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Unexpected pro-injury effect of propofol on vascular smooth muscle cells with increased oxidative stress*

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Abstract

Objectives—Propofol is a widely used intravenous anesthetic agent with antioxidant properties. However, the effect of propofol on reactive oxygen species-induced injury in vascular smooth muscle cells is still unknown. In this study, the authors determined the effect of propofol on hydrogen peroxide-induced injury in vascular smooth muscle cells and the potential molecular mechanisms involved.

Design—Prospective cell and animal study.

Setting—University research laboratory.

Subjects—Sprague-Dawley rats.

Interventions—For the *in vitro* study, rat vascular smooth muscle cells pretreated with vehicle or hydrogen peroxide (200 μ M) were exposed to vehicle or increasing concentrations of propofol (10–50 μ M). For the *in vivo* study, propofol (12 mg kg⁻¹/hr⁻¹, intravenous) or vehicle was administered into rats after carotid artery angioplasty.

Measurements and Main Results—The cell survival and cell death were measured by MTT and trypan blue exclusion. Cell apoptosis was evaluated by terminal deoxynucleotidyl transferase dUTP nick end labeling staining and cleaved caspase-3 expression. To further elucidate the molecular mechanisms in propofol-mediated cellular effect, the expression of programmed cell death 4 and microRNA-21 were measured. Unexpectedly, propofol exacerbated hydrogen peroxide-induced injury responses in vascular smooth muscle cells as demonstrated by a decrease in cell viability and an increase in trypan blue-stained cells, cell apoptosis, and cleaved caspase-3 expression. In addition, propofol inhibited hydrogen peroxide-induced up-regulation of microRNA-21 and increased its target gene programmed cell death 4. Propofol-mediated injury was attenuated by restoration of microRNA-21 expression. Finally, the pro-injury effect of propofol on vascular cells with increased reactive oxygen species was illustrated *in vivo* in rat carotid arteries after angioplasty.

*See also p. 896.

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Conclusions—The results revealed that propofol exacerbates cell injury in vascular smooth muscle cells with increased reactive oxygen species, at least in part, through microRNA-21 and its target gene, programmed cell death 4. Because increased reactive oxygen species is a common pathologic component in many vascular diseases, the novel findings in the current study suggest that propofol might have some application limitations.

Keywords

propofol; oxidative stress; vascular smooth muscle cells; vascular disease; apoptosis; microRNA; reactive oxygen species; free radicals; ischemia-reperfusion

Propofol (2,6-diisopropylphenol) is a widely used intravenous anesthetic agent. In addition to its anesthetic effects, propofol has a protective effect against oxidative stress-mediated cell injuries (1). Propofol inhibits reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2)-induced injury in cultured endothelial cells and cardiac cells *in vitro* (2, 3) and in hearts with ischemia/reperfusion injury *in vivo* (4). However, propofol-mediated antioxidative effect might be cell-specific. For example, in a recent study, propofol was found to facilitate the process of cell death induced by H_2O_2 in rat thymocytes (5).

ROS are reactive derivatives of O_2 metabolism, including superoxide anion (O_2^-), H_2O_2 , hydroxyl radical, and nitric oxide. All types of vascular cells produce ROS, primarily via cell membrane-associated NAD(P)H oxidase, uncoupled nitric oxide synthase, xanthine oxidase, and mitochondria. It is well-established that the increased ROS, such as H_2O_2 , is a common pathologic component in many vascular diseases, including atherosclerosis, hypertension, restenosis after angioplasty or bypass, diabetic vascular complications, transplantation arteriopathy, and vascular aneurysm (6–12). The increased oxidative stress induced by ROS is related to vascular smooth muscle cell (VSMC) injury (apoptosis) and proliferation, which are critical cellular events in the pathogenesis of the aforementioned vascular diseases (13–15). Currently, the biological effect of propofol on ROS-mediated injury on VSMCs is unclear. Propofol is widely used in patients with diverse vascular diseases with increased ROS; uncovering the effect of propofol on VSMCs is therefore very important in clinical anesthesiology and critical care medicine.

MicroRNAs (miRNAs) have emerged as a novel class of endogenous, small, non-coding RNAs that negatively regulate ~30% of genes in a cell via degradation or translational inhibition of their target mRNAs (16–19). Using microarray analysis, we demonstrated that H_2O_2 increased microRNA-21 (miR-21) expression in VSMCs and miR-21 had an anti-apoptotic effect in H_2O_2 -mediated VSMC apoptosis and death via its target gene, programmed cell death 4 (PDCD4) (20). However, the potential involvement of miR-21 in propofol-mediated effect on VSMCs is unknown.

