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Propofol, an anesthetic possessing neuroprotective action against oxidative stress, promotes the process of cell death induced by H_2O_2 in rat thymocytes

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Abstract

Propofol (2,6-diisopropylphenol) is a general anesthetic possessing a neuroprotective action against oxidative stress produced by H_2O_2. H_2O_2 induces an exposure of phosphatidylserine on outer surface of cell membranes, resulting in change in membrane phospholipid arrangement, in rat thymocytes. Since propofol is highly lipophilic, the agent is presumed to interact with membrane lipids and hence to modify the cell vulnerability to H_2O_2. Therefore, to test the possibility, we have examined the effect of propofol on rat thymocytes simultaneously incubated with H_2O_2. Although propofol (up to 30 μM) alone did not significantly affect the cell viability, the agent at 10 μM started to increase the population of dead cells in the presence of 3 mM H_2O_2 and the significant increase was observed at 30 μM. Propofol at clinically relevant concentrations (10–30 μM) facilitated the process of cell death induced by H_2O_2 in rat thymocytes. However, propofol protected rat brain neurons against the oxidative stress induced by H_2O_2 under same experimental condition. Therefore, the action of propofol may be dependent on the type of cells.

Keywords: Anesthetics; Propofol; Oxidative stress; Lymphocyte; Neuron

1. Introduction

Propofol (2,6-diisopropylphenol) is a general anesthetic possessing antioxidant action (De La Cruz et al., 1998; Bao et al., 1998; Tsuchiya et al., 2001). This anesthetic, chemically similar to ∂-tocopherol (Murphy et al., 1992; Aarts et al., 1995), exhibits potent in vitro scavenging action against superoxide anion and H_2O_2 (Gülçin et al., 2005). As to the effect against the toxicity induced by H_2O_2, the attenuation of the toxicity by propofol is observed in several types of preparations (Eriksson et al., 1992; Koike and Hara, 1996; Koike et al., 1998; Peng et al., 2004; Balyansnikova et al., 2005; Lee et al., 2005).

The incubation of rat thymocytes with H_2O_2 greatly increases the population of cells with phosphatidylserine-exposed membranes and this phenomenon is attenuated by quercetin or deferoxamine (Oyama et al., 1999). Since an intact lipid bilayer of membranes is required to maintain membrane integrity (Schreier et al., 2000), the change in arrangement of membrane phospholipids may affect the cell susceptibility to lipophilic compounds. Since propofol is highly lipophilic, the agent is presumed to interact with membrane lipids and hence to modify the cell vulnerability to H_2O_2, regardless of its antioxidant action. Therefore, to test the possibility, we have examined the effect of propofol on rat thymocytes simultaneously incubated with H_2O_2.

2. Materials and methods

2.1. Reagents

Propofol injection "Maruishi" containing 1% propofol was obtained from Maruishi Pharmaceutical. Co., Ltd. (Osaka, Japan). The vehicle for propofol contained 0.3 mg sodium oleate, 12 mg L-α-phosphatidylcholine, 25 mg glycerol, 50 mg soybean oil, and

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50 mg coconut oil in 1 ml distilled water. These agents were purchased from Sigma Chemical Co. (St. Louis, MO, USA) to prepare the vehicle.

2.2. Cell preparation

The procedures to prepare the cell suspensions of rat thymocytes and cerebellar granule cells were respectively similar to those previously reported (Chikahisa and Oyama, 1992; Oyama et al., 1992, 1995; Chikahisa et al., 1996).

Thymus glands dissected from 3- to 4-week-old Wistar rats were sliced at a thickness of 400–500 μm. The slices were triturated in chilled Tyrode's solution (in mM: NaCl 150, KCl 5, $CaCl_2$ 2, $MgCl_2$ 1, glucose 5, HEPES 5, with an appropriate amount of NaOH to adjust pH to 7.3–7.4) to dissociate thymocytes. Thereafter, the Tyrode's solution was passed through a mesh (a diameter of 10 μm) to prepare the cell suspension (about 5×10^5 cells/ml). The cells were incubated at 35–36 °C for 1 h before use.

As to cerebellar granule neurons, cerebella were dissected from brains of 2-week-old Wistar rats. The slices of cerebella were incubated with Tyrode's solution containing 1 mg/ml dispase (Godo Shusei, Tokyo, Japan) for 1 h at 35–36 °C, and then the slices were triturated in chilled Tyrode's solution to dissociate granule cells. The Tyrode's solution was passed through the mesh to prepare the cell suspension (about 5×10^5 cells/ml). The cells were also incubated at 35–36 °C for 1 h before use.

