Original Article

Edaravone, a scavenger for multiple reactive oxygen species, reacts with singlet oxygen to yield 2-oxo-3-(phenylhydrazono)-butanoic acid

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Edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one) is a synthetic antioxidant used as a drug to treat acute ischemic stroke in Japan and amyotrophic lateral sclerosis in Japan and the USA. Its pharmacological mechanism is thought to be scavenging of reactive oxygen species, which are intimately related with these diseases. Recently, the singlet oxygen ($^1O_2$) has attracted attention among reactive oxygen species. In this study, we investigated the reactivity of edaravone toward $^1O_2$ and identified its reaction products. Edaravone showed a reactivity toward $^1O_2$ greater than those of uric acid, histidine, and tryptophan, which are believed to be $^1O_2$ scavengers *in vivo*. And we confirmed that 2-oxo-3-(phenylhydrazono)-butanoic acid was formed as an oxidation product. We propose a plausible mechanism for 2-oxo-3-(phenylhydrazono)-butanoic acid production by $^1O_2$-induced edaravone oxidation. Since 2-oxo-3-(phenylhydrazono)-butanoic acid has already been identified as a radical-initiated oxidation product, free radical-induced oxidation should be seriously reconsidered. We also found that edaravone can react with not only hypochlorous anions but also $^1O_2$ that are formed from myeloperoxidase. This result suggests that edaravone treatment can be beneficial against myeloperoxidase-related injuries such as inflammation.

***Key Words*: edaravone, singlet oxygen, oxidative stress**

The formation of singlet oxygen ($^1O_2$) *in vivo* has attracted much attention in recent years. Umeno *et al.* detected $^1O_2$-specific oxidation products of linoleic acid (LA) and 10- and 12-(*Z*,*E*)-hydroxyoctadecadienoic acids (HODE) in human plasma, and the ratio of 10- and 12-(*Z*,*E*)-HODE to LA can serve as a prominent biomarker for the diagnosis of early stage diabetes symptoms such as impaired glucose tolerance (IGT).[1] Recently, we demonstrated that parabanic acid (PA) and its hydrolysate oxaluric acid (OUA) are $^1O_2$-specific oxidation products of uric acid (UA).[2] Hillered *et al.*[3] showed that PA levels are dramatically elevated in intracerebral microdialysates harvested from serious aneurysmal subarachnoid hemorrhage patients. These results indicate that $^1O_2$ is formed during severe brain ischemic damage. A possible source for $^1O_2$ generation *in vivo* is thought to be the myeloperoxidase (MPO) system. MPO catalyzes the reaction of hydrogen peroxide (H_2O_2) and chloride anions (Cl^-) to yield hypochlorous anions (ClO^-) (Scheme 1). During this process, H_2O_2 is further oxidized by the formed ClO^- to produce $^1O_2$ (Scheme 1). In fact, we have confirmed the formation of OUA during MPO-induced UA oxidation (unpublished results).

In addition, an interesting novel $^1O_2$ generation system has been proposed recently. Indoleamine 2,3-dioxygenase 1 (IDO1) is an essential enzyme for tryptophan (Trp) metabolism in which it converts Trp to *N*-formyl-kynurenine. It has been demonstrated that IDO1 produces $^1O_2$ in the presence of H_2O_2 to form stereospecific tricyclic hydroperoxides of L-Trp (WOOH), of which only *cis*-WOOH shows arterial relaxation and decreases blood pressure in model animals.[4]

$$H_2O_2 + Cl^- \xrightarrow{MPO} ClO^- + H_2O \quad (1)$$

$$ClO^- + H_2O_2 \longrightarrow {}^1O_2 + Cl^- + H_2O \quad (2)$$

Scheme 1.

