Effects of Injectable Propofol Emulsion on Singlet Oxygen Released From Activated Human Neutrophils and That Chemically Generated

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Abstract. Effects of an injectable emulsion of propofol and its emulsifier on singlet oxygen (\(1^\text{O}_2\)) were examined. \(1^\text{O}_2\) released from activated human neutrophils was detected by chemiluminescence, and chemically generated \(1^\text{O}_2\) was detected by electron paramagnetic resonance (EPR). Both the propofol emulsion and the emulsifier suppressed \(1^\text{O}_2\) release from neutrophils. However, the emulsifier did not quench chemically generated \(1^\text{O}_2\), while the propofol emulsion quenched it. These results indicated that the emulsifier did not scavenge \(1^\text{O}_2\) released from neutrophils but inhibited \(1^\text{O}_2\) generation. The suppressive effects of propofol emulsion on \(1^\text{O}_2\) release from neutrophils consist of \(1^\text{O}_2\) scavenging and inhibition of \(1^\text{O}_2\) generation.

Keywords: propofol emulsion, emulsifier, singlet oxygen

There is a growing body of evidence that propofol (2,6-diisopropylphenol), a widely used anesthetic agent, has neuroprotective effects (1). Neuroprotective activity of propofol is attributed to its ability to scavenge reactive oxygen species (ROS) (2). Superoxide anion (\(O_2^-\)), hydrogen peroxide (\(H_2O_2\)), and hydroxyl radicals (\(\cdot OH\)), are involved in neuronal damage (3), and recently, in addition to these ROS, singlet oxygen (\(1^\text{O}_2\)) has become known as an effector of neuronal damage (4). It is reported that propofol did not scavenge \(O_2^-\), \(H_2O_2\), and \(\cdot OH\) released from activated human neutrophils (5). On the other hand, propofol is reported to scavenge chemically generated \(1^\text{O}_2\) (6). However, in their reports, the clinically-used form of propofol was not tested. The clinically-used form of propofol is an injectable emulsion consisting of propofol substance and its emulsifier. Therefore, it is still unclear whether the propofol emulsion reacts with ROS like propofol substance and whether the emulsifier has any effects on ROS, especially against \(1^\text{O}_2\) released from activated human neutrophils.

In the present study, to elucidate this issue, effects of the propofol emulsion and the emulsifier on \(1^\text{O}_2\) and \(O_2^-\) released from activated human neutrophils were examined by chemiluminescence. Furthermore, effects of them on chemically generated \(1^\text{O}_2\) and \(O_2^-\) were also examined by electron paramagnetic resonance (EPR).

As the propofol emulsion and the emulsifier, Diprivan® (AstraZeneca, Osaka) and Intralipid® (Fresenius Kabi Japan, Tokyo) were used, respectively. Diprivan® contains 10 mg propofol, 100 mg soy bean oil, 22.5 mg glycerin, and 12 mg lecithin in 1 ml, and Intralipid® has the same composition but lacking propofol. Hanks’ balanced salt solution (HBSS) was purchased from Invitrogen (Carlsbad, CA, USA); \textit{trans}-1-(2'-methoxyvinyl)pyrene (MVP) was from Molecular Probes (Eugene, OR, USA); 2-methyl-6-phenyl-3,7-dihydroimidazo[1,2-a]pyrazin-3-one (CLA) was from Tokyo Kasei Kogyo (Tokyo); 2,2,6,6-tetramethyl-4-piperidone (4-oxo-TEMP) and pterin-6-carboxylic acid were from Sigma-Aldrich Co. (St, Louis, MO, USA); hypoxanthine (HPX) was from Kanto Chemical Co. (Tokyo); xanthine oxidase (XOD) was from Roche Dignostics (Basel, Switzerland); 5,5-dimethyl-1-
pyrroline-N-oxide (DMPO) was from Labotec Co. (Tokyo); and diethylenetriaminepentaacetic acid (DETAPAC) was from Nacalai Tesque (Kyoto). Other chemicals, such as zymosan, were purchased from Sigma Chemicals (St. Louis, MO, USA).

