International Journal of Hyperthermia

ISSN: 0265-6736 (Print) 1464-5157 (Online) Journal homepage: informahealthcare.com/journals/ihyt20

The effects of artery occlusion on temperature homogeneity during hyperthermia in rabbit kidneys *in vivo*

Z. Q. Jia, A. E. Worthington, R. P. Hill & J. W. Hunt

To cite this article: Z. Q. Jia, A. E. Worthington, R. P. Hill & J. W. Hunt (1997) The effects of artery occlusion on temperature homogeneity during hyperthermia in rabbit kidneys *in vivo*, International Journal of Hyperthermia, 13:1, 21-37, DOI: 10.3109/02656739709056427

To link to this article: https://doi.org/10.3109/02656739709056427

INT. J. HYPERTHERMIA, 1997, VOL. 13, NO. 1, 21–37

The effects of artery occlusion on temperature homogeneity during hyperthermia in rabbit kidneys *in vivo*

Z. Q. JIA†, A. E. WORTHINGTON†‡, R. P. HILL†‡ and J. W. HUNT†‡*

‡Division of Experimental Therapeutics, Ontario Cancer Institute and †Department of Medical Biophysics, University of Toronto, 610 University Avenue, Toronto, ON Canada, M5G 2M9

(*Received;accepted*)

To investigate the role of arterial occlusion on temperature homogeneity during hyperthermia for deep seated tissue, a renal hyperthermia animal model has been established using New Zealand white rabbits. The effects of ultrasound-induced renal hyperthermia, with or without continuous and intermittent renal artery occlusion, were compared and analysed. Both continuous and intermittent occlusion showed certain protection of surrounding tissue and demonstrated improved temperature homogeneity and heating efficiency. The benefits of continuous *vs.* intermittent occlusion are compared and discussed as well.

Key words: Hyperthermia, renal, temperature distribution, ultrasound, blood flow, vascular occlusion, kidney, animal.

1. Introduction

Studies with cells in culture have shown that thermal damage is both temperature and time dependent. *In vivo* studies have also demonstrated a close relationship between the temperature distribution achieved and hyperthermia outcome (Dewhirst *et al.* 1984; Oleson *et al.* 1989, 1993, Leopold *et al.* 1993). Many factors can affect temperature homogeneity during hyperthermia *in vivo*, including the specific absorption rate (SAR) distribution of the applicator used to heat the tissue, differential absorption of energy by tissues, thermal conduction, and localized and regional blood flow. In many cases, blood-flow cooling may be the major factor in governing the treatment outcome. Both temperature increase and temperature distribution are flow-rate dependent (DeYoung *et al.* 1987, Hynynen *et al.* 1987, 1989). Any reduction in blood circulation alters heat transport, and thus changes the temperatures produced in tissues (Jain 1984, Reinhold 1986, Hahn 1987).

What makes blood-flow cooling particularly important is that blood-flow in tumours is often not uniform. Thus, even if a homogeneous SAR distribution could be obtained in the tumour volume, the temperature in the tumour would be non-uniform owing to heterogeneous energy removal by blood flow. Localized cool regions may be found in the treatment field where tumour cells may survive, leading to regrowth of the tumour. Studies by Jain (1984), Boddie *et al.* (1985a, b), Reinhold (1986), Hahn (1987), Dewhirst *et al.* (1990), Brown *et al.* (1992a), Prescott *et al.* (1992) and Levin *et al.* (1994) have demonstrated that if blood flow is reduced or blocked, a great improvement in temperature homogeneity can be achieved.

Since stopping blood circulation both improves the homogeneous temperature

*Please address correspondence to J. W. Hunt at ‡address.

distributions in superficial tumours and may have a synergistic effect during the treatments of superficial tumours, we hypothesized that the combination of the occlusion technique and hyperthermia for deep-seated organs can also produce the same or similar result. Several experiments on liver have been conducted which demonstrated improved efficacy for hyperthermia with hepatic artery embolization (Boddie *et al.* 1985b, 1986, Erichsen *et al.* 1985, Akuta *et al.* 1987).

This paper is to test our hypothesis that occlusion in hyperthermia for deep-seated tissue should allow improved temperature homogeneity, heating efficiency, and potential protection of surrounding tissue of deep-seated tumours. Occlusion of the renal artery was used in the model since the poorly perfused medulla, surrounded by a highly perfused cortex, mimics the vasculature pattern of many tumours (Endrich *et al.* 1979). A comparison of the effect of intermittent and continuous occlusion on tissue damage was also undertaken, to explore possible enhancement of the technique in terms of further decreasing its impact on normal tissue.

2. Materials and methods

2.1. *Experimental set-up*

Ten female New Zealand white rabbits weighing from 4·6 to 5·2 kg (average 4·7 kg) were used in the study. The animals were randomly divided into three groups, hyperthermia alone (H), hyperthermia with continuous occlusion of the renal artery (HO) and hyperthermia with intermittent occlusion of the artery (HIO) (see the note in Table 1 for the detailed protocols). The left kidney was usually selected for heating, while the right kidney was selected to carry out intermittent occlusion (IO), or continuous occlusion (O) without hyperthermia.

Under general anaesthesia induced by ketamine hydrochloride (40 mg/kg), xylazine (7·5 mg/kg) and phenobarbital sodium (25–30 mg/kg) premedication and maintained by halothane inhalation under continuous control, both kidneys and the renal arteries were exposed through a median incision. Type K thermocouples made of 64 μm twisted Chromel and Alumel wires (California Fine Wire, Grover City, CA), were welded, inserted into 27 guage 37 stainless steel needles (Decton Dickinson, Toronto, ON, Canada) and fixed by cyanoacrylate glue just inside the needle's point. Double thermocouples were inserted in 23 gauge needles and separated by 1 cm. As many of 14 thermosensors were implanted in the kidney. In addition, one thermocouple was inserted into the abdominal wall between the bolus of the applicator and the kidney, and for most animals a second thermocouple was implanted into the lumbar muscle behind the kidney. In order to reduce the effect of the intense distribution by ultrasound attenuation, all the thermocouples were inserted in the same plane and depth in the kidney, and perpendicular to the ultrasound beam (Figure 1).

A surgical tourniquet consisting of a piece of 1-0 surgical silk suture and a piece of polyethylene tubing (internal diameter 1·14 mm) was mounted around the renal artery close to its origin at the aorta. The abdominal contents, which had been gently pulled out of the abdomen cavity before the exposure of the kidney and the artery, were then replaced and an adequate amount of warm saline (at about 37°C) was infused into the cavity to drive out any trapped air. At least 15 min before the heating and/or occlusion of the renal artery 500 units/kg heparin was infused intravenously.