Therefore, it is important to search for $^1O_2$ scavengers. In this study, we found that the synthetic antioxidant edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one) is a good antioxidant against $^1O_2$. Edaravone was developed as a drug against acute ischemic stroke and was approved in Japan in 2001.[5] It was further approved for amyotrophic lateral sclerosis (ALS) in Japan in 2015 and in the USA in 2017.[5] The formation of free radicals has been suggested in acute ischemic stroke,[5] and we hypothesized that edaravone may scavenge peroxynitrite.[6,7]

Edaravone reacts with many kinds of ROS. We previously examined the antioxidant activity of edaravone against free radicals derived from thermal decomposition of azo-initiators,[8] peroxynitrite ($ONOO^-$),[6] and ClO^-.[9] Edaravone strongly inhibits peroxyl radical-mediated oxidation of soy-phosphatidylcholine liposomal membranes induced by the lipid-soluble initiator 2,2'-azobis(2,4-dimethylvaleronitrile) or water-soluble 2,2'-azobis(2-amidinopropane) dihydrochloride.[8] We also showed that edaravone can react with $ONOO^-$ 30-fold faster than does UA, which is an essential scavenger of $ONOO^-$ *in vivo*.[6] Recently, we reported that edaravone has comparable reactivity to ClO^- with thiol and sulfide residues, which are endogenous scavengers for ClO^-.[9] Furthermore, we identified their reaction products (Fig. 1); free radicals-, $ONOO^-$-, and ClO^--induced edaravone oxidation yield 4-oxoedaravone and its hydrolysate 2-oxo-3-(phenylhydrazono)-butanoic acid (OPB),[8] predominantly 4-NO-edaravone and slightly 4-NO_2-edaravone,[6] and 4-Cl-edaravone and (*E*)-2-chloro-3-[(*E*)-phenyldiazenyl]-2-butenoic acid (CPB),[9] respectively.

In this study, we evaluated the reactivity of edaravone toward $^1O_2$ in comparison with established $^1O_2$ scavengers such as UA, histidine (His), methionine (Met), and Trp. Edaravone showed higher reactivity than the reference antioxidants, and the rate constant was estimated at 10^8–10^9 $M^{-1}s^{-1}$. However,

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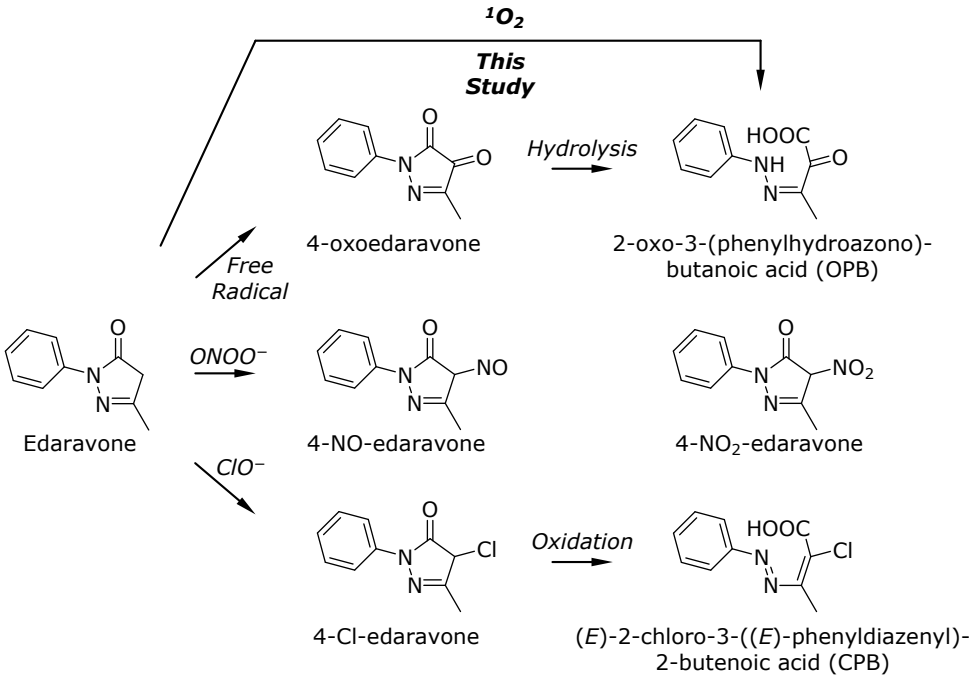

Fig. 1. Chemical structures of edaravone and its oxidative metabolites formed by reactions with free radicals (upper), $ONOO^-$ (middle), and ClO^- (lower). In this study, $^1O_2$-induced edaravone oxidation led to OPB production without forming 4-oxoedaravone.