2.3. Fluorescence measurements of cellular parameters

The methods for measurements of cellular parameters using a flow cytometer equipped with an argon laser (CytoACE-150, JASCO, Tokyo, Japan) and fluorescent probes to monitor cellular parameters were similar to those described previously (Chikahisa and Oyama, 1992; Oyama et al., 1992; Chikahisa et al., 1996). Fluorescence was analyzed by JASCO Ver.3XX software (JASCO, Tokyo, Japan).

To assess the cell viability, propidium iodide (Molecular Probe Inc., Eugene, OR, USA) was added to the cell suspension to achieve a final concentration of 10 μM. Since propidium stains dead cells, the measurement of propidium fluorescence from the cells provides a clue to estimate the viability. The fluorescence was measured at 1–2 min after the start of application by a flow cytometer. The excitation wavelength for propidium was 488 nm and the emission was detected at 600±20 nm.

The exposure of phosphatidylserine on outer membranes or rat thymocytes was detected by annexin V-FITC (1 mg/100 μl, Sigma Chemical Co., St. Louis, MO, USA) (Nakata et al., 1999; Oyama et al., 1999). The fluorescence of FITC was monitored from the cells at 20–30 min after the start of application of annexin V-FITC. The excitation wavelength was also 488 nm. The emission was detected at 530±20 nm for FITC fluorescence to estimate annexin V binding to membranes.

2.4. Statistics

Statistical analysis was performed by a paired Student's *t* test and/or an overall test of significance using an *F*-ratio derived from analysis of variance (ANOVA). Significance between test groups was also confirmed by Dunnett's test. *A* P value of <0.05 was considered significant.

3. Results

3.1. Effect of propofol on the viability of rat thymocytes simultaneously incubated with or without hydrogen peroxide

Propofol alone at clinically relevant concentrations (up to 30 μM) did not increase the population of rat thymocytes stained with propidium, presumably dead cells, indicating no change in cell viability by propofol when the cells were incubated with the agent for 3 h (Fig. 1). Incubation with 3 mM H_2O_2 for 3 h significantly increased the population of dead cells. The simultaneous incubation with H_2O_2 and propofol (3 μM or more) for 3 h further increased the population of dead cells. The increase in dead cell population by 30 μM propofol in the presence of 3 mM H_2O_2 was

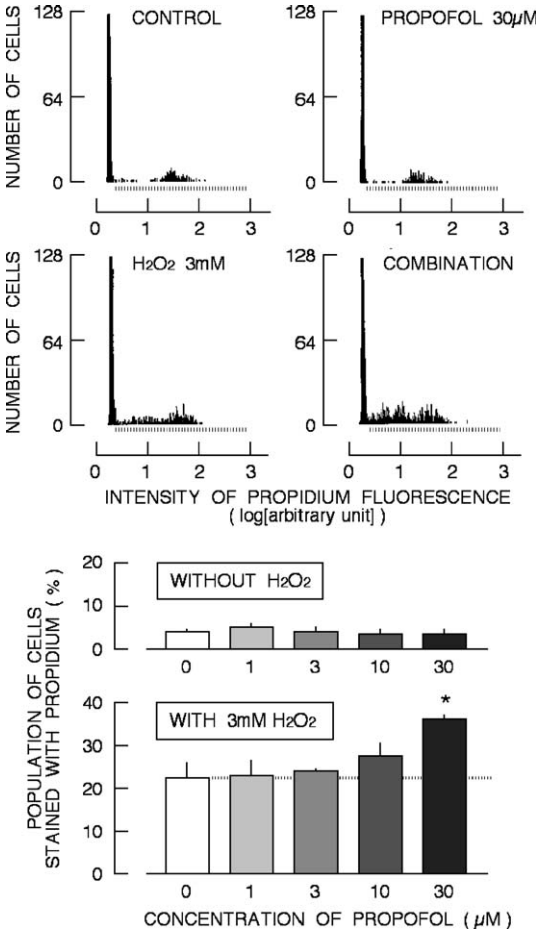

Fig. 1. Effect of propofol on rat thymocytes in absence or presence of hydrogen peroxide. Upper panels: the effect on the histogram of propidium fluorescence monitored from 2000 cells. The dotted line indicates the cells exhibiting propidium fluorescence, indicating the dead cell population. Effects were examined at 3 h after the start of respective applications. Lower panels: the concentration-dependent change by propofol in percentage population of cells stained with propidium, presumably dead cells. The column and bar indicate mean and standard deviation of 4 experiments.

statistically significant (Fig. 1). Therefore, it is likely that propofol augments the cytotoxicity of H_2O_2 in rat thymocytes.

