Original Article

The dose-response relationships of the direct scavenging activity of amide-based local anesthetics against multiple free radicals

Yukiko Sato,[1] Shigekiyo Matsumoto,[2] Kazue Ogata,[3] Kira Bacal,[4] Misato Nakatake,[3] Takaaki Kitano,[2] and Osamu Tokumaru[3,*]

[1]Department of Fundamental Nursing, School of Nursing and [2]Department of Anesthesiology, Oita University Faculty of Medicine, 1-1 Idaigaoka, Hasama-machi, Yufu, Oita 879-5593, Japan
[3]Department of Physiology, Faculty of Welfare and Health Sciences, Oita University, 700 Dannoharu, Oita 870-1192, Japan
[4]Medical Programme Directorate, Faculty of Medical and Health Sciences, University of Auckland, Bldg 507, 28 Park Ave, Grafton, Auckland 1010, New Zealand

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This study aimed to illustrate the dose-response relationships of the direct scavenging activity of amide-based local anesthetics against multiple free radicals *in vitro*. We have demonstrated that amide-type local anesthetics selectively and directly scavenge some free radicals. Three kinds of free radicals were eliminated by all the four local anesthetics examined. Mepivacaine, lidocaine, bupivacaine, and dibucaine scavenged hydroxyl radicals in dose-dependent manners. Ascorbyl free radicals were also scavenged in dose-dependent manners, and lastly singlet oxygen was scavenged in dose-dependent manners. Three other free radicals were not scavenged by all of the four local anesthetics; *tert*-butoxyl radical was scavenged by all the anesthetics examined but dibucaine, nitric oxide by mepivacaine but not by the other three, and tyrosyl radical by mepivacaine and lidocaine but not by the other two. Some free radicals (superoxide anion, *tert*-butyl peroxyl radical, DPPH) were not scavenged by any of the four local anesthetics. The local anesthetics were also shown to inhibit lipid peroxidation by TBARS assay. These results suggest that local anesthetics have antioxidant properties through their free radical scavenging activities.

***Key Words*: local anesthetics, free radical species, radical scavenging activity, oxidative stress**

In Japan, the number of elderly cases involving surgical procedures is expected to increase as we enter a super-aged society. Elderly patients are at high risk of perioperative complications from general anesthesia, and the need to use peripheral nerve blocks as an alternative to general anesthesia is increasing. Recently a number of papers have described the anti-inflammatory/anti-oxidative activity of local anesthetics. For example, a recent study demonstrated that lidocaine exhibited anti-inflammatory activity.(1) Das and Misra(2) reported that lidocaine scavenged hydroxyl radical and quenched singlet oxygen, but not superoxide anion. However, the relationship between local anesthetics and oxidative stress remains veiled.

Previous studies(1,2) only investigated metabolites or oxidized substances produced in oxidative reactions and did not investigate whether amide-based local anesthetics directly scavenge free radical species. Accordingly, the present study focused on the dose-response relationships of direct scavenging activity of amide-based local anesthetics against multiple free radicals *in vitro*.

Materials and Methods

Materials. The amide local anesthetics (mepivacaine, lidocaine, bupivacaine, and dibucaine, Fig. 1) were purchased from Sigma-Aldrich (St. Louis, MO). *tert*-Butyl hydroperoxide and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich. 5,5-Dimethyl-1-pyrroline-*N*-oxide (DMPO), *N*-methyl-3-(1-methyl-2-hydroxy-2-nitrosohydrazino)-1-propanamine (NOC7), and 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl 3-oxide (carboxy-PTIO) were purchased from Dojindo (Kumamoto, Japan). 2-(5,5-Dimethyl-2-oxo-2λ5-[1,3,2]dioxaphosphinan-2-yl)-2-methyl-3,4-dihydro-2H-pyrrole 1-oxide (CYPMPO) was purchased from Mikuni Pharmaceutical Industrial (Osaka, Japan). Sodium ascorbate, dimethyl sulfoxide, and hydrogen peroxide were purchased from Wako Pure Chemical Industries (Osaka, Japan). 2,2'-Azobis (2-amidinopropane) dihydrochloride (AAPH), Acid Red 94, and 4-hydroxy-2,2,6,6-tetramethylpiperidine (4-OH TEMP) were purchased from Tokyo Chemical Industry (Tokyo, Japan). Other reagents used were of the highest commercially available quality.

Electron spin resonance (ESR) spectrometry. ESR spectrometry was conducted as previously described.(3,4) Free radicals were quantified using an X-band ESR spectrometer (JES-RE1X; JEOL, Tokyo, Japan) with the operation software WIN-RAD ver. 1.20b (Radical Research Inc., Tokyo, Japan). The ultraviolet (UV)/visible (VIS) light source was a 200 W medium-pressure mercury/xenon arc (UVF-203S; San-Ei Electric, Osaka, Japan) with either a UV-transmitting-VIS-absorbing or a VIS-transmitting-UV-absorbing filter, where UV or VIS light was guided through a quartz light guide into the ESR sample cavity. Free radicals were produced in disposable glass micro-hematocrit capillary tubes (CS-HMT-502; Kimble Chase, Vineland, NJ). The typical instrument settings were as follows: room temperature (23°C); frequency 9.45 GHz with 100 kHz modulation; modulation width, 0.1 mT; time constant, 0.1 s; center field, 335.8 mT; sweep width, 7.5 mT; sweep time, 1 min. The microwave power was set such that the ESR signals were not saturated (4 mW).

