Propofol versus Midazolam Regarding Their Antioxidant Activities

MASAHIKO TSUCHIYA, AKIRA ASADA, KEIKO MAEDA, YASUYO UEDA, EISUKE F. SATO, MITSUO SHINDO, and MASAYASU INOUE

Department of Biochemistry, and Department of Anesthesiology and Intensive Care Medicine, Osaka City University Medical School, Abenoku, Osaka, Japan

Propofol and midazolam are commonly used as sedatives for critically ill patients. These patients usually suffer from the pathologic effects of oxidative stress, predominantly caused by an imbalance between the generation of reactive oxygen species and the antioxidant defense system. Therefore, the antioxidant activities of propofol and midazolam may be of clinical importance. We investigated the activities of these two sedatives against hydrophilic or lipophilic peroxyl radicals in a homogeneous solution and in the presence of erythrocyte membranes. A chemical analysis of the homogeneous solution revealed that propofol efficiently scavenged hydrophilic peroxyl radicals (50% inhibitory concentration [IC_{50}] = 1.3×10^{-4} M), whereas midazolam efficiently scavenged lipophilic radicals (IC_{50} = 1.5×10^{-5} M). Further, in membrane systems, propofol inhibited the oxidative damage induced by either hydrophilic or lipophilic radicals (IC_{50} = 1.5×10^{-5} M for hydrophilic radicals and IC_{50} = 3.0×10^{-4} M for lipophilic radicals), whereas midazolam did very little. In previous studies, we demonstrated that antioxidant activity is highly affected by the location and properties of the reaction site. The discrepancy in antioxidant activity between a homogeneous condition and in the presence of membranes can be well explained by this concept, and again emphasizes the importance of membranes in determining antioxidant activity. To further understand the biologic significance of these antioxidant properties, the effect of the two agents on endothelium-dependent relaxation was studied. Application of oxidative stress to aortic rings by treating them with peroxyl radicals led to a significant blockade of acetylcholine-induced relaxation after submaximal contraction with phenylephrine. Propofol pretreatment greatly attenuated the impairment in comparison with midazolam, which agrees with the concept of antioxidant activity in the presence of membranes. The results of the present study suggest that propofol has a greater potential to reduce oxidative stress than midazolam.

The induction and maintenance of appropriate sedative conditions are important for critically ill patients in the intensive care unit. A recent study reported that the respiratory and hemodynamic effects, sedation quality, and cost–benefit relationship of propofol are almost identical to those of midazolam (1–4). However, other important factors should be taken into account when determining sedatives for critically ill patients. These patients often suffer from severe oxidative stress caused by infection, hemodynamic instability, repetitive hypoxia, and multiple organ dysfunction; thus, it is important to normalize their redox status with drugs that have antioxidant activities (5, 6). We previously performed a screening study for the antioxidant activities of various drugs administered to critically ill patients, and found that, generally, sedatives had a moderate level of activity whereas most other kinds of drugs did not have an adequate amount (6). However, only limited information is available regarding the precise ability of propofol and midazolam to suppress oxidative stress (6–9).

We have developed highly sensitive and convenient methods for measuring the antioxidant activities of compounds with a small molecular weight, allowing us to quantitate the scavenging activity of a compound toward peroxyl radicals in either an aqueous or nonaqueous homogenous phase (10, 11). Because the biologic system consists of both lipophilic and aqueous components, it is of critical importance to understand the antioxidant activities in both phases. The importance of the amphipathic nature of antioxidants has been well demonstrated with α-tocopherol and ascorbic acid; lipophilic α-tocopherol preferentially inhibits the oxidative injury occurring in membranes and lipoproteins, whereas ascorbic acid effectively suppresses oxidative stress in the aqueous phase (12). For the present set of experiments, we further developed our assay system to analyze antioxidant activities against peroxyl radicals in a membrane system, as well as in aqueous and nonaqueous homogenous systems. The aim of the present study was to elucidate and compare the antioxidant natures of propofol and midazolam using this assay system, and then to confirm their activities in aortic ring preparations exposed to peroxyl radicals–induced oxidative stress, as an example of a biologically functioning system.

METHODS

Chemicals

B-phycoerythrin and *cis*-parinaric acid were purchased from Sigma Chemical Co. (St. Louis, MO), and 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH) and 2,2′-azobis(2,4-dimethylvaleronitrile) (AMVN) were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). α-Tocopherol was from Nacalai Tesque (Kyoto, Japan), and 2,6-diisopropylphenol (propofol) and Trolox were from Aldrich Chem. Co. (Milwaukee, WI). Diprivan and midazolam were from Astra-Zeneca (Osaka, Japan) and Yamanouchi (Tokyo, Japan), respectively. Other chemicals used were the highest grade commercially available.

Preparation of Erythrocyte Ghosts

Erythrocyte ghosts were prepared as previously described (13). Fresh human erythrocytes from healthy volunteers were centrifuged for 10 min at 750 × *g* and 4° C, and then washed three times with phosphate-buffered saline (PBS) (pH 7.4) containing 100 μM ethylenediaminetetraacetic acid (EDTA). The washed erythrocytes were lysed in an 8-fold volume of hypotonic phosphate buffer (pH 7.4) containing 100 μM EDTA. After 45 min, the ghosts were centrifuged at 30,000 × *g* and 4° C for 20 min, and subsequently washed 5 times with the same hypotonic buffer. The ghost samples were stored at −80° C until used for the experiments.