edaravone reactivity depended on its ionization; it was elevated with increasing ionization. Moreover, the oxidation product was confirmed to be OPB, which we identified as a radical-induced oxidation product. Therefore, the formation of OPB in acute ischemic brains should be reexamined. We are also planning to study the plasma of ALS patients. Before doing these studies, we conducted MPO-induced edaravone oxidation, since the MPO system is a plausible source of $^1O_2$ *in vivo* as mentioned above. In addition to 4-Cl-edaravone and CPB, OPB was formed with edaravone degradation, indicating that $^1O_2$ generated from the MPO system reacted with edaravone to produce OPB. Since edaravone oxidative metabolites are specific to the reacted ROS, analysis of those metabolites can identify the ROS formed *in vivo*.

Materials and Methods

Chemicals. Edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one), His, Met, Trp, UA, rose bengal (RB), and other chemicals were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). MPO was purchased from Sigma-Aldrich Japan (Tokyo, Japan). Methanolic solutions of edaravone were prepared and stored at −20°C until use. OPB was a kind gift from Mitsubishi Tanabe Pharma Corporation (Osaka, Japan).

4-NO_2-edaravone or 5-acetyl-edaravone (3-methyl-1-phenyl-1*H*-pyrazol-5-yl acetate, 5-AE) were synthesized by reactions of edaravone and $ONOO^-$ or acetic anhydride, respectively. To prepare the reaction, $ONOO^-$ was formed as described by Kato *et al.*[10] A 0.6 M HCl, 0.7 M H_2O_2 aqueous solution (10 ml) was prepared and stirred well on ice. An ice-cold 0.6 M $NaNO_2$ aqueous solution (10 ml) was added, immediately followed by 1.5 M NaOH (20 ml). Then manganese dioxide (MnO_2) was added to the solution to eliminate H_2O_2 remaining in excess. The $ONOO^-$ was concentrated by frozen fractionation, and the concentrated $ONOO^-$ was added to the edaravone solution to synthesize 4-NO-edaravone and 4-NO_2-edaravone.[6] Next, 5-AE was also synthesized. Briefly, edaravone (5 g) was dissolved in acetic anhydride (20 ml) containing triethylamine (500 µl) and sulfuric acid (500 µl) and heated at 90°C for 24 h. The formation of 4-NO_2-edaravone or 5-AE was confirmed with optimized LC/TOFMS, and both were isolated by HPLC. Methanolic solutions of 4-NO_2-edaravone and 5-AE were also stored at −20°C until use.

Photooxidation of edaravone and its derivatives. Photooxidation of edaravone was induced by RB addition and UVA irradiation. Edaravone was dissolved in a 50% methanolic phosphate buffer solution (40 mM, pH 7.4) containing 100 µM diethylenetriaminepentaacetic acid (DTPA) and 10 µM RB as a photosensitizer. To examine edaravone reactivity, UA, His, Met, or Trp was co-dissolved in the solution as an internal reference. The final concentrations of the edaravone and the internal references were approximately 25 µM. The photooxidation was started by UVA irradiation. Changes in concentrations of the edaravone and the references during the photooxidation were determined by HPLC equipped with a UV detector monitoring at 210 nm or an electrochemical detector (ECD) as described in the HPLC analysis section. In order to estimate edaravone reactivity to $^1O_2$, the ratio of the rate constants of edaravone and the internal reference was evaluated as described previously.[6,9] Briefly, the edaravone and co-existing internal reference competitively decreased where both rate constants were defined as k_E and k_R respectively (equations 3, 4). The ratio of the rate constants (k_E/k_R) is given by equation 5 as a solution of simultaneous differential equations.

$$\text{Edaravone} + {}^1O_2 \xrightarrow{k_E} \quad (3)$$

$$\text{Reference} + {}^1O_2 \xrightarrow{k_R} \quad (4)$$

$$k_E/k_R = \frac{\log([\text{Edaravone}]/[\text{Edaravone}]_0)}{\log([\text{Reference}]/[\text{Reference}]_0)} \quad (5)$$

Scheme 2.

