The widely used anesthetic agent propofol can replace α-tocopherol as an antioxidant

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Abstract The cell membrane is protected against lipid peroxidation through endogenous antioxidants such as the lipid soluble α-tocopherol. The anesthetic agent propofol (2,6-diisopropylphenol) has a chemical structure which is similar to α-tocopherol, since it also contains a phenolic OH-group. The transient protection of GSH against lipid peroxidation in control liver microsomes is not observed in microsomes deficient in α-tocopherol. Introducing propofol (2 and 5 μM) restored the protective effect of GSH. Similar to the control microsomes the GSH-protective effect did not occur in previously heated microsomes. These results suggest that propofol acts similarly to α-tocopherol as a chain breaking antioxidant in liver microsomal membranes.

Key words: Lipid peroxidation; α-Tocopherol; Glutathione; Propofol; Antioxidant

1. Introduction

Tissue damage caused by the activity of reactive oxygen species (ROS) plays a role in many clinical situations such as ARDS, sepsis, multiple organ failure and ischemia-reperfusion [1,2]. As a consequence of the activity of ROS, lipid peroxidation can occur. The polyunsaturated fatty acids in the cell membrane are protected against lipid peroxidation through endogenous antioxidants such as the lipid-soluble chain-breaking antioxidant (α-tocopherol) [3,4]. α-Tocopherol terminates the chain reaction of lipid peroxidation by scavenging lipidperoxyl radicals (LOO·) by a process of hydrogen donation. In this reaction α-tocopherol itself becomes a radical which is, however, much less reactive than LOO· (reaction 1) [3]:

$$\alpha\text{-Tocopherol-OH} + \text{LOO}^{\cdot} \rightarrow \alpha\text{-tocopherol-O}^{\cdot} + \text{LOOH} \quad (1)$$

The hydroxyl (OH) group of the chroman moiety of α-tocopherol donates the hydrogen atom. The anesthetic agent propofol (2,6-diisopropopylphenol) also contains a phenolic OH-group (Fig. 1) and has been reported to be an antioxidant [5,6]. We hypothesized that propofol might act similarly to α-tocopherol as a chain-breaking antioxidant and could substitute for α-tocopherol. It has already been shown that propofol, used in clinically relevant concentrations, could inhibit lipid peroxidation in liver microsomes and mitochondria [5,6]. In order to keep the levels of α-tocopherol high enough to protect the membranes against lipid peroxidation, the antioxidant is continuously recycled in the liver. In this process of recycling, via a so-called free radical reductase in liver-microsomes, the α-tocopherol radical, formed in reaction 1, is regenerated to α-tocopherol by the cytosolic thiol GSH [7]. This system is excellently suited to test our hypothesis that propofol can replace α-tocopherol.

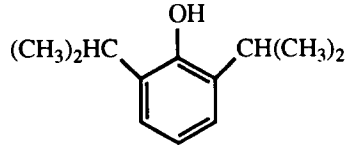
2. Materials and methods

2.1. Microsomes

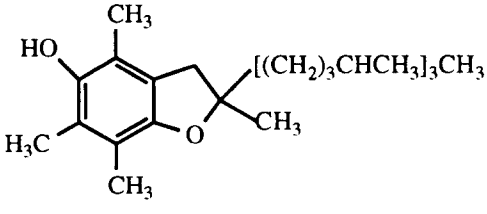
The microsomes were prepared from male Wistar rats, 200–250 g, as described before, and stored at −80°C [7]. Control microsomes were prepared by washing the stored microsomes twice by centrifugation [7]. α-Tocopherol was extracted from the microsomes according to Maiorino et al. [10]. In short, the stored microsomes were thawed and mixed with 2 vols. of ice-cold acetone and shaken vigorously. After 5 min the microsomes were washed twice by centrifugation. Care was taken to remove all the acetone, since it was observed that acetone interfered with the method used to detect lipid peroxidation (acetone reacts with thiobarbituric acid). The pellet obtained after centrifugation of the α-tocopherol-extracted microsomes was resuspended in 50 mM Tris-HCl (pH 7.4, 37°C) containing 150 mM KCl. Propofol was added to the microsomes. After the addition of propofol, the microsomes were washed twice and harvested again by centrifugation.

2.2. Incubation conditions

Microsomes (final concentration derived from 1/8 g liver/ml) were incubated at 37°C, with shaking, and oxygen was freely admitted in Tris-HCl/KCl (50 mM/150 mM, pH 7.4). Ascorbic acid and GSH were neutralized with KOH before addition. Reactions were started by adding a freshly prepared $FeSO_4$ solution.



