Japanese Journal of Veterinary Research 67(3): 209-214, 2019

JJVR

SHORT COMMUNICATION Experimental Research

Hydroxyl radical formation in lactated Ringer's solution and BSS Plus® intraocular irrigating solution

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Received for publication, November 15, 2018; accepted, February 14, 2019

Abstract

Free radicals generated during phacoemulsification, a method of cataract surgery, have been shown to damage corneal endothelial cells. We compared hydroxyl radical formation (HRF) in lactated Ringer's solution (LRS) and BSS Plus® intraocular irrigating solution, as well as physiological saline solution (PSS) and Ringer's solution (RS). Hydroxyl radicals were generated by a phacoemulsification machine or UV irradiation in the presence of hydrogen peroxide. We measured DMPO-hydroxyl radical adducts using ESR. HRF did not significantly differ between LRS and BSS Plus®, and tended to be smaller in LRS and BSS Plus® than in PSS or RS. Even if corneal damage after phacoemulsification in LRS is different from that in BSS Plus®, the influence of HRF on this difference may be small.

Key Words: free radical, hydroxyl radical, phacoemulsification

Phacoemulsification is a common cataract surgery technique for both animals and humans. In phacoemulsification, a fine metal needle-like tip (US tip) vibrates at ultrasonic frequency, thereby emulsifying and aspirating the crystalline lens. However, a possible complication is corneal endothelial damage, which can lead to corneal opacification; this may be caused by collisions of nuclear fragments or surgical instruments with the corneal endothelium and heat or free radicals generated by vibration of an US tip in the anterior chamber. In vitro and in vivo studies showed that free radicals are generated by phacoemulsification[5,9,19–21] and can damage corneal endothelial cells[6,12,16].

Ultrasound irradiation in liquid can cause cavitation, involving the formation, growth, and collapse of small gas bubbles, associated with the generation of extremely high temperatures and pressures and leading to the generation of hydroxyl radicals and hydrogen atoms via the dissociation of water[17–19]. Hydroxyl radicals are highly reactive free radicals, and various studies have examined their generation and elimination during phacoemulsification[5,20,21]. Hydroxyl radicals are unstable in solution at room temperature, making them difficult to detect; however, nitrone or nitroso compounds can react with free radicals

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doi: 10.14943/jjvr.67.3.209

to form more stable spin adducts. 5,5-Dimethyl-1-pyrroline N-oxide (DMPO) is a widely used reactive nitrone that reacts with hydroxyl radicals to form DMPO-hydroxyl radical adducts (DMPO-OH) (Fig. 1), which can then be detected by electron spin resonance (ESR).

In phacoemulsification, the anterior chamber is irrigated with intraocular irrigation solution, of which BSS Plus® is commercially available and widely used. BSS Plus® has ion composition, osmolarity and pH similar to those of aqueous humor and also contains glucose, bicarbonate and oxiglutathione. In veterinary medicine, in addition to such commercially available irrigating solutions, use of lactated Ringer's solution (LRS) in phacoemulsification surgery is also documented[7]. LRS is somewhat less expensive than commercially available irrigating solutions, such as BSS Plus®, and has been used in phacoemulsification[1,7,11,15,22].

A clinical study in humans showed that corneal damage after phacoemulsification was greater when LRS was used as intraocular irrigating solution compared to when BSS Plus® was used[22]. Another study with human subjects showed no significant difference in postoperative corneal changes between BSS Plus® and LRS in uncomplicated phacoemulsification surgeries[11]. However, the author also mentioned that when LRS was used, a trend was seen toward lower endothelial cell density in surgeries with longer phacoemulsification time and higher irrigation volumes[11].

As LRS is used in phacoemulsification surgery, its characteristics compared with commercially available irrigating solutions warrant investigation in several respects. In the present study, we compared LRS with BSS Plus® for production of hydroxyl radicals, a highly reactive free radical. If free radical formation is greater in LRS, its use might be made safer by taking measures to reduce free radicals. In addition, as LRS is usually used for fluid therapy, we also tested hydroxyl radical formation (HRF) in other solutions used for fluid therapy, such as

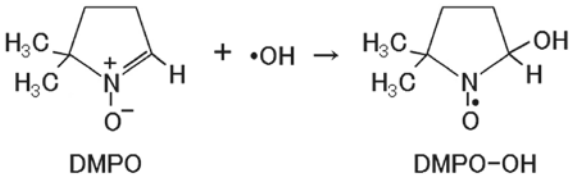

Fig. 1. DMPO-OH formation from DMPO and •OH.

physiological saline solution (PSS) and Ringer's solution (RS), which were used as intraocular irrigation solutions[1,11].

