THE POTENTIAL USE OF LASER ENERGY IN THE MANAGEMENT OF MALARIA

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Summary

A feasibility study was conducted to illustrate that malarially parasitized erythrocytes from infected ducks and man, when treated with methylene blue and exposed to ruby laser energy, show a wavelength specific photolysis of only the parasitized erythrocytes. On the strength of this preliminary study, continuing investigation is being conducted to evaluate other parameters of laser treatment in the management of malaria in experimental animals. These studies suggest that it is feasible to design a protocol for the management of malaria in human patients.

Evidence indicates that a biological effect can be produced by laser energy if the absorption characteristics of the target molecule match the emission frequency of the laser source (Giese, 1964; Blum, 1964; Rounds, 1967). This conformance to the Beer-Lambert law suggests that laser energy might be considered in the management of malarial infections in circulating erythrocytes.

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One might utilize either a natural constituent as a chromophore or might apply a vital dye to specifically sensitize the parasite. There are two apparent possibilities for practical implementation. One would be to use the by-products resulting from hemoglobin metabolized by Plasmodium (Russell, 1963; Sherman, 1968). The second consideration, application of a photosensitizing dye, must meet certain criteria: (1) the dye must be non-toxic; (2) it must have greater specific uptake by the malaria parasite than by cells of the host system, and (3) it must absorb adequately at an emission wavelength of one of the standard lasers. Silverman (1944) reported that methylene blue is effective in the management of Plasmodium galinaceum. This dye is non-toxic and has appropriate absorption characteristics in the emission regions of the ruby and helium-neon lasers.

It can, therefore, be hypothesized that if methylene blue were administered to a malaria-infected patient, the dye would specifically photosensitize circulating infected erythrocytes to both ruby and helium-neon laser emissions. The present study was undertaken to determine the effect of laser energy on unstained and methylene blue stained duck erythrocytes infected with Plasmodium lophurae.

Materials and Methods

Blood was drawn from the jugular vein of white Pekin ducklings in a heparinized syringe and divided into several aliquots. Methylene blue was dissolved in Gey's balanced salt solution to yield serial concentrations ranging from 0.00005 percent to 0.5 percent. Equal volumes of the avian blood and the methylene blue dilutions were mixed and drops of each sample were placed on a slide, overlaid with a coverslip and sealed with mineral oil to form a wet-mount. Laser energy from a pulsed ruby laser (pulse width 2.5 msec) was applied in a range from 10 j/cm^2 to 100 j/cm^2.

Individual cell types found within these smears were examined with oil-immersion phase-contrast optics both before and immediately after laser treatment. The total erythrocyte population was counted and plotted

according to cell condition. Cells without evident parasites and with typically normal morphology were considered normal. Cells showing any evidence of contained Plasmodium lophurae were scored as parasitized. Hemolyzed cells were also recorded. Damage to the leukocyte population was evaluated in terms of morphological alteration as seen with oil-immersion phase-contrast optics.

Results

Stained smears of duck blood reveal that the erythrocytes are

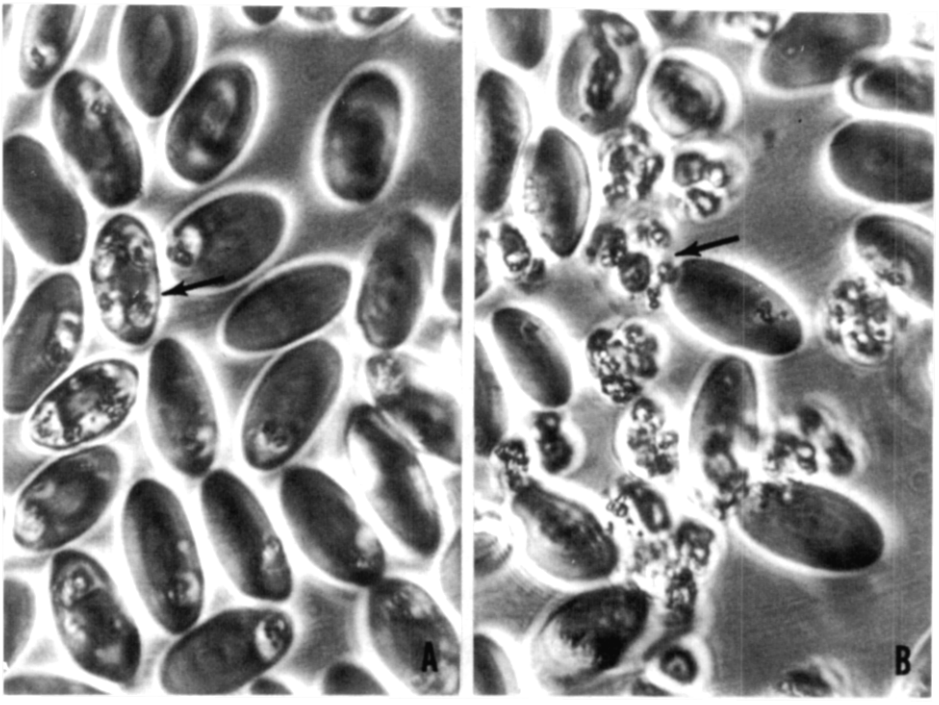

Figure 1a: Erythrocytes from duck after treatment with 0.00025 % methylene blue. Note peripherally located Plasmodium lophurae parasites.
Figure 1b: Duck erythrocytes after 0.00025 % methylene blue treatment followed by 70 joules/cm^2 ruby laser irradiation. Parasitized erythrocytes have been hemolyzed, while normal elements remain intact.