Table 1 lists the production and trapping methods for the nine free radicals examined. Briefly, hydroxyl radicals were produced using a Fenton-type reaction and trapped with CYPMPO.(5) Superoxide anions were generated by the mixture of hypoxan-

*To whom correspondence should be addressed.
E-mail: ostokuma@oita-u.ac.jp

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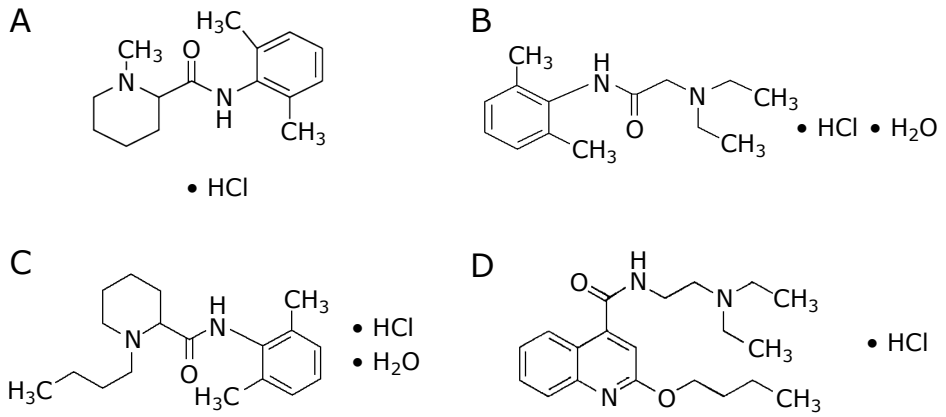

Fig. 1. Chemical structures of amide-based local anesthetics. (A) Chemical structures of mepivacaine (Sigma-Aldrich, MW 282.81, CAS 1722-62-9), (B) lidocaine (Sigma-Aldrich, MW 288.81, CAS 6108-05-0), (C) bupivacaine (Sigma-Aldrich, MW 342.90, CAS 73360-54-0), (D) dibucaine (Sigma-Aldrich, MW 379.92, CAS 61-12-1).

Table 1. Generation of free radicals

Free radical species	Precursor/sensitizer	Solvent	Spin trap/quencher
Hydroxyl radical	0.15% (49 mM) hydrogen peroxide + 0.05 mM $FeSO_4$	H_2O	0.25 mM CYPMPO
Superoxide anion	0.04 mM hypoxanthine + 5 μM DTPA + 0.1 U/ml XOD	Phosphate-buffer	0.5 mM CYPMPO
tert-Butyl peroxyl radical	80 mM *tert*-butyl hydroperoxide + 0.1 mM DTAPA + UV 20 s	Phosphate-buffer	5 mM CYPMPO
tert-Butoxyl radical	1.5 mM AAPH + UV 4 s	Phosphate-buffer	0.4 mM CYPMPO
Ascorbyl free radical	0.03 mg/ml sodium ascorbate + 99% DMSO	Phosphate-buffer	—
Singlet oxygen	0.2 mM rose bengal + 500–600 nm light 60 s	H_2O	1.7 mM 4-OH TEMP
Nitric oxide	0.14 mM NOC7	Phosphate-buffer	0.01 mM carboxy-PTIO
DPPH	15 μM DPPH	Ethanol	—
Tyrosyl radical	0.1 mM myoglobin + 0.002% (0.7 mM) hydrogen peroxide	H_2O	0.7 M DMPO

thine dissolved with phosphate buffer (pH 7.4), xanthine oxidase, and CYPMPO as a spin-trap agent.[5] The *tert*-butyl peroxyl was produced by UV irradiation of *tert*-butyl hydroperoxide and trapped with CYPMPO.[5] The *tert*-butoxyl radical was generated by UV irradiation of 1 mM AAPH and trapped with CYPMPO.[5] Ascorbyl free radicals were produced from sodium ascorbate by adding 99% dimethyl sulfoxide.[6] Singlet oxygen was generated by VIS light irradiation of Acid Red 94 with its quencher 4-OH TEMP, forming 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl radical (TEMPOL).[5] The nitric oxide radical was produced from NOC7 reacted with carboxy-PTIO, generating carboxy-PTI, which was measured 60 min after mixing.[5] DPPH was directly observed using ESR. Tyrosyl radical was generated from hemoglobin by adding hydrogen peroxide and trapped with DMPO.[7]

The third and fourth ESR signals of manganese (II) oxide (MnO) were used as external references for the ascorbyl free radical, singlet oxygen, DPPH, nitric oxide, and tyrosyl radical, and the second and fifth for CYPMPO-spin adducts. Ratios of the height of the target signal of the free radical to that of MnO were calculated, and then standardized relative to the control ESR signal with no local anesthetics added.

Calculation of 50% inhibitory concentration. Dose-response curves were nonparametrically calculated by fitting data to a sigmoid curve:[8]

$$y = \frac{1}{1 + \left(\frac{x}{a}\right)^b}$$

where a gives the estimation of 50% inhibitory concentration (IC_{50}), x is the final concentration of local anesthetics [M], and y is the observed free radical activity relative to the control.

