Hiroshima J. Med. Sci.
Vol.45, No.1, 15~22, March, 1996
HIJM 45-3

The Protective Effect of Low Power He-Ne Laser Against Erythrocytic Damage Caused by Artificial Heart-Lung Machines

Takashi ITOH, Hironobu MURAKAMI, Kazumasa ORIHASHI, Taijiro SUEDA and Yuichiro MATSUURA

First Department of Surgery, Research Institute of Replacement Medicine, Hiroshima University School of Medicine, Kasumi 1-2-3, Minami-ku, Hiroshima 734, Japan

ABSTRACT

We studied the protective effects of low power He-Ne laser irradiation on erythrocytes against the damage caused by experimental artificial heart-lung machines.

The erythrocyte suspension in a closed circuit was perfused with a roller pump. One of two circuits was used for laser treatment and the other for control. The laser group demonstrated significantly higher erythrocyte deformability and erythrocyte ATP levels, and lower free hemoglobin and lipid peroxide levels. A laser output power of 8.5 mW was most effective in protecting the erythrocytes. A further morphological finding using a scanning electron microscope showed fewer echinocytes and more discocytes in the laser group. This finding was consistent with the results of physiological and biochemical tests.

Low power He-Ne laser irradiation was shown to protect human erythrocytes from the damage caused by an experimental artificial heart-lung machine, raising the possibility of the clinical application of low power He-Ne laser treatment during extracorporeal circulation in cardiovascular surgery.

Key words: *Artificial heart-lung machine, Low power He-Ne laser, Erythrocyte deformability, Free hemoglobin*

Extracorporeal circulation (ECC) by means of an artificial heart-lung machine, which is the main measure for providing circulatory assistance in cardiovascular surgery, has markedly improved recently. However, by its nature it is a non-physiological process and affects the body in various manners[4,6,20]. Direct contact with the materials of ECC by means of an artificial heart-lung machine significantly damages the patient's blood, which in turn affects the patient's entire body via the circulatory system[5]. Thus, reducing the hematologic damage has been one of the most serious concerns in open heart surgery. Notably, hemolysis remains an especially serious unsolved problem during ECC, although improvements in the structure and composition of ECC with an artificial heart-lung machine as well as protection through the administration of various drugs have been attempted. Protection of the erythrocytes during ECC should contribute to the alleviation of the invasiveness of cardiovascular surgery.

The field of laser medicine has progressed markedly in recent years. Various low power lasers, such as the He-Ne laser, have been developed and applied clinically in both analgesia and wound healing. Although the precise mechanism by which low power laser facilitates treatment is not yet understood, low power laser irradiation is thought to activate the cellular function[2]. At present, drug administration is the general means used for protection againsts the effects of ECC. However, it sometimes causes host reactions and side effects. Therefore, the application of a low power laser instead of administering drugs was studied as a novel means of protecting erythrocytes from the damage caused by ECC using an artificial heart-lung machine.

MATERIALS AND METHODS

Preparation of leukocyte-excluded human erythrocyte suspension

In this experiment, only the damage to erythrocytes caused by the experimental artificial heart-lung machine was evaluated. Leukocytes, platelets and plasma were excluded, because these might cause a complex reaction to ECC. From six units of conserved or concentrated red cell (supplied by Japan Red Cross Society, 21 to 25 days after collection), leukocytes were excluded by a leukocyte removal filter (Sepacell® R-500 A, Asahi Medical Co. Ltd., Tokyo, Japan). The concentrated red cell was then rinsed with saline and centrifuged ($1000 \times g$, 5 min at 4°C). The supernatant was discarded, and 300 ml of

saline was again added for re-centrifugation. This process was repeated three times to exclude the plasma components. The precipitated erythrocytes were finally re-suspended in saline at a concentration equivalent to the physiological hematocrit. The suspension was inspected microscopically to confirm the absence of leukocytes and platelets in every field, and was stored at 4°C for 48 hours until the experiment to damage the erythrocytes.

Preparation of the experimental circuit

A hollow-fiber, membranous-type oxygenator made of polypropylene (type 30 EC, Senko Medical Instrument Manufacturing Company Limited, Tokyo, Japan) was connected to a tube made of vinyl chloride (diameter 4.5 mm, Senko Medical) including a reservoir in the circuit. A thermometer (diameter 4.5 mm, Senko Medical), and an OxySAT® cell (OTC–0250, Baxter Limited, Tokyo, Japan) were connected serially between the reservoir and pump and between the pump and oxygenator, respectively. Two sets of this closed circuit were set in position at the upper and lower deck of a double roller pump (MBP–107, Senko Medical). Oxygen and nitrogen were introduced through a roter flow meter and a gas mixture into the oxygenator, out of which the gas was ventilated to outside the room. The entire circuit, except for the pump, was maintained in an incubator to exclude the effects of temperature changes. (Fig. 1)

