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Changes in Local Hepatic Blood Perfusion During Interstitial Laser-Induced Thermotherapy of Normal Rat Liver Measured by Interstitial Laser Doppler Flowmetry

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Abstract. Interstitial laser Doppler flowmetry was used to measure the effect of interstitial laser-induced thermotherapy on local blood perfusion in normal rat liver in the peripheral treatment region elevated to hyperthermic temperatures. The Nd:YAG laser emitting at 1064 nm was utilised as heat generation source. The plane-cut tip of an optical fibre was placed in the middle of the exteriorised left liver lobe. Blood perfusion and temperature were measured in the liver parenchyma 4 mm from the laser fibre. The temperature at the location of the liver temperature sensor was maintained at 41 or 44°C during 30 min by regulating the power of the heating laser. The laser Doppler signal was recorded during and after heat treatment, for a total time of 60 min. At 41°C, a significant increase in perfusion up to 1.3 times the initial value was observed 2–16 min after start of treatment. At 44°C, perfusion decreased continuously during and after treatment, and was significantly different from control 40 min after start of treatment. The results may be valuable in assessing the thermal response of tissues surrounding the target in interstitial laser-induced thermotherapy of liver tumours during conditions of normal blood flow.

Keywords: Interstitial laser therapy; Liver; Perfusion

INTRODUCTION

Interstitial laser-induced thermotherapy (ILT) is a method for thermal destruction of tumours, while the surrounding healthy tissue is protected [1–5]. Protection of normal tissue is achieved mainly by the selective deposition of thermal energy in the tumour. By elevating the temperature to approximately 60°C, rapid coagulation of the tumour is accomplished. The temperature of the healthy tissue around the neoplasm will rise, although less than that of the tumour. It is of interest, therefore, to investigate not only the effect of ILT on tumour but also the effect on normal tissue.

Tissue blood perfusion is an important factor in determining local temperature increase and intratissue microenvironment. During heat treatment perfusion may increase or decrease depending on temperature level and the properties of the specific tissue. In ILT, blood flow in the central area around an optical fibre, implanted into a tumour, can be assumed to stop soon after initiation of treatment due to the high temperatures induced, whereas the influence of hyperthermic temperatures (41–44°C) on perfusion appears less predictable and consistent [6,7]. The effect of hyperthermia on blood flow in skin and muscle of animals has been studied extensively [8,9]. In these investigations, tissue blood flow has been shown to increase remarkably at heating temperatures up to approximately 45°C. At higher temperatures, the flow generally decreases in a time-dependent manner. However, little is known about the effect of heat on hepatic circulation. In previous studies of liver blood flow during local heat treatment, microwaves have been utilised as heat induction method. These studies showed decreased flow after treatment at temperatures greater than 43°C [10,11]. At lower temperatures no significant change in liver blood flow was detected, although an isolated small increase in hepatic
arterial flow was observed [12]. Thus, the increase in perfusion detected in skin and muscle during heat treatment at temperatures below 45°C has not been observed in the liver.

The purpose of the present study was to measure local changes in liver blood flow at hyperthermic temperatures using interstitial laser Doppler flowmetry (LDF) during ILT. Previously, measurement of liver blood flow during heat treatment has relied on methods such as radioactive microspheres, hydrogen clearance and thermal washout [10,12,13]. LDF is a more sensitive modality for measuring changes in tissue blood flow. This technique is based on the spectral broadening of monochromatic light due to scattering from moving red blood cells (Doppler shift). Changes in the Doppler shifted signal have been shown to correlate well with changes in hepatic perfusion as measured with other methods [14,15].

MATERIALS AND METHODS

Animals

Twenty-four inbred male Wistar FU rats, weighing 246–277 g, were used. They were housed three per cage and had free access to standard food pellets and tap water. All experiments were carried out in accordance with protocols approved by the local experimental animal ethics committee.

Surgical Procedures

Rats were anaesthetised with an intraperitoneal injection of pentobarbital sodium (50 mg/kg) and were given 0.02 mg/kg buprenorphine (Temgesic, Meda AB, Göteborg, Sweden) i.m. for postoperative analgesia. The left femoral artery was cannulated with a catheter for measurement of systemic arterial blood pressure. Mean systemic arterial blood pressure (MABP) was measured with a pressure transducer (DT-XX, Ohmeda, Helsingborg, Sweden) and recorded on a personal computer (SoftConsult, Lund, Sweden).