Estimation of reaction rate constants. According to a kinetic competition model, the following competitive reactions occur in the reaction mixture:[9]

$$\text{spin trap} + \text{free radical} \rightarrow \text{spin-adduct}\ (k_{\text{trap}})$$

$$\text{local anesthetics} + \text{free radical} \rightarrow \text{local anesthetics-radical}\ (k_{\text{local anesthetics}})$$

where k_{trap} and $k_{\text{local anesthetics}}$ are the second-order rate constants. The reaction rate constant $k_{\text{local anesthetics}}$ is expressed as follows:

$$k_{\text{local anesthetics}} = \frac{[\text{spin trap}]}{\text{IC}_{50}} k_{\text{trap}}$$

k_{trap} used were as follows: k_{CYPMPO} for hydroxyl radical 4.2×10^9 M^{-1} s^{-1},[10,11] k_{CYPMPO} for superoxide anion 48 M^{-1} s^{-1},[10,11] and k_{cPTIO} for nitric oxide 1.01×10^4 M^{-1} s^{-1}.[12,13] Because k_{CYPMPO} for *tert*-butyl hydroperoxide and *tert*-butoxyl radical have not been reported, $k_{\text{local anesthetics}}$ for those free radicals were presented as relative values to k_{CYPMPO}.

TBARS assay. Peroxidation of mouse brain homogenate initiated by hydroxyl radicals was assessed using a thiobarbituric acid (TBA) reactive substance (TBARS) assay.[14] The brain tissue was employed because it is rich in lipids. The reaction mixture contained 0.1 ml homogenate in 40 volumes Tris-HCl buffer (20 mM, pH 7.4) and 0.1 ml of saline or local anesthetic

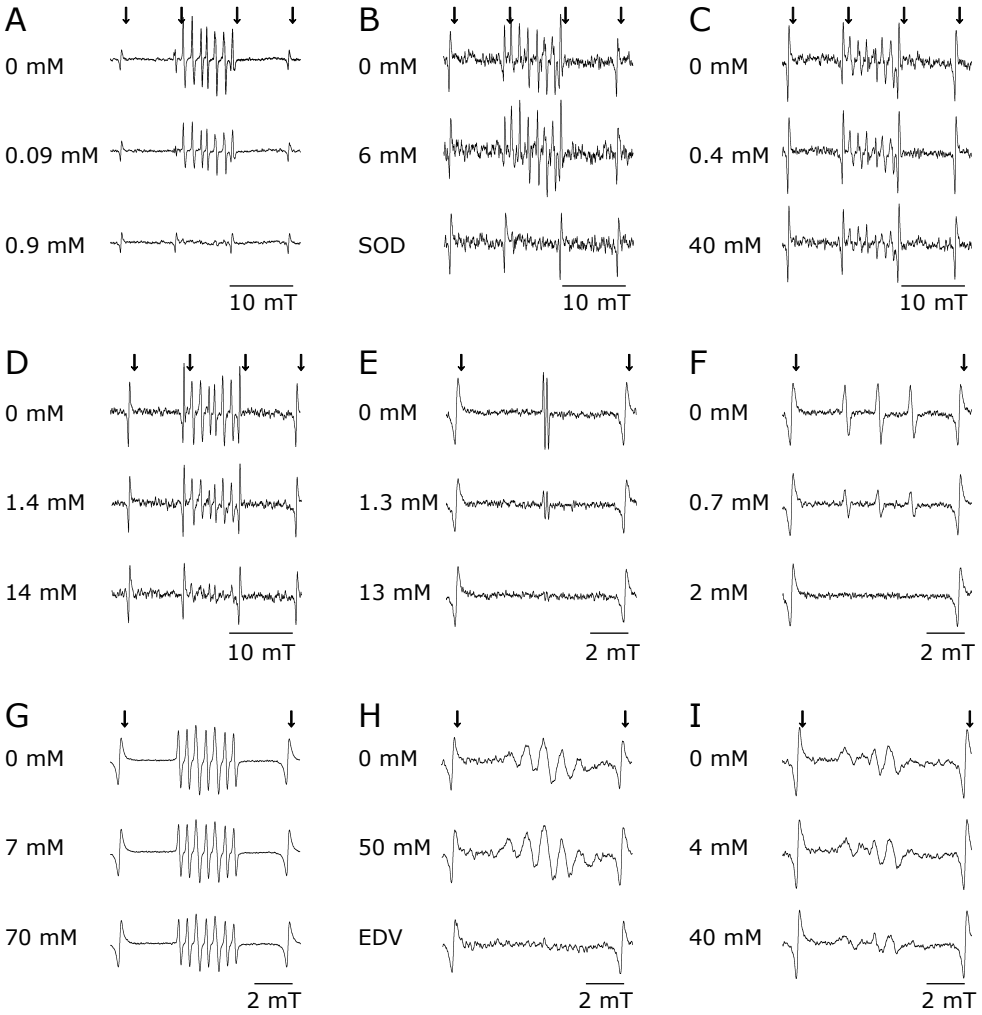

Fig. 2. Electron spin resonance (ESR) spectra of free radicals. Typical ESR spectra of free radicals with the addition of various concentrations of mepivacaine; hydroxyl radical (A), superoxide anion (B), *tert*-butyl peroxyl radical (C), *tert*-butoxyl radical (D), ascorbyl free radical (E), singlet oxygen (F), nitric oxide (G), DPPH radical (H) and tyrosyl radical (I). Signals on both ends of each spectrum, indicated by arrows, are those of the external standard of Mn^{2+}.

dissolved in saline. Peroxidation was initiated by adding 0.03 ml of $FeCl_3$ (100 mM). After incubation for 20 min at 37°C, the final product of lipid peroxidation (malondialdehyde, MDA) was reacted with TBA at 65°C for 45 min as indicated (TBARS Microplate Assay Kit, FR40; Oxford Biomedical Research, Oxford, MI). The MDA was quantified at 532 nm (Thermo Scientific™ Multiskan GO microplate spectrophotometer; Thermo Fisher Scientific, Vantaa, Finland). TBARS assay was conducted only on peroxidation initiated by hydroxyl radicals. Use of the brain tissue was approved by the Oita University Animal Ethics Committee (approval number 205801).