Filling the circuit with erythrocyte suspension and heating

Nitrogen was used to flush the circuit before filling, to producing an unaerobic situation. After 100% nitrogen was introduced into the two sets of circuits at the flow rate of 3 L/min for three minutes, the circuits were filled with 350 ml of the erythrocyte suspension, followed by de-airing. Under the nitrogen flow, the incubator was heated for 30 min to warm the blood to 37°C, and this temperature was maintained throughout the

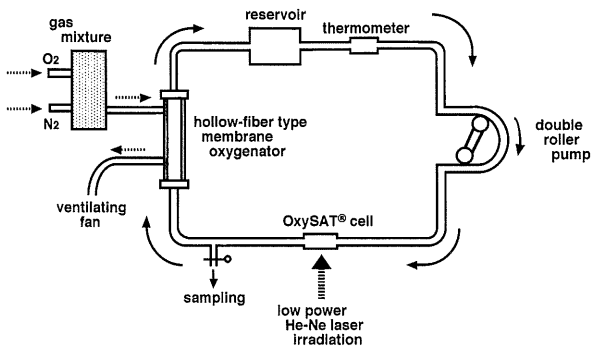

Fig. 1. Schematic illustration of a closed circuit experimental artificial heart-lung machine

experiment. In order to keep the distribution and temperature of the blood uniform, the blood pump was driven in advance at a low rate. The gas was then switched to 100% oxygen, with a flow rate of 3 L/min, and the blood pump was driven to maintain a flow rate of 330 ml/min for 240 min (228 cycles).

Laser irradiation to the circuit

The low power He-Ne laser used in this experiment was set at 632.8 nm wavelength, 8.5 mW output power and 1.96 eV quantum energy (MERA COLD LASER PDT, Senko Medical). The laser beam was irradiated to the object from 22 mm above the OxySAT cell, perpendicularly to the blood flow. The diameter of the laser beam was maintained at 4 mm. Laser output of three different intensities was evaluated: 4.25 mW (group I, n=7), 8.5 mW (group II, n=7), 17.0 mW (group III, n=8). The lower deck of the double roller pump was used for the laser group, and the upper deck without laser irradiation was used for the control group. The pumps for both groups were driven concomitantly, and both groups were tested simultaneously.

Sampling and measurement of the specimens

Samples were collected after 0 (pre-initiation value), 60 min (57 cycles), 120 min (114 cycles) and 240 min (228 cycles) from a region preceding the entrance to the oxygenator. Before the samples were centrifuged, erythrocyte ATP, which is a regulatory factor of erythrocyte deformability[14,16], was measured by the firefly luciferase assay[10], and was expressed as a percentage of the pre-initiation value. The partial oxygen pressure of the pre-centrifuged samples was also measured, by an electrode method (ABL510, Radiometer A/S, Copenhagen, Denmark), and was expressed as mmHg. After centrifugation, the free hemoglobin and lipid peroxide levels were measured in the supernatant by the hemoglobin-cyanide method[8], and Yagi method[21], respectively. These values were expressed as the rate of the increased fraction divided by the pre-initiation value to minimize the influence of individual variations, and were used as an index of hemolysis and erythrocyte membrane damage[15]. Following sedimentation, erythrocyte deformability was measured by the modified Reid method[19,22], and expressed as the RFR (Red cell filtration rate: μl/sec), an index of stress on the erythrocyte.

Morphologic observations

The erythrocytes in the control group and laser group (0utput 8.5 mW) were examined. The oxygenator was removed immediately after termination of the experiment, and rinsed with 200 ml of saline with a hydrostatic pressure of 20 cm. It was then fixed by perfusing 200 ml of 1.25% glu-

taraldehyde (in a 0.1 M phosphate buffer, pH 7.4). The polypropylene hollow fibers inside the oxygenator were then extracted and fixed by 1.25% glutaraldehyde for one day. Then they were lightly washed in a 0.1 M phosphate buffer, postfixed for 30 min in a 1% solution of O_SO_4 dissolved in a 0.1 M phosphate buffer, and dehydrated through a graded series of ethanol. Next they were immersed in isoamyl acetate, and critical pointdried using liquid CO_2. The specimens were evaporation-coated with gold-palladium and viewed in a field-emission type of scanning electron microscope (SEM: S–430, Hitachi, Tokyo, Japan)[12]. Three fields were randomly selected at ×1000 magnification and 1000 erythrocytes per each field were morphologically observed. The ratio of erythrocytes presenting a normal morphology (discocytes) was compared in both groups.

Statistical analysis

All values are expressed as mean ± S.E.. The values at each point were compared between the laser and control group by the Mann-Whitney U-test. A P-value less than 0.05 in the non-parametric test was considered to be statistically significant. The effect of laser output on each parameter was evaluated by an analysis of variance using a general linear model of SAS® (Statistical Analysis System). This model included the specimen factor, passage period and laser output power, and the effect of each factor was estimated quantitatively. Each value was calculated as a mean of Estimate ± S.E., and a P-value less than 0.05 was considered significant.

