Harmful Singlet Oxygen can be helpful

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Abstract-Highly reactive harmful singlet oxygen O_2 can be helpful while relaxing to its triplet ground state O_2. The energy emitted during this relaxation from excited energy stare is discernable at 634nm. We report here on the effect of this energy as photon illumination and as energy transfer in air on the production of reactive oxygen species (ROS) by human monocytes, measured as isoluminol-enhanced chemiluminescence. We demonstrate up to 60% decrease in the secretion of ROS after 2-min illumination of the monocytes stimulated with phorbol myristate acetate (PMA). The results provide in vitro documentation of the utility of singlet oxygen energy in modifying cellular behavior. © 1999 Elsevier Science Inc.

Introduction

Oxygen is an abundant element with multiple faces. Its most common and important one is the dioxygen (O_2), witch is a prerequisite for all aerobic cell metabolism. Another face of oxygen is the one with an unpaired electron: the free radical derivate with its highly unstable and reactive forms. These reactive forms have been implicated in a wide range of toxic mechanism in biological organisms. When the ground state O_2 is exited to a higher energy state, singlet O_2 is formed. This form of oxygen is also a harmful species in biological systems [1]. Singlet oxygen can be formed photochemically by energy transfer from an excited photosensitizer.

Numerous studies have focused on the use of antioxidants and scavenger enzyme systems. The purpose of these studies has been to prevent or attenuate toxicity from various oxygen species [2-6]. The natural scavenger enzymes, such as superoxid dismutase (SOD) and catalase, show effectiveness in vitro but not in vivo. The basic problem that remains is the administration of materia and how to make it reach its target through cellular barriers. This difficulty has dominated the debate for many years and most efforts have focused on synthesizing new substances that could lure the biological hindrance system.

Although singlet oxygen is a highly reactive species, with documented toxicity in biological systems, the energy it emits might be beneficial. Photosensitivity to various light sources can occur in biological systems at various levels [7].

An attractive idea is the use of energy transfer from one site to another in order to modify a particular system. The energy emitted by excited singlet oxygen during its relaxation to ground state oxygen will be denoted the singlet oxygen energy (SOE).

In the present study we have investigated the effect of SOE transfer as light illumination or as energy transfer in air at a wave length of 634 nm on the production of reactive oxygen species (ROS) by human monocytes, using isoluminol-enhanced chemiluminescence [8,9].

Material and Methods

Isolation of human monocytes

Mononuclear cells were collected from buffy coats by the Ficoll-Hypaque procedure [10]. Monocytes were isolated by a modified adhesion-step technique [11]. In brief, culture flasks (Nunclon, Nunc. Naperville, IL, USA) were pretreated with 5-ml heat-inactivated human serum at room temperature for 1 h. The serum was decanted and the flasks were air dried. Mononuclear cells were plated at a density of

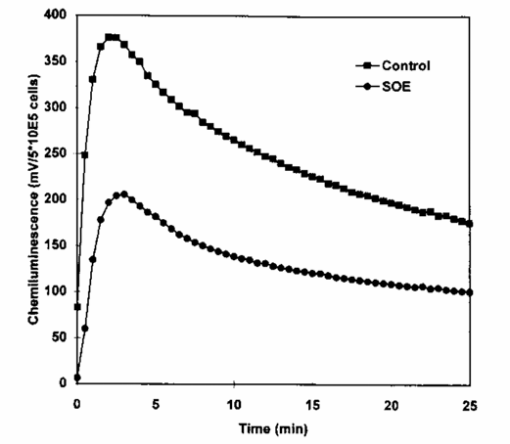
10^7 cells/ml. After 1 h incubation at 37°C in 5% CO_2, the nonadherant cells were washed away with phosphate-buffered saline (PBS). To detach the monocytes sticking to the surface, the cells were incubated with 5-ml PBS containing 5-mM ethylenediaminetetraacetate and 2% heat-inactivated human serum for 20 min at 4°C. Detached monocytes were collected, washed, and resuspended in Krebs Ringer phosphate buffer with glucose (KRG; Sigma Chemical Company, St. Louis, MO, USA). The monocytes were kept in a bath of melting ice immediately after preparation an samples were wthdawn to determine the cellular chemiluminescence.