Human neutrophils were isolated from peripheral blood of healthy adult volunteers by sedimentation through two-step Percoll (Pharmacia, Uppsala, Sweden) gradients. Healthy adult volunteers provide written informed consent for participation in an institutional review board-approved protocol at Kyoto University Hospital. Freshly purified cells were cultured in HBSS and kept on ice until use. Zymosan was first activated by boiling, and then activated zymosan was incubated and reacted with fresh AB human serum. Zymosan was resuspended in HBSS and used as opsonized zymosan (OZ). Effects of the propofol emulsion and the emulsifier on \( ^1\text{O}_2 \) and \( \text{O}_2^\cdot \) released from activated human neutrophils were examined by chemiluminescence using \( ^1\text{O}_2 \)- and \( \text{O}_2^\cdot \)-specific probes, MVP and CLA, respectively. After mixing the neutrophils (2 × 10⁶ cells) and 40 μM MVP or 2.5 μM CLA with the appropriate concentrations of the propofol emulsion and the emulsifier in HBSS, and luminescence was monitored every 10 s for 10 min. OZ (1 mg/ml) was added to the mixture 30 s after the measurement was started. The findings of representative experiments for \( ^1\text{O}_2 \) and \( \text{O}_2^\cdot \) are shown in panels A and B, respectively. The total luminescence of OZ-induced \( ^1\text{O}_2 \) (C) and \( \text{O}_2^\cdot \) (D) release for 10 min at each concentration of the propofol emulsion is shown as a percentage when the control was assigned as 100%. Values were each shown as the mean ± S.D. (n = 6 for \( ^1\text{O}_2 \) and n = 5 for \( \text{O}_2^\cdot \)). *P<0.05 and **P<0.01, show significant differences in values compared to the value of the control. Note that the IC₅₀ of the propofol emulsion against OZ-induced \( ^1\text{O}_2 \) release was approximately 0.005%.

To determine if the propofol emulsion and the emulsifier quench chemically generated \( ^1\text{O}_2 \) and \( \text{O}_2^\cdot \), EPR spectroscopy was used with an \( ^1\text{O}_2 \)- and \( \text{O}_2^\cdot \)-specific spin traps, 4-oxo-TEMP and DMPO, respectively. \( ^1\text{O}_2 \) was generated by long-wavelength ultraviolet light (UV-A) irradiation of pterin-6-carboxylic acid. Pterin derivatives, such as pterin-6-carboxylic acid, produce \( ^1\text{O}_2 \) under UV-A radiation (7). EPR spectroscopy was used with 4-oxo-TEMP, which reacts with \( ^1\text{O}_2 \) and produces a stable nitrooxide radical, 2,2,6,6-
tetramethyl-4-piperidone-N-oxyl (4-oxo-TEMPO) (8). The solution containing 50 μM pterin-6-carboxylic acid and 1 mM 4-oxo-TEMP with or without the propofol emulsion or emulsifier in phosphate-buffered saline (PBS) was prepared and transferred to a flat quartz EPR aqueous cell, which was fixed in the cavity of the EPR spectrometer; then the EPR spectrum recording was started exactly 60 s after adding XOD.

When neutrophils were stimulated with OZ, ¹O₂ and O₂⁻ were released. The propofol emulsion suppressed OZ-induced ¹O₂ release in a dose-dependent manner (Fig. 1: A and C), while it did not affect the O₂⁻ release (Fig. 1: B and 1D). The IC₅₀ of the propofol emulsion against OZ-induced ¹O₂ release was approximately 0.005% (0.5 μg/ml propofol), one tenth of the clinically relevant concentration of propofol, 5 μg/ml (5).

When the effects of the emulsifier were examined, the emulsifier also suppressed ¹O₂ release to a lesser extent than the propofol emulsion (Fig. 2: A and C), while it did not affect O₂⁻ release as well as the propofol emulsion did (Fig. 2: B and D).

