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European Journal of Pharmacology 499 (2004) 29–33

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Propofol scavenges reactive oxygen species and inhibits the protein nitration induced by activated polymorphonuclear neutrophils

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Received 1 September 2003; received in revised form 17 May 2004; accepted 25 May 2004
Available online 25 August 2004

Abstract

Activated polymorphonuclear neutrophils may damage tissues through the release of biochemical mediators. Among them, peroxynitrite is responsible for hydroxylation reactions and nitration of proteins, or is metabolised into nitrate. We investigated the effect of propofol on the production of reactive oxygen species, the nitration of proteins and the formation of nitrate by activated human polymorphonuclear neutrophils. Propofol dose-dependently inhibited chemiluminescence, nitration of proteins and nitrate production in a concentration range from 10^{-3} to 10^{-6} mM. A significant correlation was observed between the logarithm of propofol concentration and the intensity of chemiluminescence ($r^2 = 0.90$), the nitration of proteins ($r^2 = 0.67$) and the production of nitrate ($r^2 = 0.79$). Those results are consistent with the scavenging effect of propofol on peroxynitrite and could confer a protective property to propofol in pathological situations involving polymorphonuclear neutrophils activation.

Keywords: Propofol; Reactive oxygen species; Activated polymorphonuclear neutrophils

1. Introduction

There is a growing body of evidence that uncontrolled activation of polymorphonuclear neutrophils substantially contributes to tissue damage associated with ischaemia and reperfusion, severe trauma and other pathological states including inflammatory processes, sepsis, acute pulmonary failure and degenerative disorders (Chang et al., 2002; Fujishima and Aikawa, 1995; Huang et al., 2002; Santos-Silva et al., 1995). Besides polymorphonuclear neutrophils activation, nitric oxide is also produced by neuronal, glial and endothelial cells in traumatic and ischaemic injuries of the central nervous system, and is responsible for secondary insult, partially attributed to the production of peroxynitrite (Bolanos and Almeida, 1999; Liu et al., 2000).

Inhibition of polymorphonuclear neutrophils activation to protect cells against those deleterious effects may appear as a double-edged sword because activated polymorphonuclear neutrophils play also a key role in innate immunity. Hence, a more appropriate strategy to protect tissues against oxidative stress would be to neutralize reactive oxygen species excessively produced in pathological situations. Several compounds, roughly termed antioxidants, are investigated in this field.

The anaesthetic agent propofol has properties related to its phenolic structure. It has been acknowledged to scavenge reactive oxygen species and inhibit lipid peroxidations for several years (Aarts et al., 1995; Eriksson et al., 1992; Green et al., 1994; Hans et al., 1996; Murphy et al., 1993; Musacchio et al., 1991). It is also known to protect macrophages and bronchial epithelial cells against nitric oxide-induced toxicity (Chang et al., 2002; Huang et al., 2002). Its capacity to scavenge peroxynitrite has been reported for the first time in 1997, in an in vitro experiment using the enhanced chemiluminescence technique (Kahraman and Demiryurek, 1997). In 1998, propofol has been demonstrated to react with peroxynitrite, using an electron spin resonance technique that showed the production of a phenoxyl radical partially moving towards a phenolic structure

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doi:10.1016/j.ejphar.2004.05.043

(Mouithys-Mickalad et al., 1998). Propofol has also been shown to protect cultured endothelial cells subjected to a peroxynitrite donor (Mathy-Hartert et al., 2000). In an attempt to complete those investigations, we designed a study to evaluate the effects of pure propofol on the global production of reactive oxygen species, the nitration of protein and the formation of nitrate resulting from peroxynitrite production by activated human polymorphonuclear neutrophils.