The objectives of the current study were to determine the effect of propofol on H_2O_2 -induced injury on VSMCs and vascular walls, and to elucidate the molecular mechanisms involved. Surprisingly, in contrast to its antioxidative effect on other cells, we found propofol exacerbated injury responses in VSMCs and in vascular walls with increased H_2O_2 , at least in part, through miR-21 and its target gene, PDCD4.

MATERIALS AND METHODS

All protocols were approved by the Institutional Animal Care and Use Committee at the University of Medicine and Dentistry of New Jersey and were consistent with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health publication 85-23, revised 1985).

Cell Culture

VSMCs were obtained from the aortic media of male Sprague-Dawley rats (specific pathogen free, 5 wks old) using an enzymatic dissociation method as described (20). VSMCs were cultured with DMEM containing 10% fetal bovine serum. Cells between passage 3 and 6 were applied for the experiments.

Cell Treatments With H₂O₂, Propofol, or premiR-21

Confluent rat VSMCs cultured with serum-free medium were treated with vehicle (phosphate-buffered saline) or different concentrations of H₂O₂ (0–300 μ M; Sigma, St. Louis, MO). In our preliminary study, we found that the cell viability after treatment with H₂O₂ at 200 μ M was ~60%. Obviously, 200 μ M of H₂O₂ could allow us to test either a protective or an injury effect of propofol. H₂O₂ (200 μ M) was therefore to be used in our subsequent experiments. Briefly, confluent rat VSMCs cultured with serum-free medium were treated with vehicle (phosphate-buffered saline) or H₂O₂ (200 μ M; Sigma) for 30 mins. Then, the cells were treated with vehicle (0.05% dimethyl sulfoxide or intralipid emulsion) or different concentrations of propofol (10, 30, or 50 μ M; Sigma) for 6 and 24 hrs. In a subsequent experiment, the VSMCs pretreated with H₂O₂ (200 μ M; Sigma) for 30 mins and premiR-21 (30 nM; Ambion, Austin, TX) or control oligo (30 nM; Ambion) for 4 hrs were also treated with propofol (50 μ M) for 6 hrs.

Assessment of Cell Viability and Cell Death

Cell survival was measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay as described in our previous study (21). In brief, VSMCs were cultured in 96-well plate and 10 μ L of MTT (Sigma) were added to each well for 4 hrs. The product was then dissolved in isopropanol and quantified spectrophotometrically at a wavelength of 570 nM. Cell death was determined by trypan blue exclusion test. In this experiment, the VSMCs were detached by trypsinizing and were collected after centrifugation. The cells were resuspended in phosphate-buffered saline and incubated with equal volume of 0.4% trypan blue for 5–10 mins at room temperature. The number of dead cells (stained cells) were counted using a hemocytometer and expressed as a percentage of the total cells counted.

Evaluation of Cell Apoptosis

VSMC apoptosis was determined by terminal deoxynucleotide transferase dUTP nick end labeling (TUNEL) staining and the expression of the cleaved caspase-3 (the active form of caspase-3) (20–22). TUNEL staining was performed using the *in situ* cell death detection kit (Roche, Indianapolis, IN) according to the manufacturer's protocol. The number of TUNEL-positive cells was counted under a fluorescence microscope. The cleaved caspase-3 and

intact caspase-3 were examined by Western blotting (see “Western Blot Analysis of Caspase-3 and PDCD4” below).

Oligo Transfection and miR-21 Up-Regulation in Cultured VSMCs

Oligo transfection was performed according to an established protocol (20–22). Briefly, cells were transfected using a transfection re-agent (Qiagen, Valencia, CA) 24 hrs after seeding into the well. Transfection complexes were prepared according to the manufacturer's instructions. For the miR-21 up-regulation, premiR-21 (Ambion) was added directly to the complexes at a final oligonucleotide concentration of 30 nM. The transfection medium was replaced 4 hrs after transfection by the regular culture medium. Vehicle control and oligo control (Ambion) were applied.

Measurement of mir-21

The miR-21 levels were determined by quantitative real-time polymerase chain reaction as described (20–22). Briefly, RNAs from VSMCs were isolated with mirVana miRNA Isolation Kit (Ambion). Quantitative real-time polymerase chain reaction for miR-21 was performed on cDNA generated from 50 ng of total RNA using the protocol of the mirVana quantitative real-time polymerase chain reaction miRNA detection kit (Ambion). Amplification and detection of specific products were performed with a Lightcycler 480 Detection System (Roche). As an internal control, U6 was used for miR-21 template normalization. Fluorescent signals were normalized to an internal reference, and the threshold cycle (Ct) was set within the exponential phase of the polymerase chain reaction. The relative gene expression was calculated by comparing cycle times for each target polymerase chain reaction. The target polymerase chain reaction Ct values were normalized by subtracting the U6 Ct value, which provided the Ct value. The relative expression level between treatments was then calculated using the following equation: relative gene expression = $2^{-(Ct_{\text{sample}} - Ct_{\text{control}})}$.