Fluorescence Study on Antioxidant Activity in a Homogeneous Environment, Aqueous Buffer, and Nonaqueous Solution

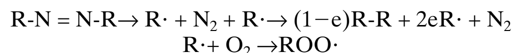
Methods for determining the antioxidant activities of a compound in both homogenous aqueous and nonaqueous phases have been described in several studies by Tsuchiya and coworkers (6, 10, 11). In sum-

(*Received in original form November 30, 1999 and in revised form June 8, 2000*)

Correspondence and requests for reprints should be addressed to Dr. Masahiko Tsuchiya, c/o Prof. Masayasu Inoue, Department of Biochemistry, Osaka City University Medical School, 1-4-3 Asahimachi, Abenoku, Osaka 545-8585, Japan. E-mail: oxymasa@ea.mbn.or.jp

Am J Respir Crit Care Med Vol 163. pp 26–31, 2001
Internet address: www.atsjournals.org

mary, our assay system contained a hydrophilic diazo compound (4 mM AAPH) and fluorescent protein (17 nM B-phycoerythrin) in PBS at pH 7.4, or a lipophilic diazo compound (200 mM AMVN) and fluorescent polyunsaturated fatty acid (100 nM *cis*-parinaric acid) in heptane. AAPH and AMVN each thermally decompose to yield peroxyl radicals at constant rates, depending on the temperature, as follows:



$$\text{R-N} = \text{N-R} \rightarrow \text{R}\cdot + \text{N}_2 + \text{R}\cdot \rightarrow (1-\text{e})\text{R-R} + 2\text{eR}\cdot + \text{N}_2$$
$$\text{R}\cdot + \text{O}_2 \rightarrow \text{ROO}\cdot$$

where R-N = N-R represents the structure of AAPH or AMVN, and e the efficiency of free radical production ($1 > e > 0$). R· rapidly reacts with surrounding oxygen to generate peroxyl radicals. The rate constants of radical generation throughout this reaction can be calculated by the effect of α-tocopherol and its hydrophilic analogue, Trolox, both of which trap exactly two moles of peroxyl radicals per one of their own moles. B-phycoerythrin and *cis*-parinaric acid (F) have their fluorescence decreased by the attack of peroxyl radicals generated from AAPH or AMVN as follows:

$$\text{ROO}\cdot + \text{F} \rightarrow \text{nonfluorescent product}$$

Antioxidants in the reaction mixture inhibit this oxidation process according to their activity, which can be measured by monitoring the reduction of the rate of fluorescence decay using a fluorescence spectrophotometer (Hitachi F-4500, Tokyo).

Study on Antioxidant Activity in the Presence of Membranes Measured by Oxygen Consumption

The antioxidant activity of the two sedatives in the presence of membranes was evaluated by measuring the rate of oxygen consumption caused by lipid peroxidation of human erythrocyte ghosts (11, 14–18). Lipid peroxidation is induced by either hydrophilic AAPH or lipophilic AMVN. AAPH generates peroxyl radicals outside the membranes, whereas AMVN generates oxidative stress within. Oxygen consumption was monitored using a Clark-type oxygen electrode equipped with an ultrathin Teflon membrane and temperature control system. The activity of each test compound to inhibit lipid peroxidation was measured by monitoring the change in the rate of oxygen consumption. The reaction mixture contained 50 mM AAPH or 20 mM AMVN and erythrocyte ghosts (0.6 mg protein/ml) in PBS (pH 7.4). The erythrocyte ghosts and AMVN were mixed, and then sonicated for 20 s at 4° C to incorporate the generator into the membranes.

Study on Antioxidant Activity in Aortic Ring Preparation Exposed to Peroxyl Radicals

Rat aortic rings were prepared according to the method previously described by Tsuchiya and coworkers (19). In brief, male Wistar rats (200–300 g) were anesthetized with ether and killed by decapitation. The thoracic aorta was rapidly excised, and cleaned of adherent fat and connective tissue. It was then cut into 3-mm ring segments and mounted between two tungsten triangles, of which the lower was fixed and the upper was attached to a force-displacement transducer, in an organ bath filled with 20 ml of Krebs-Henseleit solution (KHS: 118 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.19 mM KH_2PO_4, 1.19 mM $MgSO_4$, 25 mM $NaHCO_3$, and 5.6 mM glucose) bubbled with 95% oxygen and 5% carbon dioxide at 37° C. The preparations were equilibrated for 90 min at a resting tension of 0.5 g and then kept under a constant tension of 1 g throughout the experiment. Each ring was preincubated with KHS plus 40 mM AAPH for 20 min at 37° C, and washed three times with KHS, before being contracted with phenylephrine (10^{-6} M). When the contraction reached a plateau (after ap-