2. Materials and methods

2.1. Materials

Analytical grade Na^+, K^+, NH_4^+, Ca^+ and MgCl, glucose and diethyleneglycolmonoethylether (carbitol) were purchased from Merck (Germany). Trypan blue, 3-aminophtalhydrazide (luminal), phorbol-12-myristate-13-acetate, diphenyleneiodonium chloride, nitrate reductase and Tris were from Sigma Aldrich (Belgium). Polymorphprep™ was from PharmaciaBiotech (Belgium). Propofol was a gift from Astra Zeneca (Belgium). Phosphate-buffered saline was composed of Na_2HPO_4 1.15 g/l, KH_2PO_4 0.2 g/l, KCl 0.2 g/l, and NaCl 8 g/l.

2.2. Isolation of polymorphonuclear neutrophils

Human polymorphonuclear neutrophils were isolated from buffy coats of healthy donors (Blood Transfusion Centre, University Hospital, Liege, Belgium) by density gradient centrifugation on Polymorphprep™ (vol/vol) at room temperature ($500 \times g$, 30 min). The supernatant was diluted with 0.5 volume of 0.9% NaCl and centrifuged ($1000 \times g$, 20 min). The collected cells were washed with a hypotonic solution (155 mM NH_4Cl, 170 mM Tris–HCl, pH 7.4) to lyse the remaining erythrocytes, and then centrifuged ($800 \times g$, 15 min) (Borgeat and Samuelsson, 1979). Cells were washed again and adjusted to $5{\cdot}10^5$ cells/ml batches in phosphate-buffered saline containing 20 mM $CaCl_2$ and 20 mM $MgCl_2$ (phosphate-buffered saline CaMg) (stock solution) and used immediately. In those conditions, the percentage of viable cells assessed by the exclusion of trypan blue was higher than 98%.

2.3. Chemiluminescence assays

Luminol-enhanced chemiluminescence was measured on microplates with a multiscan luminometer (Multiscan Ascent, Thermolabsystems, Belgium). Each assay was performed on a $5{\cdot}10^5$ cell population (1 ml of the stock solution), and each group of assays including control and several propofol concentrations was repeated on 13 polymorphonuclear neutrophil batches, each batch pertaining to one single donor. Polymorphonuclear neutrophils were activated by adding 25 μl of $4{\cdot}10^{-5}$ M phorbol-12-myristate-13-acetate in phosphate-buffered saline CaMg, leading to a final phorbol-12-myristate-13-acetate concentration of 10^{-6} M. Propofol was dissolved in carbitol at 10^{-1}, 10^{-2}, 10^{-3}, $5{\cdot}10^{-3}$, and 10^{-4} M. The cell suspensions were then added 2.5 μl of the propofol solutions in carbitol to achieve the final propofol concentrations of 10^{-3}, 10^{-4}, $5{\cdot}10^{-5}$, 10^{-5}, and 10^{-6} mM. Insofar as carbitol has a partial quenching effect on chemiluminescence of activated polymorphonuclear neutrophils, assays were first performed with an equivalent volume of carbitol alone (2.5 μl) on activated polymorphonuclear neutrophils and the resulting chemiluminescence was taken as 100% control value. Chemiluminescence of non-activated polymorphonuclear neutrophils was monitored in each group of assays to rule out any spontaneous polymorphonuclear neutrophil activation during the density gradient preparation. The effect of an inhibitor of nicotinamide adenine dinucleotide phosphate (NADPH) oxydase and nitric oxide synthase (diphenyleneiodonium chloride 10^{-4} M) was evaluated in each group of assays to confirm the essential role of NADPH oxydase in the chemiluminescence response of activated polymorphonuclear neutrophils (Hancock and Jones, 1987; Vasquez-Vivar et al., 1998). All those manipulations led to the following experimental conditions: control (phosphate-buffered saline CaMg-carbitol without propofol or phorbol-12-myristate-13-acetate), phorbol-12-myristate-13-acetate (phosphate-buffered saline CaMg + phorbol-12-myristate-13-acetate, 100% control value), diphenyleneiodonium chloride (phosphate-buffered saline CaMg + phorbol-12-myristate-13-acetate + diphenyleneiodonium chloride), propofol 10^{-6} (phosphate-buffered saline CaMg-carbitol + phorbol-12-myristate-13-acetate + propofol 10^{-6} mM), propofol 10^{-5} (phosphate-buffered saline CaMg-carbitol + phorbol-12-myristate-13-acetate + propofol 10^{-5} mM), propofol $5{\cdot}10^{-5}$ (phosphate-buffered saline CaMg-carbitol + phorbol-12-myristate-13-acetate + propofol $5{\cdot}10^{-5}$ mM), propofol 10^{-4} (phosphate-buffered saline CaMg-carbitol + phorbol-12-myristate-13-acetate + propofol 10^{-4} mM) and propofol 10^{-3} (phosphate-buffered saline CaMg-carbitol + phorbol-12-myristate-13-acetate + propofol 10^{-3} mM). Chemiluminescence values, which reflect the total production of reactive oxygen species, were monitored for 15 min and computerized. The total emitted light was calculated as the area under the curve and the results obtained at various propofol concentrations were expressed as the percentage of the control value. In order to exclude a direct effect of propofol on the chemiluminescence of polymorphonuclear neutrophils, chemiluminescence was measured under the same experimental conditions in the absence of phorbol-12-myristate-13-acetate, except for the propofol 10^{-3} mM concentration (four batches).