After the treatment procedure (Table 1) of either H, HO or HIO was completed on the target kidney, O or IO occlusion was carried out on the opposite kidney. The kidneys, surrounding tissues, and the locations of the thermocouple were then

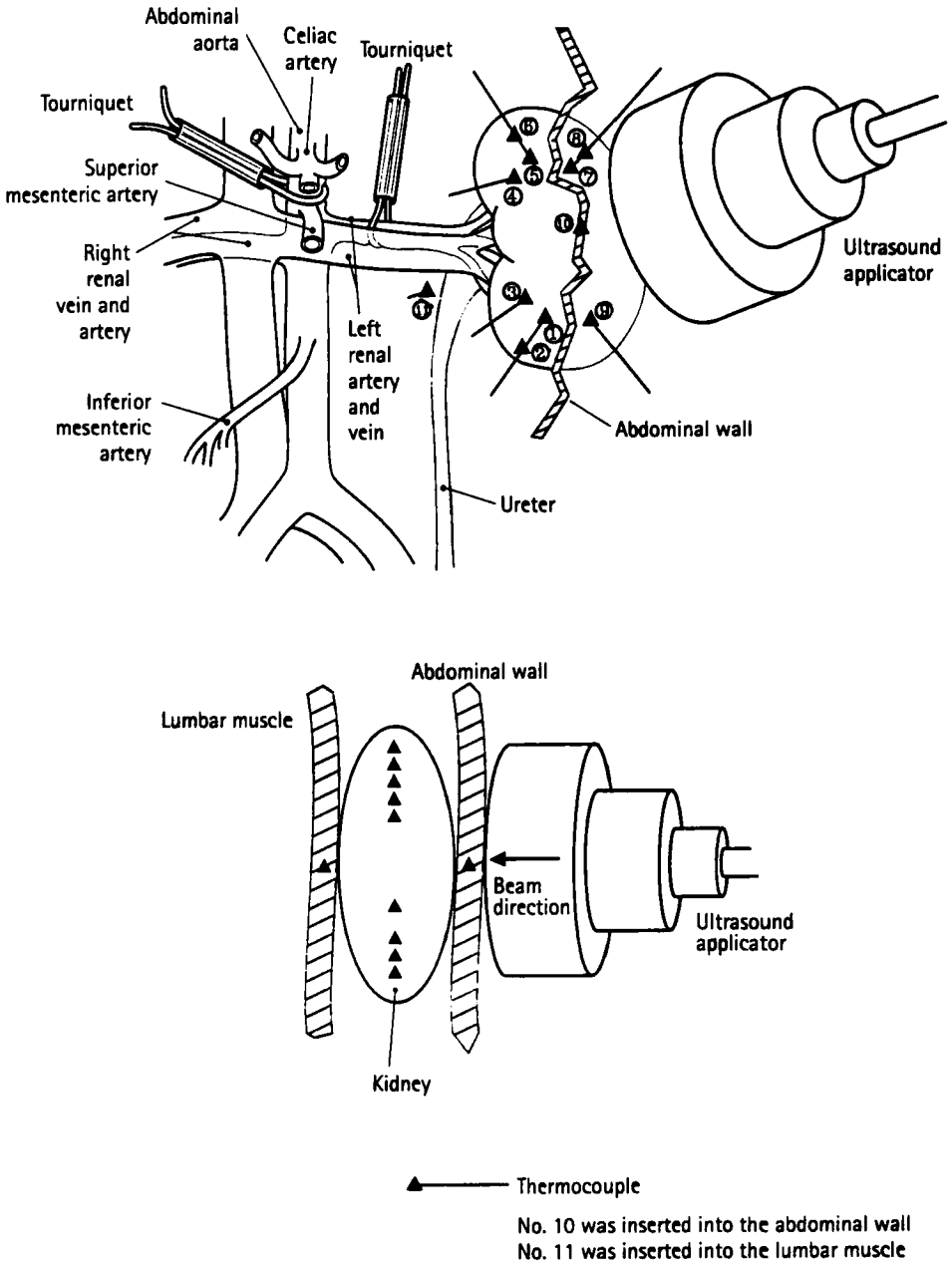

Figure 1. A typical experimental set-up. The upper section shows the arrangement of an ultrasound beam passing through the abdominal wall, the tourniquet used to occlude the renal artery, and the abdominal aorta. Up to 9 thermocouples, P1 to P9, are inserted in the kidney, while P10 and P11 are inserted in the abdominal wall and lumbar muscle respectively. The lower section describes the orientation of the thermocouples in the kidney at similar distances from the ultrasound applicator.

Table 1. Occlusion studies of the rabbit kidney during hyperthermia treatments.

Animal number Left = L, Right = R		Experiment protocol (See footnote)	Muscle temperature measurement	Kidney size (cm)
1	L	HIO	No	4×3×2
	R	O		4×2·8×1·8
2	L	HIO	No	4·5×3×2·3
	R	IO		4·5×3×2·3
3	L	HIO	No	Not available (NA)
	R	IO		NA
4	L	HIO	No	3·7×2·5×1·2
	R	O		3·7×2·8×1·5
5	L	HO	Yes	3·5×2·5×2
6	L	HO	Yes	3·8×2·8×2·2
	R	O		3·5×2·5×2·0
7	L	H	Yes	NA
	R	IO		NA
8	L	HO	Yes	3·8×2·5×2·3
	R	IO		NA
9	L	HIO	Yes	3·7×2·7×2·0
10	L	H	Yes	4·0×3·0×2·0
	R	O		NA

H: The target temperature was 41–43°C, to be maintained at this level for 12 min. (Owing to the extreme difficulty in achieving this temperature in the kidney and an unacceptably high temperature in the surrounding tissue, only two animals were studied using this protocol).

HO: The kidney was heated close to the target temperature, and then a tourniquet was applied and the power level adjusted to maintain the temperature within 41–43°C for 12 min.

HIO: The kidney was heated close to the target temperature then a tourniquet was applied for 3 min. The power was adjusted to maintain the temperature within the target temperature range; the occlusion was released for 4 min with the power on. These steps were repeated three more times so that the total occlusion time was also 12 min.

IO: Similar to the procedure of HIO except that there was no heating.

O: Similar to HO but no heating.

examined grossly and the finding recorded. Finally the animal was sacrificed using the respiratory inhibitor, T61 (DIN 00294039, Hoechst, Canada Inc.), injected intracardiacly or intravenously, and the kidneys were harvested and fixed immediately with 10% formalin to prepare for histological examination. During the entire period of an experiment a heating pad and a heating lamp were used to maintain animal body temperature.

2.2. *Heating technique*

Hyperthermia was performed using a home-made ultrasound applicator with a planar PZT4 transducer (5 cm diameter), driven at a frequency of 1·43 ± 0·058 MHz. A Hewlett Packard 8656A frequency synthesizer (Palo Alto, C. USA) provided the frequency modulated sine wave which was amplified by an EN 300A (Electronic Navigation Instruments, Rochester, NY, USA) 300 Watt (W) linear amplifier. The applicator was oriented so that the centre of the kidney was located on the axis of the ultrasound beam. Acoustic gel was used to couple the water bolus of the applicator and the shaved abdominal skin (Figure 1). The bolus temperature was maintained at 37–38°C.

2.3. *Temperature monitoring*

During the whole period of an experiment the temperatures in the kidney, the surrounding tissue and the rectum were recorded continuously using a multifunction 16 channel data acquisition system (Labmate, Sciemetrics, Nepean, ON, Canada). The accuracy of the system was $\pm 0{\cdot}1$°C and it was stable with time (Brown *et al.* 1992b for further details). Before each individual experiment, all thermocouples to be used in the experiment were calibrated in a waterbath with a secondary standard glass mercury thermometer (Fisher, Canada).

2.4. *Data analysis*

Three parameters were employed to analyze the thermal data. Coefficient of variation (CV), heating efficiency factor (HEF °C/W)), and cooling coefficient, $k_c(sec^1)$. The CV is the standard deviation of all the temperatures (at equilibrium state in HO group and at maximum temperature in H and HIO groups) measured in the kidney divided by the mean temperature increase. This measure reflects the homogeneity of the temperature distribution. The HEF is the mean of the maximum temperature increase for all thermocouples in the kidney divided by the power employed during the heating. Thus HEF reflects the heating efficiency for a certain excitation power. According to our protocol, the average temperature in the kidney was not allowed to go >43°C. Therefore an equilibrium state was not possible without adjusting the electrical output accordingly, especially in HO experiments. Consequently, an estimated maximum temperature increase (used to calculate the HEF value) was extrapolated for both heating with and without the occlusion, using a modified program described by Provencher (1976) and Brown *et al.* (1988).

