Propofol Anesthesia Compared to Awake Reduces Infarct Size in Rats
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Background: Propofol has not been studied directly in animals subject to cerebral ischemia in the conscious state. Strokes are usually induced in animals while they are anesthetized, making it difficult to eliminate anesthetic interactions as a complicating factor. Therefore, to compare the neuroprotective effects of propofol to the unanesthetized state, experiments were performed using a model that induces a stroke in the conscious rat.

Methods: Cerebral ischemia was induced in awake Wistar rats by a local intracerebral injection of the potent vasoconstrictor endothelin. Four days before the strokes were induced, a guide cannula was implanted for the injection of endothelin. On the day of the experiment, endothelin (6.0 pmol in 3 μl) was injected into the striatum. Propofol (25 or 15 mg · kg⁻¹ · h⁻¹) or intralipid (vehicle) were infused for 4 h starting immediately after the endothelin injection. In another series, the propofol infusion was begun 1 h after the endothelin injection and continued for 4 h. Three days later, the animals were killed, and the brains were sectioned and stained.

Results: The propofol group (25 mg · kg⁻¹ · h⁻¹) had a significantly reduced infarct size (0.7 ± 0.21 mm³, first 4 h; 0.27 ± 0.07 mm³, started 1 h after initiation of infarct) compared with the intralipid controls (3.40 ± 0.53 mm³). To exclude a direct interaction between propofol and endothelin, in thiobutabarbital anesthetized rats, endothelin-induced cerebral vasoconstriction was examined using videomicroscopy, with or without propofol. Propofol had no effect on the magnitude or time course of the endothelin-induced vasoconstriction.

Conclusions: The results show that concurrent or delayed administration of propofol is neuroprotective.

ADDITIONAL research is needed to identify which anesthetics are most effective at conferring neuroprotection. Anesthetic agents are a diverse group of compounds with the capability of producing many simultaneous extracellular and intracellular effects. Recent work has established the concept that anesthesia contributes to neuroprotection against ischemic injury by mechanisms such as decreasing body temperature, cerebral metabolism, and antioxidant activity. It also is necessary to discover how potently, how rapidly, and in what time frame the desirable effects are achieved because sedatives and anesthetics, especially at low doses, could be ideal in many applications, such as before, during, and after surgery, neuroradiology, and intensive care.

Propofol (2,6-disopropylphenol) has properties that suggest it could be beneficial in preventing or ameliorating focal cerebral ischemia. These properties include reduction in cerebral metabolism, potentiation of γ-aminobutyric acid-mediated inhibition, altered cerebral blood flow (which may beneficially redistribute flow), and its antioxidant ability. Propofol contains a phenolic hydroxyl (OH) group, which confers antioxidant activity by scavenging free radicals.

Previous experiments using animal models of stroke found evidence suggesting that propofol is neuroprotective. Neuroprotection by propofol anesthesia has been compared with halothane⁷ and pentobarbital⁸ anesthesia, but these studies did not directly establish neuroprotection by propofol because propofol-anesthetized rats were not compared with rats undergoing the injury while awake. Therefore, to eliminate potential anesthetic interactions and to compare the neuroprotective effects of propofol in the unanesthetized state, a series of experiments was performed using a model that induces stroke in the conscious rat via an intracerebral injection of endothelin. This is a novel model of focal ischemia modified from the method of Sharkey and Butcher⁹ and using the potent vasoconstrictor peptide, endothelin-1. Microinjections of endothelin-1 result in a reproducible pattern of focal cerebral infarction in the conscious animal. It also remains to be determined which dose and duration of administration of propofol are protective because only deep anesthetic doses that lowered arterial blood pressure have been considered.

In the studies reported herein, we addressed the following questions: (1) Does a light surgical depth of propofol anesthesia reduce infarct size? (2) Does a sedative dose of propofol reduce infarct size? (3) If administration of propofol is delayed 1 h, does it still reduce infarct size?