Statistical analysis. Statistical tests were performed by a statistical software R ver. 4.2.1 (https://www.R-project.org/).[15] Values are presented as mean ± 95% confidence interval. The significance level was set at $p<0.05$.

Results

The ESR spectra of the spin adducts for each radical examined are shown in Fig. 2. Each spectrum was assigned to the corresponding free radical by the hyperfine splitting constants (Table 2). Dose-response curves of the four local anesthetics are shown in Fig. 4.

Hydroxyl radical. Mepivacaine (Fig. 3A), lidocaine, bupivacaine, and dibucaine (Fig. 4A) scavenged hydroxyl radicals in dose-dependent manners. IC_{50} were 0.12 ± 0.01 mM, 0.088 ± 0.011 mM, 0.051 ± 0.008 mM, 0.054 ± 0.009 mM, respectively ($p<0.001$). Since the reaction rate constant of CYPMPO (k_{CYPMPO}) for hydroxyl radical is already known, $k_{mepivacaine}$, $k_{lidocaine}$, $k_{bupivacaine}$, $k_{dibucaine}$ were estimated to be 8.8×10^9 M^{-1} s^{-1} ($k_{mepivacaine}/k_{CYPMPO} = 2.1$), 1.2×10^{10} M^{-1} s^{-1} ($k_{lidocaine}/k_{CYPMPO} = 2.9$), 2.1×10^{10} M^{-1} s^{-1} ($k_{bupivacaine}/k_{CYPMPO} = 5.0$), 2.1×10^{10} M^{-1} s^{-1} ($k_{dibucaine}/k_{CYPMPO} = 5.0$), respectively.

Superoxide anion. Mepivacaine (Fig. 3B), lidocaine, bupivacaine, and dibucaine (Fig. 4B) did not scavenge superoxide anion at all.

***tert*-Butyl peroxyl radical.** Mepivacaine (Fig. 3C), lidocaine, bupivacaine, and dibucaine did not scavenge *tert*-butyl peroxyl radical at all (Fig. 4C).

***tert*-Butoxyl radical.** Mepivacaine (Fig. 3D), lidocaine, bupivacaine significantly scavenged *tert*-butoxyl radical in dose-dependent manners (Fig. 4D, $IC_{50} = 7.1 \pm 0.9$ mM, 0.53 ± 0.08 mM, 2.5 ± 1.0 mM, $p<0.001$). $k_{mepivacaine}$, $k_{lidocaine}$, $k_{bupivacaine}$ were estimated to be $k_{mepivacaine}/k_{CYPMPO} = 0.060$, $k_{lidocaine}/k_{CYPMPO} = 0.79$, $k_{bupivacaine}/k_{CYPMPO} = 0.16$, respectively. However, dibucaine did not scavenge *tert*-butoxyl radical significantly.

Ascorbyl free radical. Mepivacaine (Fig. 3E), lidocaine, bupivacaine, and dibucaine directly scavenged ascorbyl free radi-

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Table 2. Half-maximal inhibitory concentrations (IC_{50}) and relative reaction rate constants of local anesthetics against multiple free radicals

Free radical species	hfsc [mT] a_H, a_N, a_P	Local anesthetics	IC_{50} [M]	Relative reaction rate constants	*p* value
Hydroxyl radical	1.37, 1.37, 4.88, 1.23, 1.35, 4.70	Mepivacaine	1.2×10^{-4}	$2.1 \times k_{CYPMPO}$	<0.001
		Lidocaine	8.8×10^{-5}	$2.9 \times k_{CYPMPO}$	<0.001
		Bupivacaine	5.1×10^{-5}	$5.0 \times k_{CYPMPO}$	<0.001
		Dibucaine	5.4×10^{-5}	$5.0 \times k_{CYPMPO}$	<0.001
Superoxide anion	1.11, 1.26, 5.25, 1.04, 1.27, 5.10	Mepivacaine	—	—	—
		Lidocaine	—		
		Bupivacaine	—		
		Dibucaine	—		
tert-Butyl peroxyl radical	1.35, 1.45, 5.05	Mepivacaine	—	—	—
		Lidocaine	—		
		Bupivacaine	—		
		Dibucaine	—		
tert-Butoxyl radical	1.24, 1.36, 4.80	Mepivacaine	7.1×10^{-3}	$0.06 \times k_{CYPMPO}$	<0.001
		Lidocaine	5.3×10^{-4}	$0.79 \times k_{CYPMPO}$	<0.001
		Bupivacaine	2.5×10^{-3}	$0.16 \times k_{CYPMPO}$	0.018
		Dibucaine	—	—	—
Ascorbyl free radical	0.186, —, —	Mepivacaine	5.3×10^{-4}	$4.0 \times k_{DMSO}$	<0.001
		Lidocaine	3.6×10^{-4}	$6.0 \times k_{DMSO}$	<0.001
		Bupivacaine	3.4×10^{-4}	$6.3 \times k_{DMSO}$	<0.001
		Dibucaine	5.3×10^{-4}	$4.0 \times k_{DMSO}$	<0.001
Singlet oxygen	—, 1.50, —	Mepivacaine	7.0×10^{-4}	$0.7 \times k_{4\text{-OHTEMP}}$	<0.001
		Lidocaine	5.7×10^{-4}	$2.9 \times k_{4\text{-OHTEMP}}$	<0.001
		Bupivacaine	6.3×10^{-4}	$2.7 \times k_{4\text{-OHTEMP}}$	<0.001
		Dibucaine	2.5×10^{-4}	$6.7 \times k_{4\text{-OHTEMP}}$	<0.001
Nitric oxide	a_{N1} 0.981, a_{N2} 0.445	Mepivacaine	0.72	$0.0013 \times k_{cPTIO}$	0.003
		Lidocaine	—	—	—
		Bupivacaine	—	—	—
		Dibucaine	—	—	—
DPPH	—, 0.903, —	Mepivacaine	—	—	—
		Lidocaine	—		
		Bupivacaine	—		
		Dibucaine	—		
Tyrosyl radical	?	Mepivacaine	0.15	$0.0020 \times k_{DMSO}$	0.004
		Lidocaine	0.13	$0.0023 \times k_{DMSO}$	<0.001
		Bupivacaine	—	—	—
		Dibucaine	—	—	—