RESULTS

Erythrocyte deformability (Fig. 2)

Group I: Erythrocyte deformability of the laser group was significantly higher at 60 min (75.9 ± 5.0 vs. 59.5 ± 3.4 μl/sec). No significant differences between the two groups were observed at the other two time points.

Group II: Erythrocyte deformability of the laser group was significantly higher at all three time points after 60 min when compared to the respective control group (89.1 ± 3.8 vs. 65.1 ± 7.4 μl/sec at 60 min, 71.1 ± 4.9 vs. 51.8 ± 6.3 μl/sec at 120 min, 66.5 ± 6.2 vs. 45.9 ± 5.1 μl/sec at 240 min).

Group III: Erythrocyte deformability of the laser group was significantly higher at all three time points after 60 min when compared to the respective control group (55.2 ± 6.7 vs. 36.0 ± 4.4 μl/sec at 60 min, 41.0 ± 4.9 vs. 24.1 ± 3.6 μl/sec at 120 min, 23.5 ± 4.1 vs. 14.1 ± 0.9 μl/sec at 240 min).

Erythrocyte ATP levels (Fig. 3)

Group I: No significant differences of values were observed between the two groups at any of the three time points after 60 min.

Group II: Erythrocyte ATP levels were significantly higher in the laser group when compared to the control group at all three time points after 60 min (81.8 ± 2.9 vs. 67.5 ± 2.7% at 60 min, 71.8 ± 3.7 vs. 56.3 ± 3.2% at 120 min, 42.9 ± 3.7 vs. 29.9 ± 2.9% at 240 min).

Group III: Erythrocyte ATP levels were significantly higher in the laser group when compared to the control group at all three time points after 60 min (76.9 ± 4.2 vs. 65.8 ± 2.8% at 60 min, 65.9 ± 5.4 vs. 47.9 ± 3.9% at 120 min, 42.1 ± 3.7 vs. 30.0 ± 2.8% at 240 min).

Free hemoglobin levels (Fig. 4)

Group I: No significant differences of values were observed between the two groups at any of the three time points after 60 min.

Group II: Free hemoglobin levels were significantly lower in the laser group when compared to the control group at all three time points after 60 min (5.8 ± 2.1 vs. 11.7 ± 2.4% at 60 min, 11.5

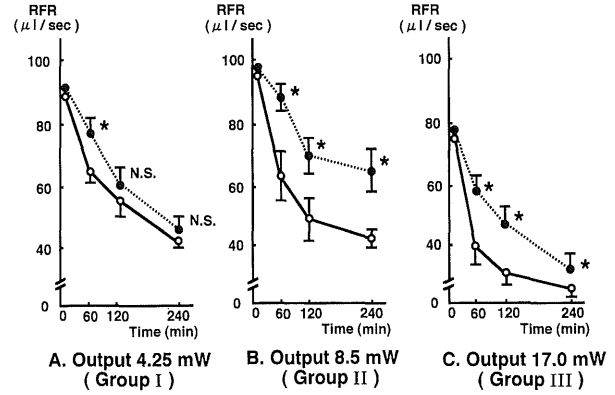

Fig. 2. Changes in erythrocyte deformability
○—○: Control Group, ●···●: Laser Group (mean ± S.E.)
*: $p < 0.05$ (Mann-Whitney U-test)

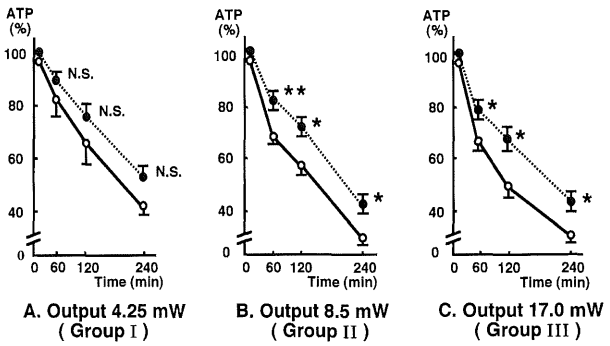

Fig. 3. Changes in the erythrocyte ATP level
○—○: Control Group, ●···●: Laser Group (mean ± S.E.)
**: $p < 0.01$, *: $p < 0.05$ (Mann-Whitney U-test)