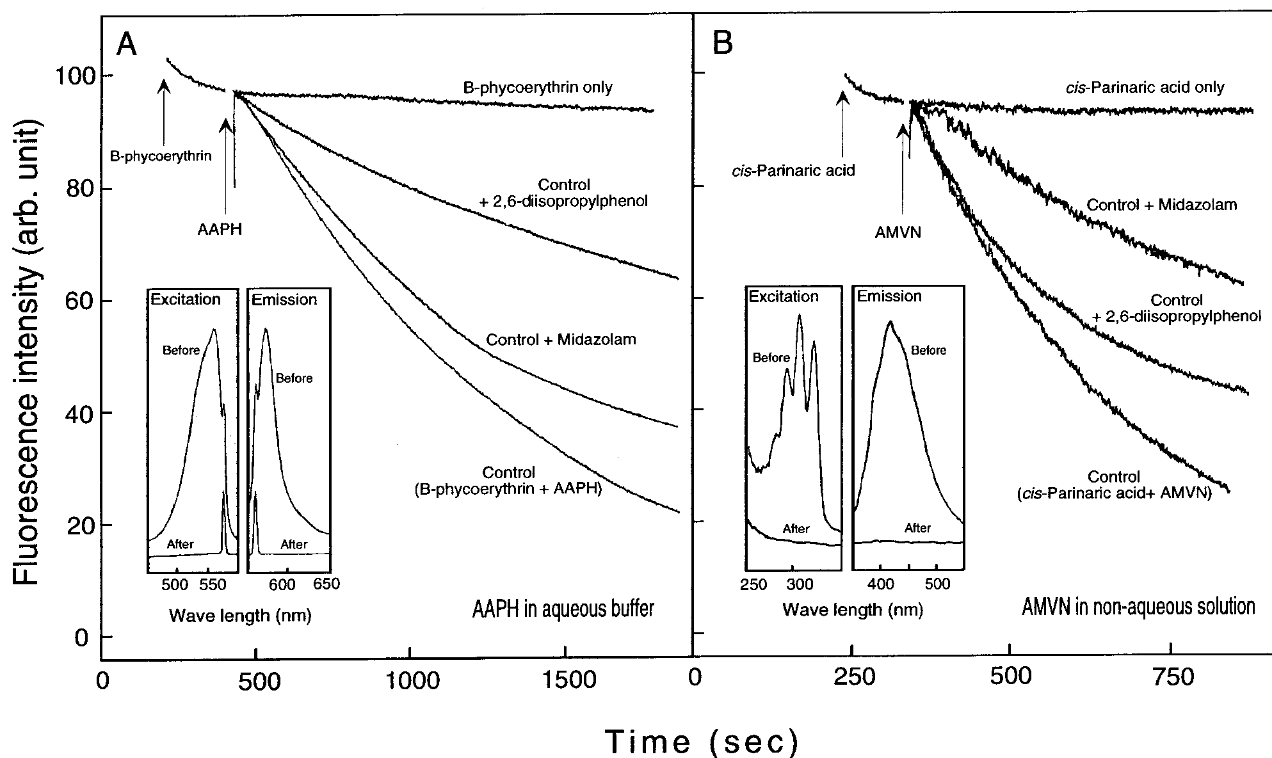


Figure 1. Effect of propofol and midazolam on AAPH-induced oxidation of B-phycoerythrin in aqueous phases, and AMVN-induced oxidation of *cis*-parinaric acid in nonaqueous phases. (*A*) The reaction mixtures contained 17 nM B-phycoerythrin and 4 mM AAPH in a final volume of 3 ml of PBS. Fluorescence intensity was measured at an emission wavelength of 571 nm using an excitation wavelength of 558 nm at 37° C. Concentrations of propofol and midazolam were 200 μM and 190 μM, respectively. The reaction was started by the addition of AAPH. The *inset* shows excitation and emission spectra, before the addition of AAPH and after complete oxidation of B-phycoerythrin. (*B*) The reaction mixtures contained 100 nM *cis*-parinaric acid and 200 mM AMVN in heptane. Fluorescence intensity was measured at an emission wavelength of 421 nm using an excitation wavelength of 305 nm at 50° C. Concentrations of propofol and midazolam were 200 μM and 30 μM, respectively. The reaction was started by the addition of AMVN. The inset shows excitation and emission spectra, before the addition of AMVN and after complete oxidation of *cis*-parinaric acid.

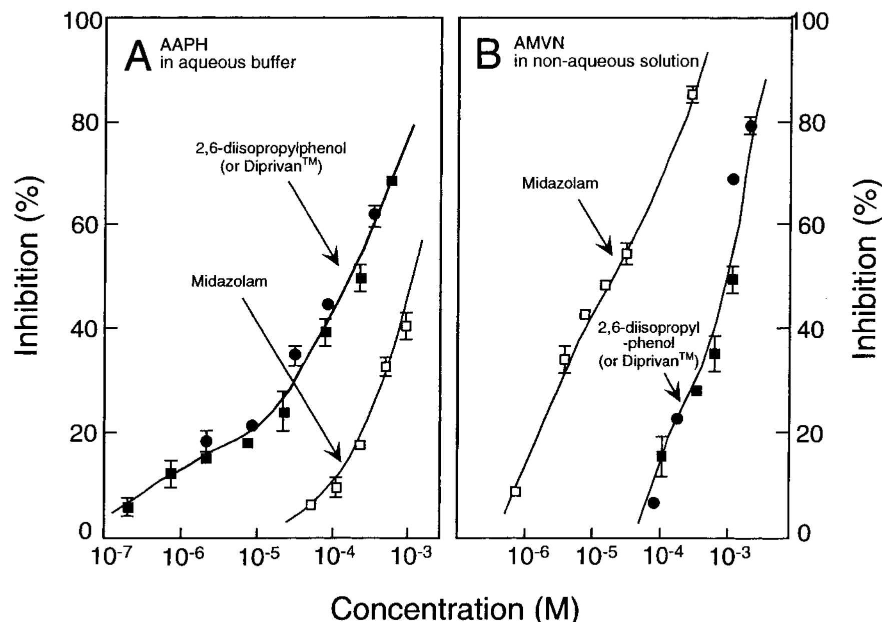


Figure 2. Dose dependency of the inhibition of propofol and midazolam against the oxidation of B-phycoerythrin and *cis*-parinaric acid by AAPH and AMVN. (*A*) The inhibitory effects of propofol and midazolam on the AAPH-induced oxidation of B-phycoerythrin in PBS were analyzed at different concentrations of the two agents. (*B*) The effects of propofol and midazolam on the AMVN-induced oxidation of *cis*-parinaric acid in heptane were also determined. Other conditions were as described in the legend to Figure 1. *Filled squares*: 2,6-diisopropylphenol; *circles*: Diprivan; *open squares*: midazolam.