Material and Methods

Conscious Endothelin Model
These experiments were approved by the Animal Care Committee at the University of Western Ontario (London, Ontario, Canada). Adult male Wistar rats (250–350 g) were anesthetized with an intraperitoneal injection of pentobarbital (40 mg/kg). Body temperature measured via a rectal thermometer was maintained at...
37°C throughout the surgical period. For subsequent propofol or intralipid infusion, the right femoral vein was cannulated, and the catheter was run subcutaneously and exteriorized at the dorsal aspect of the neck. The animals were placed in a David Kopf stereotaxic apparatus (David Kopf Instruments, Tujunga, CA), and a small burr hole (2 mm in diameter) was drilled in the skull over the striatum. A stainless steel guide cannula (23 gauge; 0.0 mm anterior, 3.0 mm lateral to bregma, 1.0 mm below the dura mater) was implanted into the brain and fixed in place to the skull with dental acrylic. The wounds were sutured closed, and the animal allowed to recover for 4 days.

After recovery from the initial surgery, an injection cannula (30 gauge) was inserted into the guide cannula with the tip 4.0 mm below the bottom of the guide cannula. This resulted in an injection approximately into the center of the striatum. An injection of 6.0 pmol endothelin (Sigma-Aldrich Canada Ltd., Oakville, Ontario, Canada) in 3 μl saline was made into the striatum over a 2-min time period. The injection cannula was left in place after the endothelin injection for a period of 5 min to prevent leakage of the endothelin up the injection cannula tract.

Propofol, 25 mg · kg⁻¹ · h⁻¹ in seven rats or 15 mg · kg⁻¹ · h⁻¹ in nine animals, was infused for a 4-h period immediately after the injection of endothelin. In five rats, an infusion of propofol at 25 mg · kg⁻¹ · h⁻¹ was made beginning 1 h after the injection of endothelin and continuing for an additional 4 h. Each of these experimental groups had a control group consisting of equal volumes of an intralipid infusion in seven, nine, and five rats, respectively. During the propofol infusion period, body temperature was continuously monitored through a rectal thermometer and maintained at 37°C using a heating pad.

Three days after the endothelin injection, the rats were reanesthetized with pentobarbital and perfused transcardially with 0.9% phosphate-buffered saline followed by 4% paraformaldehyde. After perfusion, the brains were removed, and coronal sections (40 μm) were cut on a freezing microtome and distributed sequentially into two series. One series was stained with thionine, and the other was stained with hematoxylin and eosin. The sections were examined with a microscope (Wild Leitz Canada Ltd., Willowdale, Ontario, Canada), and the area of the infarct was measured using a computerized imaging system (Mocha; Jandel Scientific, Chicago, IL). In addition, the hemispheric volumes for both sides were measured to determine whether there was any swelling of the brain after the stroke (ratio of the contralateral to ipsilateral hemisphere multiplied by the area of the infarct for each brain section). The area measurements were made by an investigator blinded to the identity of the brain sections. The volume of infarct was calculated in cubic millimeters by integrating the infarct measurements for each of the sections. The infarct size was corrected based on a comparison of size of both hemispheres to account for any differences that might have occurred because of brain swelling.

Monitoring of Physiologic Variables

Previously, we have demonstrated in 10 animals that propofol (25 mg · kg⁻¹ · h⁻¹) compared with intralipid infused for 6 h did not significantly change arterial pressure or heart rate. This finding was confirmed in two additional animals in the current experiments. A transmitter (TLHM2-C5-AXT; Data Sciences International, St. Paul, MN) for recording arterial blood pressure, heart rate, and body temperature was implanted in the abdominal cavity (with a cannula inserted into the descending aorta) at the time of the initial surgery for the insertion of the femoral cannula and the guide cannula. The abdominal wound was sutured closed, and the animal was allowed to recover for 4 days. On the day of the endothelin injection and the infusion of propofol, the animals were monitored continuously for echocardiogram, arterial blood pressure, heart rate, and temperature using telemetry. These values were then analyzed using the Dataquest LabPRO software package (Data Sciences International).