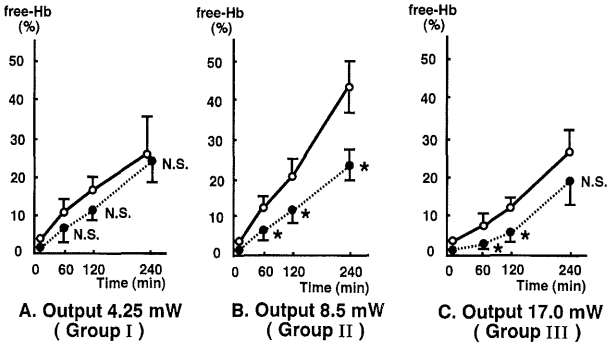

Fig. 4. Changes in the free hemoglobin level
○−○: Control Group, ●···●: Laser Group (mean ± S.E.)
*: $p < 0.05$ (Mann-Whitney U-test)

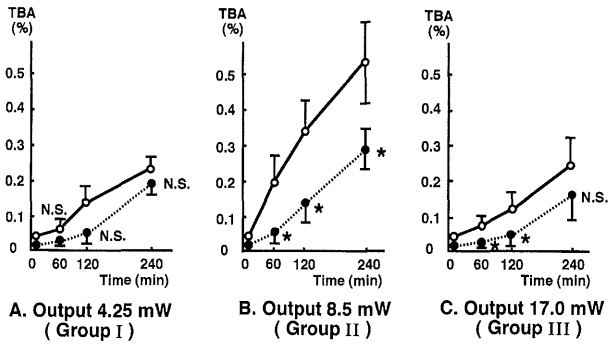

Fig. 5. Changes in the TBA level
TBA: Thiobarbituric acid reactive substance
○−○: Control Group, ●···●: Laser Group (mean ± S.E.)
*: $p < 0.05$ (Mann-Whitney U-test)

± 3.6 vs. 20.2 ± 3.4% at 120 min, 22.9 ± 3.3 vs. 42.7± 7.1% at 240 min).

Group III: Free hemoglobin levels were significantly lower in the laser group when compared to the control group at 60 and 120 min, but no statistical difference was detected at 240 min (2.4 ± 0.8 vs. 6.1 ± 1.0% at 60 min, 5.0 ± 2.0 vs. 11.0 ± 3.0% at 120 min).

Lipid peroxide levels (Fig. 5)

Group I: No significant differences of values were observed between the two groups at any of the three time points after 60 min.

Group II: Lipid peroxide levels were significantly lower in the laser group when compared to the control group at all three time points after 60 min (5.4 ± 2.5 vs. 19.3 ± 7.8% at 60 min, 13.1 ± 5.2 vs. 33.2 ± 9.6% at 120 min, 28.5 ± 5.3 vs. 53.0 ± 12.4% at 240 min).

Group III: Lipid peroxide levels were significantly lower in the laser group when compared to the control group at 60 and 120 min, but no statistical difference was detected at 240 min (0.9 ±

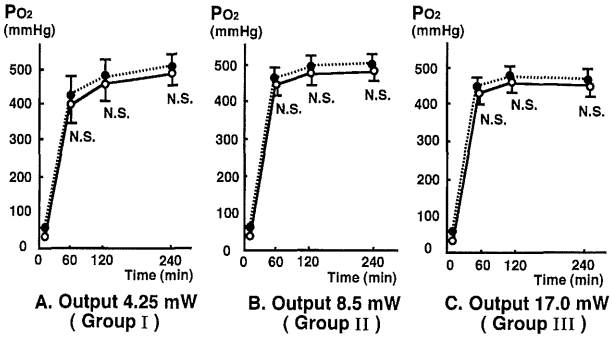

Fig. 6. Changes in the Po_2 level
○−○: Control Group, ●···●: Laser Group (mean ± S.E.)
Mann-Whitney U-test

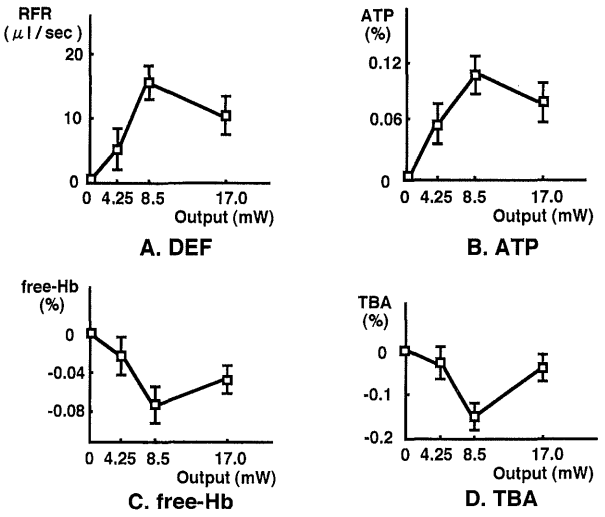

Fig. 7. The effects of Laser output on each parameter
$p < 0.0001$ (General Linear Model of SAS)
Results are expressed as Estimate ± S.E.

0.5 vs. 5.4 ± 2.1% at 60 min, 3.7 ± 2.1 vs. 11.2 ± 3.7% at 120 min).