Western Blot Analysis of Caspase-3 and PDCD4

The expressions of cleaved caspase-3 and PDCD4 were determined by Western blot analysis as described (21). The whole cell lysates were prepared in RIPA buffer (50 mM Tris-HCl, pH 8, 150 mM NaCl, 1% Nonidet P-40, 0.1% SDS, 1% Triton X-100 plus proteinase inhibitors; Sigma). Protein concentration was determined by Bradford assay, and samples containing 30 µg were separated by 12% SDS-PAGE. Proteins were transferred to nitrocellulose membranes (Hybond ECL, Amersham Pharmacia Biotech). After membranes were blocked in 5% nonfat milk in 20 mM Tris-HCl, 150 mM NaCl, and 0.05% Tween 20 for 1 hr at room temperature, the membranes were incubated overnight at 4°C with different primary monoclonal antibodies: anti-cleaved caspase-3 (1:1000; Asp175; Cell Signaling Technology, Danvers, MA, #9664), anti-intact caspase-3 (8G10) (1:1000; Cell Signaling Technology, #9665), and anti-PDCD4 (1:1500; Santa Cruz Biotechnology, Santa Cruz, CA). GAPDH antibody (1:5000 dilution; Cell Signaling Technology) was used as a loading control. The membranes were then incubated with secondary antibody conjugated to horseradish peroxidase for 1 hr at room temperature and were exposed to enhanced chemiluminescence reagents. Densitometric analysis was performed to quantify the signal intensity.

Rat Carotid Artery Balloon Injury, Propofol Treatment, and Cell Apoptosis Assay in Vascular Walls *in Vivo*

Carotid artery balloon injury was induced in male Sprague-Dawley rats (specific pathogen-free, 250–300 g) as described in our previous studies (22–24). Briefly, rats were anesthetized with ketamine (80 mg/kg)/xylazine (5 mg/kg). Under a dissecting microscope, the right common carotid artery was exposed through a midline cervical incision. A 2-Fr Fogarty catheter (Baxter Edwards, Irvine, CA) was introduced via an arteriotomy in the external carotid artery, and then the catheter was advanced to the proximal edge of the omohyoid muscle. To produce carotid artery injury, we inflated the balloon with saline and withdrew it three times from just under the proximal edge of the omohyoid muscle to the carotid bifurcation. After injury, the external carotid artery was permanently ligated with a 6-0 silk suture, and blood flow in the common carotid artery was restored. Sham-opened animals without vessel injury were used as controls. At 4 days after angioplasty, the control and balloon-injured rats were divided into two groups, propofol-treated group and vehicle-treated group. Six hours after propofol ($12 \text{ mg/kg}^{-1}/\text{hr}^{-1}$, intravenous) or vehicle treatment, the animals were euthanized and the carotid arteries were isolated for apoptosis assay. The dose we used in the rat study was based on previous reports (25, 26). Detection of apoptotic cells in the vascular walls was performed using the TUNEL method by immunofluorescence (22). Apoptotic cells were quantified by counting the percentage of TUNEL-positive cells against total nucleated cells in the tissue section. The average of the six sections was used as the parameter for one animal. The apoptosis of the vascular cells was further verified by cleaved caspase-3 via Western blot.

Statistics

All data are presented as mean $\pm$ standard error. For relative cell viability or gene expression, the mean value of the control group was defined as 1 or 100%. If normality and homogeneity of variance had been proven (Shapiro Wilks test and Levene's test, respectively), then one-way analysis of variance followed by Scheffe test were used for statistical evaluation of the differences among three or more groups data; otherwise, Kruskal-Wallis one-way analysis of variance was used and Wilcoxon-Mann-Whitney test with a Bonferroni correction was used for multiple comparisons. Two-tailed unpaired Student's *t* test was used for statistical evaluation of the differences between two groups' data. All statistics were analyzed using SPSS 17.0 (SPSS, Chicago, IL) or SAS (SAS Institute Inc, Cary, NC) statistical analysis program. A value of $p < .05$ was considered statistically significant.

RESULTS

Propofol Promotes Cell Death and Reduces Cell Viability in VSMCs Pretreated With H_2O_2

As shown in Figure 1, propofol (10–50 μM) and its vehicle have no effect on VSMC viability after 24-hr treatment (Fig. 1A). In contrast, incubation with H_2O_2 resulted in decreased VSMC viability in a dose-dependent manner (Fig. 1B). Notably, H_2O_2 -induced decrease in cell viability was significantly exacerbated by propofol at clinically relevant concentrations (10–50 μM) as demonstrated by MTT assay (Fig. 1C). Accordingly, H_2O_2 -induced cell death was facilitated by propofol (Fig. 2).

