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Inhibition of lipid peroxidation in isolated rat liver mitochondria by the general anaesthetic propofol

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Abstract—The effect of the general anaesthetic propofol (2,6-diisopropylphenol) on lipid peroxidation in rat liver mitochondria was assessed with the thiobarbituric acid (TBA) assay. Propofol was shown to inhibit the accumulation of TBA-reactive compounds after initiation of radical production by the addition of the ADP-Fe^{2+} complex. Analysis of kinetics showed that propofol caused a concentration-dependent delay as well as a decrease in the rate of the peroxidation process. ^{1}H-NMR spectra of mitochondrial lipid extracts indicated that 95% of the added propofol remained intact after 30 min incubation under conditions of low oxidative stress. The ESR spectrum of propofol incubated in the presence of EDTA-Fe^{2+} and H_2O_2 as initiators of radical production showed a radical that was most likely a decomposition product of the primary phenoxy radical of propofol. It is concluded that (a) propofol acts as a chain reaction-breaking antioxidant by forming a stable radical and (b) propofol does not seem to be metabolized in mitochondria *in vitro*.

Tissue damage due to oxidative reactions involving free radicals and singlet oxygen plays an important role in several diseases [1]. It is therefore of medical interest to find substances that could be used as antioxidants. The general anaesthetic propofol (2,6-diisopropylphenol*) [2] is structurally related to the commonly used phenolic antioxidants BHT and BHA. However, these compounds have a number of toxic effects [3, 4] that have not been described for propofol. In the present work, the antioxidant effects of propofol have been investigated in a mitochondrial system subjected to increased oxidative stress, to test whether propofol could be useful as an antioxidant in biological systems.

Materials and Methods

Preparation of mitochondria. Rat liver mitochondria were isolated from male rats as described previously [5]. Mitochondria were incubated in a medium containing 250 mM sucrose, 4 mM Pi, 2 mM succinate, 20 μM carboxyatractyloside, 6 μM rotenone, 10 mM Hepes pH 7.40 that had been freed from calcium and paramagnetic impurities by passage through a Chelex (Bio-Rad Laboratories, Richmond, CA, U.S.A.) cation exchange column prior to use. The incubations were made at 37°, and the medium was saturated with oxygen during the incubation.

Chemicals. Propofol was purchased from Lancaster (Strasbourg, France) and purified as described in [6].

Lipid peroxidation. Peroxidation was initiated by adding 75 μM Fe^{2+} and 0.50 mM ADP to the mitochondrial suspension. Aliquots were removed for the TBA test and for extraction of lipids. Measurement of TBA-reactive compounds was performed after mixing 1 mL of the mitochondrial suspension with 2 mL of TBA medium containing 0.25 M HCl, 15% trichloroacetic acid, 3 mM TBA and 0.1% BHT. The colour was allowed to develop in the dark for 12 hr according to Erdahl *et al.* [7] and the absorbance was measured at 533 nm. The amount of malondialdehyde was calculated from $E_{533} = 1.49 \times 10^5\,M^{-1}\,cm$ [8].

Liposomes. A mixture of 80 mM phosphatidyl choline (Sigma Chemical Co., St Louis, MO, U.S.A.) and 30 mM propofol was sonicated in 50 mM Pi pH 8.0 for 20 min with a Branson sonicator model B-15P (Danbury, CT, U.S.A.) under a stream of nitrogen. The production of hydroxyl radicals was initiated by incubating the liposomes in the presence of 1 mM Fe^{2+}, 1 mM EDTA and 2 mM H_2O_2.

^{1}H-*NMR spectroscopy.* Extraction of mitochondrial lipids and ^{1}H-NMR analysis were performed as described previously [9].

ESR spectroscopy. The ESR analyses (15 μL samples) were performed with a Bruker ESP 300 spectrometer. The spectrometer settings were: microwave power 20 mW; modulation amplitude 1 G; 20 G/min scanning rate. Computer simulations of the experimental ESR spectra were carried out using the EPRCALC simulation program.

