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EFFECTS OF INFRARED RADIATION ON INTRAOSSEOUS BLOOD FLOW AND OXYGEN TENSION IN THE RAT TIBIA

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INDEXING WORDS

blood flow; oxygen tension; hydrogen washout technique; infrared radiation; rat tibia

SYNOPSIS

In order to examine the effects of infrared radiation on intraosseous blood circulation, the intraosseous blood flow and oxygen tension were examined in rat tibiae. Infrared radiation was performed for 5 minutes over the ankle joint approximately 40 cm from the skin surface. The intraosseous blood flow was measured with a hydrogen washout technique. Before and after infrared radiation, the blood flow rates in the proximal metaphysis of the rat tibia were 8.7 ± 2.5 ml/min/100g and 15.6 ± 5.4 ml/min/100g, respectively, i.e. the intraosseous blood flow increased approximately 80% with the radiation. The intraosseous oxygen tension and temperature were measured with a Po_2 sensor, altered to our own specifications. The former increased from 20.4 ± 6.2 mmHg to 23.3 ± 6.7 mmHg (about 15%), and the latter from 29.0 ± 1.4 °C to 31.2 ± 1.9 °C, respectively, during the 5 minutes infrared radiation. The results of the present study show that infrared radiation remarkably improves intraosseous blood circulation, and these effects continue for at least 30 minutes. It is, therefore, suggested that infrared radiation can be used as a measure of thermotherapy in some bones to improve intraosseous blood circulation.

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INTRODUCTION

Heat has been shown to be effective for pain, muscle spasms, wound healing, frost-bite, joint stiffness, and shortness of soft tissues. Thus, various kinds of thermotherapy such as hot packs, paraffin baths and infrared, microwave, and ultrasound radiation, have been widely applied for the treatment of trauma and muscle pain, as well as bone and joint disorders. Of these thermal modalities, infrared radiation is considered to be one of the superficial heat modalities, thus its effects have been mainly studied in surface tissues, including the skin.^{1,17)} These reports noted that a rise in local temperature induces vasodilatation and an increase in blood flow. The range of infrared rays for heating superficial tissue is divided into near infrared (770-1,500 nm) and far infrared (1,500-150,000 nm). As determined by their specific wave lengths, near rays penetrate to subcutaneous tissue, whereas far rays are absorbed primarily by the superficial epidermis.¹²⁾ These findings suggest that at least near infrared radiation can be used as a heat modality for some muscles and bones if they are near the skin. To date, however, the effects of near infrared radiation in bone tissue have not yet been fully examined. Intraosseous blood circulation should be very important not only for the development and maintenance of normal bone structure and function, but also for fracture healing and improvement of ischemic bone diseases. In the present study, to clarify the importance of intraosseous blood circulation, changes in intraosseous blood flow and oxygen tension during and after near infrared radiation treatment were continuously measured in the proximal metaphysis of rat tibia.

MATERIALS AND METHODS

Twenty-two male Wistar rats, 3-6 months old, weighing 260-450g, were used in the study. These animals were anesthetized with an intraperitoneal injection of pentobarbital sodium (30mg/kg), and then heparin (50 units) was intravenously injected to prevent blood coagulation. A short longitudinal incision was made in the skin and patellar ligament along the anterior mid-line of the right knee to expose the proximal end of the tibia.

The following experiments were carried out at room temperature (20-25 °C) controlled with an air conditioner.

Infrared radiation

An infrared lamp (500-2,500 nm, SLT-1001, Sakai Medical Co. Ltd., Tokyo, Japan) was used in this study. Infrared radiation was applied for 5 minutes over the right ankle joint, approximately 40 cm from the surface of the skin.