Brain Temperature Recordings

In the previous groups of animals, body temperature was continuously monitored with a rectal thermometer and maintained at 37°C using a heating pad and an overhead lamp. However, in an additional eight rats, brain temperature was monitored during the propofol and intralipid infusions and for the next 3 days of survival. In these animals, on the same day of the surgery, for the insertion of the femoral and guide cannulas, a brain probe holder consisting of a short segment of polyethylene tubing (ID, 8 mm) was cemented to the skull over the occipital cortex contralateral to the guide cannula.

On the day of the experiment, the brain temperature probe (XM-FH-BP, Mini-Mitter, Bend, OR) was inserted into this holder with the tip of the probe 6 mm into the occipital cortex. The brain temperature was monitored for 1 h before the injection of endothelin. Then, the experiments continued as described with propofol (25 mg · kg⁻¹ · h⁻¹) or intralipid being infused immediately after the injection of endothelin for a duration of 4 h. Monitoring of brain temperature continued for an additional 1 h after the end of the drug or vehicle infusion. Brain temperature was also monitored for 1 h at 24, 48, and 72 h after the drug or vehicle infusions. Brain temperature values were analyzed using the Dataquest LabPRO software package.
Cerebrovascular Vasconstriction

It is possible that propofol might directly interact with the endothelin action on the cerebrovasculature and change the size of the infarct by inhibiting the action of endothelin. To determine whether this was the case, a separate series of experiments were performed in which the vasoconstricting action of endothelin was examined with and without administration of propofol.

Six male Wistar rats were anesthetized with Inactin (thiobutabarbitral, 100 mg/kg; RBI, Natick, MA). Inactin in the rat is a long-lasting barbiturate anesthetic agent that does not suppress arterial blood pressure and provides a steady state for several hours. Body temperature was continuously monitored and maintained at 37°C with a heating pad. The middle cerebral artery was exposed and a segment was selected and viewed on a computer screen via a video camera on a surgical microscope. Infusions of propofol or the vehicle (intralipid) were initiated through the femoral cannula. Ten minutes after the onset of the infusions, the dura mater covering the middle cerebral artery was removed, and endothelin (200 μM solution) was applied topically. A segment of the middle cerebral artery was chosen between two branches, and images of the segment were captured before, at 30 s, and at 10-min intervals after the application of endothelin. The area of the middle cerebral artery segment was measured using an image analysis program (Mocha; Jandel Scientific, Chicago, IL).

An image of the artery was obtained, a line was carefully drawn around the outside of the segment that was chosen, and the area was calculated by the computer.

Data Analysis

The volumes of the infarcts for each of the groups were subjected to an analysis of variance, and the Dunnnett multiple comparison test was used to compare among the integrated volume of infarct. Analysis of variance and the Tukey post hoc test were used to determine significant changes in the area of the middle cerebral artery segments in the second series of experiments. A probability level of 0.05 or less was considered to be significant. Data are expressed as mean ± standard error of the mean.

Results

Infarct Volumes

Injection of 6.0 pmol endothelin in 3 μl saline over a 2-min period resulted in a circumscribed infarct restricted to the striatum in all of the control animals. No injury was observed in the contralateral hemisphere. A typical example of the infarct obtained with the endothelin injections is shown in figure 1. For the control brains, the infarcts were usually oval in shape and generally seemed to be similar in size in both the rostral–caudal and medial–lateral directions. Some of the infarcts seemed to be extended slightly in the dorsal–ventral direction, which might have been due to some of the endothelin leaking upward along the injection cannula. The infarct size in animals receiving only the intralipid beginning immediately after the endothelin injection was 2.48 ± 0.69 mm³ for the 25-mg · kg⁻¹ · h⁻¹ group, 3.65 ± 0.91 mm³ for the 15-mg · kg⁻¹ · h⁻¹ group, and 3.25 ± 1.22 mm³ for the group corresponding to the 25-mg · kg⁻¹ · h⁻¹ dosage started 1 h after the stroke. The infarct sizes for the three intralipid series were not significantly different from each other and were thus pooled (fig. 2). The hemisphere volumes in both groups of animals did not differ from one side to the other, and the corrected value did not change the significance of the results.