proximately 10 min), acetylcholine (10^{-8} to 10^{-4} M) was cumulatively added. The relaxation was expressed as a percentage of the maximal phenylephrine (10^{-6} M) response. When indicated, either 2,6-diisopropylphenol (Diprivan) or midazolam was added to the KHS before the addition of AAPH.

Data Processing

Experiments were repeated at least 5 times, and data are expressed as mean ± SEM (standard error of the mean). Statistical analysis was performed using analysis of variance (ANOVA) with repeated measurements. A p value of less than 0.05 was considered significant.

RESULTS

Effects of 2,6-diisopropylphenol (Diprivan) and Midazolam on the Oxidation of B-phycoerythrin and *cis*-parinaric Acid as Measured by Fluorescence

To evaluate the chemical antioxidant activities of 2,6-diisopropylphenol (propofol) and midazolam, their effects on the AAPH-induced oxidation of B-phycoerythrin in a phosphate buffer, or on the AMVN-induced oxidation of *cis*-parinaric acid in heptane were analyzed by a fluorescence measuring method. Under these conditions, the rate constants of radical generation were 1.36×10^{-6} (s^{-1}) for AAPH at 37° C and 9.53×10^{-6} (s^{-1}) for AMVN at 50° C, which agree well with values that we have previously reported (6, 10, 11).

The fluorescence intensity of B-phycoerythrin rapidly decreased after the addition of AAPH, which generated hydrophilic peroxyl radicals in the aqueous phase (Figure 1A). The inset shows the excitation and emission spectra of B-phycoerythrin, just before the addition of AAPH and after the end of the reaction, indicating that the fluorescence change exactly reflected the oxidation process of B-phycoerythrin. The rate of decrease in fluorescence intensity was diminished by the presence of 2,6-diisopropylphenol and midazolam. The fluorescence intensity of *cis*-parinaric acid was also decreased by the addition of AMVN, which generated lipophilic peroxyl radicals in the nonaqueous phase (Figure 1B). Both 2,6-diisopropylphenol and midazolam inhibited this decrease in fluorescence intensity. The inset shows the excitation and emission spectra of *cis*-parinaric acid, again indicating the validity of measuring fluorescence change.

Figure 2 shows the inhibition dose dependency of the oxidation of B-phycoerythrin and *cis*-parinaric acid by 2,6-diisopropylphenol and midazolam, which was evaluated as the percent of inhibition against fluorescence decay; 100% inhibition indicates the rate of autofluorescence decay of B-phycoerythrin or *cis*-parinaric acid, and 0% inhibition indicates the decay rate in the complete system. In the aqueous system, 2,6-diisopropylphenol inhibited the oxidation of B-phycoerythrin at lower concentrations (50% inhibitory concentration [IC_{50}] = 1.3×10^{-4} M) than midazolam ($IC_{50} = 1.0 \times 10^{-3}$ M). In contrast, midazolam inhibited the oxidation of *cis*-parinaric acid in the lipophilic phases in a greater way ($IC_{50} = 1.5 \times 10^{-5}$ M) than 2,6-diisopropylphenol ($IC_{50} = 9.0 \times 10^{-4}$ M). Diprivan, the medical product of 2,6-diisopropylphenol dissolved in lipid emulsion, showed exactly the same effects as 2,6-diisopropylphenol in both the aqueous and nonaqueous systems.

Effects of 2,6-diisopropylphenol (Diprivan) and Midazolam on the Oxygen Consumption by Lipid Peroxidation of Erythrocyte Membranes

AAPH generates peroxyl radicals in the aqueous compartment, whereas AMVN does so in the nonaqueous compartments. Therefore, these two compounds have been used, respectively, to initiate lipid peroxidation of erythrocyte membranes from outside and within membrane lipid bilayers. As shown in Figure 3A, AAPH enhanced the oxidation of erythrocyte membranes, as measured by the decrease in oxygen concentration in the medium. The rate of oxygen consumption was markedly decreased by the presence of 2,6-diisopropylphenol (propofol), but not midazolam. On the other hand, AMVN enhanced the rate of oxygen consumption by a mechanism that was inhibited by both 2,6-diisopropylphenol and midazolam (Figure 3B).