Partial oxygen pressure (Fig. 6)

Groups I, II and III: No significant differences of values were observed between the two groups at all three time points.

The effects of laser output on each parameter (Fig. 7)

Erythrocyte deformability (Fig. 7 A): The effect of 0 mW output was determined to represent 0 μl/sec and was used as a standard for calculating the general linear model of SAS. The calculated values were 5.78 ± 2.97 μl/sec, 15.51 ± 2.56 μl/sec, and 10.82 ± 2.46 μl/sec, for 4.25 mW, 8.5 mW, and 17.0 mW, respectively. This value was maximized with a laser output of 8.5 mW.

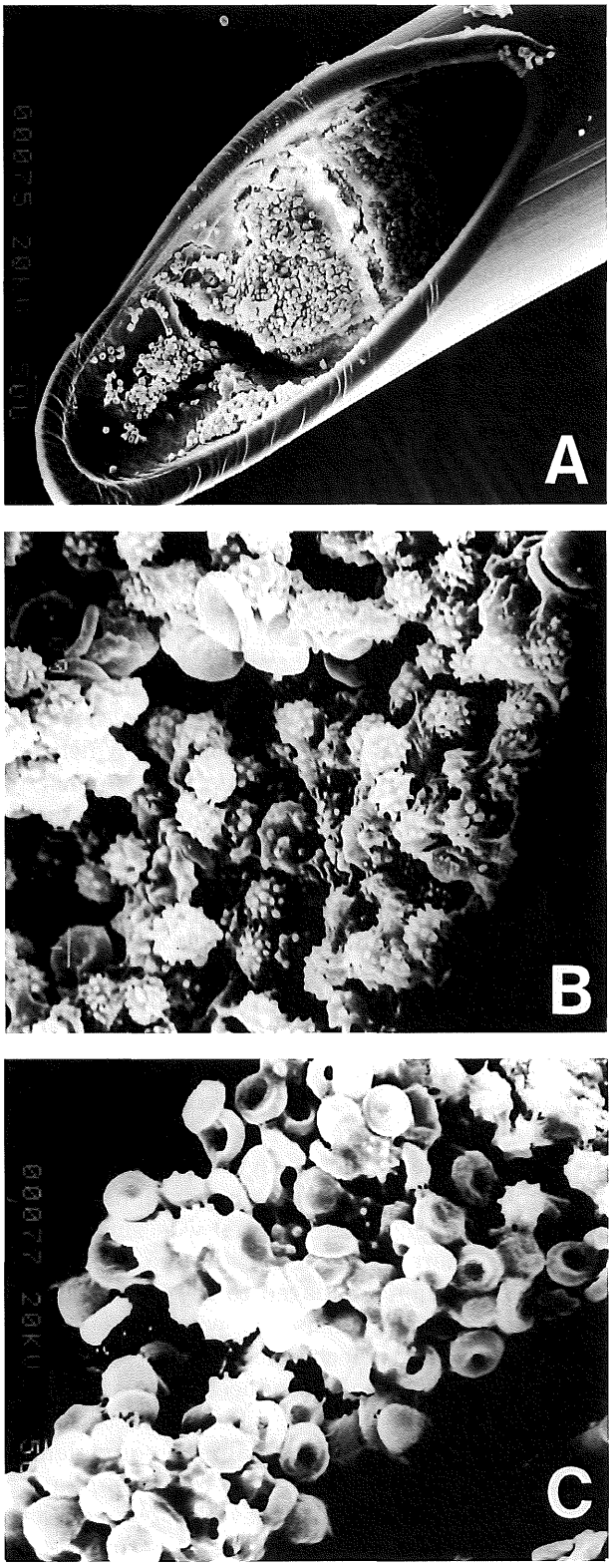

Fig. 8. Scanning electron micrographs of erythrocytes in hollow-fiber
(A) ×210
(B) ×1800: Control Group; the data of this sample are Deformability 4.76 μl/sec, free-Hb 205.9% (1.11 g/dl) and TBA 57.1% (1.10 nmol/ml)
(C) ×1800: Laser Group of output 8.5 mW; the data of this sample are Deformability 10.42 μl/sec, free-Hb 97.1% (0.63 g/dl) and TBA 10.7% (0.78 nmol/ml)

Erythrocyte ATP levels (Fig. 7 B): Similarly, the effect of 0 mW output was determined to represent 0% and was used as a standard. The calculated values were 0.06 ± 0.02%, 0.11 ± 0.02%, and 0.08 ± 0.02%, for 4.25 mW, 8.5 mW, and 17.0 mW, respectively. This value was also maximized with a laser output of 8.5 mW.

Free hemoglobin (Fig. 7 C): Similarly, the effect of 0 mW output was determined to represent 0% and was used as a standard. The calculated values were –0.03 ± 0.02%, –0.08 ± 0.02%, and –0.05 ± 0.02%, for 4.25 mW, 8.5 mW, and 17.0 mW, respectively. This value was lowest with a laser output of 8.5 mW (a negative value indicates an inhibitory effect).