3.2. Time course of propofol-induced change in the viability of rat thymocytes in absence or presence of hydrogen peroxide

The population of dead cells increased as the time for incubation with 3 mM or 10 mM H_2O_2 prolonged. Therefore, the effect of propofol on the time course of H_2O_2-induced increase in the dead cell population was examined. As shown in Fig. 2, the incubation with 3 mM H_2O_2 alone time-dependently increased the dead cell population. The population of dead cells during 2–3 h after the start of simultaneous incubation with propofol (30 μM and 100 μM) and H_2O_2 was significantly higher than that with H_2O_2 alone (Fig. 2). Thus, it is likely that the time course for H_2O_2-induced increase in dead cell population is hastened by propofol. Similar result was also obtained when 10 mM H_2O_2 was used. The time course of increase in dead cell population by 10 mM H_2O_2 was more rapid than that by 3 mM H_2O_2 (Fig. 3). The significant augmentation of H_2O_2-induced cytotoxicity by propofol (30 μM and 100 μM) was observed at 1 h after the start of simultaneous incubation (Fig. 3).

3.3. Effects of propofol, hydrogen peroxide, and their combination on cell populations classified by annexin V-FITC and propidium iodide

The incubation of thymocytes with H_2O_2 induces the exposure of phosphatidylserine on cell membrane surface (Oyama et al., 1999), one of events during early stage of apoptosis (Vermes et al., 1995). It is a possibility that propofol facilitates the process of cell death induced by H_2O_2. To test the possibility, the effects of propofol, H_2O_2, and their combination on the classification of intact living cells (area N of Fig. 4), annexin V-positive living cells (area A, living cells with phosphatidylserine-exposed membranes), and dead cells (areas P and AP) were examined at 2 h after the start of respective application. As shown in Figs. 4 and 5, propofol at 30 μM alone did not significantly affect the population. However, the incubation with 3 mM H_2O_2 alone greatly decreased the population of intact living cells (area N), significantly increased that of annexin V-positive living cells (area A), and slightly increased that of dead cells (areas P and AP). In the presence of H_2O_2, propofol further decreased the population of intact living cells and increased that of dead cells without apparently affecting that of annexin V-positive living cells. Thus, propofol accelerates the process from intact living cells to annexin V-positive living cells and that from annexin V-positive cells to dead cells.

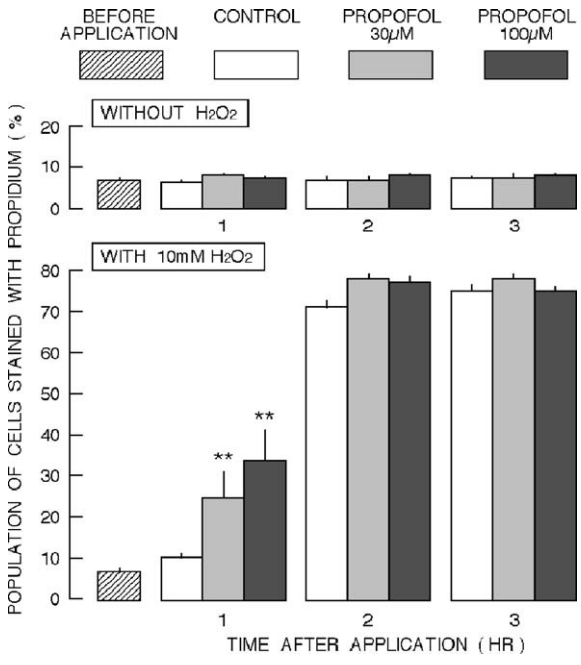

Fig. 2. Time course for propofol-induced change in the percentage population of dead cells. Effect of propofol on rat thymocytes was examined in absence or presence of 3 mM H_2O_2. The column and bar indicate mean and standard deviation of 4 experiments. Asterisks (* and **) indicate significant difference ($P<0.05$ and $P<0.01$) between the cells incubated with H_2O_2 (filled column) and those without H_2O_2 (open column), respectively.