Results and Discussion

Figure 1 shows the time course of the accumulation of peroxidation products in rat liver mitochondria incubated in the presence of ADP-Fe^{2+} as measured with the TBA assay. In the experiment with no propofol added, a rapid accumulation of peroxidation products was seen after a short lag phase (trace A). In the control experiment (ADP-Fe^{2+} not added) only a very small increase in the amount of peroxidation products was observed (trace G, dotted line). This clearly shows that the amount of ADP-Fe^{2+} added induced a production of radicals that was higher than the endogenous capacity for radical scavenging in the mitochondria. However, by adding 8 μM propofol to the suspension, the accumulation of peroxidation products (trace F) was inhibited and the amount observed was as low as in the absence of ADP-Fe^{2+}. By adding progressively smaller amounts of propofol to the suspension (traces B–E), two effects on the kinetics of the reaction could be distinguished: a delay and a decrease in the accumulation rate. At a low concentration of propofol, the delay was more pronounced (trace B) while the maximum rate of the process remained unchanged, whereas at higher concentrations a delay as well as a decreased rate were observed (traces C–E). This shows that propofol is a potent inhibitor of the lipid peroxidation. At the concentration employed in these experiments, propofol had no effect either on the respiratory chain activity or on the respiratory control ratio. At very high concentrations (>500 μM), however, propofol caused a perturbation of the mitochondrial membrane with uncoupling and large-amplitude swelling. These effects have also been described for BHT and BHA but at lower concentrations [10].

* Abbreviations: BHA, 2-*tert*-butyl-4-metoxyphenol; BHT, 2,6-*tert*-butyl-4-methylphenol; DMPO,5,5,-dimethyl-1-pyrroline *N*-oxide; Pi, inorganic phosphate; propofol, 2,6-diisopropylphenol; TBA, thiobarbituric acid.

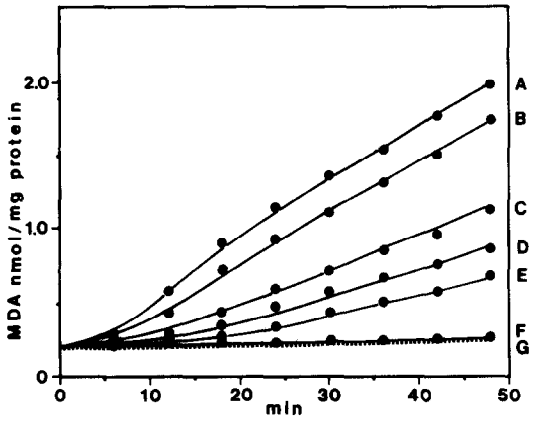

Fig. 1. The accumulation as a function of time of peroxidation products in rat liver mitochondria measured with the TBA test. Mitochondria (2.5 mg/mL) were incubated in the standard medium, and the experiment was started by addition of 75 μM Fe^{2+} and 0.5 mM ADP. Aliquots of 1 mL were mixed with the TBA medium and allowed to develop as described in Ref. 6. The absorbance was measured at 533 nm. Propofol was added prior to ADP–Fe^{2+} at a concentration of: (A) 0, (B) 0.1, (C) 0.5, (D) 1, (E) 2 and (F) 8 μM. A control incubation in the presence of ADP–Fe^{2+} and propofol was performed: (G) dotted line. Data are from one characteristic experiment of five.