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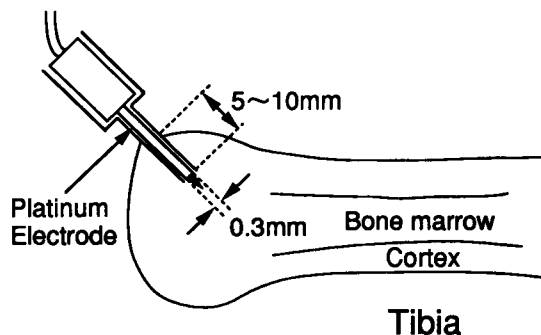


Figure 1. : Schematic drawing of the cylinder and electrode inserted into the proximal metaphysis of the rat tibia to measure blood flow.

Measurement of the blood flow

Eleven rats were used in this experiment. Intraosseous blood flow was measured with the hydrogen washout technique described by Whiteside et al.^{3,23)} A needle-like platinum electrode (Unique Medical Co. Ltd., Tokyo, Japan), 0.3 mm in diameter, was used as a sensor, and a calomel-type, silver-silver chloride electrode, was used as a reference electrode.

A hollow cylinder 0.8 mm in diameter was inserted 5-10 mm into the proximal metaphysis of the right tibia through the articular cartilage (Fig. 1). After a leakage of blood through the cylinder was confirmed, the platinum electrode was inserted into the cylinder and the reference electrode into the subcutaneous tissue of the left thigh. A mixture of 90% air and 10% hydrogen gas was administered through a face mask until the hydrogen concentration reached a suitable level (Fig. 2). The hydrogen build-up and washout curve were recorded with a pen recorder (PHG-203, Unique Medical Co. Ltd., Tokyo, Japan) until the hydrogen concentration recovered to the initial level. Then, infra-red radiation was applied for 5 minutes over the right ankle joint, the gas mixture was administered again, and the clearance curve was recorded to examine the effects of the thermotherapy on blood flow.

Measurement of oxygen tension

Eleven rats were used in this experiment. To determine the intraosseous oxygen tension, a needle-like Po_2 sensor, modified in our laboratory, was used.^{2,7,14,15,16)} This sensor

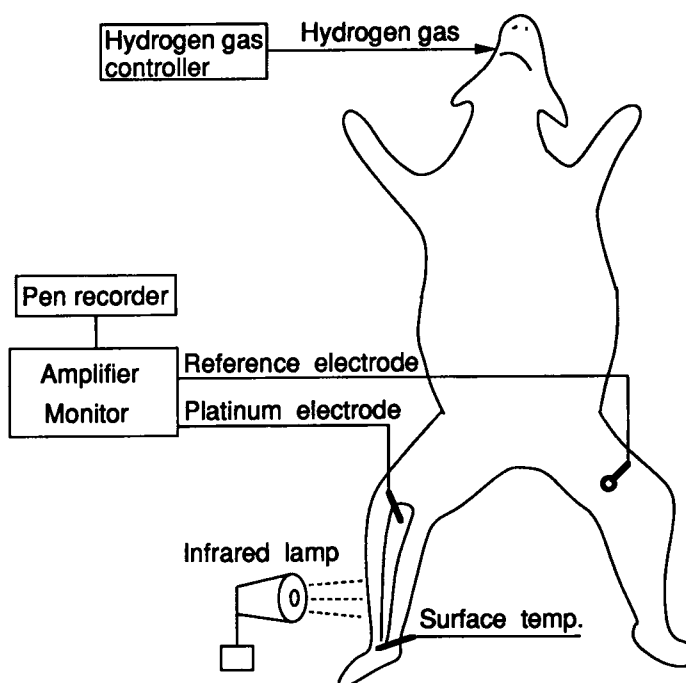


Figure 2. : Schematic diagram of the experiment to measure the effects of infrared radiation on intraosseous blood flow in the rat tibia.

was composed of an oxygen electrode and a thermocouple. Those together were covered with a Teflon tubing filled with 3 mol KCl (Fig. 3). Since this Po_2 sensor measured only 0.4 mm in diameter, we were able to continuously and quantitatively examine the oxygen tension and temperature at any point, even in the blood vessels and bones. This Po_2 sensor was placed in the cylinder inserted into the proximal metaphysis of the right tibia, as mentioned above. The intraosseous oxygen tension and temperature were measured during and after the application of infrared radiation over the right ankle joint, and continuously recorded with a digital monitor and desktop pen recorder (POG-203, Unique Medical Co. Ltd., Tokyo, Japan). Since the current induced by the oxygen electrode increases by 2 % per 1 $^{\circ}\text{C}$,⁶⁾ raw reading of the O_2 sensor placed at constant Po_2 varied with temperature (Table I). To compensate this temperature dependency, a special program was included in our oxygen tension monitoring system.