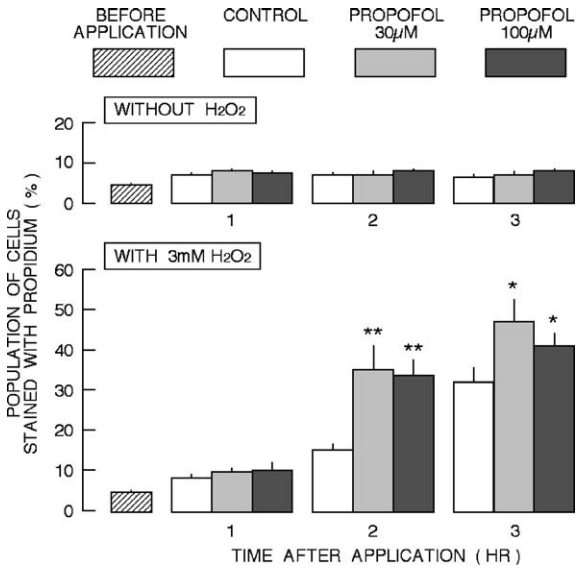

Fig. 3. Time course for propofol-induced change in the percentage population of dead cells. Effect of propofol on rat thymocytes was examined in absence or presence of 10 mM H_2O_2. The column and bar indicate mean and standard deviation of 4 experiments. Asterisks (* and **) indicate significant difference ($P<0.05$ and $P<0.01$, respectively) between the cells incubated with H_2O_2 (filled column) and those without H_2O_2 (open column), respectively.

3.4. Population of hypodiploidal cells in rat thymocytes incubated with propofol, hydrogen peroxide, and their combination

The exposure of phosphatidylserine on outer surface of cell membranes is one of events during early stage of apoptosis (Vermes et al., 1995). H_2O_2 greatly increased the population of annexin V-positive living cells (Figs. 4 and 5), indicating the increase in population of cells with phosphatidylserine-exposed membranes. Therefore, to see whether the increase in dead cell population is due to necrosis or apoptosis, the distribution of cellular DNA content was examined after respective incubation with propofol, H_2O_2, and their combination for 3 h. As shown in Fig. 6, the population of

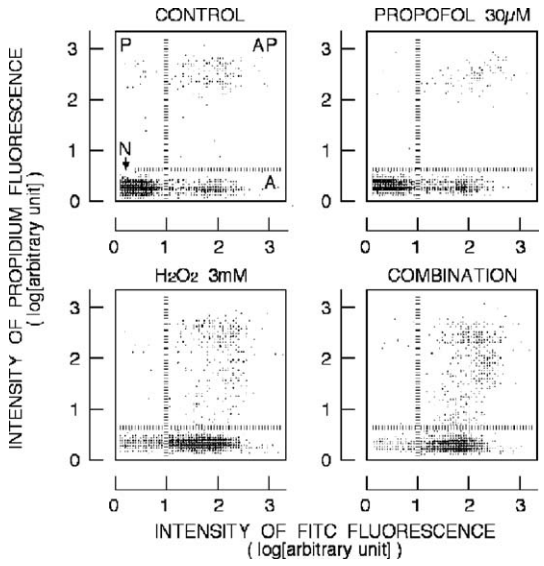

Fig. 4. Cell population classified by propidium iodide and annexin V-FITC in presence of propofol, H_2O_2, or both (COMBINATION). Fluorescence cytogram constructed from 2500 cells. Area N: intact living cells. Area A: annexin V-positive living cells (living cells with phosphatidylserine exposed on membrane surface). Area AP and Area P: dead cells with and without phosphatidylserine exposed on membrane surface, respectively. Effects of propofol, H_2O_2, or their combination were examined at 2 h after the start of application.

cells with hypodiploidal DNA was not affected by propofol, H_2O_2, and their combination when the cells were incubated for 3 h. Therefore, it is likely that the increase in dead cell population by propofol in the presence of H_2O_2 is due to necrosis.