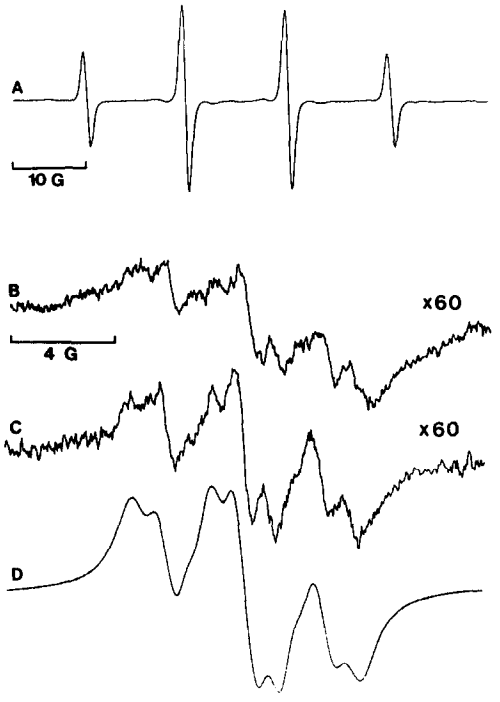

Fig. 2. The ESR spectra of a suspension of liposomes containing phosphatidyl choline and 10 mM DMPO or 30 mM propofol. (A) 10 mM DMPO incubated in the presence of 1 mM EDTA, 1 mM Fe^{2+} and 2 mM H_2O_2. (B) 30 mM propofol incubated with 1 mM EDTA, 1 mM Fe^{2+} and 2 mM H_2O_2. (C) 30 mM propofol incubated with 0.5 M $K_3Fe(CN)_6$. (D) Computer simulation of a radical with two sets of two equal protons (a1(2*H*) = 3.3, a2(2*H*) = 1.1) employing a line broadening of 1.8 G. The instrumental settings were: 20 mW power; 1 G modulation amplitude; 20 G/min scanning rate.

The inhibitory effect of propofol on peroxidation may be due to an ability to break the chain reaction of lipid peroxidation, possibly by forming a stable radical by hydrogen abstraction. However, propofol was able to suppress completely the onset of lipid peroxidation only for a limited period of time which was dependent on the concentration of propofol. One possible explanation might be that propofol is metabolized to compounds with a smaller or no antioxidative effect since enzyme systems that are able to metabolize phenols have been described in mitochondria [11]. Another explanation could be that propofol is converted to its corresponding phenoxyl radical or undergoes other reactions, and thus would no longer be active as an antioxidant.

In order to investigate this matter further, mitochondria were incubated as described whereupon the lipids were extracted and analysed with ^{1}H-NMR. Incubation of the mitochondria for 30 min in the presence of propofol but in the absence of the peroxidation initiator complex did not cause any changes in the ^{1}N-NMR spectrum (not shown). The areas of all the peaks of propofol remained constant (±5%) with respect to the major mitochondrial peaks, and no additional resonances appeared in the spectrum. It could therefore be concluded that propofol was not metabolized in rat liver mitochondria under these conditions.

The mitochondrial suspension containing propofol was also analysed with the ESR technique. However, under the conditions described it was not possible to detect any phenoxy radical in the mitochondrial suspension, suggesting a steady state concentration of these radical species below the detection limit of the technique. The oxidation of propofol was therefore studied in model liposomes, as described in Materials and Methods, in order to employ a higher concentration of propofol without causing a disruption of the membrane. In a control experiment the liposomal suspension was incubated in the presence of 10 mM of the spin trap DMPO and the EDTA–Fe^{2+} complex plus H_2O_2 as radical initiators. A clear signal of the DMPO hydroxyl radical adduct appeared (Fig. 2, spectrum A). When 30 mM propofol was added to the liposomes (in the presence of EDTA–Fe^{2+} and H_2O_2) a more complex radical signal was observed (spectrum B) while in the absence of the radical initiator complex no signal appeared (spectrum not shown). It is thus likely that the observed radical signal derived from propofol that had been oxidized by hydroxyl radicals. This hypothesis was further tested by treating the suspension of liposomes containing propofol with $K_3Fe(CN)_6$ which has been reported to be an efficient and selective one-electron oxidant of phenols in water suspension [12]. A radical signal with identical hyperfine splitting characteristics but with a better signal to noise ratio appeared (Fig. 2, spectrum C), supporting the interpretation that the observed radical was formed by the oxidation of propofol. The simulated spectrum is consistent with a radical with two sets of two equal protons having hyperfine splitting constants of 3.3 and 1.1 G (spectrum D). These most probably arise from the two isopropyl groups and the two protons at the 3- and 5-positions of the benzene ring, respectively. However, the

spectrum is not compatible with the primary phenoxy radical of propofol, due to the lack of doublet splitting expected for the proton at the 4-position of the ring. Hence, the stable radical formed under these experimental conditions is a secondary radical of propofol, possibly formed by radical–radical coupling of its less stable primary phenoxy radical [13].

The effects of propofol described in the present paper are most likely entirely unrelated to its effect as a general anaesthetic. However, the concentration of propofol causing an observable inhibitory effect on lipid peroxidation is in the same range as the concentration at which it interacts with the GABA–receptor complex [14] and it is therefore possible that propofol may act also as an antioxidant during anaesthesia and sedation. This in turn might be beneficial in disease states such as ischemia and inflammation where tissue damage due to oxygen radicals are thought to play an important role [1]. It is also possible that propofol may be potentially useful as an antioxidant in many biological systems where the GABA receptor is not present.

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