2.4. Nitrates and nitrated proteins measurements

Regarding the measurement of nitrates and nitrated proteins, 13 batches of $5{\cdot}10^5$ polymorphonuclear neutrophils

were activated as previously described for the chemiluminescence assays. After the activation procedure, the test tubes were centrifuged (1000 × *g*, 5 min at 20 °C) and the supernatants were collected and stored at −70 °C. Nitrates were measured by the Griess reaction in the presence of nitrate reductase (Green et al., 1982). Nitrated proteins were quantified using a competitive enzyme-linked immunosorbent assay (ELISA) in microplates pre-coated with a fixed amount of nitrated proteins (Protein Nitrotyrosine ELISA Kit, TCS Biologicals, UK; nitrated bovine serum albumin). The reference curve was obtained by serial dilutions (0.4 to 100 μg/ml) of the standard nitrated bovine serum albumin. The lower limit of detection was 1 μg/ml. The ELISA was specific for free nitrotyrosine and nitrotyrosine in proteins in a sequence-independent manner (Khan et al., 1998). Samples of 100 μl were used directly or after dilution in phosphate-buffered saline CaMg and measured in triplicate. Results were calculated from the reference curve in micrograms of nitrated bovine serum albumin equivalents, and expressed as a percentage of control value (activated polymorphonuclear neutrophils in the absence of propofol). The following experimental conditions were achieved for these measurements: phorbol-12-myristate-13-acetate (phosphate-buffered saline CaMg-carbitol + phorbol-12-myristate-13-acetate), propofol 10^{-6} (phosphate-buffered saline CaMg-carbitol + phorbol-12-myristate-13-acetate + propofol 10^{-6} mM), propofol 10^{-5} (phosphate-buffered saline CaMg-carbitol + phorbol-12-myristate-13-acetate + propofol 10^{-5} mM) and propofol 10^{-4} (phosphate-buffered saline CaMg-carbitol + phorbol-12-myristate-13-acetate + propofol 10^{-4} mM).

2.5. Statistical analysis

All results were expressed as a percentage of control and presented as mean values with the standard deviation. Statistical analysis was performed using Kruskal–Wallis tests and Mann–Whitney *U* tests for post hoc comparisons. A $p < 0.05$ was considered statistically significant or otherwise corrected according to Bonferroni in case of multiple comparisons. A linear relationship was sought between the logarithm of propofol concentrations and the inhibition of chemiluminescence, nitration of proteins and production of nitrates, respectively.

