

# Immune-related Effects of Local Hyperthermia in Patients with Primary Liver Cancer

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## KEY WORDS:

Local hyperthermia; HCC; Immune system; CD4; CD8; CD4/CD8; NK

## ABBREVIATIONS:

Hyperthermia (HT); Natural Killer (NK); Transcatheter Arterial Embolization (TAE); Percutaneous Ethanol Injection Therapy (PEIT)

## ABSTRACT

**Background/Aims:** To investigate immune-related effects of local hyperthermia (HT) with hepatocellular carcinoma (HCC).

**Methodology:** Immune status after 7 HT was studied in 11 patients (M/F - 9/2; 1st group). The effects were also evaluated during one HT session in 4 of those pts (M/F - 4/0; 2nd group).

The HT treatment was performed by means of an 8-MHz capacitive heating device, Thermotron RF8 (Japan). The mean time of one HT session was 60 min. HT was performed 1-2 times a week. In both groups the percentage of T and B cells, CD4+, CD8+ subsets of T cells, the CD4/CD8 ratio and activation of NK cells were evaluated.

**Results:** In the 1st group, CD4/CD8 ratio was

decreased significantly ( $p < 0.05$ ), whereas the relative amount of CD4+ T cells showed a tendency to decrease ( $p = 0.063$ ), and CD8 - to increase ( $p = 0.088$ ). An activation of NK cells was observed in patients who had a low or normal pretreatment level of activation. In the 2nd group, there was a significant decrease in the CD4/CD8 ratio by the end of the treatment ( $p < 0.05$ ) and increased activity of NK cells as early as 20 min after the onset of HT ( $p < 0.05$ ).

**Conclusions:** Our results suggest that HT stimulates the immunity of cancer patients by several means and therefore may exhibit indirect anticancer effect. In addition, activation of NK cells by HT may be associated with improved quality of life.

## INTRODUCTION

The rate of hepatocellular carcinoma (HCC) has been increasing in Japan. Most cases are associated with HCV infection. The mechanism of HCV-related carcinogenesis and disease progression are not well understood, although it is thought that not only virus-induced but also immunologically mediated mechanisms play an important role (1,2)

Such local methods as transcatheter arterial embolization (TAE) and percutaneous ethanol injection therapy (PEIT) are widely used in the treatment of inoperable HCC. However, it has been reported that the TAE procedure might suppress a host immunity response against cancer in HCC patients (3). Recently local hyperthermia (HT) has been widely used for cancer treatment as a way to increase a tumor's radio- and chemosensitivity (4,5). In contrast to most other cancer treatment modalities, HT is also known to cause an activation of the immune system (6-10).

In this study, the potential of local HT to enhance the cell-mediated immunity of HCC patients was investigated.

## METHODOLOGY

**Patients' characteristics:** Clinical characteristics are summarized in **Table 1**.

**Heating:** The HT treatment was performed by means of an 8-MHz capacitive heating device, "Thermotron RF-8" (Yamamoto Vinita Co., Ltd., Osaka, Japan). The electromagnetic power ranging from 614 to 1363 W was applied between two 30-cm electrodes placed on opposite sides of the patient's upper abdominal region. Overlying water boluses were placed between the electrode and the skin to improve the homogeneity of the heating. A saline solution maintained at 2-15°C was circulated in the boluses to avoid overheating of the skin. For premedication, 50mg indomethacin (i.r.) was administered 30 min before HT. A 1% xylocaine topical gel was applied to the skin to decrease the sensation of heat. Blood pressure and pulse were monitored during treatment. The time of one HT session was 40-60 min. HT was performed 1-2 times a week. A total of 8 sessions delivered 1-2 times a week, were performed (1 course). Thermometry was not performed for ethical reasons.

**Laboratory data:** In the first group (N= 11), the percentage of T and B cells, CD4+ and CD8+ subsets, the CD4/CD8 ratio and activation of natural killer (NK) cells were evaluated before the 1st and 8th sessions. In the 2nd group (N= 4) the same immune parameters were investigated during one 60-minute HT session, namely, before the onset of heating, 20 and 40

TABLE 1 Pretreatment Characteristics of the Patients and Parameters of the HT

Case	Sex	PS	Age	Clin. stage	Child stage	Location of tumor	AFP, ng/mL	PIVKA-II, µg/mL	TB, mg/dL	Alb, g/dL	PLT, x10000/µL	Ascitis	Previous treatment	Combination with HT	Power, W
Case 1	M	1	73	II	A	S5	56	-	0.6	4.6	12.1	-	TAE, PEIT	PEIT	659-1150
Case 2	M	2	70	IVa	B	S4, 5, 6, 8	137.6	-	3.5	3.5	7.8	+	TAE	none	814-1124
Case 3	F	2	71	II	B	S6	-	-	1.7	3.8	4.1	-	TAE, PEIT	TAE	700-1167
Case 4	M	1	62	III	A	S2, 6	6	-	2	4	6.6	-	TAE	none	624-1180
Case 5	M	1	72	II	A	S8	12	-	1.1	3.8	8	-	TAE, PEIT	none	886-1284
Case 6	M	1	69	III	C	S8	144	-	1	2.9	3.5	-	TAE, PEIT	none	853-1375
Case 7	F	2	70	III	C	S8	13	-	4.8	2	5	+	PEIT	none	550-664
Case 8	M	1	62	II	B	S6	56	22	1.6	3.3	7.6	-	surgery	none	900-1363
Case 9	M	2	77	III	C	S8	18	-	2.2	2.8	4.9	-	TAE, RT	none	694-1059
Case 10	M	0	64	IV	A	S2, 6, 8	20	-	0.7	4.5	7.8	-	TAE	none	614-1291
Case 11	M	1	65	III	B	S8	-	150	0.7	4.3	10.5	-	TAE, PEIT	TAE, PEIT	862-1273