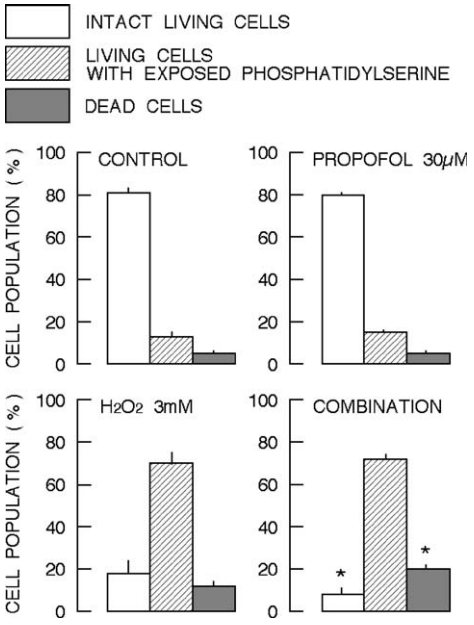

Fig. 5. Change in percentage population of cells by propofol, H_2O_2, or both (COMBINATION). Effects of propofol, H_2O_2, or their combination were examined at 2 h after the start of application. Column and bar respectively indicate mean and standard deviation of four experiments. Asterisk (*) shows significant difference ($P<0.05$) between cells incubated with H_2O_2 alone and those with propofol and H_2O_2.

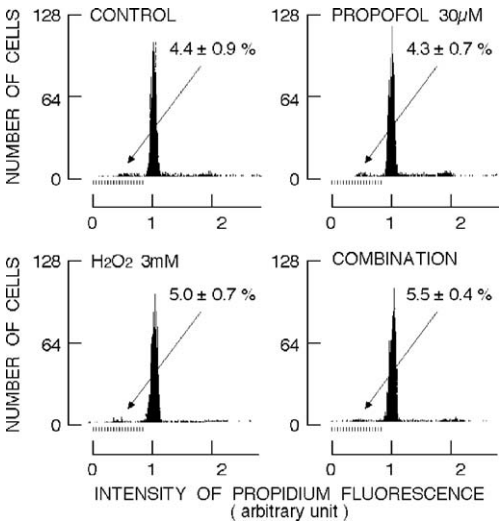

Fig. 6. Effects of propofol, H_2O_2, or both (COMBINATION) on distribution of relative DNA content (the histogram of propidium fluorescence intensity) of rat thymocytes. The histogram was constructed with 2500 cells. Effect was examined at 3 h after start of respective applications. The numbers near the histogram indicate the percentage population (mean ± S.D. of four experiments) of cells with hypodiploidal DNA.

3.5. Effect of vehicle for propofol on rat thymocytes

When the cells were incubated with 30–100 μM propofol, the cell suspension contained 0.053–0.178% vehicle. Therefore, the cytotoxicity of vehicle was examined in the absence or presence of H_2O_2. The incubation of cells with 0.053–0.178% vehicle did not affect the viability under control condition. Furthermore, the vehicle did not affect the increase in dead cell population by 3 mM H_2O_2. Therefore, it is unlikely that the vehicle contributes to the propofol-induced action on the cells.

3.6. Effect of propofol on neurons suffering from oxidative stress induced by H_2O_2

The attenuation of the H_2O_2 toxicity by propofol has been reported in several types of preparations (Eriksson et al., 1992; Koike and Hara, 1996; Koike et al., 1998; Peng et al., 2004; Balyansnikova et al., 2005; Lee et al., 2005). Furthermore, the

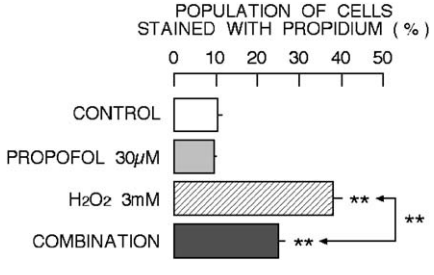

Fig. 7. Change in percentage population of dead neurons by propofol, H_2O_2, or both (COMBINATION). Column and bar respectively indicate mean and standard deviation of four experiments. Asterisks (**) indicate significant difference ($P<0.01$) between control cells (CONTROL) and the cells incubated with H_2O_2, or propofol and H_2O_2 (COMBINATION). Asterisk with arrows shows significant difference ($P<0.01$) between cells incubated with H_2O_2 alone and those with propofol and H_2O_2.

agent is used as a neuroprotective agent against brain ischemia (Arcadi et al., 1996; Ito et al., 1999; Yamaguchi et al., 1999; Shibuta et al., 2001). Therefore, it was very important to see if propofol can protect neurons against oxidative stress induced by H_2O_2 under the present experimental condition. As shown in Fig. 7, the incubation with 3 mM H_2O_2 for 3 h increased the population of dead cells. In the presence of 30 μM propofol, the increase in dead cell population by H_2O_2 was significantly attenuated. Therefore, propofol seems to protect brain neurons against oxidative stress as reported elsewhere.

