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Propofol Enhances Hemoglobin-Induced Cytotoxicity in Neurons

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DISCLOSURES

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Abstract

BACKGROUND—It has been increasingly suggested that propofol protects against hypoxic-/ischemic-induced neuronal injury. As evidenced by hemorrhage-induced stroke, hemorrhage into the brain may also cause brain damage. Whether propofol protects against hemorrhage-induced brain damage remains unknown. Therefore, in this study, we investigated the effects of propofol on hemoglobin-induced cytotoxicity in cultured mouse cortical neurons.

METHODS—Neurons were prepared from the cortex of embryonic 15-day-old mice. Hemoglobin was used to induce cytotoxicity in the neurons. The neurons were then treated with propofol for 4 hours. Cytotoxicity was determined by lactate dehydrogenase release assay. Caspase-3 activation was examined by Western blot analysis. Finally, the free radical scavenger U83836E was used to examine the potential involvement of oxidative stress in propofol's effects on hemoglobin-induced cytotoxicity.

RESULTS—We found that treatment with hemoglobin induced cytotoxicity in the neurons. Propofol enhanced hemoglobin-induced cytotoxicity. Specifically, there was a significant difference in the amount of lactate dehydrogenase release between hemoglobin plus saline ($19.84\% \pm 5.38\%$) and hemoglobin plus propofol ($35.79\% \pm 4.41\%$) in mouse cortical neurons ($P = 0.00058$, Wilcoxon Mann-Whitney U test, $n = 8$ in the control group or the treatment group). U83836E did not attenuate the enhancing effects of propofol on hemoglobin-induced cytotoxicity in the neurons, and propofol did not significantly affect caspase-3 activation induced by hemoglobin. These data suggested that caspase-3 activation and oxidative stress might not be the underlying mechanisms by which propofol enhanced hemoglobin-induced cytotoxicity. Moreover, these data suggested that the neuroprotective effects of propofol would be dependent on the condition of the brain injury, which will need to be confirmed in future studies.

CONCLUSIONS—These results from our current proof-of-concept study should promote more research in vitro and in vivo to develop better anesthesia care for patients with hemorrhagic stroke.

Propofol is a commonly used IV anesthetic and sedative-hypnotic agent. It has been reported that propofol is protective to hippocampus neurons and slices from hypoxia-/ischemic reoxygenation-induced cytotoxicity.^{1,2} Several studies involving focal stroke

models of rats and mice have also shown that propofol reduced infarction size and improved neurologic outcomes.^{3–5} Although the underlying molecular mechanisms of propofol's neuroprotection remain undefined, reducing hypoxia-/ischemia-mediated endoplasmic reticulum stress,⁶ inhibiting mitochondrial permeability transition pore opening,⁷ and antiautophagic and apoptotic signaling pathways⁸ could all contribute to the underlying mechanisms of propofol's neuroprotective effects.

Apart from ischemic/hypoxic brain damage, acute brain injuries also involve hemorrhagic brain damage, including hemorrhagic strokes and brain trauma, both of which require neurosurgery under general anesthesia.⁹ However, it remains largely unknown whether propofol could be a good anesthetic for such surgeries through its neuroprotective effects from brain hemorrhagic damage.^{10–12} Therefore, in this study, we used hemoglobin to induce neuronal damage in cultured primary cortical neurons and then determined the effects of propofol on hemoglobin-induced cytotoxicity in the neurons.

The primary hypothesis in this study was that propofol would be able to attenuate cytotoxicity induced by oxygen glucose deprivation (OGD; ischemia damage model) and by hemoglobin (hemorrhagic damage model) in the primary neurons.

METHODS

Primary Mouse Cortical Neuron Cultures

All experiments were performed in the laboratory of Dr. Xiaoying Wang at Massachusetts General Hospital, Boston, MA, following an approved protocol in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health). The animal protocol was approved by the Standing Committee on Animals at Massachusetts General Hospital, Boston, MA. A primary neuronal culture was prepared from the cortex of an embryonic 15-day-old mouse as we previously described.¹³ In brief, cortical neurons were suspended in a neuron-defined culture medium and plated onto poly-D-lysine-coated 35-mm dishes or 24-well plates. Neural basal medium (Life Technologies, Grand Island, NY) supplemented with 2% B27, 0.3 mM L-glutamine, and 1% penicillin–streptomycin was used. Half of the medium was replaced every 3 days. Neuron cultures were used for experiments 9 days after plating. Before hemoglobin exposure or OGD, the medium was changed with neural basal medium supplemented with 0.1% B27 for 1 day and then was maintained in this medium for the duration of the experiments.