Lipid peroxide levels (Fig. 7 D): Similarly, the effect of 0 mW output was determined to represent 0% and was used as a standard. The calculated values were –0.03 ± 0.04%, –0.15 ± 0.03%, and –0.04 ± 0.03%, for 4.25 mW, 8.5 mW, and 17.0 mW, respectively. This value was lowest with a laser output of 8.5 mW (a negative value indicates an inhibitory effect).

The correlation coefficient between the data and the value calculated according to the general linear model was as high as 0.84–0.96. This model was considered to accurately reflect the characteristics of the data. Based on this model, a laser output of 8.5 mW was significantly effective ($p<0.0001$) in the protection of erythrocytes, and was the most effective of the three different laser intensities evaluated.

Morphologic observations

The erythrocytes within the hollow-fiber matrix of the oxygenator from both the laser (output 8.5 mW) and control groups could be categorized as either morphologically normal cells (discocytes) or as abnormal spinous erythrocytes (echinocytes) (Fig. 8). The percentage of discocytes was approximately 25% and 45%, in the control and the 8.5 mW output laser groups, respectively. Thus, there were fewer echinocytes and more discocytes in the 8.5 mW output laser group, when compared to the control group.

DISCUSSION

Many studies have reported the attenuation of erythrocyte damage by ECC using an artificial heart-lung machine in cardiovascular surgery. However, most of these studies reported improvements in the structure and composition of ECC with an artificial heart-lung machine[18], or reported protection of the erythrocyte membrane following the administration of drugs, such as PGE_1[23], PGI_2 derivative[1], α-tocopherol[15], urea[22] and Poloxamer 188[17]. However, there have been no studies reporting the prevention of erythrocyte damage caused by ECC using an artificial heart-lung machine without changes to the

apparatus or without drug administration.

LASER is an acronym for light amplification by stimulated emission of radiation, which can be defined as light energy having special qualities, such as coherence, monochromaticity, photo-orientation. The medical laser apparatus utilizes both high and low power lasers. High power lasers have an output of several to 100 W. This output level causes light-induced reactions in the tissue such as perspiration, carbonization, coagulation and protein degeneration, according to the energy density of the laser beam. Low power lasers have an output of up to 100 mW, and induce photochemical effects (biological activation and stimulation), in addition to effects caused by photosensitization[2]. Other reports have shown that the effects of low power laser are enhanced in damaged tissues[7,24].

Although low power lasers are widely used in a clinical setting, the mechanisms underlying their clinical effects are not yet well understood. Karu[9] reported that low power laser can stimulate the respiratory chain components of mitochondria and accelerate ATP production, while Kubasova[11] reported that the laser irradiation-induced circumstances on cell surfaces can contribute to the strength of cell-to-cell contacts. Berki[3] reported the Arndt-Schultz rule: that cell activation occurs at a well-defined energy level, but that no effect occurs below this level and destruction occurs above it. Luckey[13] also reported a similar concept, that of radiation hormesis, noting that although high dose radiation was biologically harmful, low dose radiation induced positive effects. The photochemical effects of low power laser may be attributable to the fact that radiant energy and light are both properties of electromagnetic radiation. Molecular fission occurs when the quantum energy of laser light is very high[2]. However, when the quantum energy is minimal as it is in low power lasers, it only causes an expansion of the intracellular distance. In accordance with the phenomena Karu and Kubasova reported, this expansion of the intramolecular distance might allow more flexibility in the erythrocyte membrane and thereby improve erythrocyte deformability.

Based on these observations, we focused on the use of low power laser for the protection of human erythrocytes during ECC with an artificial heart-lung machine.

In our experiment, the only difference between the laser and control groups was the presence of laser irradiation. The samples were made from the same supply of conserved blood. Moreover, the circuit apparatus and the pump were precisely matched, and the experiment was performed simultaneously, in both groups. No significant differences were observed between the upper and lower decks of the double roller pump in a preliminary experiment measuring free hemoglobin levels. Furthermore, no significant difference was observed in the partial oxygen pressure, between the laser and control groups, indicating that erythrocyte oxygenation was maintained throughout the experiment. The total irradiation time and quantity were apparently appropriate since erythrocyte hemolysis, which would have occurred at the beginning at the experiment if the low power laser was damaging the erythrocytes, was not observed.