3. Results

3.1. Chemiluminescence

Polymorphonuclear neutrophils were activated neither during the isolation procedure nor during exposure to propofol alone as demonstrated by the low chemiluminescence values obtained under those conditions in the absence of phorbol-12-myristate-13-acetate. The effects of propofol at various concentrations and of 10^{-4} M diphenyleneiodonium chloride on the chemiluminescence of activated polymorphonuclear neutrophils are shown in Table 1. Chemiluminescence was completely inhibited by diphenyleneiodonium chloride 10^{-4} M. Propofol inhibited chemiluminescence in a dose-dependent way, ranging from 98% to 12% of control at 10^{-3} and 10^{-6} mM, respectively ($H_{(4)} = 95.9$, $p < 0.001$). A significant inverse correlation was observed between the logarithm of propofol concentrations and the chemiluminescence intensity ($r^2 = 0.9$, $p < 0.001$), yielding the following equation: $Y = -29.4 \log[\text{propofol}] - 83.4$).

3.2. Protein nitration and nitrate production

The effects of propofol at various concentrations on protein nitration and nitrate production by activated polymorphonuclear neutrophils are shown in Table 1. Propofol partially reduced protein nitration and nitrate production in a dose-dependent way [$H_{(2)} = 43.2$, $p < 0.001$; $H_{(2)} = 45.5$, $p < 0.001$, respectively]. Inhibition of protein nitration ranged from 50% to 17% of control at 10^{-4} and 10^{-6} mM propofol, respectively. Inhibition of nitrate production ranged from

Table 1
Chemiluminescence (CL), protein nitration (PN) and nitrates (N) as measured in the experimental conditions described in the core text and expressed in % of the 100% control condition (phorbol-12-myristate-13-acetate) (mean of 13 batches [± SD])

	Control	Phorbol-12-myristate-13-acetate	Diphenyleneiodonium chloride	Propofol 10^{-6}	Propofol 10^{-5}	Propofol $5{\cdot}10^{-5}$	Propofol 10^{-4}	Propofol 10^{-3}
CL	0.7 ± 0.7	100	0.8 ± 0.6	88[a] ± 8.3	66[b] ± 8.9	54.8[c] ± 7.2	28.1[d] ± 8.5	1.1[e] ± 1
PN		100		82.1[f] ± 7.2	68.1[g] ± 12.3		49.6[h] ± 7.9	
N		100		93.5[f] ± 2.9	78[g] ± 9.7		59.5[h] ± 8.2	

Statistical analysis (Kruskal–Wallis and Mann–Whitney *U* test for post hoc comparisons): [a] = significantly higher than control, diphenyleneiodonium chloride , propofol 10^{-5}, propofol $5{\cdot}10^{-5}$, propofol 10^{-4} and propofol 10^{-3}, significantly lower than phorbol-12-myristate-13-acetate; [b] = significantly higher than control, diphenyleneiodonium chloride, propofol $5{\cdot}10^{-5}$, propofol 10^{-4} and propofol 10^{-3}, significantly lower than phorbol-12-myristate-13-acetate; [c] = significantly higher than control, diphenyleneiodonium chloride, propofol 10^{-4} and propofol 10^{-3}, significantly lower than phorbol-12-myristate-13-acetate; [d] = significantly higher than control, diphenyleneiodonium chloride, and propofol 10^{-3}, significantly lower than phorbol-12-myristate-13-acetate; [e] = significantly higher than control, and diphenyleneiodonium chloride, significantly lower than phorbol-12-myristate-13-acetate; [f] = significantly higher than propofol 10^{-5}, and propofol 10^{-4}, significantly lower than phorbol-12-myristate-13-acetate; [g] = significantly higher than propofol 10^{-4}, significantly lower than phorbol-12-myristate-13-acetate; [h] = significantly lower than phorbol-12-myristate-13-acetate.

