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INHIBITORY EFFECT OF SUPEROXIDE-GENERATING QUINONES ON SUPEROXIDE DISMUTASE

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The one-electron reduction of quinones to semiquinones and the subsequent autooxidation of the semiquinone back to the quinone can generate large quantities of superoxide anion radical ($O_2^{\cdot-}$) (1,2). This reduction-oxidation process, known as redox cycling, is catalyzed by a variety of cellular flavoproteins and is thought to be responsible for many of the therapeutic and toxic effects of quinonoid compounds. Since quinones have been shown previously to inhibit a variety of enzymatic processes (3-5), we have investigated the effect of various quinones on the activity of superoxide dismutase (SOD), the key enzyme involved in protecting cells against the toxic effects of $O_2^{\cdot-}$. Here we report that certain quinones inhibit SOD activity *in vitro* and may, therefore, potentiate their own toxicity by this effect.

Quinones were preincubated with bovine blood SOD (50 units/ml) in 196 μl of 50 mM phosphate buffer, pH 7.8, at 25°. The quinones were added in 4 μl dimethyl sulfoxide to give a maximal final concentration of 100 μM. Control SOD was preincubated with 4 μl dimethyl sulfoxide alone. After a 30-min preincubation, the activity of the SOD was determined by dual wavelength spectrophotometry (550-557 nm), using a hypoxanthine/xanthine oxidase $O_2^{\cdot-}$ generating system with cytochrome *c* (40 μM) as the indicating scavenger (6). The final concentration of quinone in the reaction cuvette never exceeded 0.5 μM. None of the quinones tested altered the rate of $O_2^{\cdot-}$-dependent cytochrome *c* reduction at this concentration. The percent inhibition of SOD was determined by subtracting the activity of SOD incubated with quinone from the control SOD activity and dividing this number by the control activity. 1,2- and 1,4- Naphthoquinone, menadione, and 9,10-phenanthrene quinone all inhibited SOD activity to some extent (Table 1). These four quinones are potent redox cyclers with

Table 1. Concentrations of quinone that inhibited superoxide dismutase activity by 50% (IC_{50}) *

Quinone	IC_{50} (μM)
1,2-Naphthoquinone	14
1,4-Naphthoquinone	60
9,10-Phenanthrene quinone	42

* IC_{50} values were calculated from at least five different doses by regression analysis.

cellular flavoproteins. Menadione was the least effective, producing only a 15% inhibition at 100 μM. Other redox cycling quinones, such as Adriamycin, alloxan, danthron, mitomycin C, and the 1,6-, 3,6-, and 6,12-benzo(a)pyrene quinones, were without effect at concentrations up to 100 μM. Various phenolic compounds, including 1-naphthol and gossypol, were also ineffective.

We therefore conclude that some $O_2^{\cdot-}$ generating-quinones are able to directly inhibit SOD at micromolar concentrations and may potentiate their own toxicity by this effect. We are currently investigating the mechanism by which these quinones inhibit SOD and their effects on SOD in intact cells and *in vivo*.

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