Laser Heating and Regulation System

For local liver heating a laser unit and a temperature feedback control unit interfaced with the laser were used. Laser light at a power of 2 W from a continuous wave Nd:YAG laser (CL60, Surgical Laser Technologies, Malvern, PA), emitting light at a wavelength of 1064 nm, was delivered through a 0.6 mm optical fibre. The fibre cladding and jacket were stripped back 5 mm from the tip, which was plane cut before each experiment. The fibre tip was inserted into the central area of the left lateral lobe at an approximate depth of 2 mm. Tissue temperature was measured using 2K7 thermistors (Microtherm AB, Lund, Sweden) inserted into steel cannulas (outer diameter 0.6 mm) for direct puncture. One thermistor was inserted in the liver parenchyma 4 mm lateral to the laser fibre used for heating, as measured with sliding callipers. Another thermistor was placed inside the abdominal cavity. Temperatures were measured every other second. The temperature recorded by the thermistor placed in liver (master thermistor) was used to turn the laser on and off at a preprogrammed temperature level, which was achieved by an automatic thermometry/regulation system (ATS-100, Lund Science, Lund, Sweden). The performance of the system in regulating tissue temperature has been thoroughly investigated in previous studies [16,17].

Laser Doppler Flowmetry System

A commercially available LDF system was used for measuring hepatic perfusion (Perimed 4001, Perimed, Järfalla, Sweden). The operating principle of this instrument has been described in detail previously [18]. In short, light from a diode laser is guided to the tissue by means of an optical fibre (diameter 0.5 mm) placed interstitially. The same fibre transmits the backscattered light from the tissue. The output from the flowmeter in arbitrary perfusion units is derived from the Doppler-shifted part of the backscattered light. The Doppler shift occurs when light is scattered by moving red blood cells, the number and velocity of which determine the laser Doppler flowmeter output value.

The laser Doppler system was calibrated using a standard consisting of a suspension of 2 µm latex spheres (Perimed). Immersing the
Interstitial laser Doppler fibre produced a standard deflection of 250 perfusion units. The LDF system was interfaced to a personal computer, and the signals were analysed with commercially available software (Perisoft, Perimed). The interstitial laser Doppler fibre was inserted 4 mm lateral to the laser fibre used for heating (as measured with sliding callipers) and opposite to the master thermistor. Deviations by less than 0.5 mm from the intended 4 mm distance to the laser fibre from the master thermistor and laser Doppler fibre were accepted. A schematic illustration of the exposed liver lobe with the inserted probes is shown in Fig. 1.

**Experimental Procedures**

After the surgical procedures, 15 min was allowed for stabilisation. Baseline LDF values were then averaged over approximately 30 s. The influence of respiration on the LDF signal was evaluated by calculating the average of maximum differences in flux values during ten respiration cycles. Rats received treatment at a steady-state target temperature of 41 or 44°C for 30 min or served as control (n=8 in each group). Measurements of MABP and blood perfusion continued for 30 min after treatment. Because the laser light used for heating interfered with the LDF measurements, the former laser was switched off during 10 s every 2 min. Quoted LDF values, except baseline values, are means of the recorded flow signal computed over the 10 s period when the heating laser was turned off. At the end of the experiments, the control animals were given an overdose of anaesthetic and the zero-flow LDF signal was recorded. In the heat-treated animals, the abdomen was closed in two layers. Glucose solution (5%, 20 ml) was injected subcutaneously into the back of each animal for volume replacement. Rats were killed after 3 days by an overdose of ether. The heat-treated liver lobe was cut through the line made up by the insertion sites of the three probes (laser Doppler fibre, laser fibre for heating and master thermistor) and the maximum macroscopic lesion diameter was measured using sliding callipers.

**Statistical Analysis**

Results are expressed as mean values (SEM). Differences between groups were assessed using the Kruskal–Wallis test and/or Mann–Whitney U test. To account for multiple comparisons, the Bonferroni method was employed. Linear regression was used to calculate the slope of MABP as a function of time for the individual experiments and to calculate the slope of perfusion in the sham-treated group. The change in slope from zero value was evaluated by one sample t-test. p<0.05 was considered statistically significant.

**RESULTS**

The average time (SEM) to reach target temperature was 18.1(2.8) s and 20.4(5.3) s in the 41°C and 44°C groups, respectively. The abdominal temperature never rose above 39°C. Before the start of treatment, mean values of MABP were 101(3) mmHg in the sham-treated group, and 95(2) mmHg and 97(4) mmHg in the groups treated at a local temperature of 41°C and 44°C, respectively. At the end of treatment the corresponding values were 99(4), 91(3) and 95(4) mmHg. No significant difference in initial blood pressure (p=0.38) nor in slope (pressure vs. time) (p=0.26) was found between groups. Blood pressure in each group showed no significant change with time during the experiments (p>0.81).