4. Discussion

Propofol is known as an antioxidant in many chemical and biological studies as described in the Introduction. However, the protective action of propofol is dependent on the type of cells and/or the experimental condition because the agent augmented the cytotoxicity of H_2O_2 in rat thymocytes (Figs. 1–3). Thus, propofol at 10 μM started to increase the population of dead cells in the presence of 3 mM H_2O_2 (Fig. 1) and the significant increases were observed at 30 μM and 100 μM (Figs. 1–3). Furthermore, propofol facilitated the process of cell death induced by H_2O_2 (Figs. 4 and 5). Propofol at concentrations of 10–30 μM is clinically relevant for general anesthesia (Andrew et al., 1997; Doi et al., 1997; Kazama et al., 2000; Adachi et al., 2005). H_2O_2 induced the exposure of phosphatidylserine on outer surface of cell membranes (Fig. 4), one of the markers for early stage of apoptosis (Vermes et al., 1995). However, the agent did not promote the process of apoptosis since there was no increase in the population of cells with hypodiploidal DNA. Thus, propofol may affect the cells with phosphatidylserine-exposed membranes, resulting in necrotic cell death.

One may wonder if propofol, known as an antioxidant, augments the cytotoxicity of H_2O_2 in rat thymocytes. H_2O_2 increases the $[Ca^{2+}]_i$ (Okazaki et al., 1996) and induces the exposure of phosphatidylserine on membrane surface (Oyama et al., 1999). The increase in population of cells with phosphatidylserine-exposed membranes by H_2O_2 occurs within 15 min after the start of H_2O_2 application in a Ca^{2+}-dependent manner (Oyama et al., 1999). H_2O_2 may increase the $[Ca^{2+}]_i$ of rat thymocytes before propofol sufficiently scavenges H_2O_2 under present experimental condition. This explanation is plausible because of following observations. The increase in $[Ca^{2+}]_i$ by A23187, a calcium ionophore, also increases the population of cells with phosphatidylserine-exposed membranes (Nakata et al., 1999; Nishizaki et al., 2003). The cytotoxic action of A23187 was slightly augmented by propofol (our unpublished observation). Furthermore, it may be a common feature for compounds possessing a lipophilic property to promote the process of cell death in the cells with phosphatidylserine-exposed membranes. We have reported that polysorbate 80 and cremophor EL, non-ionic surfactants, hasten the process of cell death respectively induced by A23187 and H_2O_2 in rat thymocytes (Iwase et al., 2004; Tatsuishi et al., 2005; Yamaguchi et al., 2005). It is also the case for other compounds such as dodecylbenzenesulfonate, an anionic surfactant (Yamaguchi et al., in press), and lidocaine, a local anesthetic (Nishimura et al., 2006). Therefore, it can be suggested that propofol, acting as a surface-active drug (Schreier et al., 2000), promotes the process of cell death induced by H_2O_2.

It is likely that propofol affects rat thymocytes incubated with H_2O_2, resulting in necrotic cell death (Figs. 4–6), although the cells exhibit shrinkage and exposure of phosphatidylserine that are the events during early stage of apoptosis (Vermes et al., 1995; Maeno et al., 2000). However, propofol is reported to exert antioxidant action on several types of preparations, especially on brain neurons suffering from oxidative stress (Arcadi et al., 1996; Ito et al., 1999; Yamaguchi et al., 1999; Shibuta et al., 2001). In fact, propofol also protected the neurons suffering from oxidative stress induced by H_2O_2 under present experimental condition (Fig. 7). Therefore, the action of propofol on the cells suffering from oxidative stress may be dependent on the type of cells.

References

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

Adachi, Y.U., Satomoto, M., Higuchi, H., Watanabe, K., 2005. Rapid fluid infusion therapy decreases the plasma concentration of continuously infused propofol. Acta Anaesthesiol. Scand. 49, 331–336.

Andrew, D.T., Leslie, K., Sessler, D.I., Bjorksten, A.R., 1997. The arterial blood propofol concentration preventing movement in 50% of healthy women after skin incision. Anesth. Analg. 85, 414–419.

Arcadi, E.A., Rapisarda, A., De Luca, R., Trimarchi, G.R., Costa, G., 1996. Effect of 2,6-diisopropylphenol on the delayed hippocampal cell loss following transient forebrain ischemia in the gerbil. Life Sci. 58, 961–970.