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Photooxidations of edaravone alone at various pH were also conducted. Reaction mixtures had their pH adjusted to 4–11 with phosphoric acid and sodium hydroxide and were irradiated with UVA. Photooxidation of 4-NO_2-edaravone or 5-AE was also carried out at various pH conditions (pH 4–9). Changes in concentrations were obtained by HPLC analysis.

Identification of reaction products of edaravone and $^1O_2$. To search for reaction products, edaravone was photooxidized alone. Edaravone (100 μM) was dissolved in 50% methanolic phosphate buffer solution (40 mM, pH 7.4) containing DTPA (100 μM) or 100% methanol. RB (10 μM) was added to the solution, which was irradiated with UVA. The reaction solution was analyzed by optimized LC/TOFMS and HPLC every 30 min. The observed mass-to-charge ratio (*m/z*) was compensated with trifluoroacetic acid to obtain the accurate *m/z* value as described below.

HPLC analysis. In competitive photooxidation of edaravone and Met or Trp, as internal references, the changes in concentrations of those antioxidants were determined by a reverse phase HPLC system equipped with an ECD (HPLC-ECD) or a UV detector (HPLC-UV) to monitor absorption at 210 nm. A 25% methanol aqueous solution containing $NaClO_4$ (50 mM) and NaH_2PO_4 (40 mM) was delivered at 1.0 ml/min as the mobile phase. Separation was carried out by a Wakopak Navi C18 (5 μm, 250 mm × 4.6 mm, FUJIFILM Wako Pure Chemical Corporation). The applied voltage of the electrochemical detector against an Ag/AgCl reference electrode was 800 mV. When His and UA were measured, a different HPLC system was used. A formic acid aqueous solution (pH 3.0) delivered at 1.0 ml/min and a Develosil C30-UG (5 μm, 4.6 mm × 250 mm, Nomura Chemical Co. Ltd., Tokyo, Japan) were used as the mobile phase and separation column, respectively.

To obtain changes in concentration of edaravone, OPB, 4-Cl-edaravone, and CPB in MPO-initiated oxidation, or 5-AE and 4-NO_2-edaravone in photooxidation for the other HPLC-UV was used; the mobile phase and a separation column were methanol/40 mM NaH_2PO_4 aqueous solution = 60/40 (v/v) delivered at 1.0 ml/min and a CAPCELL PAK C18 (5 μm, 250 mm × 4.6 mm, Shiseido, Japan) respectively. Detection was carried out by monitoring absorbance at 210 nm.

LC/TOFMS analysis. To obtain accurate *m/z* values of OPB, HPLC combined with a TOFMS (LC/TOFMS, JMS-T100LC, JEOL Ltd., Tokyo, Japan) system was used. Negative electrospray ionization (ESI) was performed at an ionization potential of −2,000 V. The optimized applied voltages to the ring lens, outer orifice, inner orifice, and ion guide were −5 V, −10 V, −5 V, and −500 V, respectively. To obtain accurate *m/z* values, trifluoroacetic acid (TFA) was used as an internal standard for *m/z* calibration. Analysis of 4-oxoedaravone was carried out by TOFMS in positive ESI mode. The optimized conditions were as follows: ionization potential 2,500 V, ring lens 10 V, outer orifice 35 V, inner orifice 5 V, ion guide 500 V.

Results and Discussion

Competitive reaction of edaravone and *in vivo* scavengers of $^1O_2$. The reactivities of edaravone and well-recognized *in vivo* scavengers of $^1O_2$ (UA, His, Met, and Trp) to $^1O_2$ were compared. Both edaravone and the co-existing internal references were photooxidized in a homogenous solution (pH 7.4) using 10 μM RB as a photosensitizer. Edaravone decreased faster than any of the reference scavengers (Fig. 2). The ratios of rate constants of edaravone to those of references (k_E/k_R) were calculated and are summarized in Table 1. Considering the rate constants of the references,[11–16] the rate constant of edaravone was estimated on the order of 10^8–10^9 $M^{-1}s^{-1}$. Moreover, the k_E/k_R values were