Treatment with singlet oxygen energy

Singlet oxygen energy was produced by Valkion equipment (Göteborg, Sweden) as air in one device, and as photons via fiberoptics in a second device. In the Valkion equipment, singlet oxygen is generated through a photosensitization process. The photosensitizer used is well known [12]. A full-spectrum halogen lamp is used as a light source. The medium where the singlet oxygen is generated is air with a very high relative humidity. The life of singlet oxygen in this medium is about 2μs for the state. Cells were exposed to singlet oxygen energy photons at λ 634nm by bubbling air into the tube, or by illumination, before the production of reactive oxygen species were measured in the luminometer. The exposed cells, as well as the unexposed control cells, were kept at 37°C. Cell viability was determined with trypan blue dye exclusion test.

Chemiluminescence measurement

Chemiluminescence (CL) was measured in duplicates or triplicates at 37ºC in a luminometer Bio-Orbit 1251 with Multi-Use software (Bio-Orbit Oy, Turku, Finland), using disposable polystyrene tubes with 1 ml reaction mixture. Tubes used for measurement of extra cellular chemiluminescence contained cells, 56- M Isoluminol (6-amino-2,3-dihydro-1,4-phthalazinedione) and 4 units horseradish peroxidase (HRP). In some experiments, catalase 2000 U/ml and SOD 50 U/ml were added. To activate the reduced nicotinamide adcninc dinuclocotide phosphate (NADPH) oxidase, phorbol myristate acetate (PMA) was added [13] to a final concentration of 10 nM just before the tubes were placed in the luminometer. The chemiluminescence was recorded as a rate of production over time. The results were expressed as maximal rates in mill volts, produced from 5 x 10^5 viable cells, unless specified otherwise. Statistical analyses were performed with Student's t-test.



Results

Human monocytes were incubated with SOE and then subjected to stimulation by PMA. The response was measured as isoluminol-enhanced chemiluminescence. Examination of the rate of ROS production as a function of time showed that the reduction caused by SOE was seen both as a decrease in peak rate of

chemiluminescence as well as a total generated chemiluminescence. The time course of PMA-induced CL production by SOE-treated cells was similar to that of control cells, and a maximal peak value was reached 2-3 min. after addition of PMA.
When monocytes were incubated with SOE by air bubbling for 10 min. the production of reactive oxygen species was significantly decreased compared to control cells 249 ± 24,7 mV/5 x 10^5 cells vs. 346 ± 24,3 mV/5 x 10^5 cells, respectively (mean ± SE of 10 different cell donors, p=.001). SOE was also produced by illumination, which gave similar CL results. 148 ± 25,9 mV/5 x 10^5 cells after 10-min. treatment and 231 ± 17,5 MV/5 x 10^5 nontreated cells (mean ±SE of six different cell donors, p=.002). Time-response incubation of monocytes with singlet oxygen energy by illumination for 2, 5, 10 and 15 min. showed that an antioxidative effect of 60% is achieved after 2 min. Keeping the cells on ice for 4 h does not affect the cell viability, but the effect of singlet oxygen is diminished after 1 h and almost extinguished after 2 h. The observed decrease in production of free radicals from the monocytes does not seem to be an effect of decreased cell viability, because the cell viability was 91 ± 0,9% for cells treated with singlet oxygen 90 ± 1,8% (n=3) for control cells.
In a cell-free system no effect of treatment with SOE was found. The maximum peak value after 10 min. SOE treatment was 0.826 mV compared with 0.814 MV in the control.
To elucidate the relative importance of different oxygen metabolites in the CL reaction, radical scavengers or different inhibitors can be used. SOD and catalase can be used to scavenge superoxide anion and hydrogen peroxide, respectively. Because these enzymes are macromolecular proteins. Only extracellular released oxygen species are scavenged. When SOD or catalase were added to singlet oxygen treated cells no change in PMA induced CL was detected, compared to control cells (Fig. 3). Adding catalase to control cells resulted in a 30% reduction of the PMA-induced response. The inhibitory effect was most pronounced for SOD (90%), indication that superoxid anion is of major importance in the luminol-amplified chemiluminescence response. The lack of difference in CL between control cells and SOE-treated cells in the presence of SOD or catalase suggests that SOE may inhibit NADPH oxidase activity, which is the major source of superoxid anion.
The detector molecule isoluminol cannot penetrate cell membranes, and thus only extracellular released oxygen metabolites are detected [9]. The addition of SOD and catalase to the assay abolished the antioxidative effect of SOE, suggesting that production both of superoxid anion and hydrogen peroxide is decreased in response to light activation or air treatment.