Balyansnikova, I.V., Vistintine, D.J., Gunnerson, H.B., Paisansatan, C., Baughman, V.L., Minshall, R.D., Danilov, S.M., 2005. Propofol attenuates lung endothelial injury induced by ischemia–reperfusion and oxidative stress. Anesth. Analg. 100, 929–936.

Bao, Y.P., Williamson, G., Plumb, G.W., Lambert, N., Tew, D., Jones, J.G., Menon, D.K., 1998. Antioxidant effects of propofol in human hepatic microsomes: concentration effects and clinical relevance. Br. J. Anaesth. 81, 584–589.

Chikahisa, L., Oyama, Y., 1992. Tri-*n*-butyltin increases intracellular Ca^{2+} in mouse thymocytes: a flow-cytometric study using fluorescent dyes for membrane potential and intracellular Ca^{2+}. Pharmacol. Toxicol. 71, 190–195.

Chikahisa, L., Oyama, Y., Okazaki, E., Noda, K., 1996. Fluorescent estimation of H_2O_2-induced changes in cell viability and cellular nonprotein thiol level of dissociated rat thymocytes. Jpn. J. Pharmacol. 71, 299–305.

De La Cruz, J.P., Sedeno, G., Carmona, J.A., DeLa Sanchez Cuesta, F., 1998. The in vitro effects of propofol on tissular oxidative stress in the rat. Anesth. Analg. 87, 1141–1146.

Doi, M., Gajraj, R.J., Mantzaridis, H., Kenny, G.N., 1997. Relationship between calculated blood concentration of propofol and electrophysiological variables during emergence from anaesthesia: comparison of bispectral index, spectral edge frequency, median frequency and auditory evoked potential index. Br. J. Anaesth. 78, 180–184.

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.

Gülçin, I., Alici, H.A., Cesur, M., 2005. Determination of in vitro antioxidant and radical scavenging activities of propofol. Chem. Pharm. Bull. 53, 281–285.

Ito, H., Watanabe, Y., Isshiki, A., Uchino, H., 1999. Neuroprotective properties of propofol and midazolam, but not pentobarbital, on neuronal damage induced by forebrain ischemia, based on the GABAA receptors. Acta Anaesthesiol. Scand. 43, 153–162.

Iwase, K., Oyama, Y., Tatsuishi, T., Yamaguchi, J.Y., Nishimura, Y., Kanada, Y., Kobayashi, M., Maemura, Y., Ishida, S., Okano, Y., 2004. Cremophor EL

augments the cytotoxicity of hydrogen peroxide in lymphocytes dissociated from rat thymus glands. Toxicol. Lett. 154, 143–148.

Kazama, T., Takeucji, K., Ikeda, K., Ikeda, T., Kikura, M., Iida, T., Suzuki, S., Hanai, H., Sato, S., 2000. Optimal propofol plasma concentration during upper gastrointestinal endoscopy in young, middle-aged, and elderly patients. Anesthesiology 93, 662–669.

Koike, N., Hara, A., 1996. Propofol attenuates hydrogen peroxide-induced mechanical and metabolic derangements in the isolated rat heart. Anesthesiology 84, 117–127.

Koike, N., Nara, A., Abiko, Y., Arakawa, J., Hashizume, H., Namiki, A., 1998. Propofol improves functional and metabolic recovery in ischemic reperfused isolated rat hearts. Anesth. Analg. 86, 252–258.

Lee, H., Jang, Y.H., Lee, S.R., 2005. Protective effect of propofol against kainic acid-induced lipid peroxidation in mouse brain homogenates: comparison with trolox and melatonin. J. Neurosurg. Anesthesiol. 17, 144–148.

Maeno, E., Ishizaki, Y., Kanaseki, T., Hazama, A., Okada, Y., 2000. Normotonic cell shrinkage because of disordered volume regulation is an early prerequisite to apoptosis. Proc. Natl. Acad. Sci. U. S. A. 97, 9487–9492.

Murphy, P.G., Davies, M.J., Columb, M.O., Stratford, N., 1992. Effect of propofol and thiopentone on free radical mediated oxidative stress of the erythrocyte. Br. J. Anaesth. 76, 536–543.

Nakata, M., Oyama, Y., Chikahisa, L., Yamazaki, Y., Satoh, M., 1999. Flow cytometric analysis on tri-*n*-butyltin-induced increase in annexin V binding to membranes of rat thymocytes. Environ. Toxicol. Pharmacol. 7, 267–273.