Propofol Exacerbates H₂O₂-Induced VSMC Apoptosis

Apoptosis is an important cellular mechanism in H₂O₂-induced cell injury. To determine the effect of propofol on VSMC apoptosis, TUNEL staining was performed. As shown in Figure 3, propofol had no effect on apoptosis in VSMCs without H₂O₂ treatment. However, in H₂O₂-pretreated VSMCs, H₂O₂-induced cell apoptosis was strongly increased by propofol at 6 hrs after treatment (Fig. 4A). Representative TUNEL-stained photomicrographs of VSMCs treated with H₂O₂ (200 μ M) alone and treated with H₂O₂ (200 μ M) plus different concentrations of propofol are shown in Figure 4B. The effect of propofol on H₂O₂-induced VSMC apoptosis was further confirmed by cleaved caspase-3 expression because the increased cleaved caspase-3 expression was found in H₂O₂-pretreated(200 μ M) VSMCs after treatment with propofol (Fig. 5).

Involvement of miR-21 in Propofol-Mediated Pro-injury Effects on VSMCs

To test the potential involvement of miR-21 in propofol-mediated pro-injury effects on VSMCs, miR-21 levels in VSMCs treated with H₂O₂ and propofol were determined. As shown in Figure 6A, miR-21 expression was increased by H₂O₂. Although propofol itself had no significant effect on miR-21 expression in VSMCs without H₂O₂ treatment, H₂O₂-induced up-regulation of miR-21 was significantly attenuated by propofol (Fig. 6A).

To further determine the biological function of miR-21 in propofol-mediated cellular effects, the expression of miR-21 was up-regulated by premiR-21 (Ambion) to overcome propofol-mediated down-regulation of miR-21 in H₂O₂-treated cells. As shown in Figure 6B, miR-21 expression was successfully increased by premiR-21 (30 nM). Interestingly, propofol-mediated pro-injury effect on H₂O₂-pretreated VSMCs was significantly inhibited by the overexpression of miR-21 as demonstrated by the decreased VSMC apoptosis (Fig. 6C and D). In contrast, control oligo had no effect on VSMC apoptosis (Fig. 6C and D).

To further confirm the biological role of miR-21 in propofol-mediated cellular effect, the effect of propofol on the downstream molecule of miR-21, PDCD4, was determined in H₂O₂-treated cells. The expression of PDCD4 was increased by propofol, which matched the propofol-mediated expression change of miR-21 (Fig. 6E).

Propofol Exacerbates Cell Apoptosis in Rat Vascular Walls With Increased ROS

Increased ROS including H₂O₂ is an important mechanism of VSMC apoptosis in balloon-injured vascular walls. To test the effect of propofol on VSMCs with increased H₂O₂ in the vascular walls *in vivo*, balloon angioplasty was performed in rat carotid arteries. On day 4 after injury, the animals were treated with vehicle or propofol for 6 hrs. As expected, VSMC apoptosis was increased in balloon-injured vessels as demonstrated by TUNEL (Fig. 7A and B) and Western blot analysis of the cleaved caspase-3 (Fig. 7C and D). Propofol had no effect on cell apoptosis in normal vessels. Interestingly, the cell apoptosis in the balloon-injured vascular walls was significantly increased after treatment with propofol. Representative TUNEL-stained photomicrographs of normal uninjured vessel sections and balloon-injured vessel sections treated with vehicle or propofol are shown in Figure 7A.

DISCUSSION

Oxidative injury of VSMCs induced by increased ROS such as H_2O_2 in the vascular walls is an important mechanism in the development of many vascular diseases (6–12). Propofol, a widely used general anesthetic, is reported to have a protective effect on ROS-mediated injuries of cardiac cells and vascular endothelial cells via its antioxidant properties (2–4). We thus hypothesized that propofol might also have an antioxidative effect in VSMCs. Unexpectedly, although propofol has no injury effect on normal VSMCs and normal vessels, it significantly increased H_2O_2 -induced injury responses in cultured VSMCs and in arteries after angioplasty. The pro-injury effect of propofol on VSMCs with increased H_2O_2 is not limited in rat cells, because the similar effect was found in human aortic VSMCs pretreated with H_2O_2 (Supplemental Fig. 1 [Supplemental Digital Content 1, <http://links.lww.com/CCM/A202>]). The relative cell viability in H_2O_2 (200 μM) pretreated human VSMCs without propofol (0 group) was expressed as 100% ($n = 6$; $*p < .05$ compared with the control [0 group]; Supplemental Digital Content 1, <http://links.lww.com/CCM/A202>).