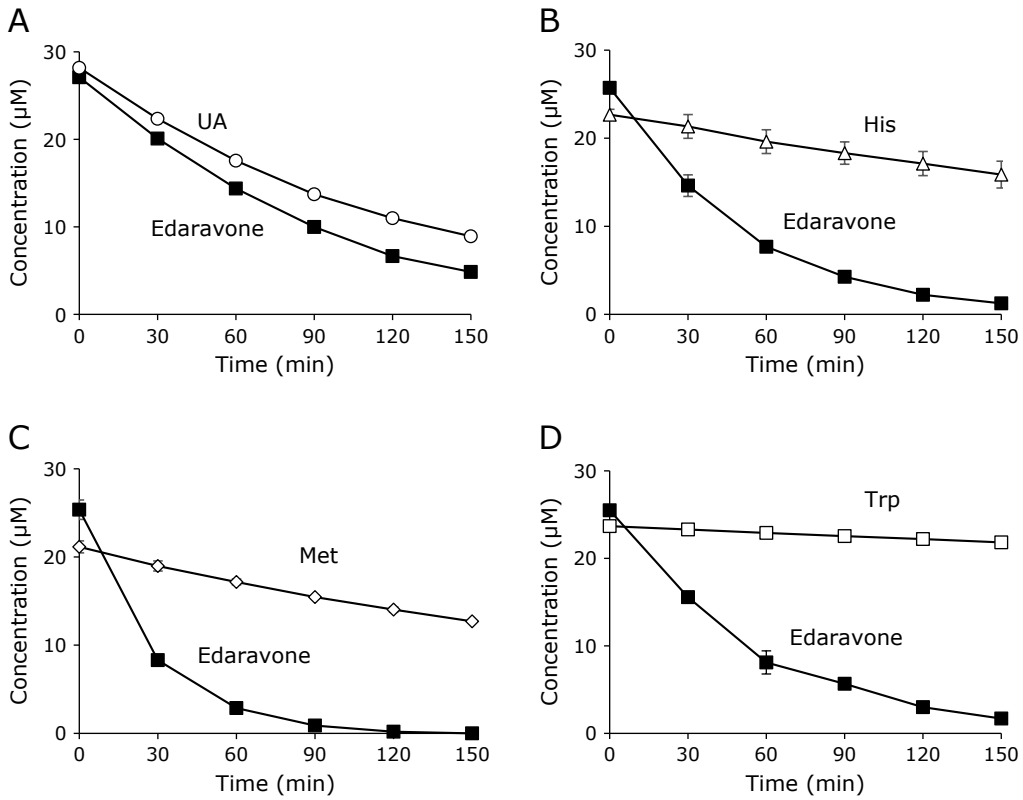

Fig. 2. Time courses of changes in concentrations of edaravone (dative each reference molecule: (A) UA (○), (B) His (△), (C) Met (◇), and (D) Trp (□), during competitive photooxidation (n = 3).

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Table 1. Ratio of rate constants of edaravone and reference antioxidants [mean ± SD (n = 3)]

Reference	In phosphate buffer (pH 7.4)		In 50% methanol	
	k_E/k_R	k_R ($M^{-1}s^{-1}$)	k_E/k_R	k_R ($M^{-1}s^{-1}$)
UA	1.76 ± 0.02	1.5×10^{8} [11,12]	0.08 ± 0.01	
His	10.0 ± 0.5	7.0×10^{7} [13]	0.56 ± 0.02	0.7×10^{7} [16]
Met	10.5 ± 0.2	2.2×10^{7} [14]	0.84 ± 0.01	0.5×10^{7} [16]
Trp	31.0 ± 2.2	3.4×10^{7} [15]	1.41 ± 0.02	0.4×10^{7} [16]

(11–16), see references.