In the present study, ESR was measured to determine HRF in various solutions, including PSS (Otsuka Normal Saline; Otsuka Pharmaceutical, Tokushima, Japan) containing (in mM) sodium chloride (154); LRS (Solulact; Terumo, Tokyo, Japan) containing (in mM) sodium chloride (102.6), potassium chloride (4.0), calcium chloride dehydrate (1.4) and sodium L-lactate (27.7); BSS Plus® (Alcon Laboratories, Inc., Texas, USA) containing (in mM) NaCl (122.1), potassium chloride (5.1), calcium chloride dehydrate (1.0), magnesium chloride hexahydrate (27.7), dibasic sodium phosphate (3.0), dextrose (4.6) and oxidized glutathione (0.3); RS (Ringer's solution Otsuka; Otsuka Pharmaceutical, Tokushima, Japan) containing (in mM) sodium chloride (147), potassium chloride (4.0) and calcium chloride dehydrate (2.2), and pure water. BSS Plus® had been mixed as two solutions according to instructions.

In experiment 1 (phacoemulsification method), hydroxyl radicals were generated by a phacoemulsification machine in a solution, and ESR was measured. We added DMPO (LABOTEC Co., Ltd., Tokyo, Japan), a spin trapping agent, to all solutions at a final concentration of 27 mM. A solution (1.5 ml) containing DMPO was put into a 1.5 ml disposable polyethylene tube. The straight part of an US tip of a phacoemulsification machine handpiece (Universal, Alcon, Texas, USA) was placed under the upper level of the solution. The machine turned on by stepping on a foot pedal for 15 seconds with ultrasound power of the machine at 90% without irrigation. Immediately after the 15 seconds of ultrasound

application, small volume of the solution in the 1.5 ml tube was sucked into a capillary tube (VC-H075P, Terumo, Tokyo, Japan) for ESR measurement. ESR measurement was performed with an X-band ESR spectrometer (JES TE-100 JEOL Co., Ltd., Tokyo, Japan) controlled by a Win-Rad ESR data analyzer (Radical Research, Inc., Tokyo, Japan) under the following conditions: microwave power 5 mW, microwave frequency 9.43 GHz, magnetic field 335.8 mT, field sweep width, ± 5 mT, field modulation 100 kHz, modulation amplitude 0.079 mT, sweep time 1 min, response time 0.1 s.

In experiment 2 (H_2O_2-UV irradiation method), hydroxyl radical was generated by UV irradiation in the presence of hydrogen peroxide, and tested with the same solutions as in experiment 1. Hydrogen peroxide (100 mM at final concentration) and DMPO (27 mM at final concentration) were added to all solutions. A solution containing hydrogen peroxide and DMPO was sucked into a capillary tube, which was irradiated with a glass fiber-type UV irradiator (RUVF-203 S, Radical Research, Inc, Tokyo, Japan; Hg-Xe lamp; power, 200 W) for 5 seconds through an RUV-29 filter (>240 nm), and its ESR spectrum was then recorded. Immediately after the irradiation, ESR was measured as described above.

Statistical analysis used the Kruskal–Wallis test followed by Dunn's multiple comparison test. $P < 0.05$ was considered significant.

Fig. 2 shows representative ESR signals consisted of four lines with a ratio of 1 : 2 : 2 : 1 after sonication in various solutions. The hyperfine coupling constants were estimated as $a_N = a_H = 1.49$ mT using computer simulation, and the spectra were assigned to DMPO-OH[3,4]. ESR signal intensities among solutions in experiment 1 are compared in Fig. 3. The ESR signal intensities did not significantly differ between BSS Plus® and LRS, but were significantly lower in BSS Plus® than in water or PSS. Signal intensities did not significantly differ among LRS, RS, PSS and water.

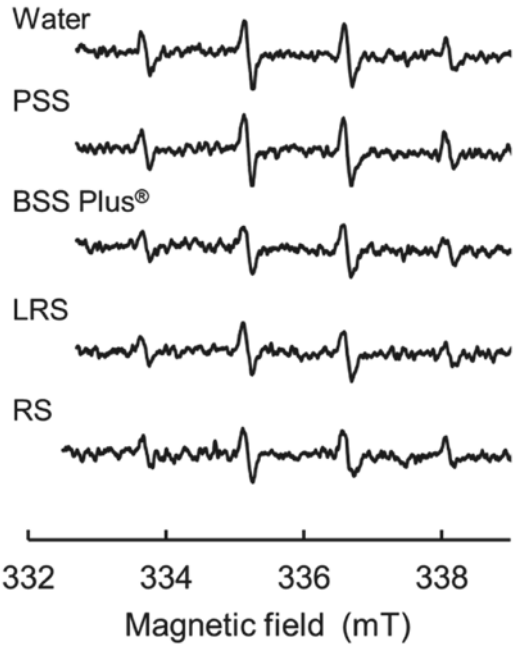

Fig. 2. Representative ESR signals consisted of four lines with a ratio of 1 : 2 : 2 : 1 after sonication in various solutions. The hyperfine coupling constants were estimated as $a_N = a_H = 1.49$ mT using computer simulation, and the spectra were assigned to DMPO-hydroxyl radical adducts (DMPO-OH).

