hepatocytes	Necrosis of liver cells	Cholestasis
1	0	+	–	+	–	–	–
	1	++	–	+	–	++	++
2	0	insufficient material (skeletal muscle)					
	1	++	–	++	–	++	–
3	0	–	–	–	+	–	–
	1	–	–	–	–	+	–
4	0	–	–	–	+	–	–
	2	+	–	–	+	–	–
5	0	–	–	–	+	–	–
	7	–	–	–	+	–	–
	14	–	–	–	+	–	–
	28	–	–	–	+	–	–

+ Focal involvement ++ Extensive involvement

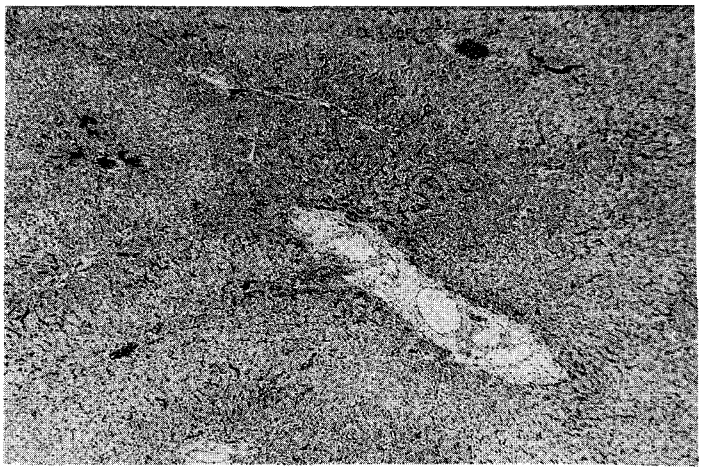


Fig. 3. Light microphotograph of liver at 7 days after 42.5°C hyperthermia for 30 minutes. Marked sinusoidal congestion and dilation of terminal hepatic venule with shrinkage of the liver cell cord. (H & E, ×40)

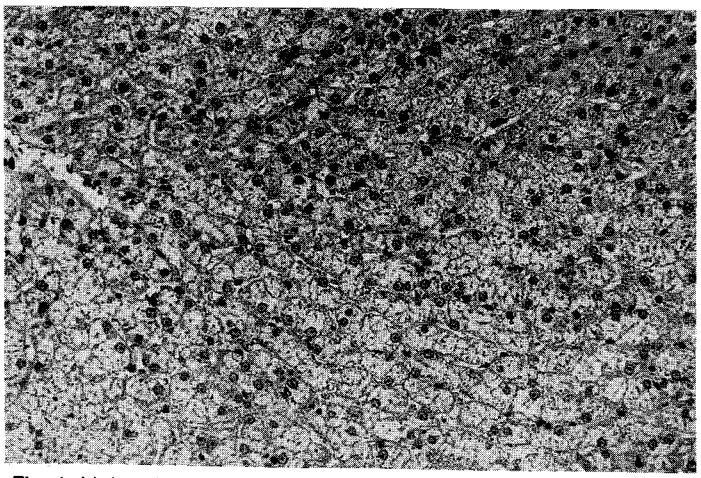


Fig. 4. Light microphotograph of liver at 7 days after 42.5 C hyperthermia for 30 minuttes. Diffuse hydropic swelling of hepatocytes with obliteration of sinusoids. (H & E, ×100)

hepatic changes were the result of a combination of hypoxia and direct thermal injury[9]. The information of the pathology of localized hyperthermia comes mainly from experimental studies in mammals[10~16]. Regardless of the heating method, vacuolization or hydropic swelling of hepatocytes was the most consistent and striking finding in most reports[1,8,10~12]. Dissociation or necrosis of hepatocytes was found after heating at a higher temperature. These changes were usually centrilobular in distribution which suggested ischemic injury was a possible cause of heat induced hepatic damage. Hydropic swelling of hepatocytes was shown even at the relatively low temperature, 41~42°C and was reversible to regenerate. Extensive necrosis caused early fatality or irreversible change which resulted in fibrosis and significant liver function insufficiency. Vacuolization of the hepatocytes is generally attributed to tissue hypoxia. Bowers et al explained this phenomenon that the inability of