The unexpected results from the current study revealed that the antioxidative effect of propofol may be cell- and oxidative condition-specific. Indeed, we did not see any injury effects on VSMCs and vessels without oxidative stress. However, in VSMCs and vessels with increased ROS, a significant pro-injury was demonstrated after treatment with propofol. In contrast, no obvious injury was found in rat endothelial cells treated with H_2O_2 and propofol (data not shown). One recent study also supports our opinion that the antioxidative effect of propofol is cell-specific. Chikutei et al (5) found that propofol promoted the process of cell death induced by H_2O_2 in rat thymocytes. However, under the same experimental conditions, in contrast to that in thymocytes, they found propofol was able to protect rat brain neurons against the oxidative stress induced by H_2O_2 , although the mechanism was not investigated (5). In addition, propofol-mediated pro-injury effect on VSMCs was likely ROS-specific, because the effect was also found in VSMCs pretreated with exogenous super-oxide (O_2^-) (generated by a xanthine/xanthine oxidase reaction [100 $\mu mol/L$ xanthine + 10 mU/L xanthine oxidase]), but not in VSMCs pretreated with exogenous reactive nitrogen species, peroxynitrite ($ONOO^-$) (produced by sodium nitroprusside [1 $\mu mol/L$ SNP] and the xanthine/xanthine oxidase reaction; data not shown).

Clinically relevant concentrations of propofol for general anesthesia are ~10–30 μM as reported previously (27–30). In the current study, we identified that the pro-injury effect of propofol could occur at as low as 10 μM (Figs. 1 and 2). Another important parameter in propofol-mediated pro-injury effect is the exposed time. In the current study we have found that exposure of propofol for 3 hrs was able to induce pro-injury effects on VSMCs. The effects of propofol at early time points should be determined in future studies. It should be noted that the exposure of propofol may be evanescent after a bolus administration because of its large volume of distribution and rapid clearance. However, propofol not only is used as an intravenous induction agent but also is commonly used as a more long-term infusion as part of a sedative–analgesic protocol. Thus, the novel finding in this study will still have clinical significance in patients with long-term exposure of propofol, especially in patients with ischemia or reperfusion or both who receive concurrent and ongoing propofol.

The molecular mechanisms involved in propofol-mediated pro-injury effect are currently unclear. In the study of Chikutei et al (5), these investigators thought that propofol might affect the cells with phosphatidylserine-exposed membranes, which could be induced by H₂O₂ in thymocytes, resulting in cell death. It is well established that although the damage stimuli are diverse, the fate of a cell under any kind of damage conditions is decided by the gene changes inside the cell (31, 32). Some of these responsive genes are pro-injury genes leading the cells to injury and death, whereas some of them are intrinsic defensive genes that protect the cells against damages (31, 32). miRNAs are a novel class of endogenous, small, noncoding RNAs that negatively regulate gene expression (16–19). Our recent studies demonstrated that miR-21 is a critical anti-injury miRNA against cell death (apoptosis and necrosis) induced by ischemic and oxidative injuries (20, 21, 33). In the current study, we identified that miR-21 is involved in propofol-mediated pro-injury effect on VSMCs based on the following three observations. First, H₂O₂-induced miR-21 up-regulation was inhibited by propofol. Second, in H₂O₂-pretreated VSMCs, cell apoptosis induced by propofol was significantly reduced by restoration of miR-21 expression. Third, propofol was able to increase the expression of miR-21 target gene, PDCD4, in H₂O₂-treated VSMCs. However, up-regulation of miR-21 can only partially inhibit propofol-mediated injury on VSMCs, suggesting that other unidentified molecular mechanisms should be investigated in future studies. It should be noted that caspase-3 is not a direct target of miR-21. However, miR-21 may indirectly affect caspase-3 expression through its target gene, PDCD4.

CONCLUSIONS

The current study identifies, for the first time, that the widely used anesthetic propofol can exacerbate cell injury in VSMCs and in vascular walls with increased ROS, at least in part, through miR-21 and its target gene, PDCD4. Because an increase in ROS is a common pathologic component in many vascular diseases, the novel findings in this study suggest that propofol might have some application limitations in patients with vascular disease, although additional clinical evidence should be provided in future studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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REFERENCES

1. Vasileiou I, Xanthos T, Koudouna E, et al. Propofol: A review of its non-anaesthetic effects. *Eur J Pharmacol.* 2009; 605:1–8. [PubMed: 19248246]
2. Xu JJ, Wang YL. Propofol attenuation of hydrogen peroxide-mediated oxidative stress and apoptosis in cultured cardiomyocytes involves haeme oxygenase-1. *Eur J Anaesthesiol.* 2008; 25:395–402. [PubMed: 18289444]
3. Wang B, Luo T, Chen D, et al. Propofol reduces apoptosis and up-regulates endothelial nitric oxide synthase protein expression in hydrogen peroxide-stimulated human umbilical vein endothelial cells. *Anesth Analg.* 2007; 105:1027–1033. [PubMed: 17898383]