The cooling coefficient, k_c, is approximated from the equation:

$$(T - T_{\min}) = (T_{\max} - T_{\min})e^{-k_c t}$$

where T is the temperature of the kidney, $T_{\min}$ is the temperature at the equilibrium state after blood flow was restored and power was turned off, and $T_{\max}$ is the temperature at the moment just before the blood flow was restored (for HIO) and/or the power was turned off (for HO/H). The k_c value reflects local tissue cooling caused by blood perfusion or blood perfusion and conduction between the kidney surface and the surrounding tissue after the power was turned off (Roemer 1990).

The Mann-Whitney rank test was employed to compare the CV from the H, the HO, and the HIO experiments. The Student t Test was used to analyse HEF and k_c from H, HO as well as the k_c from different occlusion cycles in the HIO group.

2.5. *Histological examination*

Four pieces of tissue from the identical parts of each kidney were prepared and examined under light microscopy. Three main histological changes were observed: congestion, cloudy swelling and hydropic degeneration. The findings were graded into one of five classes (according to the presence and extent of the findings) such that '$-$' = no effect, '$+/-$' = questionable, and '$+$', '$++$', and '$+++$' = increasing significance. A maximum rank of the individual scores was selected from those of four slides from each animal's kidney, then the Mann-Whitney rank test was used to analyse the findings.

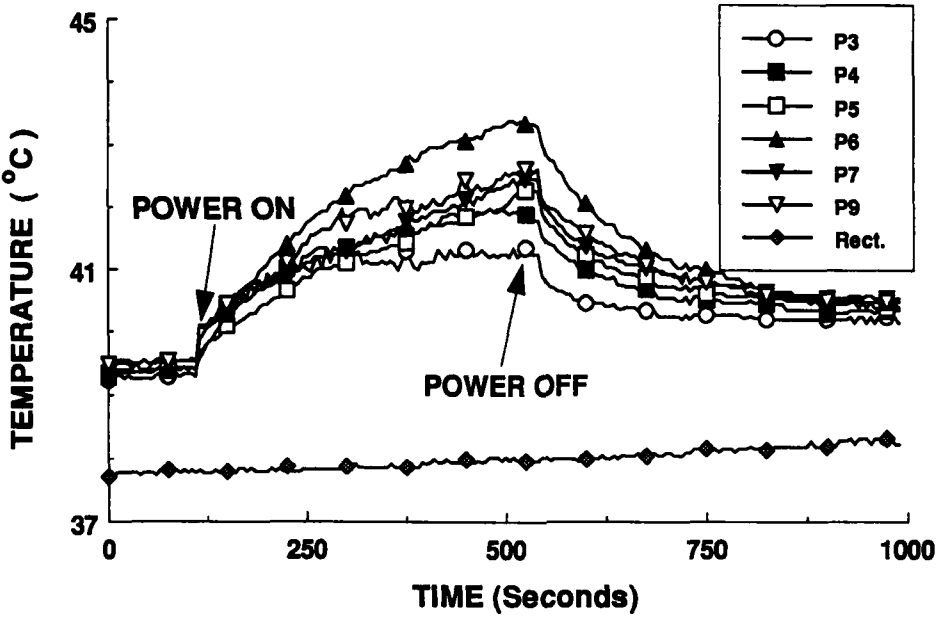

Figure 2. An example of heating without occlusion using 100W electrical power. When the power was turned on, temperatures in the kidney (P3 through P9) increased synchronously but soon diverged. By the time the power was turned off, the temperatures fell in a range of about 2°C with some of them already at an equilibrium state (P3 and P4) and the rest still rising.

3. Results

3.1. *Temperature homogeneity under different protocols*

When heated without occlusion the temperatures of different regions of the kidney initially rise synchronously. But, as seen in Figure 2, the temperatures soon diverge. At approximately 300 s, the thermocouple at position (P3), which was located in the medulla close to the hilus of the kidney (where it would be near large vessels) reached equilibrium temperature. Meanwhile the temperature at P6, which was located in the upper pole of the kidney close to the outer region of the cortex, continued to increase until the power was turned off. The difference between the maximum and minimum temperature at that time was > 2°C. As might be expected, after the power was turned off, the thermocouple at P3 went down to the equilibrium state first and that at P6 last.

In contrast, the thermal profile for heating with occlusion demonstrated a quite different pattern. As seen in Figure 3, the renal artery was occluded (downward arrow labelled as 'occlusion on') soon after the power was turned on (upward solid triangle, 120 W). Although the electric power output from the ultrasound amplifier was decreased repeatedly (from 120 to 40 W) the kidney temperatures kept rising until the power was decreased to 20 W at which point an equilibrium state was reached. The most striking observation was that the temperatures in different regions of the kidney fell in a narrow range of < 1°C; a range much smaller than that at the start of the occlusion, and much less than that seen in Figure 2 (a temperature range of > 2°C). After the power was turned off and the occlusion was released, a temperature decay profile similar to that in Figure 2 was observed.

The coefficient of variation (CV) values from five heating-without-occlusion

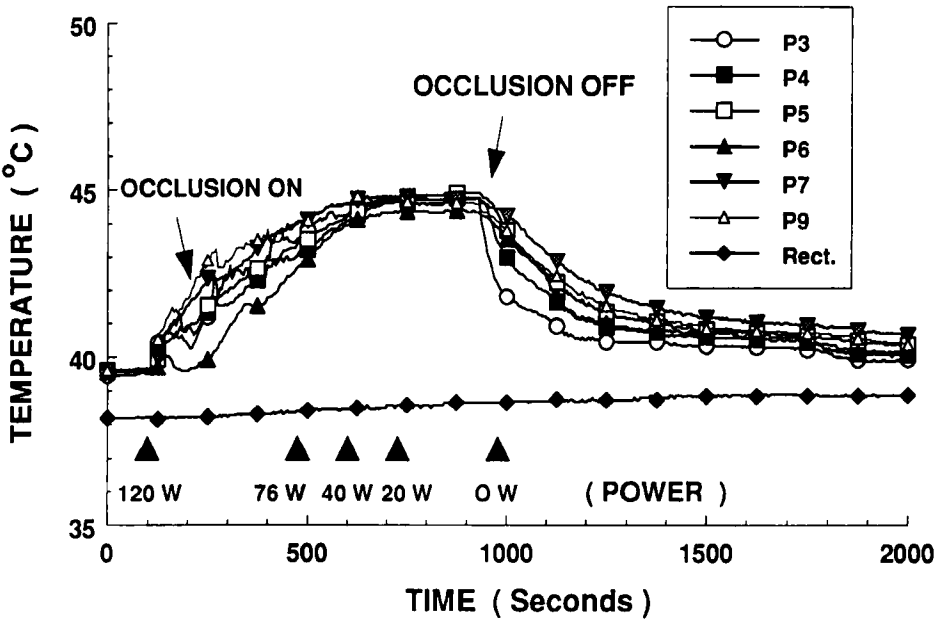

Figure 3. An example of hyperthermia with continuous occlusion (HO). The solid triangles mark the start of the power level specified. Shortly after the power was turned on (120 W), the renual artery was occluded. The kidney target temperature of 41–43°C was reached quickly and it was necessary to repeatedly decrease the power level down to 20 W so that the temperatures in the kidney did not too greatly exceed the target temperature. At this final power level, the temperatures all reached equilibrium with a spread of less than 1°C.

experiments (including data from H and data from preheating experiments† in either HO or HIO groups), three heating-with-continuous-occlusion experiments, and three HIO experiments were compared. The means (± S.E) of the CV values for H, HO and HIO were 0·37 ± 0·17, 0·08 ± 0·01, and 0·23 ± 0·03 respectively. The difference is statistically significant between H and HO. The CV values from different cycles of HIO were also compared and analyzed; there was no significant difference among the cycles (Figure 4).

