Antioxidant effects of propofol in human hepatic microsomes: concentration effects and clinical relevance


Summary

Propofol is known to possess antioxidant properties. There is controversy regarding the mechanisms by which the drug produces its antioxidant effects and the significance of these effects in relation to plasma concentrations of propofol in clinical practice. We studied the effects of increasing concentrations of Intralipid, propofol, butylated hydroxytoluene (BHT) and a vitamin E analogue (Trolox C) in 0.9% saline on non-enzymic and enzymic lipid peroxidation in human hepatic microsomes, and on concentrations of antioxidant enzymes in a Hep G2 cell line. Propofol showed significant inhibition of lipid peroxidation, but was less potent than BHT or Trolox C. IC50 values for non-enzymic and enzymic lipid peroxidation were mean 9.47 (SD 0.86) and 7.39 (0.84) μmol litre⁻¹ for propofol, 1.30 (0.57) and 0.32 (0.02) μmol litre⁻¹ for BHT and 2.34 (0.68) and 0.35 (0.04) μmol litre⁻¹ for Trolox C, respectively. The antioxidant activities of propofol were substantially retained in the presence of up to 30 g litre⁻¹ of human serum albumin. Propofol at concentrations of up to 100 μmol litre⁻¹ had no significant effect on the activities of antioxidant enzymes. Clinically relevant concentrations of propofol produced significant inhibition of both enzymic and non-enzymic lipid peroxidation in hepatic microsomal preparations, possibly as a result of accumulation in lipophilic environments. Measurement of antioxidant effects of drugs in aqueous media may have little relevance to their effects in protecting against lipid peroxidation in biological systems. (Br. J. Anaesth. 1998; 81: 584–589).

Keywords: anaesthetics i.v., propofol; enzymes, glutathione; liver, hepatocytes

Oxidant-mediated injury is thought to play an important role in several areas of critical illness, including ischaemia reperfusion injury (IRI) and secondary neuronal injury after a variety of neurological insults. Several studies have demonstrated a benefit from the use of novel 21-aminosteroid compounds in animal models of focal ischaemia, global ischaemia or concussive brain injury. These results support the investigation of other antioxidant compounds with potential neuroprotective properties.

Our previous work demonstrated that propofol (Diprivan) had antioxidant properties in vitro, and was more effective than other i.v. anaesthetic agents at reducing the abnormalities in ion fluxes produced during cerebral ischaemia in a cat model. We have also shown that propofol had acute neuroprotection in a rodent model of IRI, to an extent that cannot be accounted for by reduction of cerebral oxygen use. The possibility that these neuroprotective effects of propofol are caused by its antioxidant properties is supported by the increased resistance to oxidant injury in red blood cells and in hepatic microsomes obtained from rats anaesthetized with propofol.

Confirmation of a clinically useful effect depends on demonstration that propofol has antioxidant properties at concentrations achieved during clinical anaesthesia. In vitro studies that have addressed this issue are conflicting. Eriksson, Pollesello and Saris demonstrated that propofol protected rat liver mitochondria against lipid peroxidation and Musacchio and colleagues showed that propofol produced significant protection against lipid peroxidation in rat liver mitochondria and microsomes, and in rat brain synaptosomes, with median inhibitory concentrations (IC50) of 1.1–26.4 μmol litre⁻¹, which is similar to the clinical anaesthetic concentration range, whereas IC50 values for the established free radical scavenger butylated hydroxytoluene (BHT) were 1.6–33 μmol litre⁻¹. However, Green, Bennet and Nelson studied the antioxidant effects of propofol in an aqueous in vitro system and found little evidence of antioxidant activity at concentrations less than 60 μmol litre⁻¹ in a variety of oxidant reactions. Neither of these groups compared propofol with other clinically relevant naturally occurring antioxidant compounds such as vitamin E. Aarts and colleagues showed that propofol protected α-tocopherol-depleted rat hepatic microsomes against oxidant damage, but did not study the effects of BHT, and therefore comparison with earlier studies was not possible.