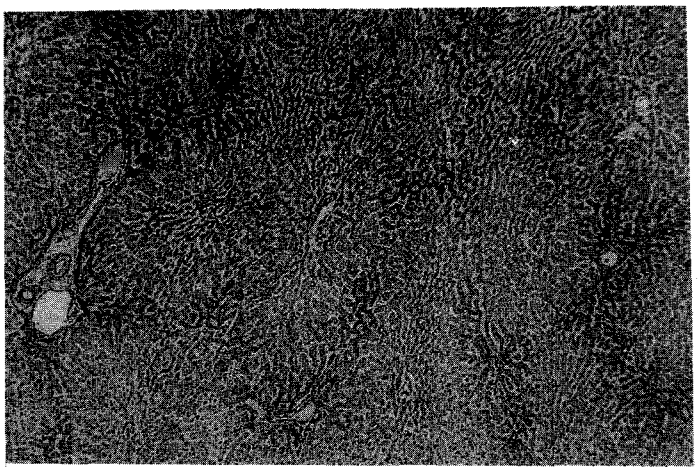


Fig. 5. Light microphotograph of liver, immediately after 45 hyperthermia for 30 minutes. Extensive coagulative necrosis of hepatocytes, especially in pericentral area with inflammatory cell infiltration. (H & E, ×40)

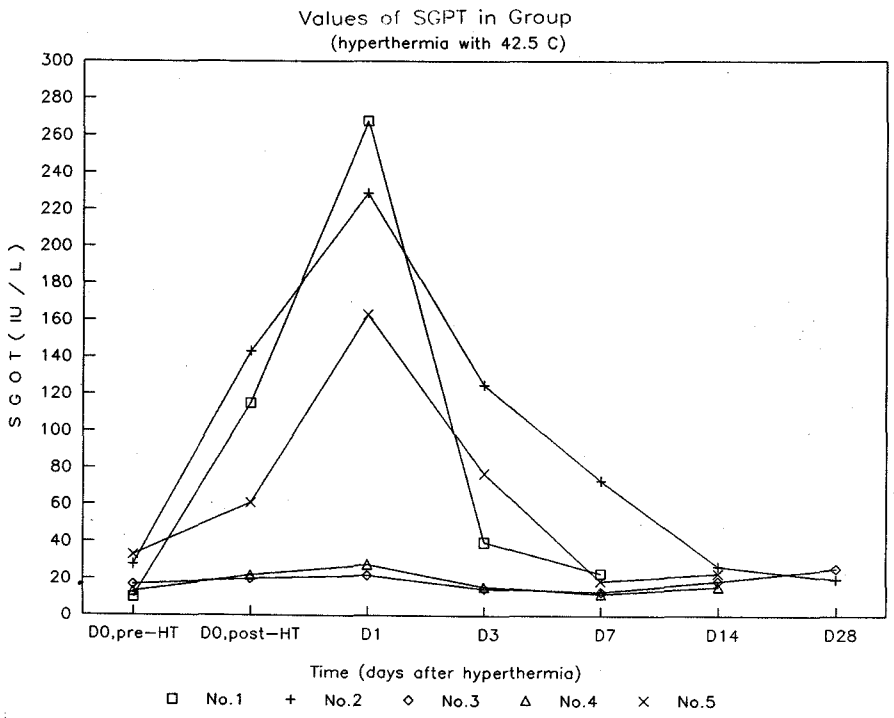


Fig. 6. Values of SGOT in groups I & II according to the time after hyperthermia.

damaged mitochondria to utilize oxygen and generate sufficient ATP may induce a intracellular hypoxic condition[11]. Alternatively, they also explained that the loss of permeability of cell membrane would allow sodium and potassium flux down an electrochemical gradient resulting in hypdropic swelling. Ultrastructural changes of liver by heat was well-described in several re-