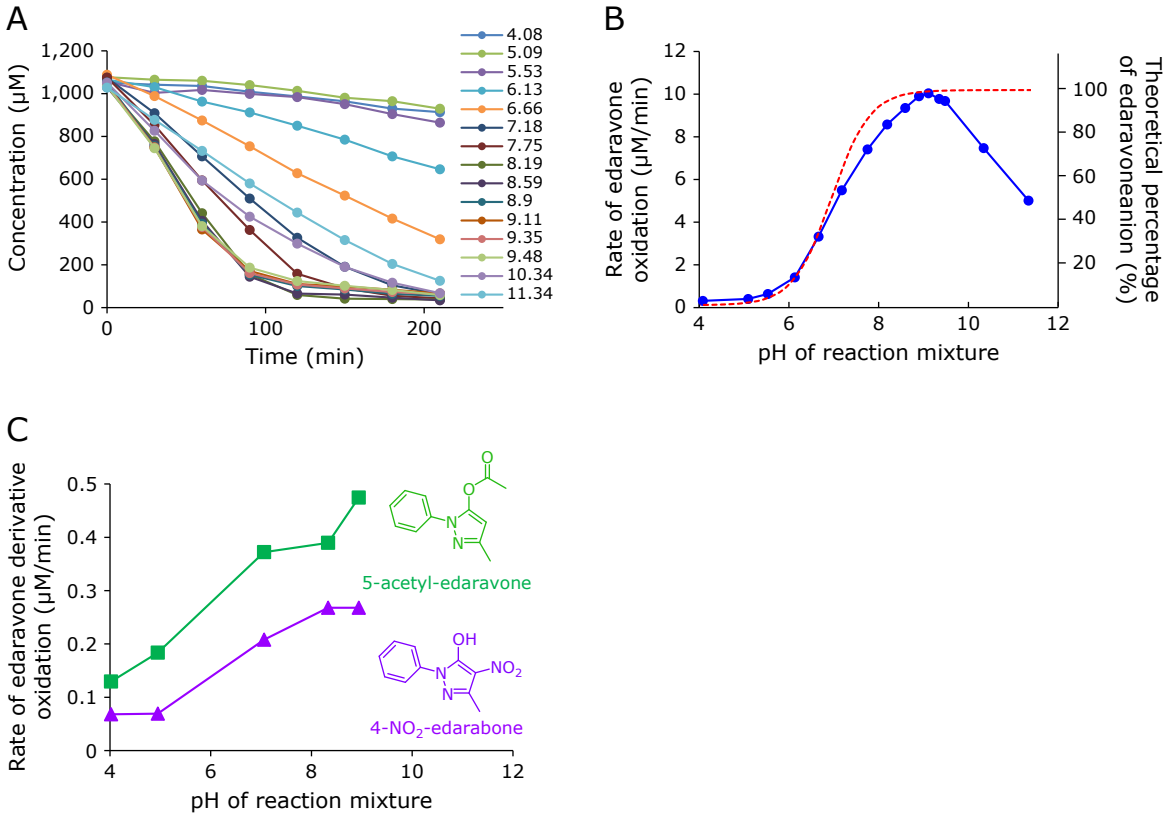

Fig. 3. Dependence of the photooxidation rate of edaravone and its derivatives on pH. (A) Time-course changes in edaravone concentration during photooxidation under different pH (4–11). (B) Observed changes in edaravone oxidation rate (blue plot and line) and the theoretical percentage of edaravone anions (red dashed line) vs pH of reaction mixtures. The electrolytic dissociation equilibrium of edaravone (pK_a) is 7.0. (C) Changes in photooxidation rate of 5-AE (green plot and line) and 4-NO_2-edaravone (violet plot and line) with pH. See color figure in the on-line version.

much reduced in 50% methanol (Table 1), suggesting that the reactivity of edaravone anions to $^1O_2$ is much greater than that of edaravone non-ions.

