Free radical and drug oxidation products in an intensive care unit sedative: Propofol with sulfite*

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Objectives: Some propofol emulsion formulations contain EDTA or sodium metabisulfite to inhibit microbe growth on extrinsic contamination. EDTA is not known to react with propofol formulation components; however, sulfite has been shown to support some oxidation processes and may react with propofol. This study compared the oxidation of propofol and the formation of free radicals by electron paramagnetic resonance analysis in EDTA and sulfite propofol emulsions during a simulated intensive care unit 12-hr intravenous infusion.

Design: Controlled laboratory study.

Setting: University laboratory.

Measurements and Main Results: Propofol emulsions (3.5 mL) were dripped from spiked 50-mL vials at each hour for 12 hrs. Two propofol oxidation products, identified as propofol dimer and propofol dimer quinone, were detected in sulfite and EDTA propofol emulsions; however, sulfite propofol emulsion contained higher quantities of both compounds. After initiation of the simulated infusion, the quantities of propofol dimer and propofol dimer quinone increased in the sulfite propofol emulsion, but the lower levels in the EDTA propofol emulsion remained constant. Sulfite propofol emulsion began to visibly yellow at about 6–7 hrs. The EDTA propofol emulsion remained white at all times. The absorbance spectra of the propofol dimer and propofol dimer quinone extracted from sulfite propofol emulsion showed that propofol dimer did not absorb in the visible spectrum, but the propofol dimer quinone had an absorbance peak at 421 nm, causing it to appear yellow. Electron paramagnetic resonance analysis of the propofol emulsion containing metabisulfite revealed that the sulfite propofol emulsion yielded a strong free radical signal consistent with the formation of the sulfite anion radical (SO₃⁻). The EDTA propofol emulsion yielded no free radical signal above background.

Conclusion: Sulfite from the metabisulfite additive in propofol emulsion creates an oxidative environment when these emulsions are exposed to air during a simulated intravenous infusion. This oxidation results in propofol dimerization and emulsion yellowing, the latter of which is caused by the formation of propofol dimer quinone. These processes can be attributed to the rapid formation of the reactive sulfite free radical. (Crit Care Med 2003; 31:787–792)

Key Words: sulfite; propofol; metabisulfite; propofol oxidation; free radicals; EDTA; propofol dimer; propofol dimer quinone

Propofol (2,6-diisopropylphenol) is an intravenous sedative that is administered continuously to a wide variety of critically ill patients, including those having coronary artery bypass grafts, adult respiratory distress syndrome, asthma, head trauma, status epilepticus, pneumonia, and sepsis (according to manufacturer prescribing information from AstraZeneca Pharmaceuticals and Gensia Sicor Pharmaceuticals). Propofol is insoluble in aqueous media (propofol octanol/H₂O partition coefficient = 6800:1) and therefore is formulated in a lipid-based vehicle. The current commercial propofol formulations are oil-in-H₂O emulsions consisting of propofol (1%, 10 mg/mL), soybean oil (100 mg/mL), egg yolk lecithin (12 mg/mL), and glycerol (22.5 mg/mL) in H₂O (1). Because these emulsions can support the growth of bacteria and yeast when extrinsically contaminated (2), an agent to retard the growth of microorganisms is deemed necessary. Sodium metabisulfite (Na₂S₂O₅, 0.25 mg/mL) (3, 4) or EDTA (0.05 mg/mL) (5) are added to some propofol emulsions for that purpose.

Drug additives are usually considered inactive formulation components; however, there is evidence that sulfite is responsible for promoting the oxidation of propofol and lipids in emulsions. For example, when propofol emulsions containing metabisulfite (sulfite propofol emulsion) are exposed to air, a propofol dimerized product appears (6). A yellow coloration is also sometimes noted after exposure of the sulfite propofol emulsion to air (7–10). In addition, a comparative analysis of the sulfite propofol emulsion and propofol emulsion containing EDTA (EDTA propofol emulsion) showed that the sulfite propofol emulsion, but not the EDTA propofol emulsion, contained variable quantities of the lipid peroxidation product, malondialdehyde, which increased over time after air exposure (11).

