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OXYGEN RADICAL PRODUCTION DURING ISCHEMIA - REPERFUSION IN THE ISOLATED PERFUSED RAT LIVER AS MONITORED BY LUMINOL ENHANCED CHEMILUMINESCENCE

Masahiro Okuda, Iwao Ikai, Britton Chance and Chellappa Kumar*

Department of Biochemistry and Biophysics, School of Medicine, University of Pennsylvania, Philadelphia, PA 19104-6089 and
The University City Science Center, 3401, Market Street, Philadelphia, PA 19104

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We have applied the Luminol enhanced chemiluminescence technique to the isolated perfused rat liver during ischemia and reperfusion to monitor the production of oxygen radicals in tissue. Livers under perfusion with Luminol-containing buffer were subjected to 30 minutes of global ischemia followed by 60 minutes of reperfusion. Their chemiluminescence was continuously monitored to obtain the time course of oxygen radical production. Transient bursts of oxygen radical production were observed in the livers as indicated by chemiluminescence changes on reperfusion. Superoxide dismutase treatment abolished while catalase treatment enhanced the reperfusion-induced chemiluminescence transient.

Current theories of ischemia - reperfusion related tissue damage suggest that the production of reactive oxygen radicals in tissue under these conditions may be a major mechanism of tissue injury (1-3). The measurement of oxygen radical concentrations in tissue under these conditions has so far been difficult due to lack of suitable methods. To this end, we have recently applied the luminol-enhanced chemiluminescence technique (4,5) to perfused hearts (6). Luminol present in the perfusion buffer reacts with any oxygen radicals produced in the tissue to generate chemiluminescence with a high quantum yield. Monitoring the

* To whom all correspondence may be addressed at the University of Pennsylvania.

Abbreviations:
Luminol, 5 Amino-2,3-dihydrophthalazine-1,4-dione; LEC, Luminol enhanced chemiluminescence.

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chemiluminescence of organs perfused with Luminol-containing buffer enables a continuous study of the time course of oxygen radical production under different physiological conditions. We have now utilized the LEC technique to monitor oxygen radical production in the isolated perfused rat liver during ischemia - reperfusion and present the results here.

Methods

Organ preparations:

Sprague Dawley rats (280 - 320 g body weight) fasted for 24 hours but allowed unlimited water were used . The rats were heparinized (Sodium Heparin, 1000 units/kg, i.p.) and anesthetized 30 minutes later (Sodium Pentobarbital, 50 mg/kg, i.p.). The livers were exposed and cannulated through the portal vein and perfusion initiated. The livers were excised under perfusion, transferred to the chemiluminescence perfusion chamber and the perfusion continued at a constant flow rate of 35 ml/min. The perfusion buffer used was a modified Krebs-Henseleit bicarbonate buffer solution, pH 7.4, consisting of the following (in mM): NaCl (118), KCl (4.7), KH_2PO_4 (1.25), $CaCl_2$ (1.25), $MgSO_4$ (1.25), $NaHCO_3$ (24.0) and glucose (11.1). The temperature of the perfusion buffer was 37 ± 1°C. The perfusion buffer was gassed continuously with a mixture of 95% O_2: 5% CO_2 and was filtered through a 0.45 μ Millipore filter. After initial perfusion with the Krebs Henseleit buffer for 10 minutes, the perfusion buffer was changed to one containing 0.2 μM Luminol in addition to the above components. Since flow through, non-recirculating perfusion was used, the Luminol and other buffer constituents in the perfusate were maintained at the specified concentrations through the entire run. Global ischemia was induced by stopping the flow of the perfusion buffer to the tissue. After thirty minutes of ischemia, the flow of the buffer was restored and reperfusion continued for sixty minutes. In separate experiments to study the effect of radical scavengers on radical production during reperfusion, either superoxide dismutase (30U/ml perfusion buffer) or catalase (100U/ml perfusion buffer) were included in the perfusion buffer during the last 10 minutes before and the first 30 minutes following the ischemic period. The enzymes were obtained from Sigma. Each experiment was repeated at least five times and typical results are presented. Variations in LEC intensity from run to run were less than 10%.

Measurement of chemiluminescence:

The organ under perfusion was enclosed in a light-tight perfusion box. The box was designed so that all necessary manipulations could be carried out from the outside thus completely eliminating artifacts arising out of exposure of the photomultiplier to external light. Chemiluminescence was measured by an EMI 9658 photomultiplier cooled to -20 °C, at an applied potential of 0.95 kV. Standard high performance photon counting electronics consisting of a low noise preamplifier, amplifier, discriminator and ratemeter were used. Dark counts were around 60 c.p.s. The distance from the perfused organ to the photomultiplier was around 5 cm.