We have studied the antioxidant effects of propofol for several reasons. First, we compared the antioxidant effects of propofol with those of BHT so that comparison with previous studies was possible, and with Trolox C (the water-soluble analogue of vitamin E).
E) so that we could compare propofol with a clinically relevant antioxidant compound. Second, we compared these compounds in a preparation of human liver microsomes to exclude effects that might arise from inter-species differences. Finally, we wished to delineate specific processes affected by propofol. Consequently, we studied the effects on both non-enzymic and enzymic lipid peroxidation, and since many redox antioxidants modulate the production of intrinsic antioxidant enzymes in vivo via interaction with an antioxidant responsive element (ARE) at transcription sites, we studied the effect of these compounds on antioxidant enzymes in a Hep G-2 hepatoma cell line.

Materials and methods

Appropriate institutional procedures were followed, but formal Ethics Committee approval was not required as tissues obtained for these studies were from donor liver that would have been discarded at the end of a “cut down” procedure at orthotopic liver transplantation.

HUMAN HEPATIC MICROSOME PREPARATION

Hepatic tissues for microsome preparations were from healthy human livers obtained at the time of liver transplantation and stored at −70°C. Tissues were available as a result of “cut down” procedures on large donor organs being transplanted to paediatric recipients. Data reported are from liver tissues obtained from three separate donors. Microsomes were prepared freshly by the method of Lambert, using a buffer of BisTris propane 20 mmol litre−1 (pH 7.0)–sucrose 0.25 mol litre−1–KCl 0.15 mmol litre−1. Microsomes were washed with Tris/HCl 150 mmol litre−1, pH 8.0, to remove extraneous cytosolic proteins. The amounts of lactate dehydrogenase and glutamate dehydrogenase in the samples showed that the vesicles were free from cytosolic and mitochondrial contamination, respectively, and the amount of acid phosphatase showed that lysosomal contamination was minimal. All assays were in triplicate, performed on microsomes from the same liver, and results shown are mean (sd) for each concentration, indexed where appropriate to lipid peroxidation in the absence of any agent in each series of experiments.

DETERMINATION OF PROTEIN CONCENTRATION IN MICROSOMAL PREPARATIONS

Microsomal protein concentrations were determined by the bicinchoninic acid method, using bovine serum albumin as standard.

ASCORBATE–FE(III)-INDUCED PEROXIDATION

Microsomes (equivalent to 0.4 mg of microsomal protein) were suspended in KCl 150 mmol litre−1 containing FeCl3 0.2 mmol litre−1 (freshly prepared in water) and various amounts of test material. Peroxidation was initiated by addition of ascorbate (to a final concentration of 0.05 mmol litre−1), in a final volume of 0.4 ml. Samples were incubated at 37°C for 40 min, and the reactions terminated by addition of 0.8 ml of 20% w/v trichloroacetic acid–0.4% thiobarbituric acid–HCl 0.25 mol litre−1 and 0.01 ml of butylated hydroxytoluene (5%) in ethanol. Thiobarbituric acid reactive substances (TBARS) were measured after incubation at 80°C for 15 min. The concentration of the indicator reaction product (malondialdehyde) was measured as the optical density at 535 nm, and converted into micromoles using an extinction coefficient of 1.56 × 105 mol litre−1 cm−1, as described by Wills. Test substances alone showed no significant reaction in the TBARS assay.

NADPH–IRON-INDUCED PEROXIDATION

Liver microsomes (equivalent to 0.4 mg of microsomal protein) were suspended in BisTris propane buffer 20 mmol litre−1, pH 7.0, and the following added in sequence to the final concentration indicated: KCl 150 mmol litre−1; ADP 0.8 mmol litre−1; FeCl3 0.2 mmol litre−1 (freshly prepared in water) and test sample. Peroxidation was initiated with NADPH (final concentration 0.4 mmol litre−1) in a total volume of 0.4 ml. Samples were incubated and processed as described above.

CALCULATION OF IC50 VALUES

Concentrations of test substance required to produce 50% inhibition of a reaction (IC50 values) were calculated by fitting a third-order polynomial curve to the data. The derived IC50 values are shown in the text and tables together with SD of the estimate.