4. Jin YC, Kim W, Ha YM, et al. Propofol limits rat myocardial ischemia and reperfusion injury with an associated reduction in apoptotic cell death in vivo. *Vasc Pharmacol.* 2009; 50:71–77.
5. Chikutei K, Oyama TM, Ishida S, et al. Propofol, an anesthetic possessing neuroprotective action against oxidative stress, promotes the process of cell death induced by H₂O₂ in rat thymocytes. *Eur J Pharmacol.* 2006; 540:18–23. [PubMed: 16730701]
6. Bonomini F, Tenggattini S, Fabiano A, et al. Atherosclerosis and oxidative stress. *Histol Histopathol.* 2008; 23:381–390. [PubMed: 18072094]
7. Delles C, Miller WH, Dominiczak AF. Targeting reactive oxygen species in hypertension. *Antioxid Redox Signal.* 2008; 10:1061–1077. [PubMed: 18321202]
8. Tardif JC, Grégoire J, L'Allier PL. Prevention of restenosis with antioxidants: mechanisms and implications. *Am J Cardiovasc Drugs.* 2002; 2:323–334. [PubMed: 14727962]
9. Movahed A, Nair KG, Ashavaid TF, et al. Free radical generation and the role of allopurinol as a cardioprotective agent during coronary artery bypass grafting surgery. *Can J Cardiol.* 1996; 12:138–144. [PubMed: 8605635]
10. Son SM, Whalin MK, Harrison DG, et al. Oxidative stress and diabetic vascular complications. *Curr Diab Rep.* 2004; 4:247–252. [PubMed: 15265465]
11. Shi Y, Patel S, Davenpeck KL, et al. Oxidative stress and lipid retention in vascular grafts: Comparison between venous and arterial conduits. *Circulation.* 2001; 103:2408–2413. [PubMed: 11352892]
12. McCormick ML, Gavrila D, Weintraub NL. Role of oxidative stress in the pathogenesis of abdominal aortic aneurysms. *Arterioscler Thromb Vasc Biol.* 2007; 27:461–469. [PubMed: 17218601]
13. Dimmeler S, Zeiher AM. Reactive oxygen species and vascular cell apoptosis in response to angiotensin II and pro-atherosclerotic factors. *Regul Pept.* 2000; 90:19–25. [PubMed: 10828488]
14. Yung LM, Leung FP, Yao X, et al. Reactive oxygen species in vascular wall. *Cardiovasc Hematol Disord Drug Targets.* 2006; 6:1–19. [PubMed: 16724932]
15. Satoh K, Nigro P, Berk BC. Oxidative stress and vascular smooth muscle cell growth: A mechanistic linkage by cyclophilin A. *Anti-oxid Redox Signal.* 2010; 12:675–682.
16. Ambros V. MicroRNA pathways in flies and worms: Growth, death, fat, stress, and timing. *Cell.* 2003; 113:673–676. [PubMed: 12809598]
17. Farh KK, Grimson A, Jan C, et al. The widespread impact of mammalian MicroRNAs on mRNA repression and evolution. *Science.* 2005; 310:1817–1821. [PubMed: 16308420]
18. Pasquinelli AE, Hunter S, Bracht J. MicroRNAs: A developing story. *Curr Opin Genet Dev.* 2005; 15:200–205. [PubMed: 15797203]
19. Bartel DP. MicroRNAs: Genomics, biogenesis, mechanism, and function. *Cell.* 2004; 116:281–297. [PubMed: 14744438]
20. Lin Y, Liu X, Cheng Y, et al. Involvement of MicroRNAs in hydrogen peroxide-mediated gene regulation and cellular injury response in vascular smooth muscle cells. *J Biol Chem.* 2009; 284:7903–7913. [PubMed: 19158092]
21. Cheng Y, Liu X, Zhang S, et al. MicroRNA-21 protects against the H₂O₂-induced injury on cardiac myocytes via its target gene PDCD4. *J Mol Cell Cardiol.* 2009; 47:5–14. [PubMed: 19336275]
22. Ji R, Cheng Y, Yue J, et al. MicroRNA expression signature and antisense-mediated depletion reveal an essential role of MicroRNA in vascular neointimal lesion formation. *Circ Res.* 2007; 100:1579–1588. [PubMed: 17478730]
23. Liu X, Cheng Y, Zhang S, et al. A necessary role of miR-221 and miR-222 in vascular smooth muscle cell proliferation and neointimal hyperplasia. *Circ Res.* 2009; 104:476–487. [PubMed: 19150885]
24. Cheng Y, Liu X, Yang J, et al. MicroRNA-145, a novel smooth muscle cell phenotypic marker and modulator, controls vascular neointimal lesion formation. *Circ Res.* 2009; 105:158–166. [PubMed: 19542014]
25. Cechetto DF, Diab T, Gibson CJ, et al. The effects of propofol in the area postrema of rats. *Anesth Analg.* 2001; 92:934–942. [PubMed: 11273930]