Figure 4 shows the inhibition dose dependency of membrane oxidation by the two compounds, which was evaluated as the percent of inhibition against oxygen consumption; 100% inhibition indicates the rate of basal oxygen consumption induced by AAPH or AMVN alone, and 0% inhibition indicates the rate of oxygen consumption in the complete system. The IC_{50} of 2,6-diisopropylphenol for the oxidation of erythrocyte membranes by AAPH was 1.0×10^{-5} M, whereas the IC_{50} of 2,6-diisopropylphenol and midazolam for AMVN-in-

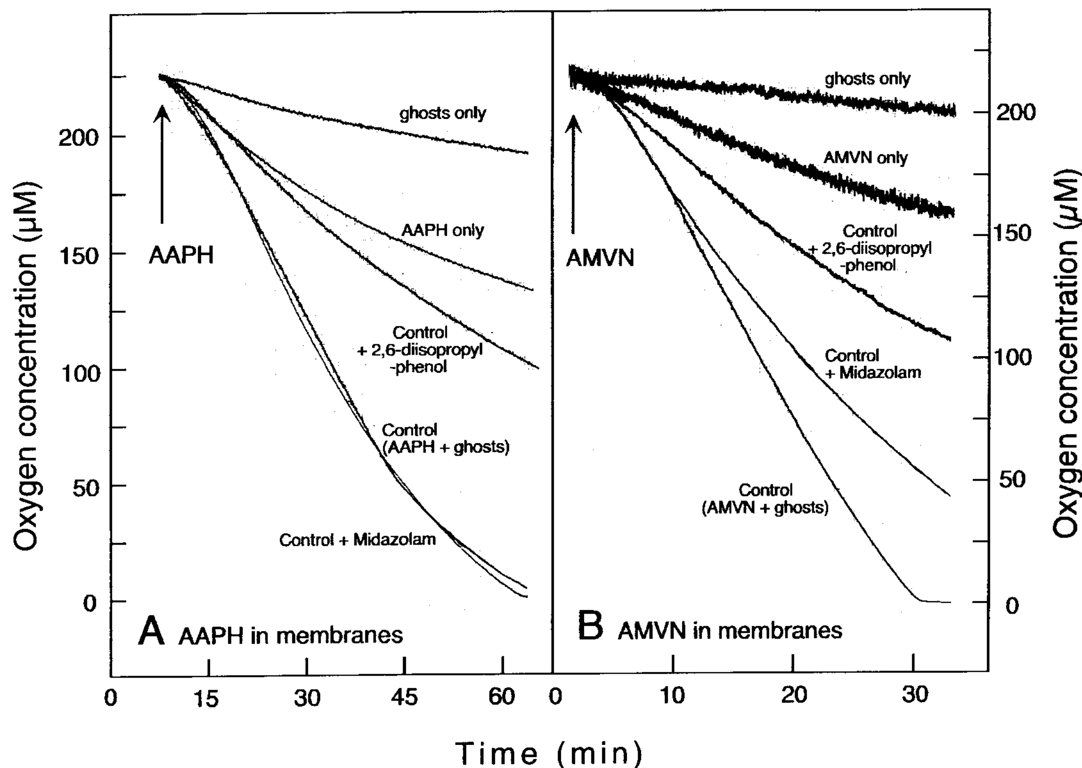


Figure 3. Effect of propofol and midazolam on the lipid peroxidation of erythrocyte membranes. (*A*) The reaction mixtures contained 50 mM AAPH and erythrocyte ghosts (0.6 mg protein/ml) in PBS (pH 7.4). Oxygen consumption was monitored using a Clark-type oxygen electrode at 37° C. Concentrations of propofol and midazolam were 50 μM and 2 mM, respectively. (*B*) The reaction mixtures contained 20 mM AMVN and erythrocyte ghosts (0.6 mg protein/ml) in PBS (pH 7.4). Erythrocyte ghosts and AMVN were mixed and sonicated for 20 s at 4° C just before their use. Oxygen consumption was monitored with a Clark-type oxygen electrode at 40° C. Concentrations of propofol and midazolam were 130 μM and 1,500 μM, respectively.

duced membrane oxidation was 3.0×10^{-4} M and 3.0×10^{-1} M (extrapolated values), respectively. Diprivan, a lipid emulsion of 2,6-diisopropylphenol, showed exactly the same effect as 2,6-diisopropylphenol in both cases.

Effects of 2,6-diisopropylphenol (Diprivan) and Midazolam on Peroxyl Radical–induced Impairment of Endothelium-dependent Relaxation of Aortic Ring Preparation

To further understand the biologic significance of these antioxidant properties, the protective effects of the two agents as observed in aortic ring preparations exposed to peroxyl radicals were compared at their near-equipotential therapeutic concentrations (50 μM of 2,6-diisopropylphenol and 5 μM of midazolam). Acetylcholine induced a dose-dependent relaxation in endothelium-intact ring preparations submaximally precontracted by phenylephrine (10^{-6} M), expressed as a percentage of the maximal phenylephrine response (Figure 5), whereas no relaxation was seen in the endothelium-denuded preparations (data not shown). Exposure of the aortic rings to peroxyl radicals by incubation with AAPH significantly inhibited this acetylcholine-induced relaxation. Pretreatment with 2,6-diisopropylphenol before peroxyl radical exposure nearly abolished the inhibitory effect, whereas midazolam did very little. Diprivan showed the same effect as 2,6-diisopropylphenol.