40% to 6% of control at 10^{-4} and 10^{-6} mM propofol, respectively. A significant inverse correlation was observed between the logarithm of propofol concentration, the production of nitrated proteins and the production of nitrate ($r^2 = 0.7$ and 0.8, respectively; $p < 0.001$), yielding the following equations: $Y = -16.2 \log [\text{propofol}] - 14.2$ and $Y = -17 \log [\text{propofol}] - 8$, respectively.

4. Discussion

This study was designed to investigate the effects of propofol on the production of reactive oxygen species by activated polymorphonuclear neutrophils and the associated nitration of proteins that results from the production of peroxynitrite. It clearly showed that propofol inhibits both of them in a dose-dependent way and similarly reduced the production of nitrate, which is the final product of peroxynitrite transformation.

We used pure propofol rather than the commercial form of the drug (Diprivan®) because Intralipid® has been demonstrated to decrease the chemiluminescence response by reacting either directly with reactive oxygen species or with cell membranes (Mathy-Hartert et al., 1998).

The luminol-enhanced chemiluminescence technique used for measuring reactive oxygen species reflects the production of superoxide anion, hydrogen peroxide, hypochlorous acid, nitric oxide, peroxynitrite and other excited oxygen species like oxygen singlet and hydroxyl radical. Insofar as the production of those species by activated polymorphonuclear neutrophils depends on cell batches, chemiluminescence results were expressed in percentage of the control value obtained in the absence of propofol. The results showed a partial and dose-dependent inhibition of chemiluminescence by propofol, which is moderately active at clinically relevant concentrations (10^{-5} mM). They confirm previous results from our team of an in vitro antilipoperoxidant effect of pure propofol (Hans et al., 1996) and are in agreement with data from the literature demonstrating the in vitro antioxidant and antiradical properties of propofol in cell-free systems of erythrocyte membranes, microsomes and mitochondria membrane lipoperoxidations (Aarts et al., 1995; Eriksson et al., 1992; Green et al., 1994; Murphy et al., 1996; Musacchio et al., 1991; Tsuchiya et al., 2002). In the conditions of the present study, the phorbol-12-myristate-13-acetate activation of polymorphonuclear neutrophils resulted in a degranulation process and the release of reactive oxygen species outside the cells. We have previously shown that propofol does not enter into the polymorphonuclear neutrophils (Mathy-Hartert et al., 1998). In those conditions, it can actually protect polymorphonuclear neutrophils and other cells by scavenging reactive oxygen species in the extracellular medium without impairing the phagocytosis activity of polymorphonuclear neutrophils.

The dose-dependent inhibition of protein nitration and nitrate production by propofol shown in the second part of this study likely results from its scavenging effect on peroxynitrite produced by activated polymorphonuclear neutrophils. Propofol has been shown to scavenge peroxynitrite in vitro and the underlying mechanism of this effect involves the formation of a phenoxyl radical (Kahraman and Demiryurek, 1997; Mouithys-Mickalad et al., 1998). The more potent the scavenging of peroxynitrite, the less the formation of nitrotyrosine and the production of nitrate which is the metabolite of peroxynitrite. When considering the cellular level, our findings confirm previous results observed with endothelial cells subjected to a peroxynitrite donor (Mathy-Hartert et al., 2000), and with macrophages and bronchial epithelial cells directly submitted to nitric oxide (Chang et al., 2002; Huang et al., 2002). They could have a seminal importance as the inflammatory reaction appears as a key response to various forms of tissue insults including trauma, hypoxia, ischaemia, and haemorrhage, in addition to infectious processes. Although this inflammatory reaction is necessary for the resolution of the primary injury, it also plays a substantial role in the development of secondary injuries through the release of several mediators. There is a growing evidence that oxygen and nitrogen reactive species are involved in mediating secondary damage, particularly in the central nervous system (Bolanos and Almeida, 1999; Chang et al., 2002; Huang et al., 2002; Liu et al., 2000; Szabo, 1996; Wilson and Gelb, 2002).