3.2. *Heating efficiency of simple hyperthermia and that of hyperthermia with occlusion*

Figure 5 shows the results of an experiment in which the kidney was first heated without occlusion of the renal artery (see first △). After the power was turned off (first ▽) and the temperatures had returned to the equilibrium state, the kidney was heated again (second △) and, about 3 min later, the artery was occluded (▲). Twelve minutes later the occlusion was released (▼). The increase of the temperatures in the kidney during hyperthermia alone (the first heating and second heating before the occlusion) was slower than that under hyperthermia with occlusion (second heating after the occlusion). In the first heating, though for a shorter period of time (about 4 min after the power was turned on), an equilibrium state had already been reached for the thermocouple at P3, and for the other two thermocouples equilibrium was

†During HIO or HO, the kidney was heated initially up to 41°C to facilitate the HO and HIO, and to supply additional thermal information. It was assumed that under this temperature there would not be any damage to the normal vasculature.

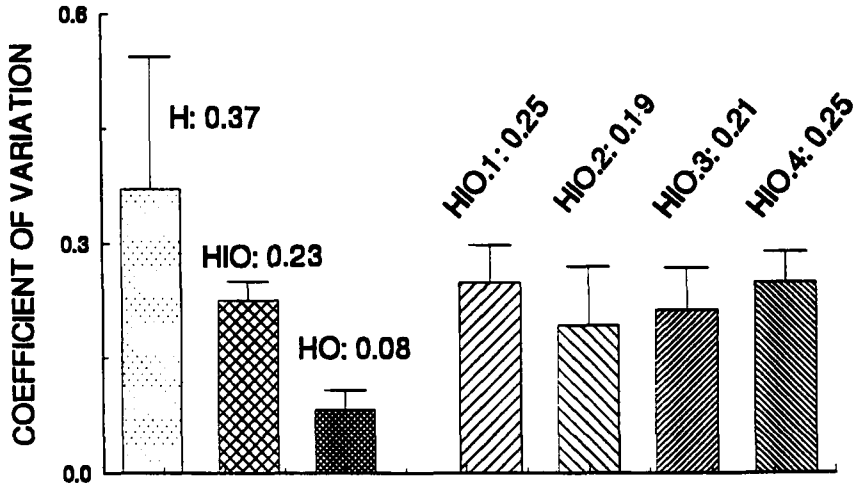

Figure 4. Coefficients of variation for H, HIO, HO and each cycle of occlusion in HIO (HIO.1, HIO.2, HIO.3 and HIO.4). The CV for H was obtained from one H experiment and four preheating experiments (note 2 in §3.1) in either HO or HIO. The CV for HO was from three HO experiments. The CV for HIO was from three HIO experiments. The CVs for each cycle of HIO were from corresponding cycles in the same HIO experiments from which the CV for HIO was obtained. The difference between HO and H was statistically significant ($P < 0{\cdot}05$, Mann-Whitney test).

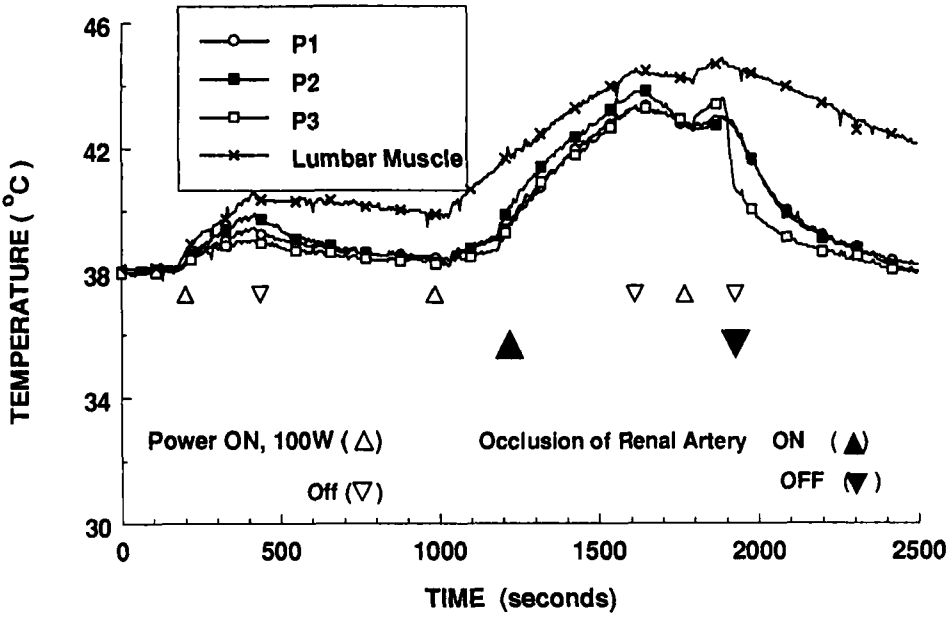

Figure 5. A example of an HO experiment demonstrating different heating efficiency of H and HO at the same power (100 W). The first heating without occlusion (t = 200 s to t = 400 s) demonstrated a slower temperature increase than the first heating with occlusion (t = 1200 s to t = 1600 s). At t = 1600 s the power had to be switched off temporarily to keep the kidney temperature from getting too high. The second heating without occlusion (t = 1000 to t = 1200) showed an even slower temperature increase than did the first one (t = 200 s to t = 400 s). Also, under heating without occlusion, the temperature increase in the lumbar muscle (the curve labelled with 'x') was faster than that in the kidney. While under heating with occlusion, similar temperature increases were observed.

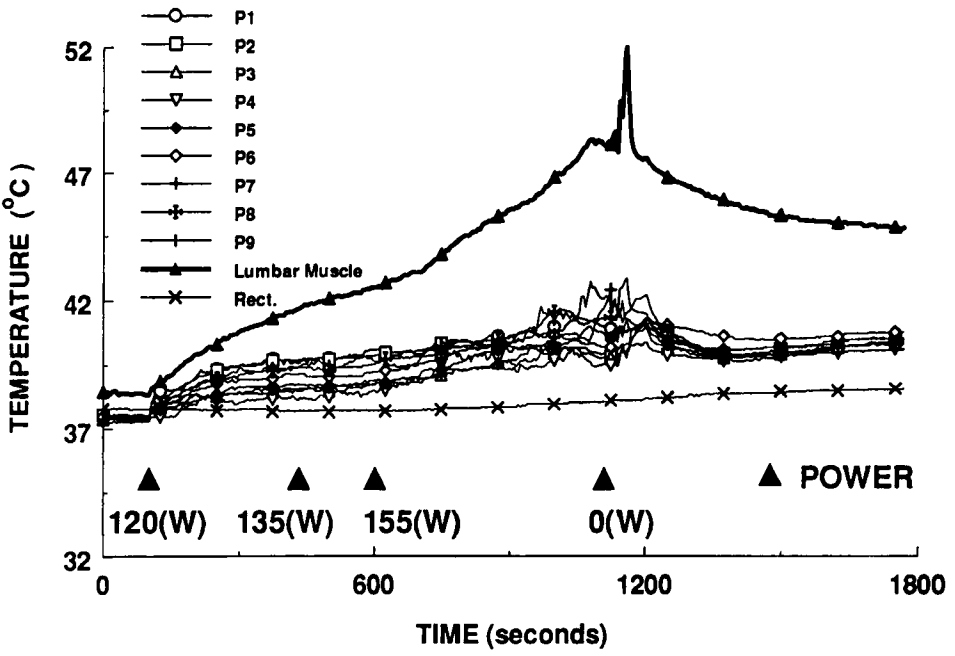

Figure 6. An example of an H experiment. Although the power was increased a number of times, the temperatures in the kidney ('P1' through 'P9') did not increase significantly. The temperature in the lumbar muscle, however, increased so high that the experiment had to be stopped.

being approached. However, for heating with occlusion, under identical power the temperatures increased quickly and continuously until the power had to be interrupted to keep the average temperature of the kidney $< 43°C$.