elliptical and nucleated. In the experimental animals infected with Plasmodium lophurae the degree of infection ranges from the small trophozoite to single or multiple schizont forms. The parasite infects primarily mature erythrocytes. The leukocytes appear to be uninfected.

Unstained wet-mount preparations of duck blood, as seen with oil-immersion phase-contrast optics, show a pattern similar to that of preparations vitally stained with methylene blue, but not treated with the ruby laser (Figure 1a). Following treatment with 70 joules/cm^2 of energy at 6943 Å surviving cells were predominantly uninfected erythrocytes or reticulocytes (Figure 1b). The cell population distribution, according to red cell condition following ruby laser treatment, is shown in Figure 2. In the sample used for this experiment, approximately 25 percent of the erythrocytes were uninfected, and this proportion remained relatively constant from the pre-irradiated to the post-irradiated condition. The 75 percent of the cells which contained mature schizonts was reduced to

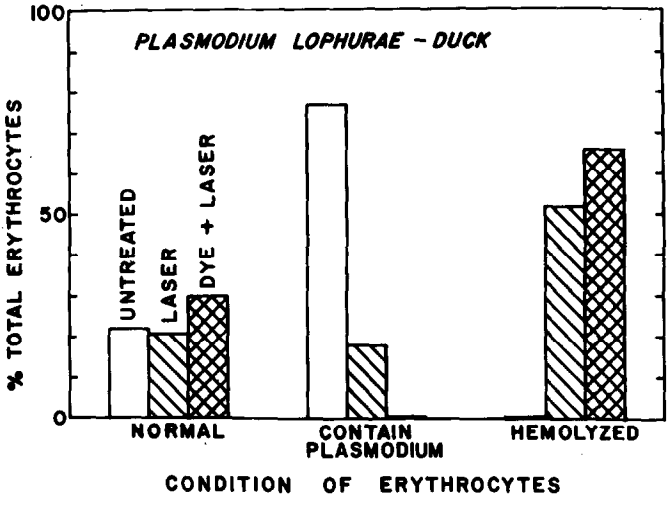

Figure 2: Distribution of erythrocyte population according to condition after treatment with laser alone (70 joules/cm^2) and methylene blue (0.00025 %) followed by laser.