Notes: Group 2 consisted of cases 1, 5, 10 and 11. TB: total bilirubin; Alb: albumin; PLT: platelets; TAE: transarterial embolization therapy; PEIT: percutaneous ethanol injection therapy; RT: radiotherapy.

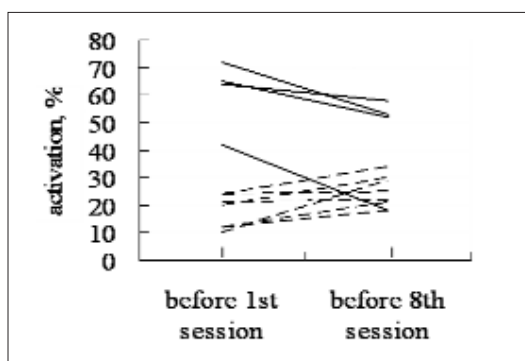


FIGURE 1 NK cells activation in the course of HT in regards to pretreatment level. Normal values of activation of NK cells are 18-40%. Solid lines indicate data for patients with pretreatment level of activation exceeding normal range. Dashed lines indicate data for patients with pretreatment level being lower or within normal range.

min after onset of heating and immediately after its completion.

All patients were informed about the nature and the results of the investigation.

**Statistics:** Data were evaluated using a paired *t*-test. *p* values < 0.05 were considered as statistically significant.

**RESULTS**

**The Effect of Seven HT Sessions on Cell-mediated Immunity**

Data are summarized in **Table 2**. As shown, the CD4+ /CD8+ T cell ratio was significantly decreased by HT (*p*=0.05) (**Table 1**). This effect was associated to a higher degree with decreased amount of CD4+ cells and to a lower degree with increased amount of CD8+ T cells (NS). The percentage of the total population of both T and B cells, as well as activation of NK cells, did not significantly change in these series. At the same time, we found that the pattern of activation of NK cells was likely to be dependent on its pretreatment status. Namely, for patients whose pretreatment level of NK activation was below or within the normal value, there was a significant additional activation caused by HT treatment (15.3± 6.7% to 24.3± 6.3%, *p*<0.05) (**Figure 1**). On the contrary, for those who demonstrated an increased pretreatment activation level, HT treatment resulted in a decrease or lack of change of that parameter (60.8± 12.9% to 45.3± 18.4, NS) (**Figure 1**).

**The Effect of a Single HT Session on Cell-mediated Immunity**

Corresponding data are summarized in **Table 3**. The CD4/CD8 ratio showed a tendency to begin decreasing already at 20 min of heating, demonstrate a significantly low value at 40 min of heating and lower further by the end of HT. The decrease in the CD4/CD8 ratio was mostly due to the decrease in CD4+ fraction and less due to the tendency for CD8+ cells to increase. The total amount of T and B cells did

TABLE 2 Immune Parameters in the Course of HT Treatment

N= 11	CD4/ CD8,%	CD4, %	CD8, %	T cells, %	B cells, %	NK activation, %
Before 1st session	2.04± 0.92	49.0± 8.6	26.8± 9.6	85.8± 9.6	10.3± 6.1	34.3± 22.6
Before 8th session	1.69± 0.75	44.4± 11.5	28.4± 9.9	85.0± 6.7	10.1± 4.6	32.7± 14.1
P value	< 0.05	NS	NS	NS	NS	
Normal values	0.6-2.4	29-55	19-41	69-89	4-13	18-40

TABLE 3 Immune Parameters in HCC Patients during a Single HT Session

N= 4	CD4/ CD8, %	CD4, %	CD8, %	T cells, %	B cells, %	NK activation, %
Before HT	1.97± 0.18	44.5± 4.5	22.5± 3.8	80.7± 5.1	9.2± 5.8	42.7± 23.2
20 min	1.7± 0.43	44.7± 0.9	25.2± 6.7	86.7± 4.3	9.0± 6.1	54.2± 20.9
40 min	1.5± 0.27	39.2± 2.6	26.2± 6.1	81.5± 4.6	8.5± 7.6	52.2± 17.0
60 min	1.4± 0.28	37.2± 5.7	27.2± 7.8	81.7± 5.9	9± 5.5	61.2± 14.2
P value	0-40< 0.05	NS	NS	NS	NS	0-20< 0.05
	0-60< 0.05					0-60< 0.05

not change during the treatment. A significant NK activation was evident as early as 20 min after the onset of treatment, and continued to increase up to the end of the treatment.