Exposure of Cultured Neurons to Hemoglobin and Propofol

Exposure of hemoglobin to cultured neurons followed the established method of our previously published study.¹⁴ Purified human hemoglobin (25 μ M; obtained from Sigma Inc., St. Louis, MO) was added into neuron cultures. Propofol used in the current experiment refers to the combination of propofol plus lipid vehicle. Estimated, clinically relevant concentrations of propofol (5–100 μ M) were used in in vitro experiments.^{15,16} To test the effects of propofol on hemoglobin-induced neurotoxicity, propofol (Diprivan, Fresenius Kabi USA, LLC, Lake Zurich, IL), ranging from 10 to 150 μ M, was added immediately after hemoglobin exposure; thus, hemoglobin exposure and propofol treatment occurred at the

same time. The time of propofol treatment was 4 hours. The medium was then replaced by medium from sister cultures treated with the same dose of only 25 μ M hemoglobin at the same time; this was followed by incubation for an additional 20 hours. To test the involvement of oxidative stress pathways, 5 μ M potent free radical scavenger U83836E (Abcam, Boston, MA) was added to cultures 60 minutes before hemoglobin exposure. The dose and efficacy of 5 μ M U83836E had been selected and validated in our previous study.¹⁴

Exposure of Cultured Neurons to OGD and Propofol

To test the effects of propofol in OGD-induced neurotoxicity, OGD was conducted as we previously described,¹⁷ by using a specialized, humidified chamber (Heidolph, Incubator 1000, Brinkmann Instruments, Westbury, NY) kept at 37°C, which contained an anaerobic gas mixture (90% N₂, 5% H₂, and 5% CO₂). To initiate OGD, the culture medium was replaced with an OGD solution containing deoxygenated, glucose-free extracellular solution–Locke’s medium (154 mM NaCl, 5.6 mM KCl, 2.3 mM CaCl₂, 1.0 mM MgCl₂, 3.6 mM NaHCO₃, 5 mM HEPES, and pH 7.2). After a 4-hour challenge, cultures were removed from the anaerobic chamber, and the OGD solution in the cultures was replaced with maintenance medium. Cells were then allowed to recover for 20 hours (for neurotoxicity assay) in a regular incubator. To test the effects of propofol on OGD-induced neurotoxicity, propofol (10–150 μ M) was added into the OGD solution; therefore, the OGD and propofol treatment started at the same time. The time of propofol treatment was 4 hours.

Assessment of Cytotoxicity

Cytotoxicity was quantified by a standard measurement of lactate dehydrogenase (LDH) release, using the Cytotoxicity Detection Kit (Roche Diagnostics, Indianapolis, IN), as we previously described.¹⁴ In brief, percent cytotoxicity was calculated by subtracting LDH content in remaining, viable cells from total LDH in undamaged controls (100% neuron survival control). Culture plate wells treated with hemoglobin, plus a 500 μ M glutamate exposure for 24 hours (induces almost 100% neuron death), were set as blank controls for the absorbance measurement by the microplate reader.

Western Blot Analysis

Activation of caspase-3 was assessed by Western blot analysis as we previously described.¹⁴ Briefly, each protein sample (25 μ g per lane) was loaded onto 4% to 20% Trisglycine gels with equal volumes of sodium dodecyl sulfate sample buffer (Life Technologies). After electrophoresis and transferring to polyvinylidene difluoride membranes (Life Technologies), the membranes were blocked in Tris-buffered saline containing 0.1% Tween 20 and 0.2% I-block (Thermo Fisher Scientific, Carlsbad, CA) for 60 minutes at room temperature. Membranes were then incubated overnight at 4°C with anticlaved caspase-3 (Asp175) antibody (1:1000, Cell Signaling Technology, Danvers, MA) and an anti- β -actin monoclonal antibody (1:10000, Sigma) after incubation with peroxidase-conjugated secondary antibodies and visualization by an enhanced chemiluminescence detection system (GE Healthcare Bio-Science, Pittsburgh, PA). The anticlaved caspase-3 antibody recognizes only cleaved caspase-3 (19 kDa). Relative caspase-3 activity (fold of normal control) was measured by optical density of the Western blot bands. β -Actin served as equal loading controls.

Statistical Analyses

Data were expressed as mean $\pm$ SD. There were 8 samples in the control group and in each of the different treatment groups according to previous studies under similar conditions,^{14,18,19} which had tested the assumption that 8 samples in each group would be able to generate sufficient power for data analysis.