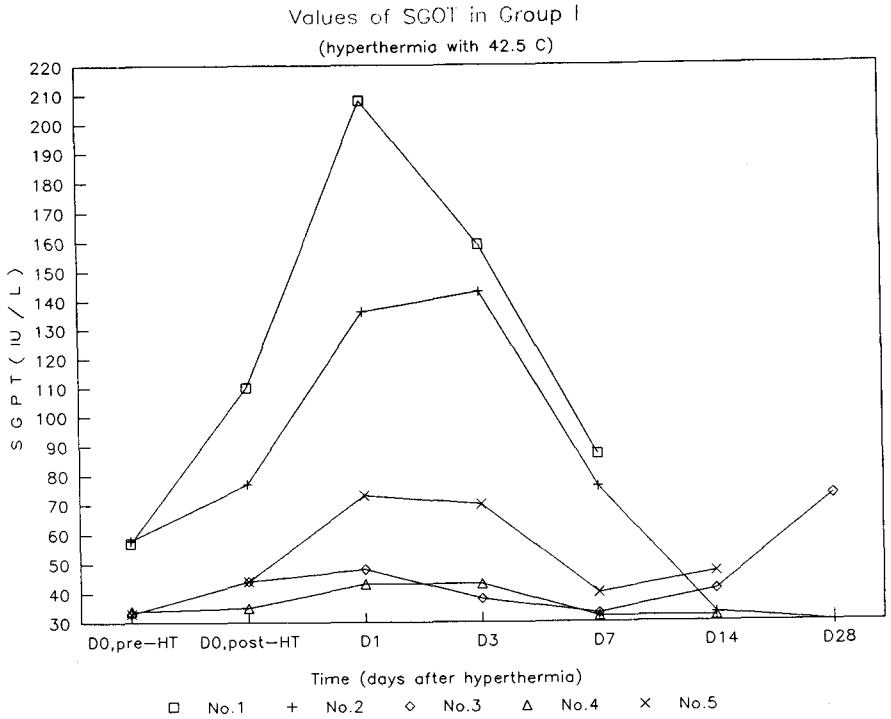


Fig. 7. Values of SGPT in groups I & II according to the time after hyperthermia.

ports[11,17,18,19]. There were numerous autophasic vacuoles, flocculent deposits in mitochondria, loss of endothelial cells, dilation of the rough endoplasmic reticulum, and dissociation of hepatocytes. These ultrastructural damages were observed even immediately after therapy in the low temperature range, 40~41°C, where there was no light microscopic change.

Although the exact mechanism of liver damage by heat is uncertain, the degree of liver damage by heat differed with the heating methods, temperature and duration of heating, and the environment of the liver, such as pH or nutrition[20,21,22]. Liver tissue tolerated heat better in localized hyperthermia than in systemic hyperthermia, where the liver is in a hypoxic condition due to such systemic effects as high oxygen consumption and decreased blood flow into the liver[23].

In localized hyperthermia, it was presumed that a direct thermal injury plays a more important role in causing liver damage than in systemic hyperthermia, because blood flow into the liver is unchanged[11]. Therefore, focal necrosis occurred without zonal distribution[19]. However, endothelial cell damage by heat-induced fibrin or platelet deposition resulted in hypoxia of surrounding liver tissue and triggered liver damage[16,19]. Nishimura et al suggested that central veins were more thermosensitive than the portal vein or hepatic arteriole because of a difference in histological structure and inflow blood[16]. Central veins carry heated blood flow whereas hepatic arterioles and portal veins carry the systemic blood. This can be a possible cause of more pronounced liver damage in centrilobular region.

Many investigators reported that liver damage by systemic hyperthermia with a temperature below 42~43°C was trivial or reversible histologically and functionally[7,8,12,23,24]. Damage was siginificant when hyperthermia with those temperatures was combined with radiation or chemotherapeutic agent[12,19]. On the other hand, systemic hyperthermia at a higher temperature often resulted in fatal outcome, even though heating only a part of liver, did not cause liver failure. So, unless there was no extensive necrosis causing early fatality, liver damage such as necrosis eventually regenerated or resulted in fibrosis[5,14,15]. In this experiment, heating of the canine liver with 42.5±0.5°C for 30 minutes was thought to be non-fatal. It caused only a mild degree of sinusoidal congestion, inflammatory cell infiltration, dilation of portal and central veins and