DISCUSSION

We previously proposed that antioxidant activity is highly dependent on the location and property of the scavenging site, which must be taken into consideration when evaluating the effectiveness of drugs that are expected to have antioxidant potency (10, 11, 20). However, there is scant information available regarding a reliable method for easy evaluation of various drugs used clinically on the basis of this concept. The present

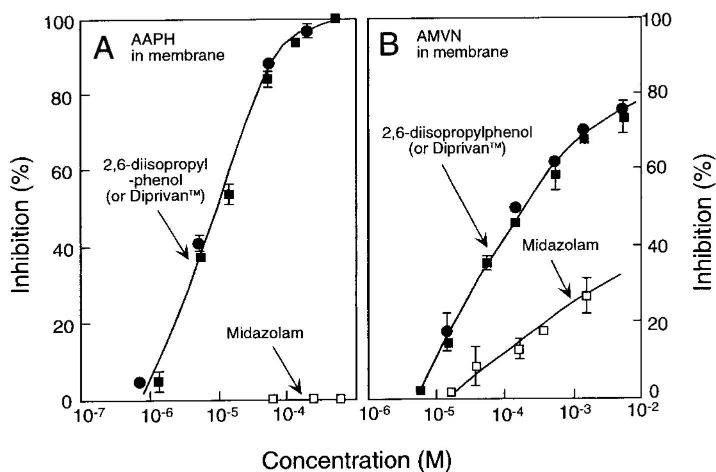


Figure 4. Dose dependency of the inhibition of propofol and midazolam on the lipid peroxidation of erythrocyte membranes induced by AAPH and AMVN. (*A*) The inhibitory effects of propofol and midazolam on AAPH-induced lipid peroxidation of erythrocyte membranes in PBS were analyzed at different concentrations of the two agents. (*B*) The effects of propofol and midazolam on the AMVN-induced lipid peroxidation of erythrocyte membranes were also determined. Other conditions were as described in the legend to Figure 3. *Filled squares*: 2,6-diisopropylphenol; *circles*: Diprivan; *open squares*: midazolam.

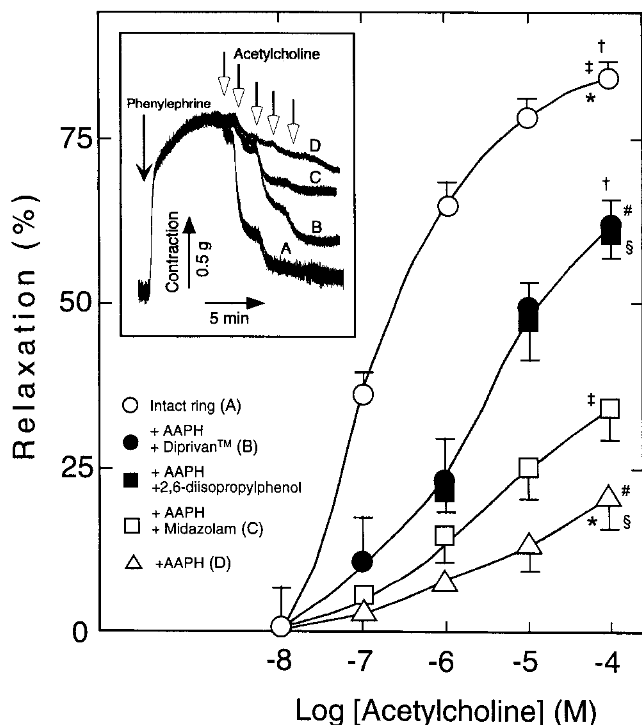


Figure 5. Concentration–response curves showing relaxation with acetylcholine on phenylephrine-contracted endothelium-containing rat aorta rings. The protective effects of the two sedatives on aortic rings were compared at their near-equipotential therapeutic concentrations (50 μM of 2,6-diisopropylphenol [or Diprivan] and 5 μM of midazolam). *Open circles*: intact ring (*A*); *filled circles*: + Diprivan in ring exposed to AAPH-derived peroxyl radicals (*B*); *filled squares*: + 2,6-diisopropylphenol in ring exposed to AAPH-derived peroxyl radicals; *open squares:* + midazolam in ring exposed to AAPH-derived peroxyl radicals (*C*); *open triangles*: ring exposed to AAPH-derived peroxyl radicals alone (*D*). *, #, †, ‡, § significantly different ($p < 0.05$). The *inset* shows an individual experimental tracing demonstrating the effect of a cumulative addition of acetylcholine (10^{-8} to 10^{-4} M) after induction of tone with phenylephrine (10^{-6} M) in an aortic ring.

integration of simple and easy analogous assays in different conditions, yields a deeper and more accurate understanding of the contribution of the reaction environment, though an individual assay may be too chemically based or provide incomplete information. In addition, the peroxyl radical is considered to be harmful, and may directly or indirectly threaten the life support system (10, 11, 20–25). Thus, the results of the present assay system could provide valuable information regarding the antioxidant potential of each of the drugs examined.

Chemical analyses in both homogeneous aqueous and nonaqueous phases revealed that propofol efficiently scavenged hydrophilic peroxyl radicals, whereas midazolam efficiently scavenged lipophilic radicals, in near-therapeutic concentrations (50 μM in propofol and 5 μM in midazolam) (26–29). However, it should be noted that heating at 50° C was applied for the measurement with heptane to allow for adequate thermal decomposition of AMVN in this assay (nonaqueous system) (11). Although this temperature is higher than the physiologic condition, our assay system does not contain any large molecules such as proteins that are affected by such heating. Furthermore, α-tocopherol, a potent natural antioxidant, has been similarly assayed using AMVN at 56° C, without affecting its antioxidant reaction or properties (30). Thus, the observed differences of antioxidant activities between the two sedatives in the present assay are considered to reflect their activity fairly well, though careful consideration should be taken.