The paired t-test was used for statistical analysis to compare the results.

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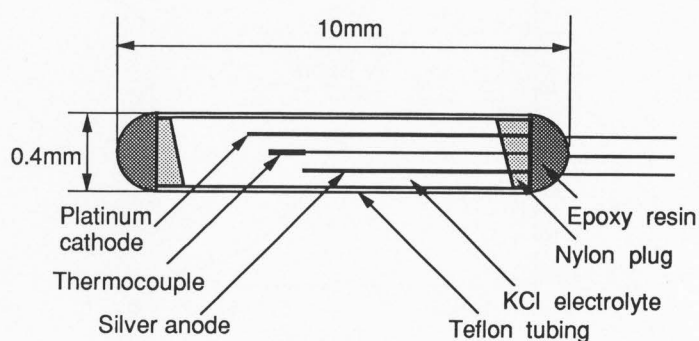


Figure 3. : Schematic drawing of the Po_2 sensor modified in our laboratory.

Table I. : Temperature dependency of the Po_2 sensor.

temperature ($^{\circ}\text{C}$)	raw reading of the O_2 (mmHg)
25	126.6
26	128.6
27	130.5
28	132.5
29	134.4
30	136.4
31	138.3
32	140.3
33	142.2
34	144.2
35	146.1
36	148.0
37	150.0
38	151.9
39	153.9
40	155.8

Note : raw reading of the O_2 tension at each temperature when the O_2 tension is kept at 150 mmHg.

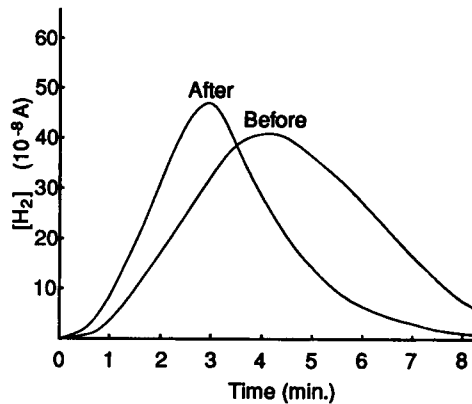


Figure 4. : Typical hydrogen build-up and washout curves before and after infrared radiation (5 minutes) in the rat tibia.

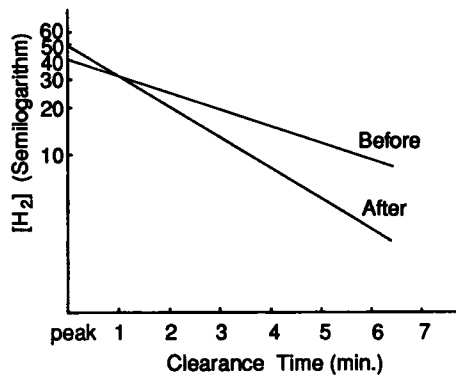


Figure 5. : Hydrogen washout curves on a semilogarithmic scale showing a monoexponential decay pattern (same data used in Fig.4).

RESULTS

Intraosseous blood flow

Figure 4 shows typical hydrogen build-up and washout curves in the proximal metaphysis of the right tibia recorded before and after the infrared radiation. The hydrogen washout curve after the radiation declined at a faster rate than before. The same data are shown on a semilogarithmic scale in Figure 5. The hydrogen washout curves before and after the radiation showed a monoexponential decay pattern on a semilogarithmic scale, but the former declined at a slower rate than the latter.