Fig. 1. Photomicrographs of the infarcts in two representative brains of animals receiving either 25 mg · kg⁻¹ · h⁻¹ propofol immediately after the endothelin-1 injection (bottom) or the intralipid control infusion (top). The photomicrographs are from coronal sections of the rat brain stained with thionine. The dashed outline shows the extent of the infarct in each of these animals.
Infarct Area

Fig. 2. Bar graph illustrating the changes in infarct size of the groups of animals treated with propofol or the control infusion with intralipid. *Significant difference from control ($P < 0.05$).

In the series in which propofol at 25 mg·kg$^{-1}$·h$^{-1}$ was infused for 4 h beginning immediately after the endothelin injection, the animals seemed heavily sedated or anesthetized with little or no movement and did not show any response to the eye blink reflex elicited by touching the cornea or any withdrawal reflex resulting from pinching the hind paw. In these animals, the infarct size (0.70 ± 0.21 mm$^3$) was significantly smaller than that of the intralipid control group. It can be seen from the photomicrograph (fig. 1) and the coronal sections (fig. 3A) illustrating these results that the infarct seems to be reduced in all directions, but particularly in the medial–lateral and rostral–caudal directions.

The animals receiving the 15-mg·kg$^{-1}$·h$^{-1}$ dosage of propofol were heavily sedated but still retained the eye blink reflex to touching the cornea or a withdrawal reflex to the pinch of the paw. The animals did exhibit some movements during the time the propofol was infused; however, these were limited in nature and usually consisted of crawling into a corner of the cage to sleep. In these animals, the shape of the infarct did not seem different from that of the intralipid control group, and the infarct volume (2.35 ± 0.86 mm$^3$) was not significantly different (fig. 2).

In the third series, which received propofol at 25 mg·kg$^{-1}$·h$^{-1}$ beginning 1 h after the endothelin injection, the animals were heavily sedated or anesthetized as in the first series. The infarct in these animals was greatly reduced in size, particularly in the medial–lateral and rostral–caudal directions (fig. 3B). The infarct size was 0.27 ± 0.21 mm$^3$, which was significantly less than that of the intralipid control group (fig. 2).

Physiologic Variables

The body temperatures of the animals infused with propofol were all maintained at 37 ± 0.5°C. It has previously been shown that regulation of body temperature is a critical factor in the determination of the effects of anesthetics on focal ischemic brain damage.\textsuperscript{12} Our previous results in 10 animals that were monitored continuously for arterial blood pressure and heart rate and that received propofol at 25 mg·kg$^{-1}$·h$^{-1}$ for 6 h were shown to have no significant change in arterial blood pressure or heart rate.\textsuperscript{10} In an additional two animals in this series, telemetry was used to assess the effects of the propofol infusion on arterial blood pressure and heart rate. As seen in the example in figure 4, the animals remained stable.

Brain Temperature Monitoring

Figure 5 illustrates the temperature of the brain in the occipital cortex contralateral to the injection of the endothelin for both propofol and intralipid infusion groups. During the infusion, when the body temperature of the propofol infusion animals is maintained with a
heating pad and lamp, there is no difference in the brain temperature compared with the intralipid group. In addition, on days 1, 2, and 3, when the brain temperature is monitored in unrestrained animals in their home cage, there is no difference between the propofol- and intralipid-treated groups.