Results and discussion

Figure 1 shows the LEC changes observed during ischemia - reperfusion from the isolated, Luminol-perfused liver and the effect of administration of superoxide dismutase or catalase on the reperfusion-induced LEC changes. The control livers showed decreased LEC throughout the period of ischemia and, on reperfusion, a large transient burst in LEC (Panel A); the LEC transient peaked about five minutes after the start of reperfusion and decayed back to near the preischemic values in the next five minutes. This reperfusion-induced transient is similar to the transient LEC increases on reperfusion of the ischemic heart reported by us(6). However, the perfused hearts showed LEC increases during ischemia(6) as well, while the livers did not. The reperfusion-induced transient increase in LEC may be ascribed to a reperfusion-induced burst in oxygen radical production, the proposed cause of reperfusion injury(1-3).

Figure 1, Panel B shows that superoxide dismutase treatment diminished the intensity of the reperfusion-induced LEC transient by more than 80%. In previous studies, superoxide dismutase was effective in

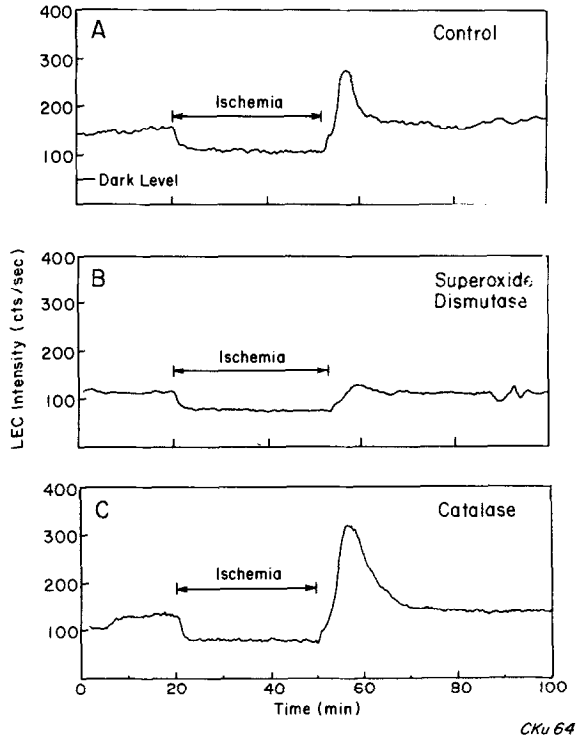

Figure 1. Luminol enhanced chemiluminescence changes in the isolated perfused rat livers during 30 minutes of ischemia followed by reperfusion: control livers (Panel A); livers under superoxide dismutase treatment (Panel B); livers under catalase treatment (Panel C).

diminishing the intensity of the reperfusion-induced LEC transient(6) and also the electron paramagnetic resonance detected burst in radical production(7,8) in the case of the heart. The high efficiency of superoxide dismutase in abolishing the reperfusion-induced LEC transient suggests that the major oxygen radical produced on reperfusion of the ischemic liver is superoxide.

Figure 1, Panel C shows that catalase treatment resulted in an increase in the intensity of the reperfusion-induced LEC transient. The peak intensity of the LEC transient (measured from the preischemic baseline) was increased by about 70% while the total oxygen radical production as measured by the area under the peak increased by more than 100%. This is an unexpected observation. In the perfused heart, catalase did not show any significant effect on the reperfusion-induced LEC transient(6). One possible explanation for this anomalous effect of catalase is that the production of oxygen radicals on the onset of reperfusion is oxygen limited and there is competition between superoxide production and peroxide production for the available oxygen; catalase, by breaking down the peroxide formed, is able to increase the total amount of oxygen available for conversion to superoxide. If this were not the case, since peroxide can be broken down to produce hydroxyl radicals in presence of transition metal ions, hemes etc., scavenging of peroxide by catalase would be expected to decrease the total oxygen radical production; the observed opposite effect of catalase suggests that the spontaneous conversion of peroxide into superoxide or the hydroxyl radical is a slow process under the conditions of the experiment in the livers.

Given the significant tissue concentrations of catalase, superoxide dismutase and glutathione peroxidase in the liver(1), the ability of the externally introduced superoxide dismutase or catalase to affect the LEC behavior of the perfused liver during ischemia - reperfusion suggests that the oxygen radicals being monitored during these LEC studies are in a tissue compartment not accessible to the intracellular enzymes but accessible to the externally added enzymes, i.e., the vascular compartment. This would be consistent with the proposal that the endothelial cells are a major site of superoxide production during reperfusion(3).

Chemiluminescence increases during reperfusion have been reported from the rat intestine(9) and Vitamin E-depleted rat hearts(10). Decreases in the spontaneous chemiluminescence of perfused liver under hypoxia also has been reported(11). Our current studies using the sensitive LEC technique are able to clearly detect the anticipated, but so far not reported reperfusion-induced burst in oxygen radical production in the perfused liver.

Acknowledgments

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