Propofol dimer is a product of a one-electron oxidation of propofol to form propofol radicals that couple with one another, generating the dimer product (6). Lipid peroxidation is a free radical process involving the formation of lipid radicals in the presence of oxygen (12). It is proposed that these sulfite-promoted chemical processes are initiated by the formation of sulfite-derived radicals.

The precise conditions for sulfite-supported oxidation of propofol in sulfite propofol emulsions remain unclear. Furthermore, the detection of free radicals in emulsions of propofol has not been examined. Twelve hours is the recommended hang time for propofol emulsion in in-
Materials and Methods

Chemicals. Fifty-milliliter vials of EDTA propofol emulsion and authentic propofol dimer and propofol dimer quinone were obtained from AstraZeneca Pharmaceuticals (Wilmington, DE). The propofol dimer and propofol dimer quinone standards had purities of >97%. Propofol emulsion containing metabisulfite, also in 50-mL vials, was purchased from Gensia Sicor Pharmaceuticals (Irvine, CA).

Simulated Intravenous Infusion. Vials of propofol emulsion were dripped by inserting a Flu-Vent vented spike and tubing (Flu Ven 0153-C, Venusa, El Paso, TX) into intact, properly stored, commercially supplied vials of propofol emulsion. The vials were inverted and hung on a ringstand at room temperature under ambient fluorescent lighting. The tubing from the vented spikes was cut to 30 cm. Emulsion release was regulated using the roller valve attached to the spike tubing. At zero time, 3.5 mL of emulsion were dripped out and collected for analysis. At each hour for up to 12 hrs, an additional 3.5-mL aliquot was released, collected, and analyzed.

Propofol Product Analysis. One milliliter of collected emulsion was treated with 100 µL of a 10% NaCl solution to crack the emulsion. Ethyl acetate (1 mL) was added to the 1-mL cracked emulsion, and the mixture was vigorously shaken, followed by centrifugation to separate the phases. Approximately 100 µL of the ethyl acetate phase was taken for liquid chromatography/mass spectral analysis.

Liquid Chromatography/Mass Spectral Analysis of Propofol Products. The ethyl acetate extracts from propofol emulsions were analyzed on a Surveyor liquid chromatography/mass spectral analysis system (LCQ Deca MS system, ThermoFinnigan, San Jose, CA) having a photodiode array detector linked in series to the mass spectrometer detector. The mass spectrometer detector utilized atmospheric pressure chemical ionization and a normalized collision energy of 40%. This instrument was equipped with a Discovery HS C-18 MS analytical column (75 × 2.1 mm ID, 3 µm) fitted with a guard column. Propofol and propofol product separation involved the use of a mobile phase gradient system. The initial mobile phase was methanol/3 mM ammonium acetate (70/30, v/v) that was run at a flow rate of 250 µL/min for 3 mins. This was followed by an increase in flow rate to 350 µL/min and a linear gradient to methanol (100%) over 2 mins and an isocratic period of 100% methanol for 4 mins. A sample size of 5 µL was injected, and fractions in the eluate were scanned from 200 to 500 nm. The data were collected using the Xcalibur Software system (Herndon, VA). Propofol dimer was quantitated by its absorbance at 266 nm and propofol dimer quinone by its absorbance at 421 nm. Standard curves were constructed using standard propofol dimer and propofol dimer quinone.

Electron Paramagnetic Resonance Spectroscopy. Electron paramagnetic resonance (EPR) spectra were recorded with a Bruker EMX EPR spectrometer (Bruker Instruments, Billerica, MA) at room temperature. Samples were prepared by adding 5,5-dimethyl-1-pyrroline-N-oxide (DMPO, final concentration of 25 mM) to the propofol emulsions (total volume of 0.5 mL) from commercially supplied vials. EPR spectra were collected immediately after sample preparation. The emulsions were maintained at room temperature at all times. All spectra were collected using identical conditions: each represents the signal-averaged result of five scans; scan rate, 80 G/84 s; microwave power, 40 mW with a TM cavity and 10 mm flat cell; frequency, 9.77 GHz; modulation amplitude, 1.00 G; time constant, 82 ms.