Propofol and Endothelin Interactions

In the control (intralipid) animals, the application of endothelin (20 μm) to the middle cerebral artery resulted in an immediate (30 s) decrease in the area of the segment. The vasoconstriction was severe enough so that only the vessel walls were apparent in the microscopic image, resulting in a total block of the artery and a complete cessation of blood flow. A progressive recovery of the vessel diameter was observed with time. The initial vasoconstriction resulted in a decrease in the measured area to 43.3 ± 1.3% of the initial. By 10 min, a small amount of recovery had occurred, with some restoration of blood flow. However, 30 min after the blockade of the artery, this value was still 66.3 ± 1.86% of the original area, indicating that substantial blockade of the artery remained at this time. The extent or recovery of the blood vessel from endothelin-induced vasoconstriction was not significantly different between propofol- or intralipid-treated groups at any of the time intervals measured (Fig. 6). At 1 h, the vessel area had recovered to 85% of the initial area, and blood flow could be observed under the microscope.

Discussion

These results indicate that propofol at a dosage of 25 mg·kg⁻¹·h⁻¹, when administered concurrent with ischemia or when delayed 1 h, significantly decreased the infarct size compared with intralipid controls when assessed 3 days after the ischemic event. Propofol at a sedative dosage, 15 mg·kg⁻¹·h⁻¹, was not effective. The decrease in infarct size is indicative of the neuroprotective effects of propofol anesthesia in focal cerebral ischemia compared with the awake state.
None of the animals were given vehicle injection only in the absence of endothelin because the purpose of the investigation was to compare the effects of propofol on the infarct. Part of the lesion may be due to trauma created by the injection procedure. An additional technical consideration is whether the body temperature or even the brain temperature of the animals was significantly different, accounting for the observed neuroprotection with propofol. Ischemia can evoke an increase in temperature, and even a modest change in temperature can significantly alter the outcome of the infarct. However, our results show that there is no significant difference in brain temperature between the propofol- and the intralipid-treated animals, either during the drug or vehicle infusion or in the 3-day survival period.

A final technical consideration is that we did not measure blood gas variables in this study. There may have been some changes in blood gas levels, especially in the propofol-treated animals. The protocol involved inducing a stroke in the conscious animal several days after the surgery. We would have had to insert an arterial cannula and maintain its viability for more than a week to obtain blood for these measures. In the awake animals, this would be a very disruptive procedure. However, it is likely that the propofol administration would have changed blood gases in such a manner as to result in a worsening of the infarct, and it is unlikely that these changes can account for the highly significant reduction in the infarct size.

Other studies have also reported similar neuroprotection mediated by propofol anesthesia during cerebral ischemia. Our data are also in agreement with those of Kochs et al., who reported that propofol, given in doses that produce electroencephalographic burst suppression, improves neurologic outcome and decreases neuronal death after incomplete ischemia compared with fentanyl–nitrous oxide anesthesia. Although we did not measure electroencephalographic activity in our study, the dosage of propofol used by Kochs et al. was 2–3 times greater than what we used, and our animals were only lightly anesthetized, so it is unlikely that there was electroencephalographic burst suppression. There remains controversy whether burst suppression is needed for cerebral protection. Based on our results and those of Warner et al., it is possible to suggest that although maximal metabolic suppression is needed with agents that produce protection only by metabolic suppression (e.g., inhaled anesthetics), with agents that produce protection by other mechanisms in addition to metabolic suppression (e.g., antioxidants), lower doses suffice, although direct evidence for this hypothesis is not available.

Previous studies have not directly compared propofol neuroprotection in the awake state. Various anesthetics have been used as the control state, and when compared with propofol, no differences in neuronal damage were observed between treatments. For example, Ridenour et al. found no protective advantage of propofol when compared with halothane during focal cerebral ischemia. It is possible that both agents were neuroprotective, and as such, no apparent neuroprotective advantage was noted with propofol. Pittman et al. found a similar result. They concluded that although the outcome from cerebral ischemia was similar for pentobarbital and propofol, their study did not directly establish neuroprotection by propofol because propofol-anesthetized rats were not compared with conscious animals. Our research contributes to defining the neuroprotective effects of propofol compared in the awake state and determining the level and timing of administration of propofol necessary to produce maximum neuroprotection.