26. Dam M, Ori C, Pizzolato G, et al. The effects of propofol anesthesia on local cerebral glucose utilization in the rat. *Anesthesiology*. 1990; 73:499–505. [PubMed: 2393135]
27. Andrews DT, Leslie K, Sessler DI, et al. The arterial blood propofol concentration preventing movement in 50% of healthy women after skin incision. *Anesth Analg*. 1997; 85:414–419. [PubMed: 9249123]
28. Doi M, Gajraj RJ, Mantzaridis H, et al. 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*. 1997; 78:180–184. [PubMed: 9068338]
29. Kazama T, Takeuchi K, Ikeda K, et al. Optimal propofol plasma concentration during upper gastrointestinal endoscopy in young, middle-aged, and elderly patients. *Anesthesiology*. 2000; 93:662–669. [PubMed: 10969298]
30. Adachi YU, Satomoto M, Higuchi H, et al. Rapid fluid infusion therapy decreases the plasma concentration of continuously infused propofol. *Acta Anaesthesiol Scand*. 2005; 49:331–336. [PubMed: 15752398]
31. Otani H. Ischemic preconditioning: From molecular mechanisms to therapeutic opportunities. *Antioxid Redox Signal*. 2008; 10:207–247. [PubMed: 17999631]
32. Das M, Das DK. Molecular mechanism of preconditioning. *IUBMB Life*. 2008; 60:199–203. [PubMed: 18344203]
33. Dong S, Cheng Y, Yang J, et al. MicroRNA expression signature and the role of microRNA-21 in the early phase of acute myocardial infarction. *J Biol Chem*. 2009; 284:29514–29525. [PubMed: 19706597]

Figure 1.

The effects of propofol on vascular smooth muscle cell (VSMC) viability. Cell viability was determined in cultured rat VSMCs by MTT assay at 24 hrs after different treatments. *A*, Propofol (10–50 μ M) and vehicle (0.05% DMSO) had no effect on cell viability in VSMCs without hydrogen peroxide (H_2O_2) treatment ($n = 8$). *B*, The dose-dependent effect of H_2O_2 on VSMC viability. The effect of H_2O_2 on VSMC viability is dose-dependent. At 200 μ M of H_2O_2 , the cell viability was only ~60% ($n = 8$; $*p < .05$ compared with vehicle control [0 group]). *C*, The effect of propofol on cell viability in H_2O_2 -pretreated VSMCs. The relative cell viability in H_2O_2 -pretreated (200 μ M) VSMCs without propofol (0 group) was expressed as 100% ($n = 8$; $*p < .05$ compared with the control [0 group]). *DMSO*, dimethyl sulfoxide.

Figure 2.

The effect of propofol on vascular smooth muscle cell (VSMC) death. Confluent rat VSMCs cultured with serum-free medium were treated with vehicle (phosphate-buffered saline) or hydrogen peroxide (200 μM) for 30 mins. Then, the cells were treated with vehicle (0.05% dimethyl sulfoxide) or different concentrations of propofol (10, 30, or 50 μM) for 24 hrs. Cell death was determined in cultured rat VSMCs by trypan blue exclusion test 24 hrs after different treatments (n = 8; * $p < .05$ compared with vehicle control [0 group]).

Figure 3.

Propofol has no effect on cell apoptosis in vascular smooth muscle cells (VSMCs) without hydrogen peroxide (H_2O_2) treatment. Rat VSMCs cultured with serum-free medium were treated with vehicle (0.05% dimethyl sulfoxide) or propofol (50 μ M) for 6 hrs. Apoptosis was measured by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining. *A*, Quantitative analysis of VSMC apoptosis. *B*, Representative TUNEL-stained photomicrographs of VSMCs treated with vehicle or propofol. *Dapi*, 4',6-diamidino-2-phenylindole.

Figure 4.