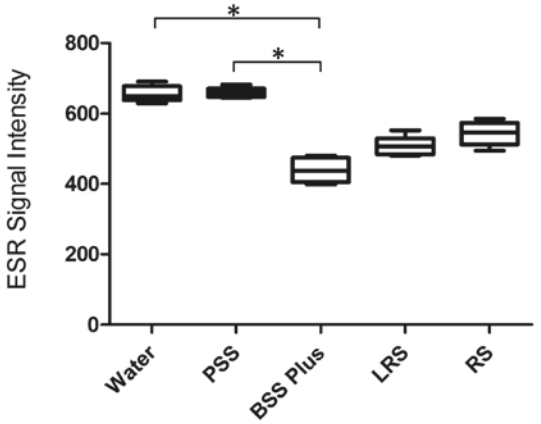

Fig. 3. Comparison of the amounts of DMPO-OH after sonication in various solutions, using five samples of each solution. *$P < 0.05$ between solutions.

Fig. 4 shows representative ESR signals consisted of four lines with a ratio of 1 : 2 : 2 : 1 after UV irradiation in the presence of hydrogen peroxide in various solutions. The hyperfine coupling constants were estimated as $a_N = a_H = 1.49$ mT using computer simulation, and the spectra were assigned to DMPO-OH[3,4]. ESR

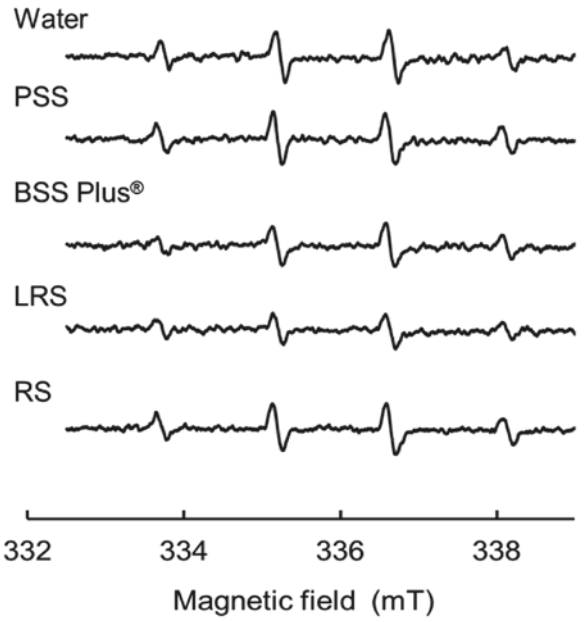

Fig. 4. Representative ESR signals consisted of four lines with a ratio of 1:2:2:1 after UV irradiation in the presence of hydrogen peroxide in various solutions. The hyperfine coupling constants were estimated as $a_N = a_H = 1.49$ mT using computer simulation, and the spectra were assigned to DMPO-hydroxyl radical adducts (DMPO-OH).

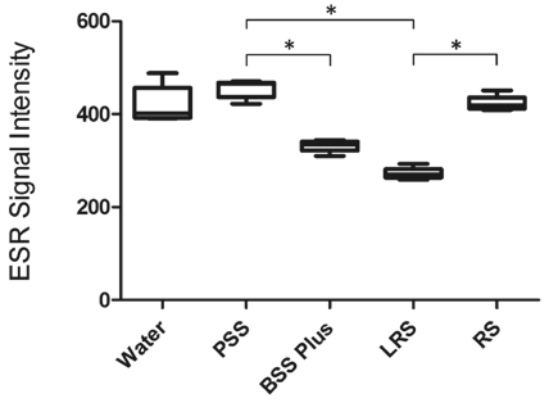

Fig. 5. Comparison of the amounts of DMPO-OH after UV irradiation in the presence of hydrogen peroxide in various solutions, using five samples of each solution. $^*P < 0.05$ between solutions.

signal intensities among solutions in experiment 2 are compared in Fig. 5. Signal intensities did not significantly differ between BSS Plus® and LRS, but was significantly less in BSS Plus® than in PSS, and significantly less in LRS than in PSS or RS.