EFFECT OF ALBUMIN ON ANTIOXIDANT EFFECTS OF PROPOFOL

In view of the fact that propofol is extensively protein bound, the antioxidant effects of propofol on ascorbate–Fe(III)-induced lipid peroxidation and NADPH–iron-induced peroxidation were assessed in the presence of human serum albumin 0.1, 1.0, 10 and 30 g litre−1. As albumin may have independent antioxidant effects, the effect of each test concentration of albumin on peroxidation was also measured in experiments where no propofol was added.

HUMAN HEP G2 HEPATOMA CELL CULTURE

Hep G2 cells were grown routinely in Eagle’s minimal essential medium with 1-glutamine, non-essential amino acids and 10% fetal calf serum at 37°C in 5% carbon dioxide in air. Confluent monolayers of cells were treated with a variety of concentrations of propofol for 24 h, after which cells were harvested using trypsin–EDTA. Cytosolic extracts of these cells were obtained by sonication in 0.1% digitonin in Tris-HCl 0.1 mol litre−1 pH 7.4, DTT 1 mmol litre−1 and phenylmethylsulphonyl fluoride 1 mmol litre−1. The supernatants (16 000 g, 30 min) were used for enzymatic assays. Phospholipid hydroperoxide glutathione peroxidase (PHGPx) activity was measured by HPLC assay using 1-palmitoyl-2-(13-hydroperoxy-cis,cis-11-octadecadienoyl)-L-3-phosphatidylcholine as substrate. Glutathione peroxidase (GPX) activity was measured using hydrogen peroxide as substrate. Glutathione S-transferase (GST) activity was mea-
Protein contents of the cell extracts were determined using the dye binding assay (Bio-Rad) protein assay reagent using bovine serum albumin as standard.

**TEST SUBSTANCES**

Diprivan (1% propofol in 10% soybean oil emulsified with egg phosphatide; Zeneca, Macclesfield, UK) was diluted to appropriate concentrations in 0.9% saline for all studies in which propofol was used. Control studies were performed with 10% Intralipid (emulsified soybean oil and egg phosphatide; Kabi Pharmacia, UK). Trolox C and BHT were obtained from Sigma Chemicals, UK.

**Results**

Intralipid had no effect on non-enzymic or enzymic lipid peroxidation in concentrations that corresponded to the amounts of Intralipid present in Diprivan solutions (results not shown). Figure 1 shows the effects of increasing concentrations of propofol, BHT and Trolox on peroxidation of human liver microsomes induced by ascorbate–Fe(III) (fig. 1) or NADPH–Fe(III) (fig. 2). Propofol showed similar inhibition curves in both assays with IC₅₀ values of 9.5 and 7.4 μmol litre⁻¹ in the aqueous phase, respectively. Clearly, propofol was equally effective at prevention of peroxidation in both enzymic and non-enzymic systems. The relative effectiveness of propofol compared with BHT and Trolox in these assays is shown in table 1 and figures 1 and 2.

In separate experiments, we found that there was no reduction in the antioxidant activity of propofol when experiments were repeated with albumin 0.1, 1.0 and 10.0 g litre⁻¹ in the medium (results not shown). Data from experiments where albumin 30 g litre⁻¹ was used were complicated by the fact that this concentration of albumin resulted in 40% reduction in ascorbate–Fe(III)-induced peroxidation and...
Table 1  IC50 values for propofol, BHT and Trolox C for inhibition of lipid peroxidation in normal human liver microsomes (mean (sd))

<table>
<thead>
<tr>
<th>Agent</th>
<th>IC50 for non-enzymic lipid peroxidation (µmol litre −1)</th>
<th>IC50 for enzymic lipid peroxidation (µmol litre −1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propofol</td>
<td>9.47 (0.86)</td>
<td>7.39 (0.84)</td>
</tr>
<tr>
<td>BHT</td>
<td>2.34 (0.68)</td>
<td>0.35 (0.04)</td>
</tr>
<tr>
<td>Trolox C</td>
<td>1.30 (0.57)</td>
<td>0.32 (0.02)</td>
</tr>
</tbody>
</table>

Figure 3  Propofol-induced inhibition of lipid peroxidation in the presence (+) and absence (−) of human serum albumin 30 g litre −1. The curves on the left (A,C) are for ascorbate–Fe(III)-induced (non-enzymic) lipid peroxidation (NELP), and those on the right (B,D) are for NADPH–iron-induced (enzymic) lipid peroxidation (ELP). The two upper curves (A,B) show the actual concentrations of lipid peroxidation, measured by generation of malondialdehyde (MDA), while the two lower curves (C,D) show the same data with values indexed to initial measurements in the absence of propofol in each set of experiments. Note that addition of albumin results in a 40% reduction in lipid peroxidation. Residual peroxidation is effectively inhibited by propofol, albeit with a change in IC50. Data are mean (SD).