The erythrocyte deformability and erythrocyte ATP levels were significantly higher in the laser treated group, when compared to the control group. Clinically, tissue capillaries are thought to be easily occluded by low pressure, non-pulsatile flow perfusion of the ECC. Therefore, maintaining erythrocyte deformability prevents thrombus and embolism formation and improves microcirculation. There is a close correlation between hemolysis and decreased erythrocyte deformability. Preservation of erythrocyte deformability is thought to prevent hemolysis[23]. In the laser group, the increase in free hemoglobin which represents the degree of hemolysis, was in fact inhibited. The inhibition of hemolysis should contribute to the prevention of complications associated with ECC, such as damage to the kidney, lung, brain and liver, and a bleeding tendency. Erythrocyte ATP levels are considered to be the regulatory factor of erythrocyte deformability, and the observation that erythrocyte ATP levels also increased significantly in laser-treated erythrocytes, is consistent with the theory. The inhibited release of lipid peroxide, which was noted in the laser group, is thought to decrease the tissue damage caused by erythrocyte-derived lipid peroxide. Morphologic observations confirm that the number of echinocytes was smaller and there were more discocytes in the 8.5 mW output laser group, when compared to the control group. These results are consistent with those of the physiological and biochemical tests. An energy output level of 8.5 mW produced the best results when the effects of three different laser output levels were compared. This value is equivalent to 0.086–0.345 J/cm^2, which is quite similar to 0.1–1.0 J/cm^2, the energy level at which Berki obtained the best results in his experiments using mouse macrophage. Therefore, a similarly low output power level of laser irradiation would appear to be effective in different species. No significant differences were observed among the 4 parameters except partial oxygen pressure between the control and laser groups at 60 min in group I (4.25 mW), with the exception of changes in the erythrocyte deformability. This observation suggests that an output level of 4.25 mW is below the requisite intensity. On the other hand, an output level of 17.0 mW is above the ideal energy

range because no significant differences were observed in the free hemoglobin and the lipid peroxide levels at 240 min, although the individuality of the samples should be taken into consideration.

In this study, strict conditions were imposed, compared with those used in clinical settings, in order to obtain accurate data. A higher laser energy output power of irradiation for as long as 8 to 12 hours, which is equal to 0.690–1.035 J/cm^2, caused the sudden onset of massive hemolysis in some cases, which is consistent with Berki's theory. Conversely, under more practical conditions with the plasma component and dilution of whole blood as in the ECC, results were improved even after 12 hours of irradiation without the sudden onset of massive hemolysis. Therefore, beneficial effects of low power He-Ne laser treatment with longer irradiation can be expected in clinical settings.

Low power He-Ne laser irradiation significantly protects the human erythrocyte from damage caused by the experimental artificial heart-lung machine during a 240 min (228 cycles) period of irradiation using a leukocyte-excluded human erythrocyte suspension within a closed circuit apparatus. This effect was maximal at an energy output level near 8.5 mW, and the clinical application of laser treatment for ECC might be indicated.

Further studies are anticipated to reveal the underlying mechanism of laser-induced effects and to develop methods suitable for clinical application.

ACKNOWLEDGMENTS

We thank Associate Professor Y. Kusumoto of the Second Department of Anatomy, Hiroshima University School of Medicine, for his invaluable support in the morphologic observation. We also thank Associate Professor M. Kakehashi of the Institute of Health Sciences, Hiroshima University School of Medicine, for his great support in statistical analysis.

This study was presented at the 6th Japan Laser Therapy Association, held on June 25–26, 1994 in Kobe, at the 32d Japanese Society for Artificial Organs, held on October 12–13, 1994 in Tokyo, at the 47th Japanese Association for Thoracic Surgery, held on October 25–27, 1994 in Chiba, at the 95th Japan Surgical Society, held on April 10–12, 1995 in Nagoya, and at the 33d Japanese Society for Artificial Organs, held on November 7–9, 1995 in Osaka.

This study was supported by The Senko Medical Instrument Manufacturing Company Limited.

(Received October 13, 1995)
(Accepted January 12, 1996)