2,6-diisopropylphenol
(propofol)



α-tocopherol

Fig. 1. Chemical structure of propofol and α-tocopherol.

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2.3. Lipid peroxidation assay

Lipid peroxidation was assayed by measuring thiobarbituric acid (TBA)-reactive material. The reaction in an aliquot of the incubation mixture (0.3 ml) was stopped by mixing with ice-cold TBA-TCA-HCl-BHT solution (2 ml; see below). After heating (15 min, 80°C) and centrifugation (15 min) the absorbance at 535 nm vs. 600 nm was determined. For the TBA-TCA-HCl-butyl-hydroxytoluene (BHT) solution, a TBA-TCA solution was prepared by dissolving 41.6 mg TBA/10 ml trichloroacetic acid (16.8% w/v in 0.25 N HCl). To 10 ml TBA-TCA-HCl 1 ml BHT (1.5 mg/ml ethanol) was added. The added chemicals did not interfere with the assay at the concentrations used. The data are the mean ± S.D. from 5 duplicate experiments.

2.4. Measurement of α-tocopherol

To measure α-tocopherol, it was extracted from the membrane by heptane according to the method of Burton et al. [11]. α-Tocopherol was quantified using HPLC (C18 nucleosil column; Chrompack, The Netherlands; eluted with 98% methanol, flow rate 0.6 ml/min) with fluorometric detection (excitation 295 nm, emission 340 nm).

3. Results

GSH (1 mM) gives a transient protection against lipid peroxidation in control liver microsomes (Fig. 2). Heating (100°C) these microsomes for 60 s abolished this GSH-protection (data not shown). After extraction with acetone the α-tocopherol concentration in the microsomes dropped from 0.68 ± 0.08 nmol/mg protein to a concentration which could not be detected anymore. In these α-tocopherol-deficient microsomes, GSH (1 mM) could not protect against lipid peroxidation anymore (Fig. 3). By introducing propofol (2 or 5 μM) into the α-tocopherol-deficient membranes the transient protective effect of GSH (1 mM) could be restored (which was dependent on the concentration of propofol used; Fig. 3). Both 2 and 5 μM propofol did not protect the α-tocopherol-deficient microsomes directly (data not shown). When the propofol was introduced in microsomes, which were heated (100°C) before propofol was introduced, propofol was not able to restore the protection by GSH (data not shown). Moreover, the latter data indicate that propofol itself, in the concentrations used, did not protect again Fe^{2+}/ascorbate-induced liver microsomal lipid peroxidation.

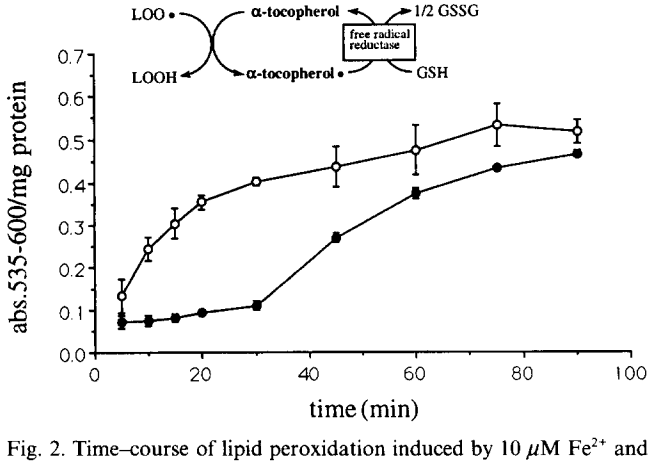

Fig. 2. Time–course of lipid peroxidation induced by 10 μM Fe^{2+} and 0.2 mM ascorbate in control microsomes (○) or in the presence of 1 mM GSH (●). The insert shows the interaction of α-tocopherol and GSH in the protection against lipid peroxidation. The α-tocopherol radicals are regenerated to α-tocopherol by GSH. This reaction is catalyzed by the heat-labile free radical reductase [7].