An example of laser Doppler signal recorded during treatment at 41°C is shown in Fig. 2. Figure 3 shows changes in blood perfusion during the heat and sham treatments expressed as values relative to the baseline LDF signal at time t=0 min. During heat treatment relative flux values increased in the group treated at a target temperature of 41°C. This increase was significant as compared to...
the sham-treated group during the first 16 min of heat treatment \((p<0.05)\). A maximum value in relative flux of 1.33 was obtained 10 min after start of heating. In the group treated at 44°C, relative LDF values decreased continuously during and after heating. The decrease was significant as compared to control animals at \(t>40\) min \((p<0.05)\). At the end of the experiment, the mean relative flux value had decreased to 0.65. In the sham-treated group, there was a tendency to a decrease in relative perfusion with time, almost reaching statistical significance \((p=0.06)\).

Three days after treatment, the macroscopic necrosis at the cut section of the heat-treated liver lobes had the shape of a semi-circle or a rectangle when the necrosis extended through the full thickness of the liver lobe. The mean maximum macroscopic necrotic diameter as measured parallel to the liver surface was 5.1(0.2) mm and 7.1(0.2) mm in the groups treated at 41 and 44°C, respectively. The difference in necrotic diameter was significant \((p=0.001)\).

The mean absolute baseline LDF value for all 24 animals was 235(20) arbitrary perfusion units (PU). The mean of the respiration-induced flux values for all 24 animals and the mean zero-flow flux value for the sham-treated animals, which were killed at the end of the experiments, were 23(1) and 26(3) PU, respectively. The influence of respiration on the LDF signal was clearly seen in the recordings (Fig. 2).

**DISCUSSION**

The present study demonstrated that heat can cause an increase in local liver perfusion. To our knowledge, this has not been described previously. An increase in liver perfusion up to 33% was found at a local tissue temperature of 41°C. Using the technique of radioactive microspheres, Uda et al. [12] measured a transient increase in hepatic arterial flow whereas portal venous blood flow did not change significantly during treatment at heating temperatures of 39–41°C. Since only 25%–30% of total liver blood flow derives from the hepatic artery [19], the authors concluded that blood flow was not significantly increased in the heated area. The increase in hepatic arterial flow measured by Uda et al. [12] during heat treatment at 41°C roughly corresponds to the increase in local liver perfusion measured at 41°C in the present study. This would imply that laser Doppler flowmetry measures arterial flow rather than combined arterial and portal flows. This appears to be true when measuring on the surface of pig liver, where the laser Doppler signal is influenced to a greater extent by a change in hepatic arterial flow than in portal flow [20]. However, measurements on the surface of rat liver show that the LDF signal accurately reflects hepatic microcirculatory blood flow during alterations of hepatic arterial and portal flows [14,15]. Also, LDF flux values measured interstitially and at the surface of rat and human liver are well correlated [21]. These two arguments lead us to reject the above assumption. The discrepancy between the two studies could be due to the different heating techniques employed.

An increase in blood flow in the periphery of the treatment region acts as a heat barrier.
Blood flow dissipates heat by convection, limiting the spread of thermal energy. Based on the results of previous studies on ILT of normal liver [22,23], it can be concluded that the increase in liver blood perfusion measured in the present study is insufficient to completely arrest the propagation of heat. By applying a constant laser power, which is common in the clinical setting, the zone of increased perfusion should move further away from the laser fibre as treatment proceeds. Nevertheless, the result is that normal tissue is protected from thermal damage to some extent. The significance of liver perfusion in reducing the thermal damage in ILT has been investigated thoroughly in previous studies [24–27]. In these studies, temporary hepatic inflow occlusion during ILT has been advocated to allow the destruction of large tumour volumes. With the development of new multifibre systems and fibre-endings capable of destroying tumours of even greater dimensions, preservation of normal tissue may become increasingly important.

At a constant laser power, decreased blood perfusion during ILT, induced either by heat damage to vessels or by mechanical interruption of blood flow, results in an increase in local temperature with less effect on temperature gradients [23,24]. In feedback temperature regulation, as in the present study, the temperature at a certain distance from the laser fibre is kept constant by regulating the laser power. Decreased perfusion then results in smaller temperature gradients, and lower temperatures, in the treatment field between the laser fibre and the point of temperature regulation [25]. Further away from the laser heat source, a reduction in perfusion is followed by an increase in tissue temperature.