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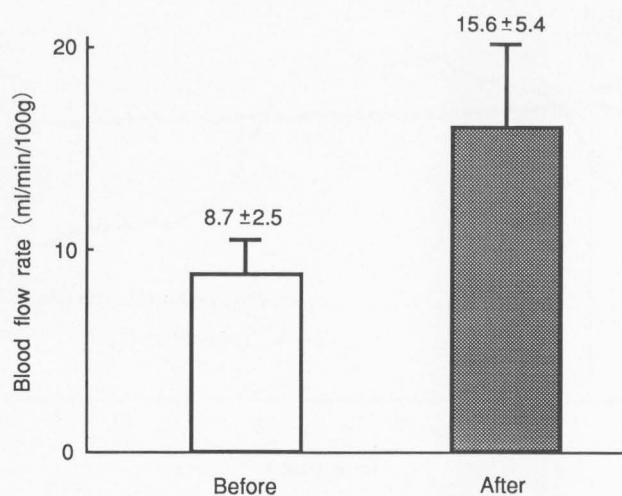


Figure 6. : Intraosseous blood flow rate in the rat tibia before and after infrared radiation (n=11, $p<0.01$). Values expressed as mean $\pm$ SD.

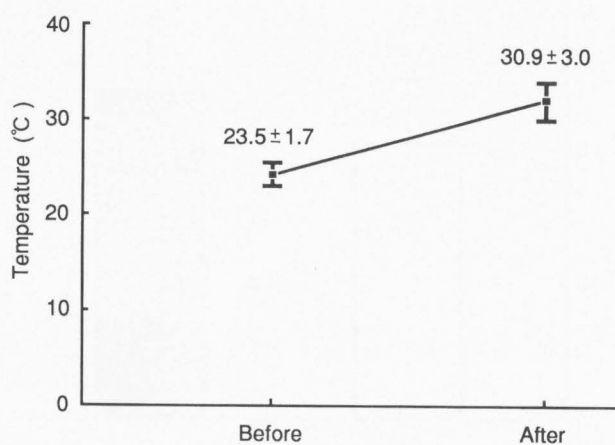


Figure 7. : Changes in skin surface temperatures before and after radiation (n=11, $p<0.01$). Values expressed as mean $\pm$ SD.

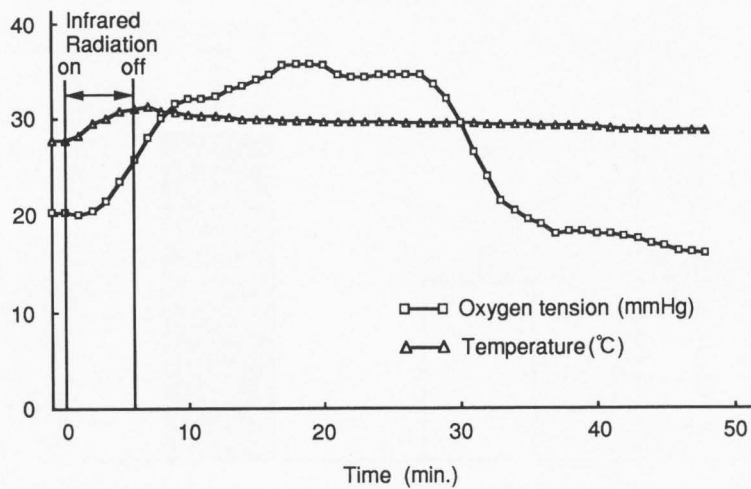


Figure 8. : Typical changes in intraosseous oxygen tension and temperature, during and after radiation, in the rat tibia.