## DISCUSSION

Both carcinogenesis and tumor progression are associated with a depressed immune status of the host. It has been suggested that a decrease of CD56+ cells and NK cells in cirrhotic livers caused by hepatitis C is related to susceptibility to HCC (11,12). Depressed NK activity was also noted in patients with HCC, cancers of the kidney and urinary bladder (13,14).

In the multimodality treatment of cancer, a local HT has been demonstrated to potentiate radiotherapy, some chemotherapies, and immunotherapy (15-17). Employment of HT in combination with other modalities used in mild regimens has also been reported. In treatment of HCC, the combination of HT with TAE and PEIT was found to be effective (18,19). Moreover, HT alone has also been reported to effectively treat liver tumors (20,21).

It has been well established that immunocompetent cells, including macrophages, T-lymphocytes and natural killer (NK) cells, in vitro, are among the most sensitive cells to temperature elevation (22,23). Therefore, both whole-body and local HT may cause favorable immune changes when employed in cancer clinic. HT-related immune effects were found to depend on a heating regimen. For instance, temperature ranging from 39 to 41°C (fever range) was shown to confer an immunoregulatory advantage by enhancing the secretion of immunoglobulins, whereas elevation of temperature to tumoricidal level (> 42°C) led to inhibition of host competence (24,25). Therefore, a whole-body HT, if used at tumoricidal temperature, may probably cause unfavorable immune response. In the case of local HT, even if temperature in the tumor exceeds 42°C, the surrounding normal tissue usually remain at fever-range temperatures. This selectivity of heat-

ing is beneficial because of its activation of immune responses. Various immune-related effects of local HT can be expected, as it has already been shown in numerous experimental and clinical investigations. Among the clinically most relevant aspects of cell-mediated immunity are CD4+ and CD8+ populations of T cells, the CD4/CD8 ratio, T and B cells, and NK cell activity. Therefore, these parameters were chosen for our study.

In the clinic, both CD4+ and CD8+ T cells were shown to play an important role in antitumor defense, although the relative clinical significance of each subset might depend on the tumor type. Stawarz *et al.* have shown an increase of the CD4/CD8 ratio by local HT in patients with advanced adenocarcinoma of the prostate (10). In contrast, we found a decrease in the CD4/CD8 ratio, which was mainly due to the increase in the fraction of CD8+ cells. Taking into account other data for ovarian cancer patients, where a decrease of CD8+ lymphocytes was shown to correlate with the progression of the disease (26), we consider the changes observed by us as favorable ones. Interestingly, HT-induced changes in CD4+ , CD8+ subsets were found in both groups, demonstrating some similarity in early and late effects of HT. Therefore, there was probably both a direct activation of immunocompetent cells by elevated temperature, and an indirect one, mediated by HT-induced heat-shock proteins, as follows from recent knowledge of the mechanisms of HT.

A significant increase of NK cytolytic activity was noted following transrectal HT of prostate, with the peak effect at 2 months and a subsequent decrease (27). In our series, there was a selective increase of NK activation in patients whose pretreatment values were lower or within normal range. These data fit the statement of Rosberger *et al.*, who noticed not only immunostimulation but also an immunomodulatory effect of local HT in patients with choroidal melanoma. They showed that local HT inverted CD4/CD8 resulting in a normalization of T-cell subset

ratios (28). In our investigation, where the affects of HT on a group of patients were studied during one 60-min HT session (second group), we found that NK cells responded first to the local heating of the liver and showed a significant increase in activation already at 20 min after the beginning of the treatment.

It was demonstrated previously that HT-related effects may also be an important step in anticancer defense as augmented immune cell traffic and its enhanced responsiveness and stimulatory activity, shown in healthy volunteer study where skin was heated in a water bath of 44°C for 3 h (6). As shown *in vivo* for murine melanoma, local HT (43°C, 15 min) induced infiltration of NK cells into the tumor (29). In patients with esophageal cancer, infiltration of lymphocytes (IL) appeared to be a prognostic predictor after preoperative chemoradiotherapy, while in addition, simultaneous HT significantly stimulated IL (30).

In addition to the above-mentioned properties of NK cells, we have noticed that its activity may be associated with quality of life. Thus, those patients in

whom quality of life was demonstrated to improve by HT had also demonstrated remarkable activation of NK cells in the course of one HT session. In contrast, those who failed to recover QOL had little change in NK activity (data not shown). We have a special interest to continue research in this field having being encouraged by previous finding by others that NK activity in patients with anxiety about cancer was significantly lower than that in those with no such anxiety (31).

In conclusion, local HT stimulates cell-mediated host immunity, involving both innate responses such as NK cell activation, and adopted responses, such as change in CD4+, CD8+ subsets. These effects take place as early as during the HT treatment and remain through the 7-session treatment schedule, lasting up to 2 months. NK cells respond first to the elevation of temperature of the lower abdomen, including the liver. However, a larger study is warranted in order to exclude the influence of such parameters as age, sex, drug administration, and psycho-emotional conditions.

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