cals dose-dependently (Fig. 4E, $IC_{50} = 0.53 \pm 0.32$ mM, 0.36 ± 0.46 mM, 0.34 ± 0.02 mM, 0.25 ± 0.01 mM, $p<0.001$). $k_{mepivacaine}$, $k_{lidocaine}$, $k_{bupivacaine}$, $k_{dibucaine}$ were estimated to be $4.0 \times k_{DMSO}$, $6.0 \times k_{DMSO}$, $6.3 \times k_{DMSO}$, $4.0 \times k_{DMSO}$, respectively.

Singlet oxygen. Mepivacaine (Fig. 3F), lidocaine, bupivacaine, and dibucaine dose-dependently scavenged singlet oxygen with IC_{50} of 0.70 ± 0.04 mM, 0.57 ± 0.02 mM, 0.63 ± 0.07 mM, 0.25 ± 0.01 mM (Fig. 4F, $p<0.001$).

Nitric oxide. Mepivacaine significantly scavenged nitric oxide in a dose-dependent manner (Fig. 3G, $IC_{50} = 0.72 \pm 2.2$ M, $p<0.01$). $k_{mepivacaine}$ was estimated to be $0.14\ M^{-1}\ s^{-1}$ ($k_{mepivacaine}/k_{cPTIO} = 1.3 \times 10^{-3}$). However, lidocaine, bupivacaine, and dibucaine did not scavenge nitric oxide significantly (Fig. 4G).

DPPH. Mepivacaine (Fig. 3H), lidocaine, bupivacaine, and dibucaine did not scavenge the artificial free radial DPPH at all (Fig. 4H).

Tyrosyl radical. Mepivacaine (Fig. 3I) and lidocaine (Fig. 4I) significantly scavenged tyrosyl radical in dose-dependent manners with $IC_{50} = 0.15 \pm 0.05$ M and 0.13 ± 0.01 M, respectively ($p<0.01$). The reaction rate constants were $k_{mepivacaine}/k_{DMPO} = 0.020$ and $k_{lidocaine}/k_{DMPO} = 3.4$, respectively. But bupivacaine and dibucaine did not scavenge tyrosyl radical (Fig. 4I).

TBARS assay. Mepivacaine, bupivacaine and dibucaine significantly inhibited peroxidation of brain tissue in a dose-dependent fashion (Fig. 5). However, inhibition of peroxidation by lidocaine was not demonstrated by TBARS assay.