REFERENCES

1. **Akemoto, K., Ueyama, T. and Koto, K.** 1988. A study of erythrocyte deformability and its prevention during cardiopulmonary bypass. J. Jap. Assoc. Thorac. Surg. **36:** 2353–2362. (in Japanese with English abstract)
2. **Atsumi, K., Nishizaka, T., Oomori, S., Sakamoto, S., Sano, K., Takemoto, T., Niijima, Y., Hirano, M. and Mishima, S.** 1980. Biological effects of low power laser, p.35–39, *In* Y. Sakurai and M. Kikuchi (eds.), Laser Igaku. Nakayama Bookseller's, Tokyo. (in Japanese)
3. **Berki, T., Nemeth, P. and Hegedus, J.** 1988. Biological effect of low-power Helium-Neon laser irradiation. Lasers in Med. Science **3:** 35–39.
4. **Galletti, P.M. and Brecher, G.A.** 1962. Heart-lung bypass. Grune & Stratton Inc, New York.
5. **Hammerschmidt, D.E., Stroncek, D.F., Bowers, T.K., Lammi-Keefe, C.J., Kurth, D.M., Ozalins, A., Nicoloff, D.M., Lillehei, R.C., Craddock, P.R. and Jacob, H.S.** 1981. Complement activation and neutropenia occurring during cardiopulmonary bypass. J. Thorac. Cardiovasc. Surg. **81:** 370–377.
6. **Hirayama, T., Roberts, D.G., Allers, M., Belboul, A., Al-Khaja, N. and Olsson, G.W.** 1988 Association between bleeding and reduced red cell deformability following cardiopulmonary bypass. Scand. J. Thorac. Cardiovasc. Surg. **22:** 171–174.
7. **Iijima, K., Shimoyama, N., Shimoyama, M. and Mizuguchi, T.** 1993. Effect of low-power He-Ne laser on deformability of stored human erythrocytes. J. Clin. Laser Med. Surg. **11:** 185–189.
8. **Kampen, E.J. and Zijlstra, W.G.** 1961. Standardization of hemoglobinometry II. The hemoglobincyanide method. Clin. Chim. Acta **6:** 538–554.
9. **Karu, T.I.** 1987. Photobiological fundamentals of low power laser therapy. IEEE J. Quantum Electronics **10:** 1703–1717.
10. **Kiyotani, K., Kanamoto, Y. and Matsuo, Y.** 1984. Firefly luciferase assay for adenosine triphosphate in cultured cells. Hiroshima J. Med. Sciences **33:** 279–285.
11. **Kubasova, T., Kovacs, L., Somosy, Z., Unk, P. and Kokai, A.** 1984. Biological effect of He-Ne laser. Investigations on functional and micromorphological alterations of cell membranes, in vitro. Lasers in Surg. Med. **4:** 381–388.
12. **Kusumoto, Y.** 1981. The effects of local anesthetics on the degranulation of rat peritoneal mast cells. Biom. Research **2:** 572–582.
13. **Luckey, T.D.** 1980. Hormesis with Ionizing Radiation. CBC Press, Boca Raton, Florida.
14. **Maeda, N.** 1989. Organization of the erythrocyte membrane skeleton and the rheology. Byoutaiseiri **8:** 965–971. (in Japanese)
15. **Murakami, H.** 1991. Influence of Heart-lung machine on lipid peroxides from human erythrocytes. Hiroshima J. Med. Sciences **39:** 265–280.
16. **Nakao, M., Nakao, T. and Yamazoe, S.** 1960. Adenosine triphosphate and maintenance of shape of the human red cells. Nature **187:** 945–946.

17. **Ohishi, K., Hoshino, Y., Isamoto, Y., Nishimura, H., Yuda, T., Miyazaki, T., Yamashita, S., Asano, K., Kaneko, H. and Tachibana, N.** 1981. Clinical evaluation of Poloxamer 188 (Exocorpol) as a prophylactic for hemolysis in excorporeal circulation by double-blind method. Jap. J. Thorac. Surg. **34:** 346–351. (in Japanese with English abstract)
18. **Onoda, K., Kondo, C., Mizumoto, T., Kusagawa, H., Katayama, Y., Hayashi, T., Komoda, T., Hirano, R., Miyamura, T., Tanaka, J., Yasuda, F., Tanaka, K., Shimpo, H., Yada, I., Yuasa, H., Aizawa, T. and Kusagawa, M.** 1994. Clinical experience with Nikkiso centrifugal pumps for extracorporeal circulation. Artificial Organs **18:** 706–710.
19. **Reid, H.L., Barnes, A.J., Lock, P.J., Dormandy, J.A. and Dormandy, T.L.** 1976. A simple method for measuring erythrocyte deformability. J. Clin. Pathol. **29:** 855–858.
20. **Wright, G.** 1986. Cardiopulmonary bypass. Chapman and Hall Ltd, London.
21. **Yagi, K.** 1976. Short communications. A simple fluorometric assay for lipoperoxide in blood plasma. Biochem. Med. **15:** 212–216.
22. **Yamaguchi, H., Allers, M. and Roberts, D.** 1984. The effect of urea on red cell deformability during cardiopulmonary bypass. Scand. J. Thorac. Cardiovasc. Surg. **18:** 119–122.
23. **Yamaguchi, H., Shimizu, T., Akutsu, H., Kawachi, K., Osada, K., Fujikawa, T., Suesada, H., Hirayama, T., Ishimaru, S. and Furukawa, K.** 1990. Hemolysis and red cell deformability during cardiopulmonary bypass. The effect of Prostaglandin E_1 for prevention of hemolysis. J. Jap. Assoc. Thorac. Surg. **38:** 625–629. (in Japanese with English abstract)
24. **Yamaguchi, N., Matono, S. and Iwase, T.** 1992. Acceleration of human lymphocytes blastoid transformation by low energy laser irradiation in vitro. J. Jap. Soc. Laser Med. **13:** 3–9. (in Japanese with English abstract)