Values are reported as mean of duplicate or triplicate determinations (±si). Statistics were performed by analysis of variance repeated measures (Sheffe’s F test). p Values of ≤.05 were considered significant.

Results

High-pressure liquid chromatography analysis of ethyl acetate extracts of propofol emulsions revealed the presence of two major fractions that could be identified as propofol oxidation products. The first fraction chromatographed at approximately 6.3–6.5 mins in the system used and was identified as propofol dimer. The absorbance spectrum from 200 to 500 nm of this peak showed an absorbance peak at 266 nm and no absorbance in the visible range. This compound chromatographed identically with authentic propofol dimer, and it exhibited the same absorbance spectrum as authentic propofol dimer, confirming that the propofol dimer is a visibly colorless compound.

The electrospray mass spectra of the propofol dimer confirms the presence of propofol dimer in these propofol emulsions. Propofol dimer (molecular weight = 354 Da) poorly ionizes in electrospray, as does propofol, due to the stability of the hydroxy groups to ionization. However, some M'- (mass-to-change ratio [m/z] 354) and MH' (m/z 355) ions are seen, as is the diagnostic m/z 313 (MH'...
The compound called propofol dimer chromatographed identically with peak absorbance of 421 nm. This product exhibited absorption in the ultraviolet and visible range, having a major peak absorbance at 228 nm and a lesser peak absorbance of 421 nm. This product chromatographed identically with the compound called propofol dimer.

Mass spectral analysis further confirmed the identity of the propofol dimer quinone (Fig. 1), a previously unreported quinone and had a similar absorbance spectrum as the standard. The standard propofol dimer quinone appeared purple in crystalline form but was yellow in solution.

Figure 2. Propofol dimer in sulfite propofol emulsion and EDTA propofol emulsion during a simulated 12-hr infusion. Values represent the mean (+SD) of triplicate determinations from three 50-mL vials of each propofol formulation. Sulfite propofol emulsions used were: one vial each of lots O0N301 (expiration date, November 2002), O1A317 (expiration date, January 2003), and O1K310 (expiration date, August 2003). EDTA propofol emulsions used were two vials of lot 4017F (expiration date, June 2003) and one vial of lot 4359B (expiration date, July 2001). *Values were significantly different from corresponding 0-hr values.

Figure 3. Propofol dimer quinone in sulfite propofol emulsion and EDTA propofol emulsion during a simulated 12-hr infusion. Values represent the mean (+SD) of triplicate determinations from three 50-mL vials of each propofol formulation. The vials used are listed in the legend to Figure 2. *Values were significantly different from corresponding 0-hr values.

There was no increase in propofol dimer quinone, and an increase in propofol dimer quinone concentration in sulfite propofol emulsion was also higher than in EDTA propofol emulsion and, like the propofol dimer, increased over time. The greatest rates of increase occurred from 6 hrs onward. The propofol dimer quinone in EDTA propofol emulsion did not increase over time.

The formation of propofol dimer quinone in sulfite propofol emulsion and EDTA propofol emulsion during the simulated infusion is shown in Figure 3. Initial propofol dimer quinone concentrations in sulfite propofol emulsion were also higher than in EDTA propofol emulsion and, like the propofol dimer, increased over time. The greatest rates of increase occurred from 6 hrs onward. The propofol dimer quinone in EDTA propofol emulsion did not increase over time, and the emulsion remained visibly white at all times.

Some of the yellowed sulfite propofol emulsion (1 mL) was treated with the chemical reductant sodium dithionite (1 mg) and examined by high-pressure liquid chromatography. Dithionite caused a bleaching of the yellow color, a decrease in propofol dimer quinone, and an increase in propofol dimer (data not shown).