In chemiluminescence, the reaction of ¹O₂ with MVP emits light output at 465 nm (9), and that of O₂⁻ with CLA emits light output at 380 nm (10). The propofol emulsion and the emulsifier are both emulsions, which might absorb these light outputs and interfere with the
measurements. To determine if this would be a problem, the absorption spectrum of 0.1% propofol emulsion in PBS, much higher concentration than that used in chemiluminescence, was measured using a UV spectrophotometer (Jasco UV/VIS Spectrometer V-550; Japan Spectroscopic Co., Tokyo). This showed that 0.1% propofol emulsion had no absorption maximum at either 380 or 465 nm (data not shown), which indicated that the emulsifier did not interfere with the measurements of $^1\text{O}_2$ and $\text{O}_2^-$ by chemiluminescence.

In the EPR study, when the solution containing pterin-6-carboxylic acid was irradiated with UV-A, signals of 4-oxo-TEMPO were observed (Fig. 3A, Control). These signals decreased when 0.1% propofol emulsion was added to the solution (Fig. 3A, +0.1% propofol emulsion), while 0.1% emulsifier did not affect the signals (Fig. 3A, +0.1% emulsifier). These results indicated that the propofol emulsion quenched chemically generated $^1\text{O}_2$, but the emulsifier did not. In the HPX/XOD reaction, signals of spin adducts of $\text{O}_2^-$ (DMPO-OOH) were observed (Fig. 3B, Control). Neither the propofol emulsion nor the emulsifier affected these signals even at a concentration as high as 10%, (Fig. 3B, +10% propofol emulsion and Fig. 3B, +10% emulsifier), which indicated that neither of them quenched chemically generated $\text{O}_2^-$. It was further confirmed by chemiluminescence that neither the propofol emulsion nor the emulsifier quenched $\text{O}_2^-$ generated by the reaction of HPX with XOD (data not shown).

In this study, it was revealed that both the propofol emulsion and the emulsifier suppressed $^1\text{O}_2$ release from activated human neutrophils. On the other hand, it was revealed that the propofol emulsion quenched chemically generated $^1\text{O}_2$, while the emulsifier did not. These results collectively indicated that the propofol emulsion had $^1\text{O}_2$ scavenging activity like propofol substance and that the emulsifier did not scavenge $^1\text{O}_2$ released from neutrophils but inhibited $^1\text{O}_2$ generation. Although it is still controversial whether the emulsifier (Intralipid®) impairs neutrophil functions, such as chemotaxis and bactericidal activity (11, 12), it has been unknown whether the emulsifier inhibits $^1\text{O}_2$ generation in neutrophils. Activated neutrophils generate $\text{O}_2^-$ by the NADPH-oxidase activity, and the generated $\text{O}_2^-$ is dismutated to $\text{H}_2\text{O}_2$. Then, $\text{H}_2\text{O}_2$ reacts with hypochlorite produced by the myeloperoxidase (MPO) activity to form $^1\text{O}_2$ (13). In this study, it is unknown how the emulsifier inhibits $^1\text{O}_2$ generation. Further study will be required to elucidate this issue.

In conclusion, we are the first to demonstrate that the propofol emulsion suppresses $^1\text{O}_2$ released from OZ-stimulated human neutrophils at much lower concentrations than clinically relevant concentrations due to direct $^1\text{O}_2$ scavenging by propofol substance and inhibition of $^1\text{O}_2$ generation by the emulsifier. Although it is still unclear if $^1\text{O}_2$ released from neutrophils is a major cause of ischemia-reperfusion injury, it was shown in rats that the depletion of circulating neutrophils inhibited the increase of MPO activity in the ischemic brain and attenuated post ischemic brain injury (14) and that neutrophils contributed to hypoxic-ischemic brain injury (15). The potent suppressive effects of the propofol emulsion on $^1\text{O}_2$ release from neutrophils might be advantageous in ischemic brain injury in clinical cases.

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References