The means ($\pm$ S.E.) of the HEF values were $0{\cdot}04 \pm 0{\cdot}01$ (°C/W) for the hyperthermia alone experiments (derived from six heating alone experiments, including those from preheating experiments in either HO or HIO group); and $0{\cdot}10 \pm 0{\cdot}01$ (°C/W) for the hyperthermia with occlusion experiments (derived from eight occlusion with hyperthermia experiments, including those from both HO and HIO). This difference is statistically significant, $P < 0{\cdot}01$.

3.3. *Temperature change in the kidney and in the surrounding tissue*

A lower temperature in the lumbar muscle behind the kidney was observed during hyperthermia with either continuous or intermittent occlusion compared to that in the hyperthermia alone group. In the H experiment, the temperature of the lumbar muscle reached as high as 52·5°C when the target temperature in the kidney was sustained between 41 and 43°C. In the HO and HIO groups the temperature of the muscle was < 45 and 42°C respectively when the same kidney temperature was obtained. A typical H experiment is shown in Figure 6. After the power was turned on (first ▲), the temperature of the kidney increased initially but soon reached an equilibrium state. Although the power was increased repeatedly, the temperatures inside the kidney increased only slightly. In contrast, the temperature in the lumbar muscle increased dramatically. In Figure 5, after the renal artery was occluded the target temperature in the kidney was reached very soon and the power level had to be turned off temporarily, which stopped the muscle temperature from continuing to rise.

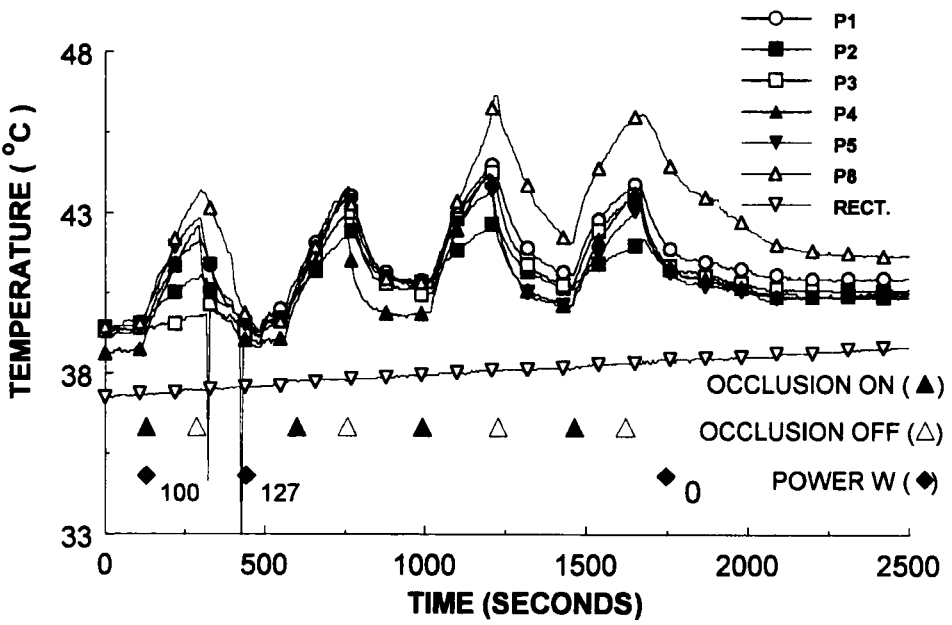

Figure 7. An example of hyperthermia with intermittent occlusion (HIO). During heating, the renal artery was occluded for 3 min and released for 4 min, and this procedure was repeated 4×. It was noticed that the temperature at P8 demonstrates an obviously decreased temperature decay after the third and fourth cycle of occlusion. This reflects a decreased local blood perfusion, which might be caused by either thermal change to local microvasculature or embolization of a local arteriole.

3.4. *Comparison of cooling coefficients*

The cooling coefficient (k_c) value for H experiments‡ was $(3{\cdot}7 \pm 0{\cdot}6) \times 10^3\ \text{sec}^{-1}$. The k_c value for HO* was $(3{\cdot}3 \pm 0{\cdot}4) \times 10^{-3}\ \text{sec}^{-1}$. The two sample t tests demonstrated no significant difference between the k_c for the H and HO groups ($p > 0{\cdot}05$). For HIO, the mean k_c values from individual thermocouples were calculated from four experiments for each cycle of occlusion (Figure 7). Then the total k_c for all cycles was calculated as the mean of those values for k_c from individual experiments. These values were then analysed using the two sample t tests. The k_c (± S.E) in first cycle of the occlusion in HIO was $(6{\cdot}3 \pm 0{\cdot}9) \times 10^{-3}\ \text{sec}^{-1}$ and significantly larger than those for H and HO, but it decreased with each subsequent cycle ($(5{\cdot}4 \pm 0{\cdot}8) \times 10^{-1}\ \text{sec}^{-1}$, $(4{\cdot}4 \pm 0{\cdot}8) \times 10^{-3}\ \text{sec}^{-1}$, and $(3{\cdot}2 \pm 0{\cdot}4) \times 10^{-3}\ \text{sec}^{-1}$ for the second, third and fourth occlusion cycles respectively). The k_c for the last cycle was not significantly different from those for H and HO (Figure 8).

3.5. *Histological findings*

A gross view did not show any obvious abnormality except that the kidney was

‡From four heating-alone experiments, including one from H, one from preheating in HIO and two from preheating in HO; the mean of the k_c values from each individual thermocouple in each experiment was determined, then the mean of these means was calculated.

*The k_c value for HO was derived from four HO experiments in a similar manner to that used to obtain the k_c values for H.

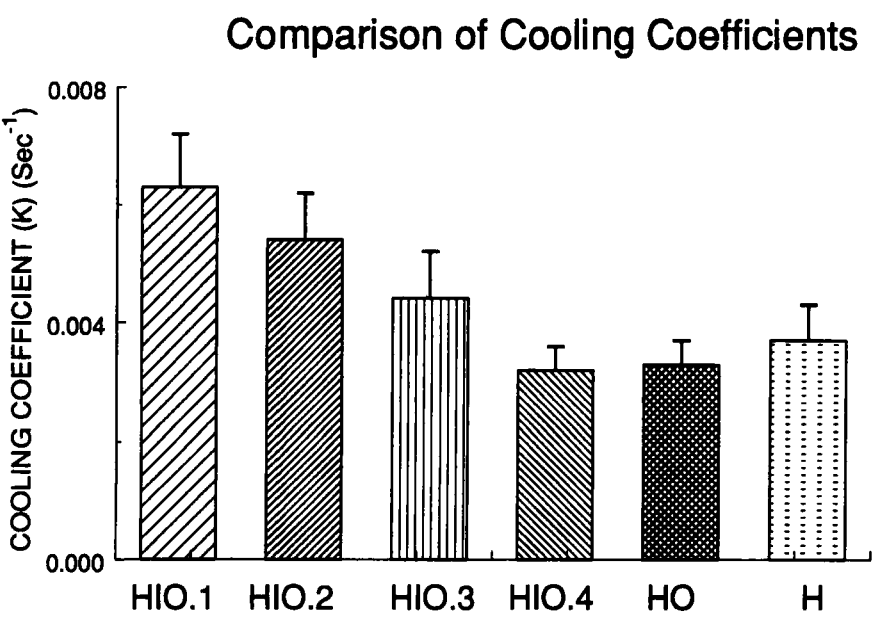

Figure 8. Cooling coeficients (k_c) for HIO (HIO.1, HIO.2, HIO.3 and HIO.4 representing the k_c for each cycle of the occlusion from 3 HIO experiments), HO (derived from 4 HO experiments), and H (from four heating alone experiments, including one form H, one from preheating prior to HIO and two from preheating prior to HO). Note that the k_c for the first cycle of occlusion in HIO was larger than those for HO and H, but it decreased with each subsequent cycle, so that the k_c for HIO.4 was not significantly different from those for HO and H.