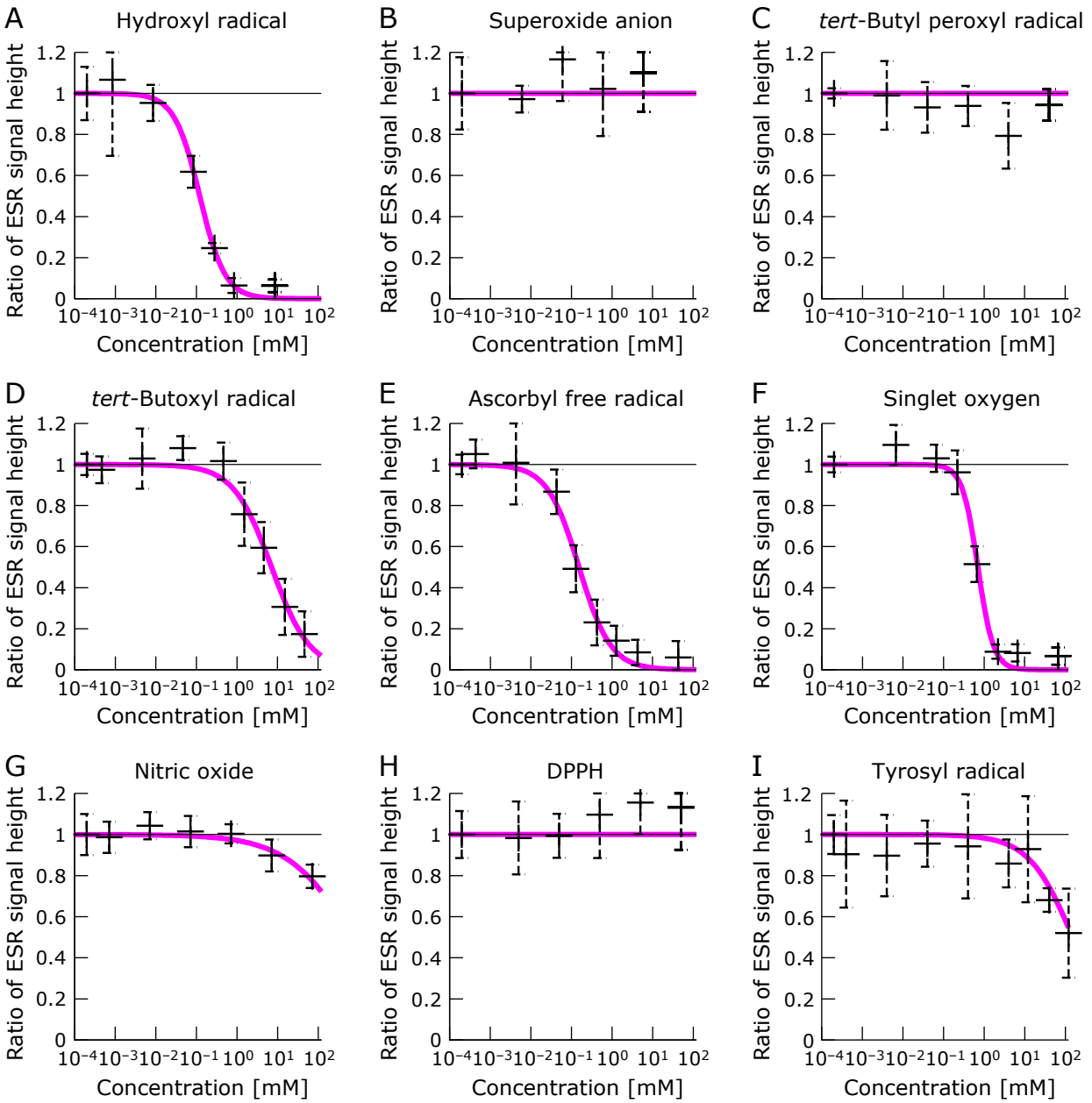

Fig. 3. The dose-response curves of direct scavenging activity of mepivacaine against multiple free radicals. Mepivacaine dose-dependently scavenged hydroxyl radical (A), *tert*-butoxyl radical (D), ascorbyl free radical (E), singlet oxygen (F), nitric oxide (G) and tyrosyl radical (I). However, it did not show radical-scavenging activity against superoxide anion (B), *tert*-butyl peroxyl radical (C) and DPPH radical (H). Error bars indicate 95% confidence intervals. The vertical axes indicate ratios of ESR signal height.

Discussion

The present study showed that the amide-based local anesthetics mepivacaine, lidocaine, bupivacaine, and dibucaine scavenged the following free radicals in dose-dependent manners; hydroxyl radicals, ascorbyl free radical, and singlet oxygen.

Using an endothelial cell model, Wohlrab *et al.*[1] demonstrated that lidocaine exhibited repression of IL-6 and IL-8 release, and early downregulation of COX2 and cell adhesion molecules. However, they did not mention contribution of free radicals in lidocaine's anti-inflammatory action.

Grandhi and Peroma[16] reviewed the anti-cancer effects of local anesthetic. It was suggested that amide-based local anesthetics reduced metastasis and recurrence in patients with breast, colon, or prostate cancer. The dose of lidocaine that some studies have shown to have a positive effect is attainable in human models; however, there are also data that some cancer types would require a dose that is higher than what is clinically feasible. None of these studies mentioned the contribution of free radicals in the anti-cancer action.

Using ESR, Das and Misra[2,17] reported that lidocaine was found to be a potent scavenger of hydroxyl radicals and singlet oxygen, but it did not scavenge superoxide anion. They speculated that the membrane stabilizing actions' of lidocaine could be explained by its scavenging activity against the hydroxyl radicals and singlet oxygen that are implicated in membrane lipid peroxidation. Free radical scavenging activity was not reported for free radicals other than the three radicals mentioned above. This study

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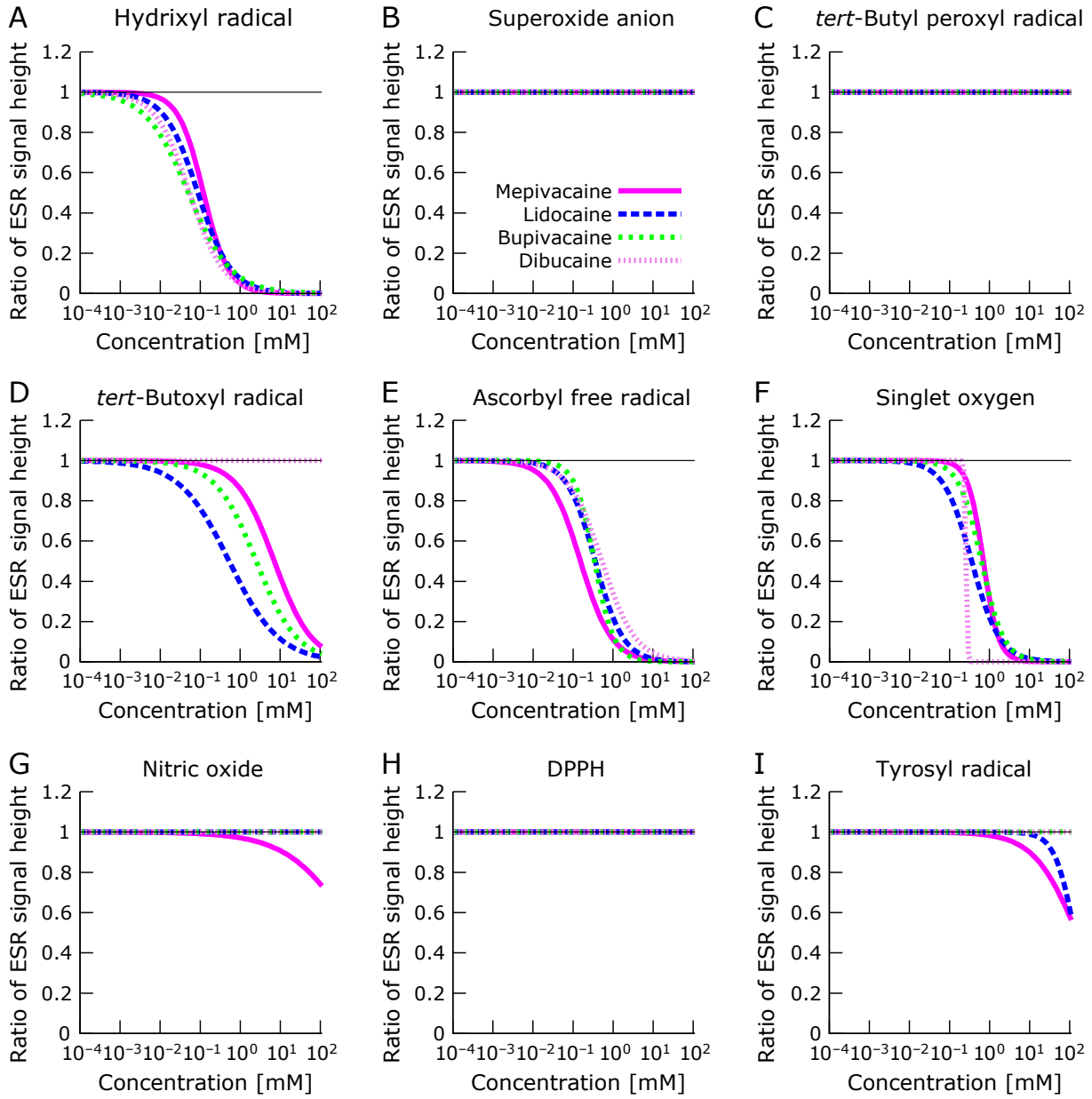