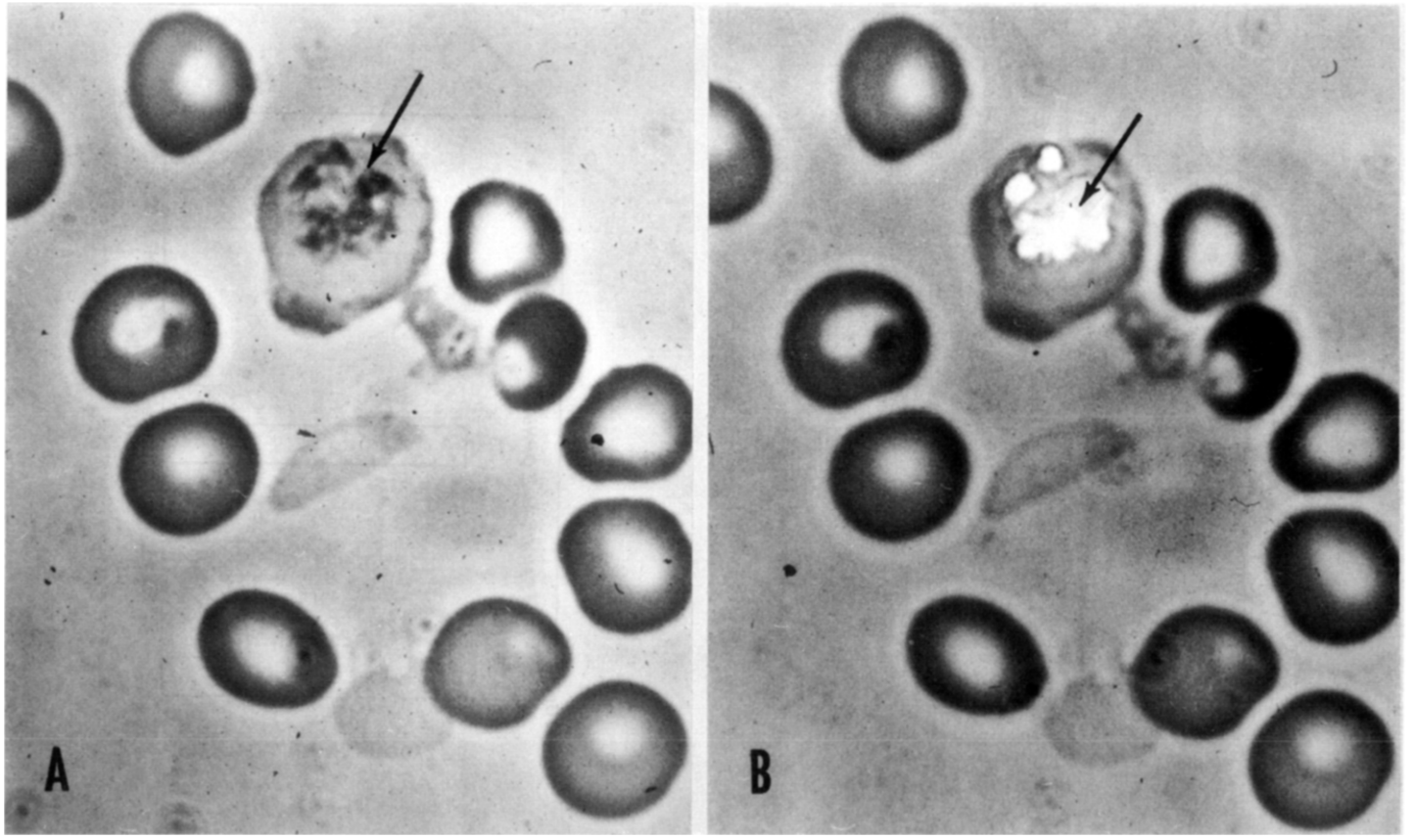

Figure 3a: Fixed and methylene blue stained human erythrocytes. Note malaria parasite in single cell (arrow).
Figure 3b: Same field following irradiation with energy from the ruby laser, demonstrating selective destruction of the parasite, which had taken up the dye. Other red cells were not stained and remain unaffected by the application of laser energy.

20 percent in the absence of methylene blue, but was reduced to 0 if a final concentration of 0.00025 percent methylene blue was present in the irradiated suspension. The number of hemolyzed cells following laser treatment paralleled the parasitized cell number before treatment. Images of human infected and non-infected erythrocytes shown in Figures 3a and 3b demonstrate that if the parasite is specifically stained to absorb the ruby laser frequency, there is a specific action imposed upon only this structure. While this study did not include the application of methylene blue to parasitized fresh blood from human subjects, the principle of action suggests that any of the four Plasmodium species found in the human would be subject to the same specific action as demonstrated with Plasmodium lophurae in avian blood.

Conclusions

The results of this preliminary study have confirmed that the laser can impose wavelength-specific damage to malaria-infected erythrocytes from both the duck and man. The observation that the ruby laser can impose severe damage on parasitized erythrocytes in unstained preparations suggests that a natural chromophore is responsible for the hemolytic effect. The nature of this chromophore was not investigated within the scope of this study; however, one of the breakdown products of hemoglobin may be responsible. The nature of the breakdown products of hemoglobin remains unknown. This product, or products, if present even in low concentration, could conceivably produce photosensitization of the parasitized erythrocytes to the ruby laser wavelength of 6943 Å. Recalling that the Beer-Lambert law implicates both the absorption characteristics and the concentration of the chromophore suggests also that a substance present in sufficient quantity, even with only slight absorbance at 6943 Å, could absorb sufficient energy to cause hemolysis. Under these circumstances, the lack of the requirement for staining with methylene blue would eliminate the potential hazard to circulating leukocytes.

Nevertheless, it was observed that the extremely low concentrations of methylene blue used in the study (0.00025 percent) demonstrated a more effective destruction in the total parasitized erythrocyte population (Figure 2), while appearing to produce minimal damage to the leukocyte population. On the strength of these results, it would appear that this general protocol should be continued to be considered in terms of the potential clinical management of the disease. As a result, continuation studies are being conducted to investigate the possibility that the ruby laser might be replaced by either high-powered or focused helium-neon continuous wave lasers or highly filtered non-coherent light sources.

In addition, whole animal studies are being conducted to establish the degree of penetration of light from these sources to determine whether it would be necessary to exteriorize the blood supply through a transparent cuvette for in vivo laser treatment. It is attractive to consider the possibility that the exteriorized blood might be subjected to centrifugal force in order to selectively direct the laser energy toward the erythrocyte population and prevent potential damage to leukocytes. Cellular debris might also be removed before the blood were returned to the host circulation. It has been anticipated that the removal of this debris would reduce the toxemia sustained by the patient following laser treatment and, as a result, more intense laser energy might be applied in the eradication of parasitized erythrocytes.

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