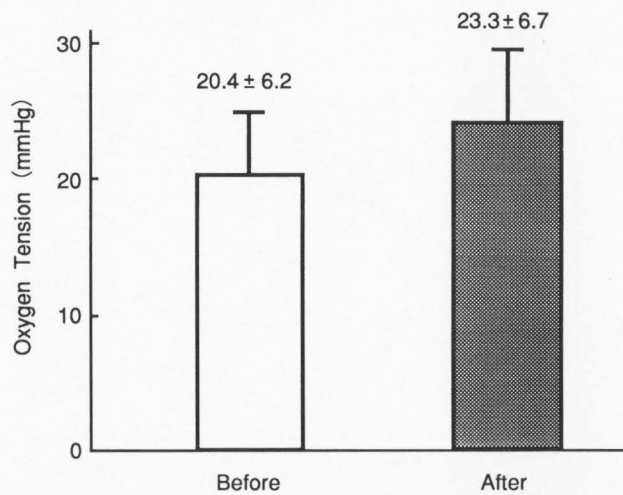


Figure 9. : Intraosseous oxygen tension before and after radiation (n=11, p<0.05).

Values expressed as mean ± SD.

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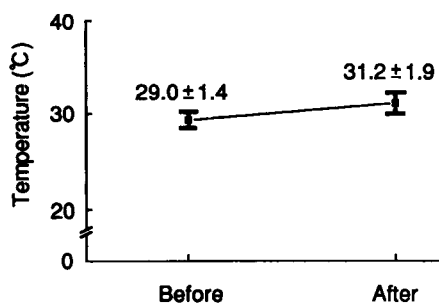


Figure 10. : Intraosseous temperature, before and after radiation, in the rat tibia (n=11, $p<0.01$).

Values expressed as mean $\pm$ SD.

The half-life obtained from the decay pattern was used to determine the intraosseous blood flow rate, using the following equation.

$$F = 69.3 \lambda / t_{1/2}$$

where

F =intraosseous blood flow rate (ml/min/100g)

λ =partition coefficient of hydrogen between blood and bone

$t_{1/2}$ =half-life of decay

The partition coefficient value for the rat metaphysis has not yet been reported, so the rabbit metaphysial bone value ($\lambda = 0.74$) reported by Whiteside et al.²³⁾ was utilized instead. The mean intraosseous blood flow rates before and after the radiation were 8.7 ± 2.5 (mean $\pm$ SD) ml/min/100g and 15.6 ± 5.4 ml/min/100g (n=11), respectively (Fig. 6). There was a statistically significant difference between them ($p<0.01$).

The skin surface temperature as measured on the right ankle joint is shown in Figure 7. The skin surface temperature rose 7.4 ± 1.3 °C after the radiation. This change in temperature was also statistically significant ($p<0.01$).

Intraosseous oxygen tension

Figure 8 shows typical changes in the intraosseous oxygen tension and temperature in the rat tibiae during and after the infrared radiation, which was performed for 5 minutes. At 2-3 minutes after the start of radiation, the oxygen tension began to increase rapidly and reached a plateau at about 10 minutes. The plateau continued for approximately 20 minutes and then rapidly declined. On the other hand, the intraosseous temperature rose slightly during the radiation and then began to decline very slowly after the radiation. The means of the intraosseous oxygen tensions before and after the radiation were 20.4 ± 6.2 mmHg and 23.3 ± 6.7 mmHg (at the maximum), respectively (Fig. 9). There was a statistically significant difference between them ($p<0.05$). The intraosseous temperature also rose from 29.0 ± 1.4 °C to 31.2 ± 1.9 °C (Fig. 10). The difference in temperature before and after the radiation was also statistically significant ($p<0.01$).

DISCUSSION

In the present study, the effects of near infrared radiation on intraosseous blood flow and oxygen tension were examined in the rat tibia. The intraosseous blood flow increased approximately 80 %, and oxygen tension approximately 15 %, when near infrared radiation was applied for 5 minutes over the ankle joint. Furthermore, these effects continued for about 30 minutes after the radiation. These findings indicate that near infrared radiation significantly improves blood circulation, and suggest that this can be used as a measure of thermotherapy in some bones.

The intraosseous blood flow rate in the proximal metaphysis of the rat tibia reported by Takahashi et al.²⁰⁾ was about 13 ml/min/100g, as measured in a rat whose body temperature was kept 36.5 °C. They reported that the regional bone blood flow did not change at 6 hours after the sham operation.