Besides the degree of toxicity of the inflammatory reaction and the involvement of reactive oxygen and nitrogen species in pathophysiological situations, the clinical relevance of these experimental results depends on propofol concentrations that can be safely achieved in patients. We have already shown an increase in the anti-oxidant capacity of plasma in patients under propofol anaesthesia (Hans et al., 1997). In the present study, propofol respectively provoked a 32% reduction of chemiluminescence and a 31% inhibition of protein nitration at $5 \cdot 10^{-5}$ and 10^{-5} mM, respectively, which are clinically relevant concentrations. On the other hand, the degree of reactive oxygen and nitrogen species toxicity varies according to the pathophysiological situations and the endogenous anti-oxidant status. It can reasonably be hypothesized that propofol, alone or associated with other drugs, could be helpful in situations characterized by a major oxidative stress when the endogenous anti-oxidative capacity is overwhelmed.

In conclusion, we demonstrated that pure propofol inhibits partially and dose-dependently the production of active oxygen species and the nitration of proteins associated with polymorphonuclear neutrophils activation. It also significantly reduces the production of nitrate. Those effects are observed at clinically relevant propofol concentrations. They are consistent with the anti-oxidative property of propofol and its capacity to scavenge peroxynitrite. The findings of this study could confer a potential benefit to propofol in clinical situations characterized by an excessive polymorphonuclear neutrophils activation.

References

Aarts, L., van der, H.R., Dekker, I., de Jong, J., Langemeijer, H., Bast, A., 1995. The widely used anesthetic agent propofol can replace alpha-tocopherol as an antioxidant. FEBS Lett. 357, 83–85.

Bolanos, J.P., Almeida, A., 1999. Roles of nitric oxide in brain hypoxia-ischemia. Biochim. Biophys. Acta 1411, 415–436.

Borgeat, P., Samuelsson, B., 1979. Metabolism of arachidonic acid in polymorphonuclear leukocytes. Structural analysis of novel hydroxylated compounds. J. Biol. Chem. 254, 7865–7869.

Chang, H., Tsai, S.Y., Chang, Y., Chen, T.L., Chen, R.M., 2002. Therapeutic concentrations of propofol protects mouse macrophages from nitric oxide-induced cell death and apoptosis. Can. J. Anaesth. 49, 477–480.

Eriksson, O., Pollesello, P., Saris, N.E., 1992. Inhibition of lipid peroxidation in isolated rat liver mitochondria by the general anaesthetic propofol. Biochem. Pharmacol. 44, 391–393.

Fujishima, S., Aikawa, N., 1995. Neutrophil-mediated tissue injury and its modulation. Intensive Care Med. 21, 277–285.

Green, L.C., Wagner, D.A., Glogowski, J., Skipper, P.L., Wishnok, J.S., Tannenbaum, S.R., 1982. Analysis of nitrate, nitrite, and [15N]nitrate in biological fluids. Anal. Biochem. 126, 131–138.

Green, T.R., Bennett, S.R., Nelson, V.M., 1994. Specificity and properties of propofol as an antioxidant free radical scavenger. Toxicol. Appl. Pharmacol. 129, 163–169.

Hancock, J.T., Jones, O.T., 1987. The inhibition by diphenyleneiodonium and its analogues of superoxide generation by macrophages. Biochem. J. 242, 103–107.

Hans, P., Deby, C., Deby-Dupont, G., Vrijens, B., Albert, A., Lamy, M., 1996. Effect of propofol on in vitro lipid peroxidation induced by different free radical generating systems: a comparison with vitamin E. J. Neurosurg. Anesthesiol. 8, 154–158.

Hans, P., Deby-Dupont, G., Deby, C., Pieron, F., Verbesselt, R., Franssen, C., Lamy, M., 1997. Increase in antioxidant capacity of plasma during propofol anesthesia. J. Neurosurg. Anesthesiol. 9, 234–236.