Kruskal-Wallis test was used to analyze the data representing the effects of the different concentrations of propofol on cytotoxicity (Fig. 1), data representing the effects of different concentrations of propofol on OGD-induced cytotoxicity (Fig. 2), and data representing the time-dependent effects of propofol on the hemoglobin-induced cytotoxicity in primary cortical neurons (Fig. 3).

Wilcoxon Mann-Whitney *U* test was used to test the difference in the amount of LDH release between hemoglobin plus saline and hemoglobin plus propofol in mouse cortical neurons (Fig. 4). Two-way analysis of variance (ANOVA) was used to assess the interaction of hemoglobin and propofol on caspase-3 activation (Fig. 5) and the interaction of propofol and U838336E on cytotoxicity (Fig. 6). Wilcoxon Mann-Whitney *U* test was used to compare the difference for subset analysis, e.g., comparing the cytotoxicity between hemoglobin plus saline and hemoglobin plus U838336E (Fig. 6, right). The cutoff *P* value was Bonferroni adjusted to correct for subset analysis. Hypothesis testing was 2 tailed. *P* values <0.01 were considered statistically significant. Prism 6 software (GraphPad Software, Inc., La Jolla, CA) was used for data analysis.

RESULTS

Propofol Alone (Without OGD or Hemoglobin) Did Not Induce Cytotoxicity in Mouse Cortical Neurons

We first examined whether propofol alone was able to cause cytotoxicity in mouse cortical neurons. The neurons were treated with propofol (0, 10, 35, 70, and 150 μ M) for 4 hours. Cytotoxicity was determined by using a Cytotoxicity Detection Kit and measuring the amount of LDH released from the neurons. We found that there were no significant differences in the LDH amounts among these treatments (overall $P = 0.1945$, Kruskal-Wallis test, Fig. 1). These data suggested that treatments with clinically relevant concentrations (10–70 μ M) of propofol for 4 hours did not induce cytotoxicity in mouse primary neurons. Treatment with a higher concentration of propofol (150 μ M) for 4 hours caused greater cytotoxicity in the neurons than treatments with lower concentrations of propofol.

Propofol Attenuated OGD-Induced Cytotoxicity in Mouse Cortical Neurons

Given the findings that clinically relevant concentrations (10–70 μ M) of propofol might not induce cytotoxicity in mouse primary neurons, we next asked whether these propofol treatments could protect the neurons from cytotoxicity induced by ischemia and hemorrhage.

Previous studies have reported that propofol is neuroprotective in OGD-induced neuron death.^{1,2} Therefore, we set up an in vitro ischemia model in the neurons using OGD. We

were able to show that OGD/reoxygenation caused cytotoxicity in the neurons (Fig. 2). Treatments with propofol significantly attenuated cytotoxicity induced by OGD (each $P < 0.01$). Specifically, the LDH amount for 0 (control group), 10, 35, and 70 μM propofol was $38.16\% \pm 3.04\%$, $29.68\% \pm 3.17\%$, $25.22\% \pm 3.59\%$, and $19.96\% \pm 3.68\%$, respectively. Treatment with a higher dose (150 μM) of propofol did not attenuate OGD-induced cytotoxicity in the neurons (Fig. 2). These data suggested that propofol was able to prevent OGD-induced cytotoxicity in the neurons and could be used to attenuate ischemia-induced brain damage. These data are consistent with findings from previous studies and further validate our established system for detecting cytotoxicity in the neurons.

Propofol Enhanced Hemoglobin-Induced Cytotoxicity in Mouse Cortical Neurons

Next, we assessed whether propofol could also be neuroprotective in hemorrhage-induced brain damage by determining the effects of propofol on hemoglobin-induced cytotoxicity in mouse cortical neurons. Contrary to our original hypothesis, we found that treatment with propofol (70 μM , 4 hours) enhanced 12 μM : $19.84\% \pm 5.38\%$ vs $35.79\% \pm 4.41\%$ ($P = 0.00058$, Wilcoxon Mann-Whitney U test; Fig. 4A), 25 μM : $33.09\% \pm 5.15\%$ vs $50.15\% \pm 6.64\%$ ($P = 0.00062$, Wilcoxon Mann-Whitney U test; Fig. 4B) and 50 μM : $45.24\% \pm 4.05\%$ vs $61.45\% \pm 5.30\%$ ($P = 0.00058$, Wilcoxon Mann-Whitney U test; Fig. 4C) hemoglobin-induced cytotoxicity in neurons. These data demonstrated that propofol had different effects on OGD- and hemoglobin-induced cytotoxicity in the neurons and suggested that propofol might enhance hemorrhage-associated brain damage, pending further investigation.