In the present study, the mean intraosseous blood flow rate in the proximal metaphysis of the rat tibia was 8.7 ± 2.5 ml/min/100g when it was measured at room temperature (20-25 °C). After near infrared radiation for 5 minutes, the intraosseous temperature in the proximal metaphysis rose from 29.0 ± 1.4 °C to 31.2 ± 1.9 °C, and the intraosseous blood flow rate increased to 15.6 ± 5.4 ml/min/100g. Since Takahashi et al.²⁰⁾ did not measure the intraosseous temperature, we could not compare all our results with them. Our results revealed that the intraosseous blood flow rate increases with an increase of intraosseous temperature. There are some possibilities which can explain why the intraosseous blood flow increases as a result of near infrared radiation.

Wright²⁴⁾ and Crockford et al.^{8,9)} stated that temperature elevation appears to have a direct effect on the state of dilatation of the capillaries, arterioles and venules. Crockford et al.⁹⁾ also suggested that temperature elevation in tissue acts directly on the smooth musculature of the vessels. In the present study, the surface skin temperature at the ankle joint was elevated approximately 7 °C, and the intraosseous temperature, approximately 2 °C, after the radiation. It is possible that with the elevation of the skin surface and intraosseous temperature, the artery which enters the tibia and the intraosseous blood vessels become dilated, thus increasing the intraosseous blood flow.

Duncan and Shim¹¹⁾ reported the existence of an autonomic nerve supply to bones. Several authors have reported an increase of blood flow in the denervated bone after paralysis induced by sciatic neurotomy or sympathectomy.^{10,18,19,21)} These findings suggested that the intraosseous blood flow might be controlled by the sympathetic nervous system. Walther et al.²²⁾ and Iriki et al.¹³⁾ stated that when the heat was locally applied to the skin, spinal cord, or hypothalamus, the sympathetic activity in the skin was suppressed (regional differentiation of sympathetic efferents). It is possible that with near infrared radiation the sympathetic activity in the skin and bone is suppressed, causing the intraosseous blood flow to increase. It is also possible that, owing to the relaxing of the muscles in the hind

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limb from the near infrared radiation, the venous return from the hind limb may increase, thus improving the blood circulation in the whole hind limb. At present, the direct effects of infrared radiation on vasoactivity is unknown.

The present study showed that both the intraosseous blood flow and intraosseous oxygen tension increased after radiation. Tissue oxygen tension has been examined by several authors. However with the electrodes used in their studies, oxygen tension could not be accurately or continuously measured during thermotherapy, because the sensitivity of a Po_2 sensor is considerably affected by temperature. Thus, if the tissue temperature changes, the Po_2 tension obtained with the sensor must be properly compensated, as it could be calculated higher than the actual tension with the increasing temperature. In the present study, we used a Clark-type polarographic electrode^{2,7)} slightly modified for our own use.^{15,16)} It consists of a Po_2 sensor cathode, a silver-silver chloride anode, and a thermocouple, which can continuously monitor the tissue temperature.

The oxygen tension in the proximal metaphysis of the rat tibia has not yet been reported. Cater⁶⁾ reported that the oxygen tension in rat bone marrow was 17.5 ± 2.8 mmHg. Brighton and Heppenstall^{4,5)} reported that in the metaphysis of the rabbit proximal tibia, the oxygen tension was 19.8 ± 3.2 mmHg. They also reported that the oxygen tension in the areas where cartilage and bone formation were actively occurring during fracture healing was relatively low, and suggested that oxygen consumption might increase in these areas. In the present study, the intraosseous oxygen tensions before and after infrared radiation were 20.4 ± 6.2 mmHg and 23.3 ± 6.7 mmHg, respectively. The increase of oxygen tension by the application of radiation was statistically significant ($p < 0.01$). The near infrared radiation might be effective for fracture healing in some bones through the increase of intraosseous blood flow and oxygen tension.

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