Under heterogeneous conditions involving biologic membranes, the fluorescence decay of reporting molecules involved with oxidative stress is also able to be measured, as reported by Tsuchiya and coworkers (11). However, the addition of propofol to the membrane and diazo-radical generator increases the reaction mixture turbidity, making it impossible to detect the fluorescence. Thus, the antioxidant activities of the two sedatives in the presence of membranes in the present study were evaluated by measuring oxygen consumption, which is another specific method for the detection of oxidative stress that is unaffected by the turbidity of the reaction mixture (11, 15–18, 30, 31). Our study demonstrated that propofol strongly inhibited the oxidative damage to membranes induced by either hydrophilic or lipophilic radicals, whereas midazolam did very little. This might be due to generation of a highly reactive intermediate in the polar surface region of the lipid bilayer during membrane oxidation, which only propofol would be able to eliminate and cause a delay in the rate of oxygen consumption. The importance of this region is also supported by the fact that α-tocopherol locates in membranes with active sites in this region (21, 24). Furthermore, the significant discrepancies between a homogeneous condition and in the presence of membranes indicate the importance of the reaction environment in determining antioxidant activity.

In the aortic ring preparations, application of oxidative stress by treatment with peroxyl radicals led to a significant inhibition of its acetylcholine-induced relaxation response, indicating an easier impairment of endothelial function by oxidative stress, in accordance with previous studies (32–34). Greater attenuation of this impairment by propofol than midazolam agrees better with the observed antioxidant activity in the presence of membranes, which might be the first evidence to suggest the protective effect of propofol against vascular endothelium oxidative injury. Our results do not directly demonstrate the clinical benefit of propofol as an antioxidant drug; however, they do suggest that the antioxidant properties of propofol might have certain biologic implications.

The simplicity of our mechanism for measuring antioxidant activity in homogenous aqueous and nonaqueous phases, as well as the heterogeneous conditions involving membranes, favors its accuracy and reliability, but may restrict the interpretation of results, because other possible antioxidant mechanisms, such as the induction of antioxidant enzymes, inhibition of radical production, and regeneration of antioxidants are not considered. In addition, such pharmacokinetic considerations as intake, distribution, metabolism, excretion, and drug–drug interaction have not been included. Thus, the antioxidant activities of the drugs discussed in this study are mainly due to their direct reaction against peroxyl radicals. In the case of propofol, hindered phenolic structures seem to exert the antioxidant activities (8), as in the case of butylated hydroxytoluene, butylated hydroxyanisole, and tocopherols (21), whereas for midazolam, the responsible structure is unclear. However, in a previous study on stobadine, a pyridoindole derivative that possesses potent antioxidant activity, Kagan and coworkers noted the possibility that intrinsic nitrogens might donate electrons to radicals as antioxidants (25). This might also be the case with midazolam.

In conclusion, it is conceivable that propofol has some greater potential for the reduction of oxidative stress than midazolam, though it is still impossible to definitely state that the oxygen radical scavenging activity measured in the present assay systems adequately reflects the phenomena in complex *in vivo* conditions.