Dependence of edaravone reactivity to $^1O_2$ on pH. We conducted photooxidation of edaravone at various pH. The rate of edaravone decline increased with increasing pH, leveled off at pH 9, and decreased with increasing pH higher than 9 (Fig. 3A). The initial rate of decline was plotted against the pH of the reaction mixture (Fig. 3B, blue plot and line). The pK_a of edaravone is 7.0,[5] so the percentages of edaravone anions at various pH were calculated (Fig. 3B, red dashed line). Interestingly the pH dependency of edaravone reactivity is fitted well with the theoretical curve from pH 4–9, confirming that the edaravone anion is the true reactant for $^1O_2$ in that pH range. This fits well with the concept that $^1O_2$ is an electrophile. The pyrazoline ring of the edaravone enol form is aromatic, and its electron density becomes high upon ionization because of the strong electron-donating effect of its oxido group (-O^-). In order to confirm that edaravone reactivity to $^1O_2$ depends on its ionization, we conducted photooxidation of 5-AE. Since 5-AE has an enolic hydrogen of the edaravone enol form replaced with an acetyl group, it does not ionize. The photooxidation rate of 5-AE was clearly low at pH 4–9 (Fig. 3C), indicating that ionization of 5-enol is essential to the reaction of $^1O_2$ and edaravone. Furthermore 4-NO_2-edaravone was also photooxidized at various pH. The nitro group is a typical electron-withdrawing group that can reduce electron density on the pyrazoline ring of edaravone. As expected, the photooxidation rate was also significantly lowered compared with that of edaravone (Fig. 3C). Taken together, $^1O_2$ is postulated to react with the pyrazoline ring of the edaravone anion whose electron density is raised by the oxido group formed upon ionization. In fact, $^1O_2$ reactivity toward phenol (pK_a = 9.8) was elevated with increasing pH of the solution, and it dramatically increased at pH 9–10.[17,18]

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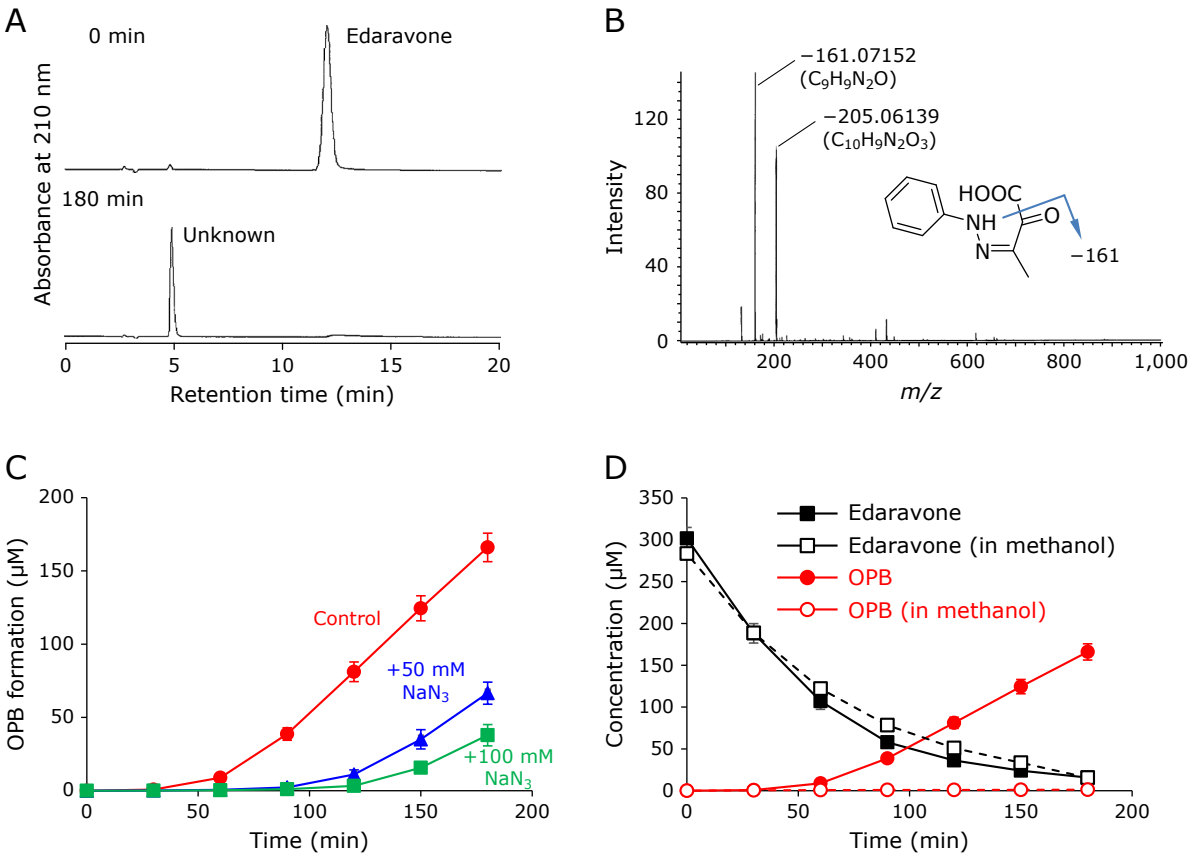