In the present study, in addition to the phacoemulsification method, we evaluated HRF in various solutions using H_2O_2-UV irradiation method, since this is a commonly used method to study HRF. UV irradiation of hydrogen peroxide leads to the formation of hydroxyl radicals via the following reaction[23]:

$$H_2O_2 \xrightarrow{h\nu} 2\ {}^{\bullet}OH$$

In both experiments, the amounts of DMPO-OH did not significantly differ between BSS Plus® and LRS. We therefore considered that HRF in LRS was comparable to BSS Plus®.

In experiment 2, the amounts of DMPO-OH was smaller in LRS than in PSS and RS (Fig. 5), indicating less HRF in LRS than in PSS or RS. In experiment 1, the amounts of DMPO-OH in LRS was not greater than in PSS or RS (Fig. 3). Thus, if we use a solution for fluid therapy other than commercially available intraocular irrigation solution, LRS would be reasonable choice among solutions we tested here.

The presence of substances with hydroxyl radical-scavensing activity in the solution decreases the amount of hydroxyl radicals, and hence the amount of DMPO-OH. The effect of lactate to scavenge hydroxyl radicals has been shown. Lactate ions at 10–60 mM decreased the amounts of DMPO-OH[8]. The LRS we used included 27.7 mM lactate. Thus, lactate in LRS may have contributed to lower HRF in LRS than in PSS and RS when hydroxyl radical was generated by UV irradiation in the presence of hydrogen peroxide in the present study. However, the amounts of DMPO-OH did not significantly differ between LRS and RS when hydroxyl radicals were generated by a phacoemulsification machine; the reason for this is unknown.

BSS plus® contains oxiglutathione and dextrose. It was reported that dextrose at 30–100 mM decreased the amounts of DMPO-OH[10]. It was shown that oxiglutathione at 1–100 mM significantly suppressed the amounts of DMPO-OH in a dose dependent manner during phacoemulsification[21]. This study also showed that the amounts of DMPO-OH in BSS Plus® was

only slightly less than that in BSS®, which did not contain oxiglutathione and dextrose[21]. The author mentioned that the antioxidative value of BSS Plus® was relatively low. Gardner *et al.*, however, showed that HRF by a phacoemulsification machine was significantly less for BSS Plus® than for BSS® or deionized water[5]. In the present study, the amounts of DMPO-OH in BSS Plus® was significantly less than in PSS when hydroxyl radicals was generated by UV irradiation or by a phacoemulsification machine. Oxiglutathione or dextrose in BSS Plus® may have scavenged hydroxyl radicals in the present study.

Ultrasound irradiation in liquid can lead to the generation of hydroxyl radicals and hydrogen atoms via the dissociation of water [17,19]:

$$H_2O \rightarrow H\bullet + \bullet OH \quad (1)$$

Successive reactions of these radical species are as follows:

$$H\bullet + O_2 \rightarrow HO_2\bullet \quad (2)$$

$$HO_2\bullet + HO_2\bullet \rightarrow H_2O_2 + O_2 \quad (3)$$

$$HO_2\bullet \rightarrow H^+ + O_2\bullet^- \quad (4)$$

$$\bullet OH + \bullet OH \rightarrow H_2O_2 \quad (5)$$

Equations 3 and 5 indicate the disproportionation of these radical species. Equation 4 indicates that superoxide anion radical ($O_2\bullet^-$) should be more stable in alkaline solution.

The reaction scheme indicates both hydroxyl radical and superoxide anion radical (hydroperoxyl radical). Shimmura *et al.* also described the singlet oxygen formation[19]. Superoxide radical anion causes radical and disproportionation reactions. The former should be generally weaker than that of hydroxyl radical[13,14]. Singlet oxygen easily transfers to triplet oxygen losing its energy in aqueous systems[2]. Although these reactive oxygen species exert various biological effects by reacting with biomolecules (e.g., lipids, proteins and DNA[24]), to the best of our knowledge, the degree of involvement of each substance against corneal damage during phacoemulsification remains unknown. Hydroxyl radicals are highly reactive free radical species, and several studies have examined their generation and elimination during phacoemulsification[5,20,21]. In the present study, we therefore concentrated on the formation of hydroxyl radicals during phacoemulsification using the widely used nitrone-type spin trap, DMPO. DMPO reacts with hydroxyl radicals to form DMPO-OH (Fig. 1), which can then be detected by ESR. The effects of other reactive oxygen species should be study in the following work.

In conclusion, we found no significant difference in HRF between BSS Plus® and LRS when hydroxyl radicals were generated by a phacoemulsification machine or UV irradiation in the presence of hydrogen peroxide. Thus, even if corneal damage after phacoemulsification in LRS is different from that in BSS Plus®, the influence of HRF on this difference may be small. In addition, HRF in BSS Plus® and LRS were less or at least not greater than that in PSS or RS.

Conflict of interest

The authors have no conflict of interest to declare.

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