hydropic swelling of hepatocytes. Although the authors did not observe a chronic change of longer than one month, these histologic changes should be reversible. In the authors other hyperthermic experiment with rabbit liver, there were no late pathologic changes in the long-term surviving rabbit after whole liver hyperthermic treatment[19]. Both dogs heated with 45°C for 30 minutes (No. 1 & 2 in group II) died immediately after hyperthermic treatment and showed extensive coagulative type liver cell necrosis. However, in the cases heated with 45°C for 15 minutes, there did not appear to be extensive liver cell necrosis. This finding confirmed the fact that a degree of biological effect by heat is positively correlated with both heating duration and temperature[7,25]. Because there is an exponential relationship between temperature and exposure time for a given isoeffect[7,26] and due to difficulties in assessing and comparing hyperthermic treatment results using different protocol, some investigators proposed a standardized thermal dose[27,28].

Biochemcal changes after hyperthermia were also observed in the patients with heatstroke or whole body hyperthermic treatment[3,4,29] and animal studies with either systemic hyperthermia or localized heating[11,12,15,19]. SGOT, SGPT, LDH (lactic dehydrogenase), serum CPK (creatinine phosphokinase), ornithine carbamyl transferase were frequently elevated after hyperthermia or heatstroke episodes. Prothrombin time and BSP retension were abnormal in 50% of patients, and serum bilirubin was elevated in 5.3~17% of patients[2,4]. Kew et al reported that the levels of SGOT, SGPT and LDH were of prognostic significance, being much greater in the severe cases[4]. SGOT, SGPT, and LDH usually elevated within a short time after hyperthermia, and the maximum values of SGOT and LDH were usually reached 48 hours after hyperthermia and returned to normal values within 7 days. SGPT was usually elevated for a longer period than SGOT, being at a high level until 3 days after hyperthermia[4,19]. The author's previous experiment also demonstrated that the incidence of SGOT & SGPT elevation increased according to the temperature and correlated with degree of liver damage. In some rabbits whose livers were heated at 41°C, SGOT and SGPT were elevated even though there was no histopathologic change, providing reasonably good evidence of liver damage.

In conclusion, according to our results with literature review, it is hard to determine the critical temperature and duration of heating which produces irreversible damage to the liver. However, it can be concluded that liver damage with localized heating at 42~43°C for less than one hour, still effective in tumor cell killing, is reversible. Heating at a temperature of more than 43°C can be fatal or result in irreversible change. Therefore, in order to safely apply hyperthermic treatment on a human liver tumor, close observation of temperature with proper thermometry is mandatory. Hyperthermic treatment should be confined to the tumor area while sparing the normal liver as much as possible.

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= 국문초록 =

라디오 파를 이용한 국소 온열치료 : 정상 개의 간에 미치는 영향

연세대학교 의과대학 치료방사선과학교실, 해부병리과학교실*

서창옥 · 노준규 · 신현수 · 이형식 · 문성록 · 성진실
추성실 · 김귀언 · 한은경* · 박찬일*

간조직에 온열치료를 시행시 출현하는 조직병리학적 소견 및 혈액의 생화학적 소견을 관찰하고자 13마리의 정상 간에 8 MHz 라디오파를 이용한 온열치료를 시행하였다. 42±0.5°C로 30분간 온열치료를 받은 군(제 1 군, n=5), 45±0.5°C로 30분간 온열치료를 받은 군(제 2 군, n=5) 및 온열치료를 받지않은 대조군(n=3)으로 나누어 분석하였을때, 혈액의 SGOT의 SGPT는 온열치료를 시행한 두 군 공히 증가된 소견을 보였고 제 1군에서는 간세포의 부종소견외 특이한 조직병리학적소견이 관찰되지않아 가역성 변화로 생각되었지만 제 2 군에서는 간 세포의 심한 괴사소견이 관찰되어 있는 불가역성의 가조직 손상으로 생각되었다. 이상의 결론으로 유추할때 임상에서 행하여지는 간암의 온열치료시에 정상 가조직의 손상을 가능한 방지하기위하여는 정확한 종괴의 구역에 치료온도의 주의깊은 관찰이 요구된다.