We further investigated the effects of propofol with different treatment times on hemoglobin-induced cytotoxicity in mouse cortical neurons. We found that with a 2-hour treatment time, only 70 and 150 μM propofol enhanced hemoglobin-induced cytotoxicity in the neurons (each $P < 0.01$; Fig. 3A), whereas with a 4-hour treatment time, 35, 70, and 150 μM propofol all enhanced hemoglobin-induced cytotoxicity in the neurons (each $P < 0.01$; Fig. 3B). These data indicated that propofol-enhanced hemorrhage-associated brain damage could be time dependent (4 vs 2 hours).

Propofol Did Not Attenuate Hemoglobin-Induced Caspase-3 Activation in Mouse Cortical Neurons

Given the findings that propofol enhanced hemoglobin-induced cytotoxicity in mouse cortical neurons, we next investigated the underlying mechanisms. Our previous study found that hemoglobin induces caspase-3 activation in cultured cortical neurons, which plays a dominant role in hemoglobin-induced neurotoxicity.¹⁴ It has been reported that propofol has both antioxidative and anticaspase-3 activation effects.²⁰ Therefore, we examined whether propofol could interface with hemoglobin-induced caspase-3 activation. The neurons were treated with 25 μM hemoglobin, with or without 70 μM propofol, for 4 hours, and the neurons were harvested at the end of the experiment. Cleaved caspase-3 expression was determined by Western blot analysis. Treatment with 25 μM hemoglobin for 4 hours increased expression of cleaved caspase-3 compared with the control condition (lanes 1 and 3, Fig. 5). We then found that there was no interaction between propofol and hemoglobin on caspase-3 activation, and the treatment with 70 μM propofol for 4 hours did not affect

hemoglobin-induced changes in the appearance of cleaved caspase-3 expression in the neurons (Fig. 5; $F = 0.0275$, $P = 0.8694$ for interaction term in 2-way ANOVA). These data suggested that the changes in caspase-3 activation might not have been the mechanism by which propofol enhanced hemoglobin-induced cytotoxicity in neurons.

Antioxidant Did Not Affect the Enhancing Effects of Propofol on Hemoglobin-Induced Cytotoxicity

Finally, we determined whether oxidative stress was the mechanism by which propofol enhanced hemoglobin-induced cytotoxicity. The free radical scavenger U83836E (5 μM) was pretreated for 1 hour before exposure of cultured neurons to hemoglobin (25 μM) or hemoglobin plus propofol (70 μM). Two-way ANOVA showed that there was no interaction between propofol and U83836E ($F = 0.2858$, $P = 0.5972$; Fig. 6). U83836E was able to attenuate hemoglobin-induced cytotoxicity with or without propofol treatment. These data suggested that oxidative stress might not be the mechanism by which propofol enhanced hemoglobin-induced cytotoxicity.

DISCUSSION

Propofol is a commonly used anesthetic, and its clinically relevant concentration is between 10 and 70 μM .^{15,16} Several studies have reported that propofol is neuroprotective to hypoxia-/ischemic reoxygenation-induced cytotoxicity in the hippocampus neurons and slices.^{1,2} However, whether propofol is also neuroprotective for hemorrhage-induced cytotoxicity in the neurons remains to be determined. Therefore, we assessed and compared the effects of propofol on cytotoxicity induced by OGD (ischemia damage model) and hemoglobin (hemorrhagic damage model) in mouse cortical neurons.

We first found that treatment with propofol 10, 35, 70, and 150 μM did not significantly induce cytotoxicity in the neurons, although 150 μM propofol caused greater cytotoxicity in neurons than other concentrations of propofol (Fig. 1). These data suggested that clinically relevant concentrations of propofol might not be cytotoxic in the primary neurons.

We then found that these clinically relevant concentrations of propofol could attenuate OGD-induced cytotoxicity in the neurons (Fig. 2), but enhanced hemoglobin-induced cytotoxicity in the neurons (Figs. 3 and 4). Moreover, the effects of propofol for enhancing hemoglobin-induced cytotoxicity were time dependent in the neurons (Fig. 3). These findings suggested that propofol might have different effects (protection versus enhancement) on ischemia- and hemorrhage-induced neurotoxicity, pending further investigation. However, although the in vitro propofol concentrations used in the current experiments were clinically relevant, future studies to systematically investigate the potential enhancing effects of propofol on hemoglobin-induced cytotoxicity in animals are warranted. Note that the “propofol” used in our studies was actually the combination of propofol plus a lipid vehicle, which is used clinically. Future studies may include the determination of whether propofol or a lipid vehicle itself can affect OGD or hemoglobin-induced cytotoxicity in the neurons.