EPR Analysis. Figure 4 shows the EPR spectra obtained from the two different propofol formulations on exposure to air. The propofol emulsion that contained metabisulfite yields an EPR spectrum consistent with the spin trapping by DMPO of the sulfite-radical anion (SO$_3$$^-$): $a^H = 16.0$ G, $a^N = 14.5$ G (13, 14). The propofol emulsion containing EDTA rather than metabisulfite yielded no radical EPR signal above background. The intensity of the EPR spectrum of the sulfite anion radical spin adduct on a simulated intravenous infusion remained relatively constant over 12 hrs (Fig. 5).
DISCUSSION

This study demonstrates that the sulfite propofol emulsion, but not the EDTA propofol emulsion, undergoes chemical changes during a simulated intravenous infusion. Compounds identified as propofol oxidation products, propofol dimer and propofol dimer quinone, increased during the simulation. The occurrence and identities of propofol dimer and propofol dimer quinone are confirmed by the facts that they are extractable from propofol emulsions with an organic solvent, they chromatograph identically with authentic compounds by high-pressure liquid chromatography, and they have identical mass spectral and ultraviolet-visible absorbance profiles as the standards.

The sulfite free radical is confirmed by the detection of the DMPO/SO$_3^-$ radical adduct that results from the spin trapping of the sulfite radical by the spin trap DMPO (Fig. 6) (13, 15). This radical was detected at a relatively constant level for the entire 12-hr time frame of the simulated intravenous infusion.

The increases of propofol dimer and propofol dimer quinone in sulfite propofol emulsion demonstrate that sulfite from metabisulfite is in some manner creating a strong oxidative environment when air is introduced. Although sulfite primarily functions as an antioxidant (4), its ability to cause oxidation in certain environments is consistent with several lines of evidence. For example, sulfite has been shown to cause the peroxidation of lipids in various lipid and fatty acid emulsions (16–18). It has also been shown to cause the peroxidation of lipids in propofol emulsions (11). Mechanisms have been proposed that involve sulfite-facilitated formation of lipid radicals (17). These lipid radicals, generated at unsaturated moieties, result in the formation of lipid peroxides. These subsequently
Sulfite causes oxidation of propofol after exposure of the drug to air during a simulated intravenous infusion.

In addition, chemical studies of sulfite in the presence of oxygen have revealed that several sulfite-derived oxidant species form (13, 15, 22, 23). Sulfite undergoes two types of oxidation, a two-electron oxidation leading to the sulfate anion ($\text{SO}_3^{2-} \rightarrow \text{SO}_4^{2-}$) and a one-electron oxidation leading to the formation of the reactive sulfite radical ($\text{SO}_3^{-} \rightarrow \text{SO}_4^{-}$). The sulfite radical has been shown to react with oxygen, forming two strong oxidant species, the sulfite peroxy radical ($\text{SO}_3^{-} \rightarrow \text{SO}_2\text{O}$) and the sulfite radical ($\text{SO}_2^{-} \rightarrow \text{SO}_4^{-}$). Lesser quantities of superoxide are also generated (13). These species, and the sulfite radical itself, may react with lipid to cause lipid radical formation. Furthermore, any of these species may also directly or indirectly initiate a one-electron oxidation of propofol, yielding the propofol radical (24) and, subsequently, propofol dimer and propofol dimer quinone.

Although propofol dimer and propofol dimer quinone required some time to increase in quantity in this study, EPR analysis shows that the sulfite radical is rapidly formed when emulsion is withdrawn from the vial. The delayed oxidation of propofol relative to sulfite radical formation could be due to oxygen requirements needed to form sufficient quantities of products, or due to physical factors such as phase separation of reactants. Sulfite-derived radicals are $\text{H}_2\text{O}$ soluble and localize in the aqueous phase of the emulsion, whereas propofol and lipid localize in the soybean oil microdroplets (1). The lack of detection of other proposed radical species (e.g., lipid radical or propofol radicals) may be due to the presence of large quantities of the sulfite radical, which would diminish any other radical signals, or may be due to the fact that the DMPO, when added to emulsion, traps the sulfite radical and thereby inhibits secondary radical processes.