Table 2. Histological data analysis.

	Congestion					Cloudy swelling					Hydropic degeneration				
Group	H	IO	O	HIO	HO	H	IO	O	HIO	HO	H	IO	O	HIO	HO
Number of specimen	2	4	3	5	3	2	4	3	5	3	2	4	3	5	3
Rank															
I(−)															
II(+/−)															
III(+)		2		1	3				2	1	1	3	2	4	3
IV(++)	2	2	3	4		2	4	2	3	2	1	1	1	1	
V(+ + +)								1							
Median	4	3·5	4	4	3	4	4	4	4	4	3·5	3	3	3	3

slightly swollen. Under microscopic examination, expansive congestion existed, and cloudy swelling and hydropic degeneration appeared sporadically on the specimens examined. There was no significant difference (the Wilcoxon rank sum test) between kidneys treated with HO and HIO (Table 2).

4. Discussion

4.1. *Deletion of blood flow cooling is the main reason for the improved temperature homogeneity*

In this study we have shown that renal artery occlusion combined with renal hyperthermia improves temperature homogeneity. Theoretically, when heating without occlusion, the energy deposited would be dissipated more effectively in regions closer to large vessels than in regions farther away from such vessels. Therefore, temperature heterogeneity would develop as demonstrated in Figure 2. In the clinical practice of hyperthermia without occlusion, highly perfused regions such as that around thermocouple 'P3' in Figure 2 might be under-heated and cause regrowth of tumour. Also, poorly perfused regions, such as that around thermocouple 'P6' in Figure 2, might form 'hot spots' leading to patient pain and to potential complications. In contrast, under conditions of occlusion, only the differential intensity attenuation in the kidney itself (Sarvazyan *et al.* 1983) and thermal conductivity occurring mainly between the surface of the kidney and the surrounding tissues would contribute in heterogeneous temperature distribution. In our experimental protocol, all the thermocouples were inserted at the same depth with respect to the ultrasound applicator in the attenuating tissues, thus the improvement of the temperature homogeneity obtained here mainly reflects a change of temperature homogeneity in one plane. The temperature homogeneity for different planes can also be improved as we have demonstrated using a kidney phantom. (Jia *et al.* 1994, 1995).

4.2. *Occlusion increases heating efficiency for the target organ*

A difference of a factor of 2·5 in the HEF between the occlusion and non-occlusion experiments clearly demonstrates that occlusion can make heating of the target organ more efficient. This is as expected and agrees with the study of Hynynen *et al.* (1989), where a difference factor of five was observed when the normalized temperature index (which we call HEF) was compared between temperature increase under fully perfused and non-perfused conditions. A similar heating efficiency improvement was also observed by Levin *et al.* (1994) when a tourniquet was applied to a patient's leg. An increased heating efficiency means shorter time and/or lower power will be needed to reach the target temperature. Thus, side effects such as skin burns and unexpected overheating of overlapping tissue (discussed below) would be less likely to occur.

4.3. *Occlusion can decrease unwanted heating of the surrounding tissue*

The prolonged duration of heating caused the temperature in the lumbar muscle to rise to a much higher level in the hyperthermia alone experiments (Figure 6). The difference between the lumbar muscle temperatures during heating with and without the occlusion, however, was due to the difference between the blood perfusion of the kidney and that of the muscle. For example, the blood perfusion rate for resting skeletal muscle is from 2 to 20 ml/min/100 g, while that for kidney can be from 350 to 550 ml/min/100 g (Rowell 1974). This differential blood flow would result in poorly perfused surrounding tissues, such as the lumbar muscle, being overheated by the time the target temperature in the kidney was reached. Under occlusion conditions, this differential effect would be diminished.

This protection of surrounding tissues with lower blood perfusion than the target organ could have significant importance in clinical hyperthermia because, for deep-seated tumours, tissues with different perfusion rates are likely to overlap each other

in the heating beam. Often, overheating of the surrounding tissue can be a limiting factor for regional hyperthermia (Feldmann 1991). The fact that the lumber muscle was situated behind the kidney where the ultrasound intensity would have been attenuated further emphasizes the importance of the potential protection by the occlusion technique for surrounding tissues during hyperthermia.

4.4. *Damage to the renal artery*

The unexpected change of the cooling coefficient with the number of occlusions in the HIO groups (Figure 7, 8) could be due to the reaction of normal tissue to increased blood perfusion in response to the increased surrounding temperatures. Also, the development of acidic conditions caused by temporary occlusion would cause local blood vessels to dilate. Both of these effects would lead to an increased renal artery perfusion after the release of the occlusion, and would explain the significantly increased cooling coefficient after the first cycle of the occlusion. Alternatively, there might have been damages to or spasm of the renal artery or embolization of its branches due to the repeated use of the surgical tourniquet. This could eventually decrease perfusion. This possibility is supported by the following observations: (a) In a non-heating experiment, we measured the perfusion rate of the kidney before and after occlusion of the aorta or renal artery respectively. We found that, after release of the occlusion of the renal artery, the perfusion rate did not reach the pre-occlusion level for a substantial period of time; (b) in an HIO experiment we observed an obvious decrease in the rate of temperature decay in one region of the kidney (Figure 7, thermocouple 'P8') relative to that in other regions. This suggests damage to localized blood perfusion caused either by the embolism of a local arteriole or by hyperthermia damage to local vasculature.

The lack of difference between the cooling coefficient for the last cycle of the occlusion in HIO and that for HO was noticed. Repeated use of the tourniquet, which could be a main reason for the damage to the renal artery during HIO, was not the case in HO, but a much longer continuous occlusion as well as a higher average temperature in the kidney throughout the occlusion might have impaired renal blood circulation and resulted in thermal decay similar to that seen at the end of HIO.

These observations indicate the likelihood that damage was caused by the occlusion technique itself which was at least partially responsible for the reduction of the renal blood perfusion. It is anticipated that this damage would be significantly reduced if a balloon catheter, instead of a tourniquet, is used. Studies in interventional radiology, especially the employment of balloon catheters in angioplasty and therapeutic embolization, have indicated that selective transient occlusion of arteries in deep-seated organs is technically feasible and reliable (Gerlock and Mirfakhraee 1985). It can be applied to almost all major arteries and their branches (Gerlock and Mirfakhraee 1985). Theoretically, it can be easily and more safely (compared with the use in angioplasty where over-dilation of pathologically narrowed vessels is necessary) used in hyperthermia treatment to block the blood supply and thus to facilitate hyperthermia. Furthermore, transcatheter infusion of anticancer drugs to treat malignant disease has been practiced for many years all around the world (Coldwell and Mortime 1989). Therefore it would be possible to combine local chemotherapy, ischemia, and hyperthermia together to treat malignant tumours.

4.5. *Intermittent versus continuous occlusion during hyperthermia*

In clinical practice there are more cases where the tumours are supplied by

multiple blood vessels than by single ones, such as colon cancer, malignant disease in pelvic organs and metastatic diseases. Consequently, a regional, instead of a localized, blockage of blood supply (such as transient occlusion of the abdominal aorta for treatment of abdominal tumours) would be necessary to facilitate hyperthermia treatment. Thus during the combination of hyperthermia with occlusion the importance of protection for normal tissues cannot be over-emphasized. Baker and Wright (1983) and Morris *et al.* (1977) reported that the incidence of heat-induced necrosis in the feet or tails of rats and mice respectively was increased by obstruction of the blood supply. However, Baker and Wright (1983) also reported that when intermittent occlusion of the blood supply to the tumour in the legs or tails of the mice was employed with hyperthermia a high tumour cure rate was maintained with damage to normal tissue virtually eliminated.