Studies comparing the effects of anesthetics with the nonanesthetic state in focal ischemia have been limited by methodologic and ethical difficulties. More than 30 yr ago, a few studies that examined the effects of various anesthetics in global ischemia demonstrated that all except nitrous oxide improved outcome. However, current interpretation of these studies is confounded by poorly controlled parameters, such as temperature and blood pressure, as well as outcome measures, e.g., survival. A limited number of focal ischemia studies have been recently performed using a filament threaded up the carotid artery during a brief anesthetic administration. Both halothane and sevoflurane were found to be protective. The protection provided by propofol in our study, both during and after ischemia, is similar in magnitude to that of halothane with mild hypothermia. This may suggest that general anesthetics produce a similar amount of protection based on a common mechanism, e.g., γ-aminobutyric acid receptor type A potentiation, and that additional benefits reflect additional protective properties.

This study did not directly investigate the mechanisms by which propofol may reduce infarct size. However, we can speculate on a number of mechanisms. Propofol, like most other general anesthetics, reduces cerebral metabolic rate for oxygen consumption (CMRO₂), probably reflecting γ-aminobutyric acid receptor type A potentiation. However, there is not a close correlation between the reduction in CMRO₂ and the extent of cerebral protection. Furthermore, because the delayed administration of halothane did not reduce the infarct size, CMRO₂ reduction may only have an effect, if any, if achieved prophylactically. Propofol has been shown to be a potent antioxidant in microsomal suspensions. Other investigations have also shown the antioxidant properties of propofol against reactive oxygen species in which propofol has been shown to inhibit lipid peroxidation during oxidative stress in cell-free systems and in peripheral tissues. In astrocytes, glutamate uptake was restored by an effect on the outside but not on the inside of the astrocyte. Therefore, it is likely that the
phenolic OH group of propofol may be neuroprotective by scavenging active oxygen species released into the extracellular medium. Propofol also inhibits the mitochondrial permeability pores and causes suppression of sodium influx through voltage-dependent sodium channels. Any or all of these mechanisms could be relevant in explaining the beneficial effects found in the current study.

In the studies examining the interaction of endothelin vasoconstriction and propofol, we demonstrated that at 1 h, the arterial vessel diameter had recovered to levels greater than 85% of the initial value. This suggests that reperfusion occurs by 1 h after endothelin-induced vasoconstriction. This time point also coincides with the delayed onset of the propofol infusion at 25 mg·kg\(^{-1}\)·h\(^{-1}\) in the one group and could help to explain why the delayed administration of propofol yielded such good results. During ischemia and especially during reperfusion, there is an increase in free radicals, which can cause neuronal damage unless they are scavenged by antioxidant enzymes. Propofol, through its free radical antioxidant properties or by rescuing endogenous antioxidant enzymes, could thus be more beneficial when it is administered 1 h after ischemia at the onset of reperfusion because this would allow for the entire 4 h of infusion to occur during reperfusion. Similarly at this time, as the endothelin-induced vasoconstriction dissipates, the cerebral vasoconstriction in normal brain associated with propofol anesthesia would be expected to redirect blood flow into the focal ischemic area, thereby preserving more penumbral regions.

The method we used to produce ischemia has previously been used. A major advantage over the more frequently used techniques of coagulating or of advancing a fine suture up the carotid artery is that anesthesia is not required at all for the induction of ischemia and is therefore eliminated as a potential confound. The stereotaxic placement of the catheter creates the potential to study the effects of ischemia in brain regions of interest. The injection of endothelin creates intense vasoconstriction, which, as shown by our video microscopy, lasts approximately 1 h. More prolonged ischemia could be produced by repeated injections. A potential confound is that pharmacologic agents, such as propofol, could directly interact with endothelin and prevent vasoconstriction, which could be misinterpreted as cerebral protection. Our video microscopy study shows that this is not the case.

In summary, this study showed that a light surgical depth of anesthesia substantially reduced cerebral infarct size relative to the awake state. Even when administration of propofol was delayed 1 h after the onset of ischemia, a significant benefit was still apparent. Such a benefit became inconsistent and therefore not statistically significant when the dose was reduced to a light sedative concentration.

References