Huang, C.J., Slovin, P.N., Nielsen, R.B., Skimming, J.W., 2002. Diprivan attenuates the cytotoxicity of nitric oxide in cultured human bronchial epithelial cells. Intensive Care Med. 28, 1145–1150.

Kahraman, S., Demiryurek, A.T., 1997. Propofol is a peroxynitrite scavenger. Anesth. Analg. 84, 1127–1129.

Khan, J., Brennand, D.M., Bradley, N., Gao, B., Bruckdorfer, R., Jacobs, M., 1998. 3-Nitrotyrosine in the proteins of human plasma determined by an ELISA method. Biochem. J. 332 (Pt. 3), 807–808.

Liu, D., Ling, X., Wen, J., Liu, J., 2000. The role of reactive nitrogen species in secondary spinal cord injury: formation of nitric oxide, peroxynitrite, and nitrated protein. J. Neurochem. 75, 2144–2154.

Mathy-Hartert, M., Deby-Dupont, G., Hans, P., Deby, C., Lamy, M., 1998. Protective activity of propofol, Diprivan and intralipid against active oxygen species. Mediat. Inflamm. 7, 327–333.

Mathy-Hartert, M., Mouithys-Mickalad, A., Kohnen, S., Deby-Dupont, G., Lamy, M., Hans, P., 2000. Effects of propofol on endothelial cells subjected to a peroxynitrite donor (SIN-1). Anaesthesia 55, 1066–1071.

Mouithys-Mickalad, A., Hans, P., Deby-Dupont, G., Hoebeke, M., Deby, C., Lamy, M., 1998. Propofol reacts with peroxynitrite to form a phenoxyl radical: demonstration by electron spin resonance. Biochem. Biophys. Res. Commun. 249, 833–837.

Murphy, P.G., Bennett, J.R., Myers, D.S., Davies, M.J., Jones, J.G., 1993. The effect of propofol anaesthesia on free radical-induced lipid peroxidation in rat liver microsomes. Eur. J. Anaesthesiol. 10, 261–266.

Murphy, P.G., Davies, M.J., Columb, M.O., Stratford, N., 1996. Effect of propofol and thiopentone on free radical mediated oxidative stress of the erythrocyte. Br. J. Anaesth. 76, 536–543.

Musacchio, E., Rizzoli, V., Bianchi, M., Bindoli, A., Galzigna, L., 1991. Antioxidant action of propofol on liver microsomes, mitochondria and brain synaptosomes in the rat. Pharmacol. Toxicol. 69, 75–77.

Santos-Silva, A., Castro, E.M., Teixeira, N.A., Guerra, F.C., Quintanilha, A., 1995. Altered erythrocyte membrane band 3 profile as a marker in patients at risk for cardiovascular disease. Atherosclerosis 116, 199–209.

Szabo, C., 1996. The pathophysiological role of peroxynitrite in shock, inflammation, and ischemia-reperfusion injury. Shock 6, 79–88.

Tsuchiya, M., Asada, A., Kasahara, E., Sato, E.F., Shindo, M., Inoue, M., 2002. Antioxidant protection of propofol and its recycling in erythrocyte membranes. Am. J. Respir. Crit Care Med. 165, 54–60.

Vasquez-Vivar, J., Kalyanaraman, B., Martasek, P., Hogg, N., Masters, B.S., Karoui, H., Tordo, P., Pritchard Jr., K.A., 1998. Superoxide generation by endothelial nitric oxide synthase: the influence of cofactors. Proc. Natl. Acad. Sci. U. S. A. 95, 9220–9225.

Wilson, J.X., Gelb, A.W., 2002. Free radicals, antioxidants, and neurologic injury: possible relationship to cerebral protection by anesthetics. J. Neurosurg. Anesthesiol. 14, 66–79.