There have been no guidelines or set protocols in terms of standard anesthesia care for surgeries or procedures involving stroke patients. Given the findings that the same anesthetic (propofol) might have opposite effects on OGD- and hemoglobin-induced cytotoxicity in the neurons, it is important to further study and identify a better anesthetic(s) to provide anesthesia care for patients with ischemia strokes or hemorrhage strokes.

We have previously established a simplified in vitro brain hemorrhage model by hemoglobin exposure to primary cortical neurons. Both caspase cascades and oxidative stress are activated, but caspase activation is mainly triggered by hemoglobin exposure-mediated oxidative stress, which is primarily attributed to hemoglobin-induced neurotoxicity.¹⁴ In this study, we found that propofol had no effect on hemoglobin-mediated caspase-3 activation (Fig. 5), suggesting that the enhancing effect of propofol on hemoglobin-induced cytotoxicity was not dependent on caspase-3 activation. One report showed that 50 or 100 μ M propofol exposure for 24 hours induced neurotoxicity of embryonic neural stem cells, presumably by increasing oxidative damage.²¹ Interestingly, the potent free radical scavenger U83836E did not specifically attenuate propofol's enhancing effects on hemoglobin-induced cytotoxicity (Fig. 6). These data suggested that the enhancing effect of propofol on hemoglobin-induced cytotoxicity might not be dependent on oxidative stress. Although we do not know the mechanism for the neurotoxic effect of propofol in the presence of hemoglobin, we speculate that, in addition to oxidative stress and caspase activation, propofol might interact with hemoglobin-associated hemolysis and a neuronal heme oxygenase-1 expression increase that, in concert, contributes to underlying neurotoxic mechanisms.^{22,23} This needs to be investigated further in both in vitro cell cultures and in vivo animal models of brain hemorrhage. We believe that the overlying significance of this study was not that we showed that propofol was not toxic to normal neurons but that, for the first time we found that clinically relevant concentrations of propofol had the opposite effects on 2 different types of neurotoxicity. Plainly, propofol was protective against OGD cytotoxicity but toxic in relation to hemoglobin cytotoxicity.

These opposite effects of propofol raised concern regarding its safety and its efficacy as a potential anesthetic for surgical care of stroke patients. Our findings postulate that the interactions of propofol with various factors might lead to different final outcomes in animal models of brain injuries. Future preclinical investigations to test this hypothesis are warranted.

Note that <2% of propofol is free, and the rest of propofol is bound to blood in patients.²⁴ In the current studies, a serum-free medium was used, and it was not known how much of the propofol was free from the lipid; thus, the applied concentrations of propofol could be beyond the clinical range. However, the objective of the current proof-of-concept study was to establish a system that could lead to a more systematic investigation to further determine whether propofol could affect hemorrhage-induced neurotoxicity, in vitro and in vivo.

The findings from the current in vitro studies might not totally reflect the same cellular mechanisms that occur in intact animal models or humans. However, the data obtained from the current studies should raise a concern that the same anesthetic (e.g., propofol) may have different outcomes (protection versus enhancement of neurotoxicity) for different brain

injuries, e.g., brain ischemia versus hemorrhage. More research to determine the in vivo relevance and clinical implication of the current in vitro findings is warranted.

There are a few caveats associated with this study. First, this model system of hemoglobin-induced neuronal injury was highly simplified. After intracerebral hemorrhage in vivo, a multitude of factors in extravasated blood could trigger brain cell death, although hemoglobin remains a major component of blood, and is highly neurotoxic.²⁵ Clearly, these in vitro findings will have to be carefully extended into in vivo systems before data can be translated into a clinical context. A second caveat is that this study focused only on the neurons. Hemoglobin-induced oxidative stress will affect nonneuronal cells, so responses to propofol in other types of brain cells will need to be investigated in the future.¹² Finally, a third caveat involves the fact that the hemoglobin model cannot replicate vascular trauma and tissue ischemia that take place after hemorrhage or hemorrhagic transformation in vivo. These interactions between multiple pathologic factors or cascades and their interactions with propofol may be critical. Thus, further experimental investigations of propofol's therapeutic effects for acute brain injury, especially testing the interaction effects of propofol with different pathogenic factors in vivo in different brain injury models and using different propofol dose regimens, should be considered as timely and translationally significant pursuits.^{12,26}