Fig. 4. The dose-response curves of direct scavenging activity of local anesthetics against multiple free radicals. Amide-type local anesthetics selectively and directly scavenge some free radicals. The three kinds of free radicals were eliminated by all the four local anesthetics [hydroxyl radicals (A), ascorbyl free radical (E), singlet oxygen (F)] examined. Other three free radicals [(*tert*-butoxyl radical (D), nitric oxide (G), tyrosyl radical (I)] were scavenged by not all the four local anesthetics. Concentrations of anesthetics are the same as those used for mepivacaine in Fig. 3. The vertical axes indicate ratios of ESR signal height.

is the first to report scavenging activity against *tert*-butoxyl radical, ascorbyl free radical and nitric oxide.

Hattori *et al.*[18] examined the effects of eight local anesthetics on O_2^- generation in human neutrophils and searched for a potential relationship between the biological activities and the physiochemical properties of eight local anesthetics. Eight local anesthetics were ranked with the concentration that produced 50% inhibition of peak chemiluminescence (IC_{50}) in the following order: dibucaine < tetracaine < bupivacaine < ropivacaine < procaine < mepivacaine < lidocaine = prilocaine. While the study by Hattori *et al.*[18] examined the production of superoxide anion, this study evaluated the local anesthetics' activity to scavenge the radical.

Ascorbate is a water-soluble antioxidant located at the most downstream of oxidative chain reactions. Ascorbate in the extracellular fluid receives unpaired electrons from intracellularly generated free radicals via vitamin E in the cell membrane, and transforms itself to the stable ascorbyl free radical (AFR). AFR is considered the final product of intracellularly produced free radicals, protecting tissue from oxidative stress. Vergely *et al.*[19] suggested that AFR liberation in coronary effluents could represent a marker of oxidative stress during ischemia and/or reperfusion of hearts.

Three kinds of free radicals (hydroxyl radical, ascorbyl free radical, and singlet oxygen) were eliminated by all the four local anesthetics examined. Another three free radicals (*tert*-butoxyl radical, nitric oxide, and tyrosyl radical) were scavenged by some but not all of the four local anesthetics. The rest of the free radicals (superoxide anion, *tert*-butyl peroxyl radical, and DPPH) were not scavenged by any of the four local anesthetics. These

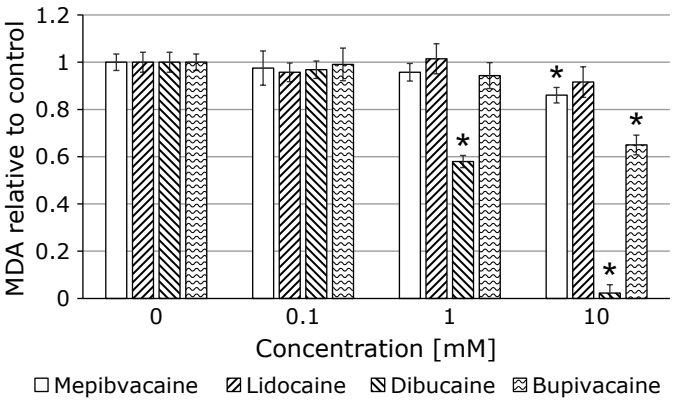

Fig. 5. Antioxidative activity of local anesthetics by thiobarbituric acid reactive substance (TBARS) assay initiated by hydroxyl radical. Mepivacaine, bupivacaine, and dibucaine significantly inhibited peroxidation of brain tissue dose-dependently (*p<0.05 with Bonferroni's correction). Error bars indicate 95% confidence intervals.

results suggest that local anesthetics have antioxidant properties.