Table 2  Effect of propofol on antioxidant enzyme activity in a Hep G2 cell line (mean (sd)). PHGPx = phospholipid hydroperoxide glutathione peroxidase; GPX = glutathione peroxidase; and GST = glutathione S-transferase

<table>
<thead>
<tr>
<th>Propofol concn (µmol litre −1)</th>
<th>PHGPx (nmol of substrate min −1 mg protein −1)</th>
<th>GPX (nmol of substrate min −1 mg protein −1)</th>
<th>GST (nmol of substrate min −1 mg protein −1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.40 (0.17)</td>
<td>5.37 (0.57)</td>
<td>25.6 (2.7)</td>
</tr>
<tr>
<td>1</td>
<td>2.48 (0.20)</td>
<td>6.00 (0.26)</td>
<td>25.8 (1.5)</td>
</tr>
<tr>
<td>10</td>
<td>2.44 (0.14)</td>
<td>6.00 (0.40)</td>
<td>22.1 (1.7)</td>
</tr>
<tr>
<td>100</td>
<td>2.44 (0.25)</td>
<td>4.87 (0.51)</td>
<td>21.6 (1.7)</td>
</tr>
</tbody>
</table>

NADPH–iron-induced lipid peroxidation. However, the residual peroxidation reaction was effectively inhibited by propofol (fig. 3), with IC50 values of 9.54 (0.02) and 12.43 (0.41) µmol litre −1 (as 1.74 (0.39) and 5.54 (0.59) µmol litre −1 in the absence of albumin), respectively.

The effect of propofol on the activity of key antioxidant and detoxifying enzymes in Hep G2 cell lines is shown in table 2. There were no significant changes with propofol for all concentration ranges.

Discussion
We have shown that clinically relevant concentrations of propofol inhibited both enzymic and non-enzymic lipid peroxidation, suggesting that it does so via mechanisms independent of cytochrome P450. Previous studies have reported that propofol inhibits microsomal cytochrome P450 activity. However, the IC50 values for this effect are considerably higher than the typical clinical concentrations (< 30 µmol litre −1) that we chose to examine in our studies. Thus Baker,
Chadam and Ronnenberg showed an IC<sub>50</sub> value of approximately 350 μmol litre<sup>-1</sup> in rat hepatic microsomes. Janicki, James and Erskine showed an IC<sub>50</sub> of approximately 60–100 μmol litre<sup>-1</sup> in human hepatic microsomes and Chen and colleagues showed an IC<sub>50</sub> of 300–1000 μmol litre<sup>-1</sup> in human liver microsomes. Lower IC<sub>50</sub> values of 25–30 μmol litre<sup>-1</sup> were reported by Baker, Chadam and Ronnenberg, but these were in hepatic microsomes obtained from phenobarbital treated rats. In contrast, Chen and colleagues showed that doses as high as 100 mg kg<sup>-1</sup> i.p., twice daily for 2 weeks, failed to produce significant effects on cytochrome P450 activity. However, we cannot exclude the possibility that the antioxidant effects we observed were related in part to inhibition of NADPH cytochrome P450 reductase, although there are no data in the literature to support such an effect either for propofol or for other closely related phenolic compounds.

The IC<sub>50</sub> for propofol in both enzymic and non-enzymic lipid peroxidation reactions was 7–9 μmol litre<sup>-1</sup>. These results agree with those of Musacchio and colleagues, who found an IC<sub>50</sub> of 1–25 μmol litre<sup>-1</sup>, and Aarts and colleagues who demonstrated significant antioxidant protection by propofol at concentrations of 11.2 and 28 μmol litre<sup>-1</sup>. We found that both BHT and Trolox C were more potent antioxidants than propofol. The IC<sub>50</sub> values for enzymic and non-enzymic lipid peroxidation were 0.35 and 2.3 μmol litre<sup>-1</sup>, respectively, for BHT, and 0.32 and 1.3 μmol litre<sup>-1</sup>, respectively, for Trolox C. Our comparative data for BHT and propofol differed from those observed by Musacchio and colleagues, who demonstrated similar antioxidant potencies for the two compounds in their assay. These differences may be related to species (rat vs human hepatocytes) or to differences in experimental methods.