In our opinion, the full advantage of ILT will be exploited only if treatment is performed with the lowest possible temperatures associated with local eradication of tumour cells [16,28,29]. Results in our laboratory following ILT of rat liver adenocarcinoma suggested involvement of immunological mechanisms when treatment is performed at these low temperatures [29]. This is consistent with previous observations that tissue breakdown products obtained after local tumour destruction may give rise to a favourable increase of the immune defence [30]. This view was corroborated by the demonstration of resistance to rechallenge following cryodestruction, or laser-photosensitiser assisted immunotherapy, of chemically induced breast adenocarcinomas in the rat [31,32]. An efficient immune response may be attained only if there is perfusion of tumour cells.

Treatment at a local tissue temperature of 44°C resulted in a continuous decrease in perfusion. The observed decrease was somewhat lower than would be expected from previously reported results [10,33]. The shape of the perfusion curves is well-known from experiments on other normal tissues, including skin and muscle.

Laser Doppler flowmetry differs from most previously employed methods in its ability to measure perfusion locally [34]. The laser Doppler signal emanates from a tissue volume less than 1 mm³. Previously, the laser Doppler probe has most frequently been applied at the tissue surface. Interstitial placement of the laser Doppler probe causes tissue damage that may influence the perfusion measurements. However, the tissue damage associated with the insertion of interstitial laser Doppler probes has been measured to be confined to a distance of less than 0.12 mm away from the probe [7]. Assuming the same degree of damage in the present study, this was unlikely to have affected the laser Doppler measurements. The quite large standard errors in the heat-treated groups can be explained by large tissue temperature gradients at the location of perfusion measurements (see below) and by different responses to heat by individual vessels [35,36].

Systemic arterial blood pressure was measured because the LDF signal has been shown to be linearly dependent on blood pressure [15]. No significant difference in MABP was found between the heat-treated groups and the sham-treated group. MABP showed a tendency to a decrease during the experiments in all groups, which is possibly reflected by the observed tendency to a decrease in relative laser Doppler flux in the sham-treated group.

Baseline LDF values varied considerably from one animal to another, indicating that laser Doppler flow values from a single site do not allow quantification of tissue perfusion in adjacent parts of the liver. The rat liver is subject to considerable respiration-related movement. The fluctuations on the LDF signal due to respiration were found to constitute less than 10% of the total laser Doppler flow signal and was very stable between experiments. Probably, part of this fluctuation originates from a true change in perfusion due to respiration-induced changes in caval and hepatic blood flow [37,38].
Another problem is that laser Doppler flowmetry not only registers genuine blood flow but also tissue motions relative to the probe. The sham-treated animals were killed at the end of the experiment to evaluate the zero-flow signal, which averaged 11% of the total LDF signal. Possible causes of zero-flow signal are tissue micromotions and Brownian motion of blood cells. Under the assumption that the respiration-related flow signal and the zero-flow signal remained constant during the experiments, the true relative change in hepatic perfusion as measured in this study may have been underestimated because no attempt to correct for movement artefacts and zero-flow signals was made.

Near-infrared light from the Nd:YAG laser penetrates only a few millimetres into tissue [39] which results in large temperature gradients [40–42]. At the temperatures used in the present study, temperature gradients of 3–4°C/mm at a distance of 4 mm from the laser fibre were generated [17,28]. With respect to the present study, it follows that the placement of the master thermistor and laser Doppler probe in relation to the heating laser fibre is crucial to the result when heat treatments that differ by only 3° (here 41 and 44°C) are given. Therefore, special attention was paid to measuring the distances between the laser fibre and the master thermistor and laser Doppler fibre, respectively. A distance of 3.5–4.5 mm was considered acceptable.

Maximum diameter of necrosis as evaluated macroscopically after 3 days was found to be less than 8 mm in both groups, indicating that the necrosis did not extend to the site where the master thermistor was placed during experiment. This agrees with previous studies on laser-induced heating [24,25]. The master thermistor and the laser Doppler fibre were placed at equal distances from the laser fibre used for heating. The temperature at these two locations was considered equal because it has been shown that laser thermotherapy gives a symmetric temperature distribution and symmetric necrosis around interstitial fibres [16,17,28,43].

This experimental study has shown that interstitial laser Doppler flowmetry is useful for detecting changes in local liver blood perfusion during heat treatment of experimental animals. Heating at 41°C gave a transient increase in local hepatic perfusion whereas heating at 44°C resulted in a gradual decrease in local perfusion.

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