IN VIVO AND IN VITRO HeNe LASER EFFECTS ON PHAGOCYTE FUNCTIONS

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Abstract—The goal of this work was to evaluate the effect of helium-neon (HeNe) laser irradiation on immunocompetent cells. We used the in vivo skin window method and in vitro granulocyte function tests. The study of cellular migration showed a marked decrease in vitro and in vivo in a dose-independent manner. Superoxide release was not modified by laser irradiation. The granulocyte's aggregation, when using PHA and PMA, presented a reduction that was statistically very significant, not as a subordinate dose. An increase of the release of ATP was demonstrated only at 4 joules and precedes granulocyte aggregation. When using Ca2+ ionophore A23187 as stimulus, laser irradiation at 1, 2 or 4J did not show any modification of granulocyte aggregation. The monoclonal antibody 60.1, which identifies a membrane antigen fundamental for aggregation and chemotaxis, is expressed in normal amounts on granulocyte membranes both before and after irradiation with a HeNe laser. In fact, laser irradiation preferentially attacks the area of the cellular centrosome that determines a modification of cellular morphology. The electron microscope and immunofluorescence study with a monoclonal antibody have pointed out a disorganization of the microtubules. The alteration of some of the granulocyte functions is correlated to the damage in the centrioles. The granulocyte mitocondrial system and surface membrane remain intact, and this explains the normal production and release of free radicals. Further experiments are necessary to evaluate the clinical application of lasers in various diseases with immunophagocytic pathogenesis.

INTRODUCTION

Phagocytic cells have an important role in host defense (1-4). The major phagocytic cells in the circulation are neutrophils and monocytes. The adherence and aggregation of these cells to vascular endothelium may be an essential first
step for other phagocytic functions, including diapedesis, chemotaxis, phagocytosis, and enzyme and ATP release (5–11). Thus, patients with primary deficiency of neutrophil aggregation and adherence suffer from severe recurrent pyogenic infections (12–23). Cell movement is a fundamental process of particular relevance to problems in biology, oncology, immunology, and dermatology. Furthermore, the phagocytes are fundamental in various noninfectious diseases (3, 24, 25).

Recently the laser has been very useful in the operating room and dentist’s office; in endoscopy, dermatology, rheumatology, ophthalmology, gynecology, otorhinolaryngology and in the laboratory (26–44). Various authors have studied its application in human pathology and estimated its effects from an electron microscope, biochemical, immunological, and clinical point of view. The interaction between a laser beam and biological material causes effects that we classify as thermal or photochemical in relation to characteristics of exposure. The final biological effects consist of the global alteration of all the biophysical parameters of the irradiated cells and their metabolism, but there are contrasting data in the literature about the relationship between the laser and the immune system.

The aim of this investigation is to evaluate the effect of helium–neon (HeNe) laser irradiation on immunocompetent cells using two methods: in vivo study by the skin window method and in vitro study by evaluation of PMN aggregation, ATP and anion release, random migration, chemotaxis, and surface membrane glycoprotein with monoclonal antibodies.

MATERIALS AND METHODS

Separation of Neutrophils. Peripheral blood leukocytes were obtained from 30 ml of healthy volunteer venous blood, to which we added 500 units of heparin in a sterile plastic test tube. Twenty milliliters of 6% dextran was then added. The mixture was left standing for 30–60 min at room temperature, after which time the leukocyte-rich supernatant was withdrawn. This procedure was done simultaneously with both patient and control blood. The polymorphonuclear leukocytes were isolated using the Isopaque-Ficoll system (45). The purity of the patient cell fractions obtained was 87 ± 1.3% PMNs and the corresponding purity of the control fractions was 94 ± 1.7%.

Contaminating erythrocytes in the PMN fraction were removed by lysis with 0.75% ammonium chloride solution, containing 20 mM Tris HCl buffer (final PH 7.4) and 0.25% autologous plasma. This was then gently shaken for 10 min at 37°. PMN viability was tested by trypan blue exclusion and was greater than 96%. Only platelet-free suspensions were used.

Measurement of Migrating Activity. Migration was measured using the modified Boyden chamber method (19, 46, 47). PMNs were suspended at a concentration of 1 × 10^7/cells/ml in Hanks’ balanced salt solution (HBSS), then incubated in a Boyden chamber (Bio-Rad Lab, Richmond, California) for 45 min at 37°C under 5% CO₂. The migration distance (μm/min) from the surface of a Millipore filter (3.0 μm pore size, Millipore, Bedford, Massachusetts) to the leading front of the cells was measured. The resulting value was used to determine random migration and