References

1. Sanchez-Izquierdo-Riera JA, Caballero-Cubedo RE, Perez-Vela JL, Ambros-Checa A, Cantalapiedra-Santiago JA, Alted-Lopez E. Propofol versus midazolam: safety and efficiency for sedating the severe trauma patient. *Anesth Analg* 1998;86:1219–1224.
2. Roman KP, Gallgher TJ, George B, Hamby B. Comparison of propofol and midazolam for sedation in intensive care unit patients. *Crit Care Med* 1995;23:286–293.
3. Weinbroum AA, Halpern P, Rudick V, Sorkine P, Freedman M, Geller E. Midazolam versus propofol for long-term sedation in the ICU: a randomized prospective comparison. *Intensive Care Med* 1997;23:1258–1263.
4. Barrientos-Vega R, Sanchez-Soria MM, Morales-Garcia C, Robas-Gomez A, Cuena-Boy R, Ayensa-Rincon A. Prolonged sedation of critically ill patients with midazolam or propofol: impact on weaning and costs. *Crit Care Med* 1997;25:33–40.
5. Goode HF, Cowley HC, Walker BE, Howdle PD, Webster NR. Decreased antioxidant status and increased lipid peroxidation in patients with septic shock and secondary organ dysfunction. *Crit Care Med* 1995;23:646–651.
6. Kang MY, Tsuchiya M, Packer L, Manabe M. In vitro study on antioxidant potential of various drugs used in the perioperative period. *Acta Anaesthesiol Scand*, 1998;42:4–12.
7. De La Cruz J, Villalobos M, Sendeno G, Sanchez De La Cuesta F. Effect of propofol on oxidative stress in an in vitro model of anoxia-reoxygenation in the rat brain. *Brain Res* 1998;800:136–144.
8. Murphy P, Myers D, Webster N, Jones J. The antioxidant potential of propofol (2,6-diisopropylphenol). *Br J Anaesth* 1992;68:613–618.
9. Green T, Bennett S, Nelson V. Specificity and properties of propofol as an antioxidant free radical scavenger. *Toxicol Appl Pharmacol* 1994; 129:163–169.
10. Tsuchiya M, Scita G, Freisleben HJ, Kagan VE, Packer L. Antioxidant radical-scavenging activity of carotenoids and retinoids compared to alpha-tocopherol. *Methods Enzymol* 1992;213:460–472.
11. Tsuchiya M, Kagan VE, Freisleben HJ, Manabe M, Packer L. Antioxidant activity of alpha-tocopherol, beta-carotene, and ubiquinol in membranes: cis-parinaric acid-incorporated liposomes. *Methods Enzymol* 1994;234:371–383.
12. Niki E. Antioxidants in relation to lipid peroxidation. *Chem Phys Lipid* 1987;44:227–253.
13. Steck TL, Kant JA. Preparation of impermeable ghosts and inside-out vesicles from human erythrocyte membranes. *Methods Enzymol* 1974; 31:172–180.
14. Hsiao G, Teng C, Wu C, Ko F. Marchantin H as a natural antioxidant and free radical scavenger. *Arch Biochem Biophys* 1996;334:18–26.
15. Barclay L, Ingold K. Autoxidation of biological molecules: 2. The autoxidation of a model membrane: a comparison of the autoxidation of egg lecithin phosphatidylcholine in water and in chlorobenzene. *J Am Chem Soc* 1981;103:6478–6485.
16. Niki E, Takahashi M, Komuro E. Antioxidant activity of vitamin E in liposomal membranes. *Chem Lett* 1986;1573–1576.
17. Niki E, Saito M, Yoshikawa Y, Yamamoto Y, Kamiya Y. Oxidation of lipids: XII. Inhibition of oxidation of soybean phosphatidylcholine and methyl linoleate in aqueous dispersions by uric acid. *Bull Chem Soc Jpn* 1986;59:471–477.
18. Darley-Usmar V, Hersey A, Garland L. A method for the comparative assessment of antioxidants as peroxyl radical scavengers. *Biochem Pharmacol* 1989;38:1465–1469.
19. Tsuchiya M, Tokai H, Takehara Y, Haraguchi Y, Asada A, Utsumi K, Inoue M. Interrelation between oxygen tension and nitric oxide in respiratory system. *Am J Respir Crit Care Med* 2000;162:1257–1261.
20. Suzuki YJ, Tsuchiya M, Packer L. Determination of structure–antioxidant activity relationships of dihydrolipoic acid. *Methods Enzymol* 1994;234:454–461.
21. Kagan V, Sebinova E, Packer L. Generation and recycling of radicals from phenolic antioxidants. *Arch Biochem Biophys* 1990;280:33–39.
22. Kagan V, Sebinova E, Packer L. Recycling and antioxidant activity of tocopherol homologs of differing hydrocarbon chain lengths in liver microsomes. *Arch Biochem Biophys* 1990;282:221–225.
23. Kagan VE, Freisleben HJ, Tsuchiya M, Forte T, Packer L. Generation of probucol radicals and their reduction by ascorbate and dihydrolipoic acid in human low density lipoproteins. *Free Radic Res Commun* 1991;15:265–276.
24. Suzuki YJ, Tsuchiya M, Wassall SR, Choo YM, Govil G, Kagan VE, Packer L. Structural and dynamic membrane properties of alpha-tocopherol and alpha-tocotrienol: implication to the molecular mechanism of their antioxidant potency. *Biochemistry* 1993;32:10692–10699.
25. Kagan VE, Tsuchiya M, Serbinova E, Packer L, Sies H. Interaction of the pyridoindole stobadine with peroxyl, superoxide and chromanoxyl radicals. *Biochem Pharmacol* 1993;45:393–400.
26. Schwagmeier R, Alincic S, Striebel H. Midazolam pharmacokinetics following intravenous and buccal administration. *Br J Clin Pharmacol* 1998;46:203–206.
27. Crevoisier C, Ziegler W, Eckert M, Heizmann P. Relationship between plasma concentration and effect of midazolam after oral and intravenous administration. *Br J Clin Pharmacol* 1983;16:51S–61S.
28. Heinzmann P, Eckert M, Ziegler W. Pharmacokinetics and bioavailability of midazolam in man. *Br J Clin Pharmacol* 1983;16:43S–49S.
29. Gepts E, Camu F, Cockshott ID, Douglas EJ. Disposition of propofol administered as a constant rate infusion in humans. *Anesth Analg* 1987; 66:1256–1263.
30. Takenaka Y, Miki M, Yasuda H, Mino M. The effect of alpha-tocopherol as an antioxidant on the oxidation of membrane protein thiols induced by free radicals generated in different sites. *Arch Biochem Biophys* 1991;285:344–350.
31. Sato K, Niki E, Shimasaki H. Free radical–mediated chain oxidation of low density lipoprotein and its synergistic inhibition by vitamin E and vitamin C. *Arch Biochem Biophys* 1990;279:402–405.
32. Ikeda M, Suzuki M, Watarai K, Sagai M, Tomita T. Impairment of endothelium-dependent relaxation by diesel exhaust particles in rat thoracic aorta. *Jpn J Pharmacol* 1995;68:183–189.
33. Gumusel B, Tel B, Demirdamar R, Sahin-Erdemli I. Reactive oxygen species-induced impairment of endothelium-dependent relaxation in rat aortic rings: protection by L-arginine. *Eur J Pharmacol* 1996;306:107–112.
34. Dudgeon S, Benson D, MacKenzie A, Paisley-Zyszkiewicz K, Martin W. Recovery by ascorbate of impaired nitric oxide–dependent relaxation resulting from oxidant stress in rat aorta. *Br J Pharmacol* 1998;125: 782–786.