We had expected that HIO would cause less damage to kidney tissue than HO. However, our study was not able to show this difference histologically. Several factors could explain this result. First, there is the uncertainty of the release of the occlusion after each cycle during HIO, which could have prolonged the effective total occlusion time of the renal artery for HIO relative to that for HO. A second possibility could be that reperfusion injury might cause more damage with HIO than with HO. During ischemia Xanthine dehydrogenase, a NAD^+ dependent dehydrogenase (XDH), is converted to the oxidant-producing Xanthine oxidase (XO) (Parks *et al.* 1988). Xanthine oxidase has the ability to generate H_2O_2 and O_2^- during the oxidation of hypoxanthine or xanthine. Then, through their interaction (Haber-Weiss reaction) another potent oxidizing agent, the hydroxyl radical ($\cdot OH$), is formed. The result of oxygen radical formation is damage to an entire array of biomolecules found in tissues (Weiss 1986). This conversion is proportional to the duration of ischemia. A burst of oxidant formation takes place immediately after reperfusion and lasts for 2–5 min (Zimmerman and Granger 1992). Since there might be more chances for oxidants to form during HIO than during HO, reperfusion might be expected to cause more damage to kidney tissue in HIO than in HO.

Several other possibilities should also be considered, including the fact that the size of the sample may not be large enough and the method employed to assess damage may not have been sensitive enough or suitable to distinguish differences which existed. In terms of temperature homogeneity, HIO seems better than H but poorer than HO, though none of the differences were statistically significant. One of the differences between HIO and HO was that during HO the equilibrium state was obtained when the target temperature was reached and the power adjusted, while during HIO this equilibrium state was lacking owing to the very short period of occlusion. Thus the following protocols could be worth considering.

4.6. *What is the feasibility for a combination of occlusion and short heating?*

From our studies described here, and those by Levin *et al.* (1994), it seems that the best way to induce a more homogeneous temperature distribution is to apply complete occlusion in the treatment field. As discussed in §4·5, in clinical practice, a regional instead of localized occlusion might often be required. Occlusion combined with conventional heating, HO, as noted in § §3·1 to 3·3, might be too long to avoid normal tissue from being damaged. A differential reaction to increased temperature and accumulated wastes of normal tissue from that of malignant tissue might give normal tissue some advantage to lessen the damage. However, increased vulnerability to ischemia under hyperthermia has to be considered seriously. According

to Lieberthal *et al.* (1988), 1 min normal-thermic ischemia is equivalent to 40 min hypothermic (27°C) ischemia in terms of histological change of kidney tissue. It can be expected that under hyperthermia (42–45°C) the tolerance of normal tissue to ischemia will be dramatically decreased.

One possibility to improve temperature homogeneity in HIO, as has been implied by the comparison of the protocol between HIO and HO in § 4·5, could be to combine intermittent occlusion with more rapid heating techniques (Hunt *et al.* 1991). This might result in both homogenous temperature distribution and diminished impact of the hyperthermia ischemia on the surrounding tissues. Rapid heating (e.g. less 1 min) makes it possible for tissue temperature to reach the target level in a very short time and subsequent adjustment of the power would lead to an equilibrium with a good temperature homogeneity. On the other hand, occlusion makes more efficient heating, and subsequently, the power would be lowered and/or the time would be shortened to reach the target temperature, which is helpful to diminish the impact of the technique on the surrounding tissues. A limitation of this technique is the necessity of scanning the beam through the treatment field because of the small size of the high intensity region of the focused ultrasound beam (ter Haar *et al.* 1991, Hynynen *et al.* 1993).

An alternative proposal is to use an intermediate treatment time (e.g. ~4 min) and higher temperature (e.g. 48°C), which would be long enough so that the temperature plateau can be reached in the treatment volume while the surrounding tissue, which might have lower blood perfusion, would not be overheated. As well, this longer treatment time allows a significantly reduced intensity for the hyperthermia source, thus larger treatment fields can be used effectively.

5. Conclusion

In summary, we have demonstrated that apart from increasing heating efficiency, transient ischemia induced by occlusion of the renal artery during heat treatment can improve renal temperature homogeneity. Equally important, the occlusion can effectively avoid overheating of surrounding tissue, which could be very significant to clinical practice. Employment of intermittent occlusion and rapid heating might demonstrate better protection of normal tissue than continuous occlusion. More sensitive methods need to be explored to address ischemia and reperfusion injury in hyperthermia.

Acknowledgements

We would like to acknowledge the financial support of the Ontario Cancer Treatment and Research Foundation, and the National Cancer Institute of Canada. We would also like to acknowledge the support of M. Barber, Animal Facility Manager, Wellesley Hospital, and Drs J. Douglas and Badru Moloo, Division of Comparative Medicine, University of Toronto on the *in vivo* animal investigations. As well, we would like to thank Drs W. Levin and M. Sherar, and also M. Hoff for their valuable suggestions and corrections in the writing of this paper.

References

Akuta, K., Hiaoka, M., Jo, S., Ma, F., Nishimura, Y., Takahashi, M., Abe, M., Malmqvist, M., Lindbom, L. O. and Lindblom, R., 1987, Regional hyperthermia combined with blockade of the hepatic arterial blood flow by degradable starch microspheres in pigs, *International Journal of Radiation Oncology, Biology and Physics*, **13**, 239–242.

BAKER, G. M., and WRIGHT, E. A., 1983, Treatment of mouse mammary tumours using combined hyperthermia and ischemia. *Cancer Research*, **43**, 3392–3397.

BODDIE, A. W. Jr., WRIGHT, K., FRAZER, J. W., STEPHENS, L. C., MONTGOMERY, L., MCBRIDE, C. M., WALLACE, S. and MARTIN, R. G., 1986, Mechanisms of synergism between arteriolar embolization and hyperthermia in a rabbit V-2 model of solitary hepatic metastasis. *Cancer Research*, **46**, 4576–4581.

BODDIE, A. W. Jr., WRIGHT, K., FRAZER, J. W., STEPHENS, L. C., MCBRIDE, C. M., WALLACE, S. and MARTIN, R. G., 1985a, Selective occlusion and focal hyperthermia of V-2 tumours in the rabbit hind-limb. *Investigative Radiology*, **20**, 736–741.

BODDIE, A. W. Jr., WRIGHT, K., STEPHENS, L. C., YAMANASHI, W. S. C., FRAZER, J., MCBRIDE, C. M., WALLACE, S. and MARTIN, R. G., 1985b, An animal model of occlusion-hyperthermia of the liver. *Investigative Radiology*, **20**, 159–165.

BROWN, S. L., HUNT, J. W. and HILL, R. P., 1988, A comparison of the rate of clearance of xenon(^{133}Xe) and pertechnetate ion ($^{99m}TcO_4^-$) in murine tumours and normal leg muscles. *Nuclear Medicine and Biology*, **15**, 381–390.

BROWN, S. L., Hunt, J. W. and HILL, R. P., 1992a, Differential thermal sensitivity of tumour and normal tissue microvascular response during hyperthermia. *International Journal of Hyperthermia*, **8**, 501–514.

BROWN, S. L., LI, X. L., PAI, H. H., WORTHINGTON, A. E., HILL, R. P., and HUNT, J. W., 1992b, Observations of thermal gradients in perfused tissues during water bath heating. *International Journal of Hyperthermia*, **8**, 275–288.

COLDWELL, D. M., and MORTIME, J. E., 1989, Transcatheter therapy for malignant neoplasms. *Western Journal of Medicine*, **151**, 299–303.

DEWHIRST, M. W., PRESCOTT, D. M., CLEGG, S., SAMULSKI, T. V., PAGE, R. L., THRALL, D. E., LEOPOLD, K., ROSNER, G., ACKER, J. C., and OLESON, J. R., 1990, The use of hydralazine to manipulate tumour temperatures during hyperthermia. *International Journal of Hyperthermia*, **6**, 971–83.