Nishimura, Y., Kanada, A., Yamaguchi, J.Y., Horimoto, K., Kobayashi, M., Tatsuishi, T., Kanemaru, K., Ueno, S., Oyama, Y., 2006. Cytometric analysis of lidocaine-induced cytotoxicity: a model experiment using rat thymocytes. Toxicology 218, 48–57.

Nishizaki, Y., Nakao, H., Umebayashi, C., Iwase, K., Tatsuishi, T., Satoh, M., Oyama, Y., 2003. Increase in number of annexin V-positive living cells of rat thymocytes by intracellular Pb^{2+}. Environ. Toxicol. Pharmacol. 45, 45–51.

Okazaki, E., Chikahisa, L., Kanemaru, K., Oyama, Y., 1996. Flow cytometric analysis on the H_2O_2-induced increase in intracellular Ca^{2+} concentration of rat thymocytes. Jpn. J. Pharmacol. 71, 273–280.

Oyama, Y., Chikahisa, L., Noda, K., Hayashi, H., Tomiyoshi, F., 1992. Characterization of the triphenyltin-induced increase in intracellular Ca^{2+} of mouse thymocytes: comparison with the action of A23187. Jpn. J. Pharmacol. 60, 159–167.

Oyama, Y., Chikahisa, L., Ueha, T., Hatakeyama, Y., Kokubun, T., 1995. Change in membrane permeability induced by amyloid beta-protein fragment 25–35 in brain neurons dissociated from the rats. Jpn. J. Pharmacol. 68, 77–83.

Oyama, Y., Noguchi, S., Nakata, M., Okada, Y., Yamazaki, Y., Funai, M., Chikahisa, L., Kanemaru, K., 1999. Exposure of rat thymocytes to hydrogen peroxide increases annexin V binding to membranes: inhibitory action of deferoxamine and quercetin. Eur. J. Pharmacol. 384, 47–52.

Peng, Z., Luo, M., Ye, S., Critchley, L.A., Joynt, G.M., Ho, A.M., Yao, S., 2004. Antioxidative and anti-endotoxin effects of propofol on endothelial cells. Chin. Med. J. 116, 731–735.

Schreier, S., Malheiros, S.V.P., de Paula, E., 2000. Surface active drugs: self-association and interaction with membranes and surfactants. Physicochemical and biological aspects. Biochim. Biophys. Acta 1508, 210–234.

Shibuta, S., Sriranganathan, V., Inoue, T., Tomi, K., Mashimo, T., 2001. The effects of propofol on MNDA- or nitric oxide-mediated neurotoxicity in vitro. NeuroReport 12, 295–298.

Tatsuishi, T., Oyama, Y., Iwase, K., Yamaguchi, J.Y., Kobayashi, M., Nishimura, Y., Kanada, A., Hirama, S., 2005. Polysorbate 80 increases the susceptibility to oxidative stress in rat thymocytes. Toxicology 207, 7–14.

Tsuchiya, M., Asada, A., Maeda, K., Ueda, Y., Sato, E.F., Shindo, M., Inoue, M., 2001. Propofol versus midazolam regarding their antioxidant activities. Am. J. Respir. Crit. Care Med. 163, 26–31.

Vermes, I., Haanen, C., Steffens-Nakken, H., Reutelingsperger, C., 1995. A novel assay for apoptosis. Flow cytometric detection of phosphatidylserine expression on early apoptotic cells using fluorescent labeled annexin V. J. Immunol. Methods 184, 39–51.

Yamaguchi, S., Midorikawa, Y., Okuda, Y., Kitajima, T., 1999. Propofol prevents delayed neuronal death following transient forebrain ischemia in gerbils. Can. J. Anaesth. 46, 593–598.

Yamaguchi, J.Y., Nishimura, Y., Kanada, A., Kobayashi, M., Mishima, K., Tatsuishi, T., Iwase, K., Oyama, Y., 2005. Cremophor EL, a non-ionic surfactant, promotes Ca^{2+}-dependent process of cell death in rat thymocytes. Toxicology 211, 179–186.

Yamaguchi, J.Y., Kanada, A., Horimoto, K., Oyama, T.M., Chikutei, K., Nishimura, Y., Yamamoto, H., Ishida, S., Oyama, Y., Okano, Y., in press. Modification of vulnerability to dodecylbenzenesulfonate, an anionic surfactant, by calcium in rat thymocytes. Environ. Toxicol. Pharmacol. doi:10.1016/j.etap.2006.03.011.