These findings need to be assessed in the context of plasma concentrations achieved in clinical practice. Typical plasma concentrations of propofol during anaesthesia are 2.8–28 μmol litre<sup>-1</sup> which are similar to the concentrations that inhibited lipid peroxidation in our studies. While comparison with clinically relevant concentrations of α-tocopherol (approximately 33 μmol litre<sup>-1</sup>) would have been interesting, its lack of solubility in water made direct comparison in our experiments difficult. Consideration of lipid solubility and partitioning is important with reference to propofol, which is a highly lipid soluble compound with a log octanol/water partition coefficient of 17.7, and estimates of its relative concentration in lipid membranes (compared with the aqueous environment) vary from 4 to 200 (data from Zeneca, Macclesfield, UK). This fact may help to explain the discrepancy between data from Green, Bennet and Nelson and the results obtained by Musacchio and colleagues, Eriksson, Pollesello and Saris, Aarts and colleagues and ourselves. Green, Bennet and Nelson studied the antioxidant effects of propofol in an aqueous system, which may not have been relevant to an agent that prevents lipid peroxidation. The relative concentration of propofol in lipid membranes may explain its antioxidant activity in microsomal preparations in the latter three studies.

Propofol is highly protein bound, and the presence of albumin in the medium may be expected to result in significant binding and reduction in antioxidant activity. However, while human serum albumin 30 g litre<sup>-1</sup> resulted in some decrease in the potency of propofol for inhibition of both types of peroxidation reactions, IC<sub>50</sub> values were well within the range of plasma concentrations observed during clinical use of the agent. IC<sub>50</sub> values for propofol in the absence of albumin in these experiments were different from those obtained in experiments that compared propofol with BHT and Trolox C. However, these two sets of studies were performed on different liver samples, and the experiments were separated by several months, as timing of these experiments was dictated by the availability of cut down livers. In this context the observed differences are not excessive.

Propofol had no significant effects on the activities of antioxidant enzymes in the Hep G2 cell line, although there appeared to be a trend towards reducing activities of GST and GPX at high propofol concentrations (approximately 100 μmol litre<sup>-1</sup>). These enzymes are known to be induced in Hep G2 cells by antioxidant compounds such as picolines and in vivo by extracts of cruciferous vegetables. These findings also distinguish propofol from other structurally similar phenolic antioxidant compounds such as BHT, which undergo redox cycling and tend to increase concentrations of these enzymes. Consequently, administration of propofol is unlikely to be associated with antioxidant effects that persist beyond the duration of exposure to the drug. Further investigations are required to clarify if prolonged exposure to high concentrations of propofol can result in attenuation of the intrinsic cellular antioxidant defences, with an increase in vulnerability to oxidant attack after withdrawal of the agent.

In summary, we conclude that propofol exhibits useful antioxidant activities in lipid membranes when concentrations in the aqueous environment are well within the anaesthetic dose range, probably as a result of preferential partitioning into hydrophobic environments. This finding may be relevant in the context of clinical use of propofol as an antioxidant, as it may be able to inhibit or limit lipid peroxidation in cell membranes. Maximal inhibition of lipid peroxidation was achieved in our experiments at concentrations <30 μmol litre<sup>-1</sup>; and there may be unfavourable effects on cellular antioxidant enzyme levels at concentrations above the clinical range, with additional effects on cytochrome P450 becoming apparent. Optimal concentrations of propofol for antioxidant activity appear to coincide with plasma concentrations obtained during clinical anaesthesia, and these effects are seen at clinically relevant concentrations of propofol, even in the presence of physiologival concentrations of human serum albumin.

Acknowledgement
We thank the Biotechnology and Biological Research Council, UK, for funding.

References
2. Xue D, Slivka A, Buchan AM. Tirilazad reduces cortical
Antioxidant effects of propofol