The simultaneous occurrence and concurrent increases of both propofol dimer quinone and propofol dimer, indicate that these two compounds are interrelated. Indeed, the propofol dimer quinone, which always occurs in slightly lesser quantities than propofol dimer, is likely a direct oxidation product of propofol dimer. A one-electron oxidation of propofol dimer will yield a propofol dimer radical; however, a two-electron oxidation of propofol dimer would be expected to yield the quinone compound (Fig. 7).

The observation that the strong redoxant dithionite causes a loss of propofol dimer quinone and yellow color and an increase in propofol dimer confirms that these compounds can interconvert, depending on oxidative or reducing conditions; this observation suggests that the dimer may redox cycle. Furthermore, it indicates that the yellow appearance of sulfite propofol emulsions can be attributed to the presence of sufficient quantities of propofol dimer quinone after emulsion oxidation. The violet region (400–430 nm) of the visible electromagnetic spectrum is complementary to yellow; therefore, a compound having an absorbance band at 421–422 nm, as does the propofol dimer quinone, will appear yellow. Previously, it has been speculated that yellowing of sulfite-containing propofol emulsion after air exposure is due to the presence of oxidized sulfite species (8). However, sulfite and its oxidized products are $\text{H}_2\text{O}$ soluble and will not extract into ethyl acetate as does propofol dimer quinone and the yellow color from a yellowed sulfite propofol emulsion. The ability of the yellow propofol dimer quinone to convert to the colorless propofol dimer also indicates that emulsion yellowing is not always a reliable indicator of the extent of propofol oxidation.

Even though this study evaluated a simulated infusion from 50-mL vials over 12 hrs, intensive care unit patients receiving propofol sedation at the recommended rates of 1–3 mg·kg$^{-1}$·hr$^{-1}$ or less could receive propofol from a single 100-mL vial over 12 hrs. For example, a 50-kg person receiving an average dose of 1.68 mg·kg$^{-1}$·hr$^{-1}$ (28 µg·kg$^{-1}$·min$^{-1}$) (25) will require 11.9 hrs to deplete a 100-mL vial of 1% propofol. Longer hang times may occur at lower doses (lower infusion rates), with smaller patients, or with intermittent infusions from a single vial. Of interest is that the highest rates of increase in propofol dimer and propofol dimer quinone occurred from 6 to 12 hrs after initiation of the simulation. Because of this result and the continued presence of radical, these oxidation processes are expected to continue and possibly accelerate after a 12-hr period.

The immediate occurrence of sulfite radical in dripped-out emulsion and its persistence in a hanging vial over 12 hrs not only implicates sulfite radical in emulsion oxidation, but demonstrates that this radical is an infused chemical entity even during short propofol administrations. The in vivo consequences of intravenous infusion of these chemical entities are not known. The sulfite radical is a chemically reactive intermediate (26). It can, for example, cleave albumin (27) and adduct to uracil (28). Sulfite radical species are also thought to be involved in sulfite-induced lung and neuronal damage via sulfite’s ability to stimulate neutrophils and activate the respi-
The generic sulfa is rated therapeutically equivalent to the EDTA propofol emulsion (34). However, we demonstrated that there are significant differences between the formulations in regard to chemical reactivity that may be associated with detrimental processes. Other differences, including drug compatibilities, have also been observed (35). The effects of sulfite in propofol emulsions need to be further studied.

CONCLUSIONS

The results presented here show that sulfite causes oxidation of propofol after exposure of the drug to air during a simulated intravenous infusion. The reactivity of sulfite can be attributed to the formation of a reactive sulfite radical.

REFERENCES

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