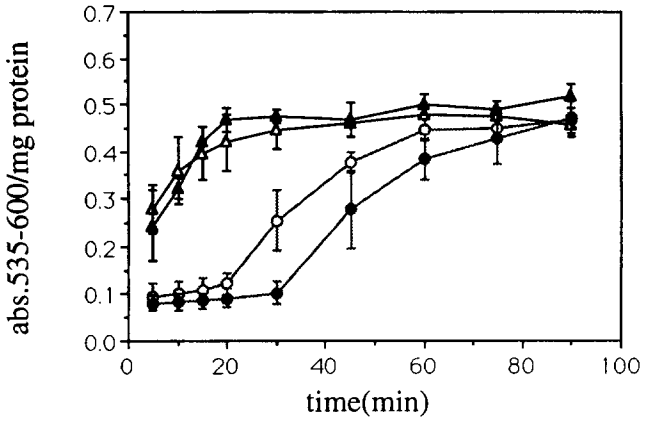

Fig. 3. Time–course of lipid peroxidation induced by 10 μM Fe^{2+} and 0.2 mM ascorbate in α-tocopherol-deficient microsomes without (△) and with (▲) the addition of GSH (1 mM). The microsomes to which GSH (1 mM) was added were enriched with 2 μM propofol (○) or 5 μM propofol (●).

4. Discussion

The protective effect of GSH against Fe^{2+}/ascorbate-induced lipid peroxidation in liver microsomes depends on the presence of α-tocopherol. In liver microsomes from animals fed a α-tocopherol-deficient diet, as well as in microsomes from which α-tocopherol was extracted with acetone, GSH did not protect against lipid peroxidation [8,9]. In the latter experiment the GSH-dependent protection could be restored by adding α-tocopherol to the α-tocopherol-depleted microsomes [10]. The anesthetic agent propofol (2,6 diisopropylphenol) chemically resembles the chain-breaking antioxidant α-tocopherol, because it also has a phenolic hydroxyl group [12]. This phenolic hydroxyl group is responsible for the antioxidant properties of propofol, as found in various in vitro experiments [5,6,12]. We show that introducing propofol in to α-tocopherol-depleted microsomes restores the GSH-dependent protection. When propofol is introduced in to α-tocopherol-depleted microsomes, which were heated (100°C) beforehand, the GSH-dependent protection is completely lost. This is similar to what is found in normal liver microsomes, i.e. the GSH-dependent protection is heat labile. This suggests that propofol can take over the role of α-tocopherol. The heat-labile free radical reductase, catalyses this recycling reaction (Fig. 4). Our data also suggest that the free radical reductase is not specific for α-tocopherol, but also acts with α-tocopherol look-alike compounds like propofol. Propofol, because of its good lipid solubility, is able to penetrate into membranes. The concentrations of propofol which are able to inhibit the lipid peroxidation and to replace the α-tocopherol function in cell membranes are in the range which is clinically used in anesthesia. Peak plasma concentrations of propofol of 40–60 μmol/l occur with the induction of anesthesia, and during the maintenance of anesthesia the anesthetic concentrations are 10–25 μmol/l [13]. Therefore the antioxidant properties of propofol can be of clinical importance. In many clinical situations where oxidative stress plays a role, the α-tocopherol content of the cellular membranes will be decreased, making these membranes even more vulnerable to oxidative stress [14–17]. Restoring the α-tocopherol concentration in acute situations is very difficult. So, during anesthesia for cardiac or vascular surgery,

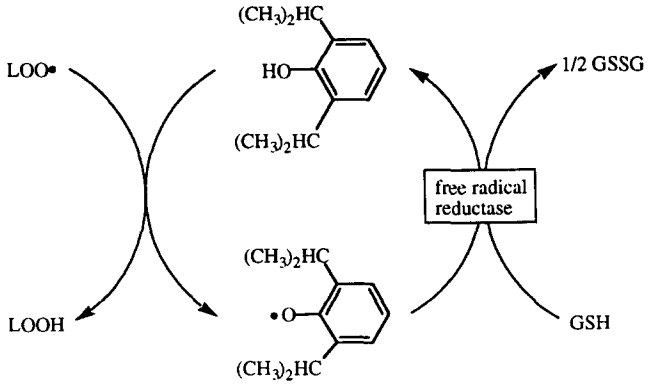

Fig. 4. Propofol probably acts as a chain-breaking antioxidant in which the propofol radical is formed. The subsequent reduction of the propofol radical by the liver microsomal GSH-dependent free radical reductase regenerates propofol in a similar way as it regenerates α-tocopherol.

for transplantation surgery or during sedation of the critically ill, artificially ventilated patients in the Intesive Care Unit (ICU) with sepsis, severe burns, etc., propofol could be an effective inhibitor of lipid peroxidation. It might support or even take over this anti-oxidant function of α-tocopherol. The clinical consequences of inhibition of membrane lipid peroxidation in these situations have never been studied in patients. Propofol being an already widely accepted drug in anesthesia as well as for sedation in the ICU, would be an interesting compound to further study the clinical consequences of pharmacological intervention of oxidative stress.

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