On clinical wards, local anesthetics are routinely used for local pain control. The results of this study would suggest that another use of local anesthetics could be to reduce oxidative injury and inflammatory damage. Active use of local anesthetics might ameliorate inflammatory injury, shortening the length necessary for the recovery of wounds after surgery.

The role of reactive oxygen species in various processes of tumorigenesis has been clarified.[20] Therefore, it is very important to select agents that inhibit oxidative stress in the perioperative period of cancer surgery. Reactive oxygen species may act as regulators of pathways related to increased risk of metastasis and cellular immune suppression after surgery. Intracellular oxidative stress impaired DNA repair for two days after surgery, and such oxidative stress sustained for 10 days after surgery.[21] In addition, reactive oxygen species reduced the survival rate of NK cells.[22]

In this study, the radical scavenging potential of local anesthetics was confirmed, i.e., local anesthetics inhibit oxidative stress. This suggests that these substances may be able to play an active role in reducing poor outcomes and promoting healing. Lidocaine, for example, was found to be a potent scavenger of hydroxyl radicals and singlet oxygen, but not superoxide anion radicals.[2] Lidocaine and dexmedetomidine together had a synergistic effect and improved the perioperative hemodynamic stability of patients with intracranial aneurysm clipping. Moreover, lidocaine pretreatment combined with dexmedetomidine can effectively improve the oxidative stress injury induced by NADPH oxidase, nNOS, and iNOS via decreasing the expression of related proteins.[23] Lidocaine was also protective against ischemia-reperfusion injury of lung[24] and brain[25] in animal models. With regard to anti-inflammatory activity, elevated plasma levels of IL-6, IL-8, complement C3a, and IL-1ra as well as expression of CD11b, L- and P-selectin, and platelet-leukocyte aggregates were significantly attenuated by systemic lidocaine.[26]

Perioperative complications such as acute kidney, postoperative delirium, and postoperative atrial fibrillation have been associated with oxidative stress.[27–29] Epidural anesthesia has been shown to be useful for these complications.[30–32] Part of the mechanism may be due to the radical scavenging ability of the local anesthetics demonstrated in this study.

This all suggests that certain anesthetics and anesthesia methods may decrease oxidative stress and reduce immune response. Combined use of regional anesthesia in the perioperative management of anesthesia suppresses the neuroendocrine stress response to surgical invasion. This would also reduce the use of general anesthetics and opioids, as well as avoid the compromise of immune function. In addition, local anesthetics can be expected to scavenge radicals, which may further improve patient prognosis. For example, combined epidural and general anesthesia effectively blocked reduced oxidative stress during robot-assisted laparoscopic prostatectomy and hip replacement for the elderly.[33,34]

Local anesthetics are lipid-soluble, and thus able to enter inside of cells through cell membrane. Therefore, in addition to its action on voltage-dependent sodium channels on cell membrane, we speculate that it may scavenge free radicals generated intracellularly in ischemia/reperfusion injury. Hattori *et al.*[18] demonstrated suppression of generation of superoxide anion of human neutrophils by eight local anesthetics which included four drugs studied here. The IC_{50} value for each local anesthetic correlated with the logarithmic value of the partition coefficient for each drug; the more lipophilic, the more inhibitory effect on radical generation. The values of partition coefficient had a rank order of dibucaine > bupivacaine > mepivacaine > lidocaine. The results of the present study also indicate such tendency (Fig. 5); the more lipophilic thus easier to enter into cells, the more antioxidative.

Quantification of free radical amount or concentration in a sample can be available by comparing the ESR signal area of the subject sample and a standard sample, which contains known amount/concentration of stable free radical. The peak area of the integral spectrum can be proportional to $H_{pp} \times W_{pp}^2$, where H_{pp} is the peak-to-peak height of the differential spectrum and W_{pp} is the peak-to-peak line width of the differential spectrum. The data of the present study showed that there were no changes observed in W_{pp} regardless of concentration of local anesthetics for each free radical (data not shown). Thus, we speculate that ratios of signal heights could be regarded as those of intensity of ESR signal, thus also as the amount or concentration of the free radicals.

The present study has some limitations. First, the present study evaluated the free radical scavenging activity of local anesthetics in solutions with only a few varieties of solute species. The possible influence of intracellular and intramitochondrial electrolytes, proteins, and other metabolites on scavenging activity were not considered in the present study. Second, the authors were only able to obtain absolute reaction rate constants for hydroxyl radical and nitric oxide; reaction rate constants for other free radicals were available only relative to reaction rate constants of spin traps.

Conclusions

We have demonstrated the dose-dependent scavenging activity of amide-type local anesthetics against specific species of free radicals. Out of nine free radical species examined, three kinds of free radicals (hydroxyl radicals, ascorbyl free radical, and singlet oxygen) were eliminated by all the four local anesthetics. Another three free radicals (*tert*-butoxyl radical, nitric oxide, and tyrosyl radical) were scavenged by some but not all the four local anesthetics, and the last three kinds of free radicals (superoxide anion, *tert*-butyl peroxyl radical, and DPPH) were not scavenged by any of the four local anesthetics. It was also confirmed that local anesthetics inhibited lipid peroxidation by TBARS assay.

Author Contributions

Study concept and design: YS, SM, and OT; acquisition of data: YS, KO, MN, and OT; analysis and interpretation of data: YS, KO, MN, and OT; drafting of the manuscript: YS and OT; critical revision of the manuscript for important intellectual content: SM, KB, and TK; statistical analysis: OT; obtained funding: TK; administrative, technical, or material support: OT; study supervision: TK.

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Conflict of Interest

No potential conflicts of interest were disclosed.

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