CYTOTOXICITY OF NITRIC OXIDE AND HYDROGEN PEROXIDE

Is There a Cooperative Action?

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INTRODUCTION

Nitric oxide (·NO) is well defined as an important effector molecule in biological systems. Secreted from various cell types, ·NO contributes to a variety of physiological and pathophysiological processes [22]. Despite of its function in the control of blood pressure, platelet aggregation and neurotransmission, which are mediated via the activation of the soluble guanylate cyclase, ·NO has been shown to be a potent modulator of the cytotoxic activity of macrophages as well [12; 21]. The mechanisms by which the bactericidal and the tumoricidal potential of ·NO is defined are still poorly understood. Diverse molecular targets have been postulated to exist for an attack of ·NO on the cell surface as well as inside the cells such as thiol groups of proteins yielding S-nitrosothiols [14]. In addition to the membrane-linked targets, ·NO is suggested to inhibit the mitochondrial respiratory chain, DNA and protein synthesis and iron metabolism [11]. Besides these effects, which could be defined as direct effects of ·NO, an increasing number of studies postulates that the cytotoxicity of ·NO is enhanced by chemical interactions with oxygen and reactive oxygen species to form other potentially toxic radicals. For example, ·NO reacts with the superoxide anion (O2−) forming the peroxynitrite anion (ONOO−) which decays, once protonated, to the very reactive hydroxyl radical (·OH) and to nitrogen dioxide (·NO2) [1]. Increased reactivity due to an interplay of ·NO with O2− via the ONOO− pathway is supported by studies in biological as well as in chemical systems [28; 25; 4; 5].

Here, we present results on the damaging effect of ·NO-releasing compounds in cooperation with reactive oxygen species generated both in the extracellular space by O2− and H2O2-generating systems, and intracellularly by inhibition of the mitochondrial respiratory chain. Since the half-life of ·NO strongly decreases with increasing concen-
trations of molecular oxygen we further studied whether hypoxic conditions might affect ·NO reactivity.

METHODS

Fu5 hepatoma cells and sinusoidal liver endothelial cells from rat as well as L929 fibroblast cells from mouse were treated and harvested with small modifications as described elsewhere [13; 26; 6]. For the experiments cells in confluency were used. On the day of experimentation, medium was removed and the cells were washed with and kept in Krebs-Henseleit buffer (pH 7.4, supplemented with 20 mM Hepes and 10 mM glucose) at 37°C (air/C0₂, 19:1). Hypoxic conditions were initiated by addition of nitrogen-saturated (CO₂/N₂, 1:19) Krebs-Henseleit buffer [2]. The experiments were started by adding the substances as indicated. S-Nitroso-N-acetyl-DL-penicillamine (SNAP) and sodium nitroprusside (SNP) were used as ·NO-donating compounds, and 3-morpholinosydnonimine-N-ethylicarbamide (SIN-1) as a compound which releases both ·NO and O₂. Glucose oxidase/glucose and xanthine oxidase/hypoxanthine were applied to produce H₂O₂ and O₂/H₂O₂, respectively. KCN, antimycin A and rotenone were given to inhibit cellular respiration. LDH Leakage was used to indicate cell viability [16]. Comparative experiments with trypan blue exclusion and phase contrast cell counts gave the same results.

RESULTS AND DISCUSSION

Cytotoxicity of ·NO-Donating Substances

All ·NO donors induced a concentration dependent cytotoxicity against sinusoidal liver endothelial cells. A significant loss in the viability of these cells of about 30% occurred between 2 and 4 h of incubation when the cells were exposed to 5 mM SIN-1, 5 mM SNAP and 20 mM SNP (Fig.1). Similar results were obtained in incubations with Fu5 hepatoma cells. The toxicity of SIN-1, SNP and SNAP is suggested to be mediated by the reactivity of ·NO since this molecule is known to be released during the decomposition of these compounds [14]. The denitrosylated substances had no influence on cell viability in agreement with data reported previously [19]. In contrast to endothelial cells and to Fu5 hepatoma cells, SNP and SNAP exhibited only slight effects against L929 fibroblast cells even at high

![Figure 1. Toxicity of ·NO-donating compounds against sinusoidal liver endothelial cells. Cells (10⁵/ml) were exposed to 5 mM SIN-1, 5 mM SNAP and 20 mM SNP. Cell injury was estimated by LDH Leakage. Data represent means ± S.E.M. from 3-6 experiments.](image-url)