Pro-injury effect of propofol in vascular smooth muscle cells (VSMCs) pretreated with hydrogen peroxide (H_2O_2). Rat VSMCs cultured with serum-free medium were treated with vehicle (phosphate-buffered saline) or H_2O_2 (200 μ M) for 30 mins. Then, the cells were treated with vehicle (0.05% dimethyl sulfoxide) or different concentrations of propofol (10, 30, or 50 μ M) for 6 hrs. Cell apoptosis was measured by terminal deoxynucleotide transferase dUTP nick end labeling (TUNEL) staining. *A*, Quantitative analysis of VSMC apoptosis. *B*, Representative terminal deoxynucleotide transferase dUTP nick end labeling-stained photomicrographs of VSMCs treated with H_2O_2 or H_2O_2 plus different concentrations of propofol ($n = 8$; $*p < .05$ compared with H_2O_2 -alone group). *Dapi*, 4',6-diamidino-2-phenylindole.

Figure 5.

The effects of propofol on the expression of cleaved caspase-3 in vascular smooth muscle cells (VSMCs). Rat VSMCs cultured with serum-free medium were treated with vehicle (phosphate-buffered saline) or hydrogen peroxide (H_2O_2) (200 μ M) for 30 mins. The cells were treated with vehicle (0.05% dimethyl sulfoxide) or different concentrations of propofol (10, 30, or 50 μ M) for 6 hrs. The expression of cleaved caspase-3 and intact caspase-3 were measured by Western blot analysis. *A* and *B*, Propofol had no effect on the expression of caspase-3 in VSMCs without H_2O_2 treatment. *A*, Representative Western blots. *B*, Quantitative analysis of protein levels. *C* and *D*, Propofol increased the expression of both cleaved caspase-3 and intact caspase-3 in VSMCs pretreated with H_2O_2 . *C*, Representative Western blots. *D*, Quantitative analysis of protein levels ($n = 8$; * $p < .05$ compared with H_2O_2 -alone group). *GAPDH*, glyceraldehyde 3-phosphate dehydrogenase.

Figure 6.

miR-21 is involved in propofol-mediated pro-injury effects on vascular smooth muscle cells (VSMCs). Rat VSMCs cultured with serum-free medium were treated with vehicle (phosphate-buffered saline) or hydrogen peroxide (H_2O_2) (200 μ M) for 30 mins. Then, the cells were treated with vehicle (0.05% dimethyl sulfoxide) or propofol (50 μ M) for 6 hrs. Before the treatment of H_2O_2 , one group of VSMCs was pretreated with premiR-21 (30 nM) for 4 hrs to up-regulate the expression of miR-21. Oligo (30 nM) was used as the control of premiR-21. **A**, miR-21 expression was increased by H_2O_2 . Although propofol itself had no significant effect on miR-21 in VSMCs without H_2O_2 treatment, H_2O_2 -induced up-regulation of miR-21 was significantly attenuated by propofol ($n = 8$; $*p < .05$ compared with vehicle; $\#p < .05$ compared with H_2O_2 -alone group). **B**, miR-21 was increased by premiR-21 ($n = 6$; $*p < .05$ compared with vehicle). **C**, Propofol-mediated increase in VSMC apoptosis was inhibited by premiR-21, but not by control oligo ($n = 8$; $*p < .05$ compared with vehicle; $\#p < .05$ compared with H_2O_2 -alone group). **D**, Representative terminal deoxynucleotide transferase dUTP nick end labeling (TUNEL)-stained photomicrographs of VSMCs with different treatments. **E**, Propofol increased programmed cell death 4 (PDCD4) expression in VSMCs pretreated with H_2O_2 . Dapi, 4',6-diamidino-2-phenylindole.

Figure 7.

Propofol exacerbates cell apoptosis in rat vascular walls with increased reactive oxygen species. Balloon angioplasty was performed in rat carotid arteries to increase hydrogen peroxide (H_2O_2) in vascular walls. Sham-opened group was used as the normal uninjured control. At 4 days after surgery, the animals were treated with vehicle or propofol ($12 \text{ mg/kg}^{-1}\text{hr}^{-1}$, intravenous) for 6 hrs and the apoptosis was determined in vessel sections by terminal deoxynucleotide transferase dUTP nick end labeling (*TUNEL*) staining and the expression of the cleaved caspase-3. *A*, Representative *TUNEL*-stained vessel sections from animals with different treatments. *B*, Quantitative analysis of *TUNEL*-stained apoptotic cells in vessels from animals with different treatments. *C*, Representative Western blots of the cleaved caspase-3 and intact caspase-3 in vessels from animals with different treatments. *D*, Quantitative analysis of the expression of cleaved caspase-3 and intact caspase-3 in vessels from animals with different treatments ($n = 8$; $*p < .05$ compared with uninjured control; $\#p < .05$ compared with balloon-injury treated with vehicle). *GAPDH*, glyceraldehyde 3-phosphate dehydrogenase.