Discussion

The aim of this study was to investigate how treatment with energy transfer by 634-nm illumination, or SOE bubbling, affects the production of reactive oxygen species from human monocytes with activated NADPH oxidase. When monocytes were exposed to SOE for the short period of 2 min, the production of superoxid anion and hydrogen peroxide was significantly decreased compared with control cells. SOE produced by illumination of monocytes gave similar CL results as air bubbling with SOE. The greatest effect of SOE was seen on the youngest cells (i.e., less than 1 h after isolation). And there was no effect on cell viability by SOE treatment, suggesting physiological conditions during the experiment. This energy transfer attenuates oxidative stress by inhibition of the respiratory burst of NADPH oxidase activated monocytes.
These findings suggest that treatment with SOE by air diffusion or illumination can decrease the production of reactive oxygen species from monocytes, thus limiting excessive tissue damage during reperfusion or inflammation.

Reactive oxygen metabolites generated by the phagocyte NADPH oxidase are critically important components of host defence. These highly toxic oxidants can cause significant tissue injury during inflammation or postischemic reperfusion, however; thus it is essential that their generation and inactivation are thightly regulated.
The NADPH oxidase is normally inactive. It can be stimulated by PMA, leading to the generation of superoxid anions. It is known that superoxid and hydrogen peroxide may be generated in the cell via the

action of NADPH oxidase. The toxicity of superoxid is further mediated through the secondary generation of HOCL from $H2O_2$ and CL by myeloperoxidase [14].
The dismutation of the superoxid dismutase, Catalase, on the other hand, catalyzes the following reaction:

2H2O2 → 02 + 2H2O

It should be pointed out that catalase may absorb photons by electronic excitation at 633 nm [15].
When the ground state dioxygen is excited to singlet oxygen, energy is emitted as solvent vibrations when it relaxes back. The fact that the transfer of 1 g singlet O_2 to 3 -g, triplet O_2 is spin forbidden, makes the lifetime of singlet oxygen relatively long, 2-;7□s or more depending on the solvent. The lifetime of singlet oxygen is expected to be shorter in biological systems than in pure H_2O, i.e., < 2 □s [16].
Depending on the various possible singlet oxygen states, emission at 381, 476, 634,762 and 1269 nm are possible. According to the literature, emission at 634 and 1269 nm occur most frequently. These two frequencies have been implicated in the reactions with biological process. Because the energy can be transported through glass, we believe that 634 nm is the actual wavelength in the used equipment and not 1269 nm.
The paradox that singlet oxygen, a highly reactive electrophilic molecule, has an antioxidative effect may thus be explained by the inactivation of NADPH oxidase. It should be pointed out that the dose response occurs in a narrow dose range. Additionally, it has been proposed that treatment with low doses of singlet oxygen modify cell behavior, where high doses are cytotoxic [17].
Low intensity ultraviolet and visible radiation are known to induce specific photo biologic effects in cells [7,18,19]. The SOE photons can be emitted as a light source (634 nm) via optic fiber. There are similarities between these findings and photodynamic therapy (PDT) being proposed as a possible treatment modality for being proposed as a possible treatment modality for arteriosclerosis [20, 21] and restenosis after angioplasty [22 - 25], as well as malignancies []The SOE photons are the result of singlet oxygen relaxing to the ground state. In the case of PDT however, singlet oxygen is generated and is partly responsible for the destructive effect on biological systems. The exact mechanisms by which PDT operates have yet to be elucidated, but the formation of cytotoxic oxidizers, such as singlet oxygen, probably has a key role.
It has been shown that the inhibition of intimal hyperplasia by PDT is selective to the media smooth muscle cells. We propose that other cells in the arterial wall may be protected from the cytotoxic effects of oxidative molecules, such as photophrin or 5-amino-levulinic acid. Light of the wavelength 630 nm without using a photosensitizing agent, as used in the experiment to produce singlet oxygen, is sufficient for effect in vivo [22, 24].
We thus conclude that singlet oxygen energy treatment of human monocytes decrease the generation of reactive oxygen species by NADPH oxidase. This may be of importance for explaining the positive effects of PDT on restenosis after angioplasty. **Establishment at the cellular level of the salvage effect of SOE treatment may have a wide range of medical applications involving oxidative stress.**

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