In conclusion, we established a simplified in vitro model of hemoglobin-induced cytotoxicity in the neurons and tested the effects of propofol on hemoglobin-induced cytotoxicity. The major finding was that although propofol was not toxic to cultured neurons under normal resting conditions, propofol did significantly enhance hemoglobin-induced cytotoxicity. For translational purposes, further investigations are imperative to clarify the effects of propofol in animal models of hemorrhagic brain injury and elucidate its underlying mechanisms.

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Figure 1.

Propofol does not induce cytotoxicity in primary cortical neurons. Propofol (0–150 μM) itself does not induce cytotoxicity compared with the control condition in the neurons (overall $P = 0.1945$, Kruskal-Wallis test). One hundred fifty micromolar propofol induces greater cytotoxicity than other concentrations of propofol but still does not reach significant levels. $n = 8$ in control or treatment group. Error bar represents SD.

Figure 2.

Propofol attenuates the oxygen glucose deprivation (OGD)–induced cytotoxicity in primary cortical neurons. Treatments with 10, 35, and 70 μM propofol decrease the cytotoxicity of the neurons induced by OGD compared with the control condition in the neurons (each $P < 0.01$ using Wilcoxon Mann-Whitney U test). High (150 μM) dose of propofol does not protect the OGD-induced cytotoxicity in the neurons. $n = 8$ in control or treatment group. Error bar represents SD. * $P < 0.01$.

Figure 3.

Propofol potentiates the hemoglobin-induced cytotoxicity in primary cortical neurons in a time-dependent manner. A, Treatments with 70 and 150 μM (but not 10 or 35 μM) propofol for 2 hours potentiates the hemoglobin-induced cytotoxicity in the neurons (each $P < 0.01$ using Wilcoxon Mann-Whitney U test). B, Treatments with 35, 70, and 150 μM propofol for 4 hours all potentiate the hemoglobin-induced cytotoxicity in the neurons (each $P < 0.01$ using Wilcoxon Mann-Whitney U test). $n = 8$ in control or treatment group. Error bar represents SD. * $P < 0.01$.

Figure 4.

Propofol potentiates the hemoglobin-induced cytotoxicity in primary cortical neurons. A, Treatment with 70 μ M propofol decreases the cytotoxicity induced by 12 μ M hemoglobin in the neurons ($P = 0.00058$, Wilcoxon Mann-Whitney U test). B, Treatment with 70 μ M propofol decreases the cytotoxicity induced by 25 μ M hemoglobin in the neurons ($P = 0.00062$, Wilcoxon Mann-Whitney U test). C, Treatment with 70 μ M propofol decreases the cytotoxicity induced by 50 μ M hemoglobin in the neurons ($P = 0.00058$, Wilcoxon Mann-

Whitney *U*Test). Hb = hemoglobin. $n = 8$ in control or treatment group. Error bar represents SD. * $P < 0.01$.

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Figure 5.

Propofol does not potentiate the hemoglobin-induced caspase-3 activation in primary cortical neurons. Hemoglobin induces caspase-3 activation compared with control condition (lane 1 versus lane 3; top panel is the Western blot and the lower panel is the quantification of the Western blot). Treatment with 70 μ M propofol does not potentiate the hemoglobin-induced caspase-3 activation (lane 3 versus lane 4, $F = 0.0275$, $P = 0.8694$, 2-way analysis of variance). Hb = hemoglobin. $n = 8$ in control or treatment group. Error bar represents SD. * $P < 0.01$.

Figure 6.

U83836E does not affect the potentiation effects of propofol on the hemoglobin-induced cytotoxicity. Treatment with 5 μM antioxidant U83836E attenuates the hemoglobin-induced cytotoxicity in the neurons (black bar versus white bar on the left panel). Propofol (70 μM) potentiates the hemoglobin-induced cytotoxicity (right panel versus left panel). However, the treatment of antioxidant U83836E decreases the hemoglobin-induced cytotoxicity to similar levels with (right panel) and without (left panel) the 70 μM propofol treatment ($F = 0.2858$, $P = 0.5972$, 2-way analysis of variance). Hb = hemoglobin. $n = 8$ in control or treatment group. Error bar represents SD. * $P < 0.01$.