DEWHIRST, M. M., SIM, D. A., SAPARETO, S., and COOER, W. G., 1994, The importance of minimum tumour temperature in determining early and long term responses of spontaneous pet animal tumours to heat and radiation. *Cancer Research*, **44**, 43–50.

DEYOUNG, D. W., KUNDRAT, M. A., and CETAS, T. C., 1987, *In vivo* kidneys as preclinical thermal models for hyperthermia. *IEEE/Ninth Annual Conference of the Engineering in Medicine and Biology Society*, pp. 994–996.

ENDRICH, B., REINHOLD, H. A., GROSS, J. F., and INTAGLIETTA, M., 1979, Tissue perfusion inhomogeneity during early tumour growth in rats. *Journal of National Cancer Institute*, **62**, 387–395.

ERICHSEN, C., BOLMSJO, M., HAUGANDER, A., and JONSSON, P., 1985, Blockage of the hepatic-artery blood flow by biodegradable microspheres combined with local hyperthermia in the treatment of experimental liver tumours in rats. *Journal of Cancer Research and Clinical Oncology*, **109**, 38–41.

FELDMANN, H. J., MOLLS, M., ADLER, S., MEYER-SCHWICKERATH, M., and SACK, H., 1991, Hyperthermia in eccentrically located pelvic tumours: Excessive heating of the perineal fat and normal tissue temperatures. *International Journal of Radiation Oncology, Biology and Physics*, **20**, 1017–1022.

GERLOCK, Jr. Q. J., and MIRFAKHRAEE, M., (eds.) 1985, Percutaneous transluminal angioplasty. *Essentials of Diagnostic and Interventional Angiographic Techniques*, (Philadelphia: W. B. Saunders), pp. 174–204.

HAHN, G. M., 1987, Blood flow: physics and technology of hyperthermia, *NATO ASI Series E. 127*, edited by S. B. Field and C. Franconi (Amsterdam: Martinus Nijhoff), pp. 441–447.

HUNT, J. W., LALONDE, R., GINSBERG, H., URCHUK, S., and WORTHINGTON, A., 1991, Rapid heating: critical theoretical assessment of thermal gradients found in hyperthermia treatments. *International Journal of Hyperthermia*, **7**, 703–718.

HYNYNEN, K., DEYOUNG, D., KUNDRAT, M., and MOROS, E., 1989, The effect of blood perfusion rate on the temperature distributions induced by multiple, scanned and focused ultrasonic beams in dogs' kidneys *in vivo*. *International Journal of Hyperthermia*, **5**, 485–497.

HYNYNEN, K., ROEMER, R., ANHALT, D., JOHNSON, C., XU, Z. X., SWINDELL, W., and CETAS, T., 1987, A scanned, focused, multiple transducer ultrasonic system for localized hyperthermia treatments. *International Journal of Hyperthermia*, **3**, 21–35.

HYNYNEN, K., DARKAZANLI, A., UNGER, E., and SCHENCK, J. F., 1993, MNI-guided noninvasive ultrasound surgery, *Medical Physics*, **20**(1), 107–115.

JAIN, R. K., and WARD-HARTLEY, K., 1984, Tumour blood flow-characterization, modifications, and role in hyperthermia. *IEEE Trans Sonics Ultrasonics*, **31**, 504–526.

JIA, Z. Q., WORTHINGTON, A. E., HILL, R. P., and HUNT, J. W., 1994, The temperature distribution during heating of a kidney phantom *in vitro*: The effect of "blood flow" and use of a focused ultrasound beam. *Program and Abstracts for 42nd AMRRS and 14th NAHS*. Nashville, USA, 109 pp.

JIA, Z. Q., 1995, Temperature homogeneity and blood flow in renal hyperthermia, M.Sc. Thesis, University of Toronto, pp. 68–101.

LEOPOLD, K. A., DEWHIRST, M. W., SAMULSKI, T. V., DODGE, R. K., GEORGE, S. L., BLIVIN, J. L., PROSNITZ, L. R., and OLESON, J. R., 1993, Cumulative minutes with T_{90} greater than Tempindex is predictive of response of superficial malignancies to hyperthermia and radiation. *International Journal of Radiation Oncology, Biology and Physics*, **25**, 841–847.

LEVIN, W., SHERAR, M. D., COPPER, B., HILL, R. P., HUNT, J. W., and LIU, F.-F., 1994, Effect of vascular occlusion on tumour temperatures during superficial hyperthermia. *International Journal of Hyperthermia*, **10**, 495–505.

LIEBERTHAL, W., RENNKE, H. G., SANDOCK, K. M., VALERI, C. R., and LEVINSKY, M. G., 1988, Ischemia in the isolated erythrocyte-perfused rat kidney. Protective effect of hypothermia. *Renal-Physiological Biochemistry*, **11**(1–2), 60–69.

MORRIS, C. C., MYERS, R., and FIELD, S. B., 1977, The response of the rat tail to hyperthermia. *British Journal of Radiology*, **50**, 576–580.

OLESON, J. R., DEWHIRST, M. W., HARRELSON, J. M., LEOPOLD, K. A., SAMULSKI, T. V., and TSO, C. Y., 1989. Tumour temperature distributions predict hyperthermia effect. *International Journal of Radiation Oncology, Biology and Physics*, **16**, 669–70.

OLESON, J. R., SAMULSKI, T. V., LEOPOLD, K. A., CLEGG, S. T., DEWHIRST, M. W., DODGE, R. K., and GEORGE, S. L., 1993, Sensitivity of hyperthermia trial outcomes to temperature and time: implications for thermal goals of treatment. *International Journal of Radiation Oncology, Biology and Physics*, 25, 289–297.

PARKS, D. A., WILLIAMS, T. K., and BECKMAN, J. S., 1988, Conversion of xanthine dehydrogenase to oxidase in ischemic rat intestine: A re-evaluation. *American Journal of Physiology*, **254**, G768.

PRESCOTT, D. M., SAMULSKI, T. V., DEWHIRST, M. W., PAGE, R. L., THRALL, D. E., DODGE, R. K., and OLESON, J. R., 1992, Use of nitroprusside to increase tissue temperature during local hyperthermia in normal and tumour-bearing dogs. *International Journal of Radiation Oncology, Biology and Physics*, **23**, 377–385.

PROVENCHER, S. W., 1976, A Fourier method for the analysis of exponential decay curves. *Biophysics Journal*, **16**, 27–41.

REINHOLD, H. S., and ENDRICH, B., 1986, Tumour microcirculation as a target for hyperthermia. *International Journal of Hyperthermia*, **2**, 111–137.

ROEMER, R. B., 1990, Local tissue cooling coefficient: a unified approach to thermal washout and steady state 'perfusion' calculations. *International Journal of Hyperthermia*, **6**, 421–430.

ROWELL, L. B., 1973, Circulation to skeletal muscles, T. C. Ruch, and H. D. Patton, eds. In *Physiology and Biophysics*, (Philadelphia: W. B. Saunders), pp. 200–213.

SARVAZYAN, A. P., and KLEMIN, V. A., 1983, Study of ultrasonic topography of the kidney. *Ultrasonic Interactions in Biology and Medicine* edited by R. Millner, E. Rosenfeld and U. Cobet, (London: Plenum), pp. 99–104.

TERHAAR, G., RIVENS, I., CHEN, L., and RIDDLER, S., 1991, High intensity focused ultrasound for the treatment of rat tumours. *Physics in Medicine and Biology*, **36**, 1495–1501.

WEISS, S. J., 1986, Oxygen, ischemia, and inflammation. *Acta Physiologica Scandinavica Supplementum*, **548**, pp. 9–11.

ZIMMERMAN, B. J., and GRANGER, D. N., 1992, Reperfusion Injury. *Surgical Clinics of North America*, **72**, 65–82.