Fig. 4. Identification of OPB as an oxidation product during $^1O_2$-induced edaravone oxidation, and OPB formation under some conditions. (A) HPLC chromatograms of edaravone solution containing 10 μM RB immediately after beginning UVA irradiation (upper) and after 180 min irradiation (lower). (B) MS spectrum showing the unknown peak as measured by optimized TOFMS with negative ESI. Observed *m/z* values were compensated by TFA. Inserted is a probable fragmentation of OPB. (C) OPB formation during edaravone photooxidation and its inhibition by the addition of NaN_3: control (●), + 50 mM (▲), +100 mM (■) (n = 3). (D) Time course of edaravone degradation and OPB formation in water containing (50%) methanol (closed symbols) and water-free methanol (open symbols) (n = 3).

Product of $^1O_2$-induced oxidation of edaravone. In order to identify the oxidation product of edaravone induced by $^1O_2$, photooxidation of edaravone was conducted. We observed that edaravone was oxidized and completely degraded after 180 min of irradiation, and an unknown peak was detected on the HPLC chromatogram (Fig. 4A). Then the MS spectrum of the unknown peak was measured by an optimized LC/TOFMS system with negative ESI. Two major ions were detected, and their accurate *m/z* values were determined as −205.06139 and −161.07152, respectively, using TFA as an internal standard (Fig. 4B). The molecular formulas of those ions were also postulated to be $C_{10}H_9N_2O_3$ (calculated *m/z* −205.06132) and $C_9H_9N_2O$ (calculated *m/z* −161.07149), respectively. The $C_9H_9N_2O$ ion is the decarboxylated fragment of the $C_{10}H_9N_2O_3$ ion, indicating that $C_{10}H_9N_2O_3$ has a carboxyl group.

OPB is the end product of radical-induced edaravone oxidation, and its formula is $C_{10}H_{10}N_2O_3$, which gives the deprotonated ion $C_{10}H_9N_2O_3$. Since the $^1O_2$-induced oxidation product was postulated to be OPB, an authentic standard of OPB was analyzed by LC/TOFMS. Chromatographic retention and the MS spectrum of the OPB standard were identical with those of the unknown oxidation product (data not shown). These results suggest that the $^1O_2$-induced oxidation product of edaravone is OPB. Then, the time-course of changes in the concentrations of edaravone and OPB during photooxidation was determined. We observed that OPB was formed accompanied with edaravone degradation. Furthermore, OPB formation (Fig. 4C) and edaravone degradation (data not shown) during photooxidation were significantly inhibited by the addition of NaN_3 in a dose-dependent manner. The data also strongly suggested that edaravone degradation and OPB formation were caused by $^1O_2$. In contrast, OPB was not produced in methanolic solutions, even one containing 7.2 mM triethylamine to promote edaravone ionization, although edaravone was decomposed similarly as that in water (Fig. 4D), indicating that the presence of water was necessary for the formation of OPB.

Comparison of OPB formation for $^1O_2$- and radical-induced edaravone oxidation. In radical-induced oxidation, the mechanism for OPB formation has been fully characterized.[8] Briefly, an edaravone radical is formed by an electron donated from an edaravone enol anion to a peroxyl radical. The carbon-centered radical among three formed tautomeric radicals reacts with an oxygen molecule to form a 4-peroxyl radical. The 4-peroxyl radical is immediately converted to 4-oxoedaravone. Then, hydrolysis of the 4-oxoedaravone occurs and it finally gives OPB (Fig. 5A). Therefore, 4-oxoedaravone is the direct precursor of OPB formation.

Hence, prior to photooxidation, we oxidized edaravone with peroxyl radicals derived from thermal decomposition of 2,2'-azobis(2,4-dimethylvaleronitrile) to confirm 4-oxoedaravone formation. The reaction mixture was analyzed by optimized LC/TOFMS with positive ESI. After a 30-min incubation, 4-oxoedaravone was detected at approximately 5.5 min of retention time on an MS chromatogram monitoring the sodium adduct of

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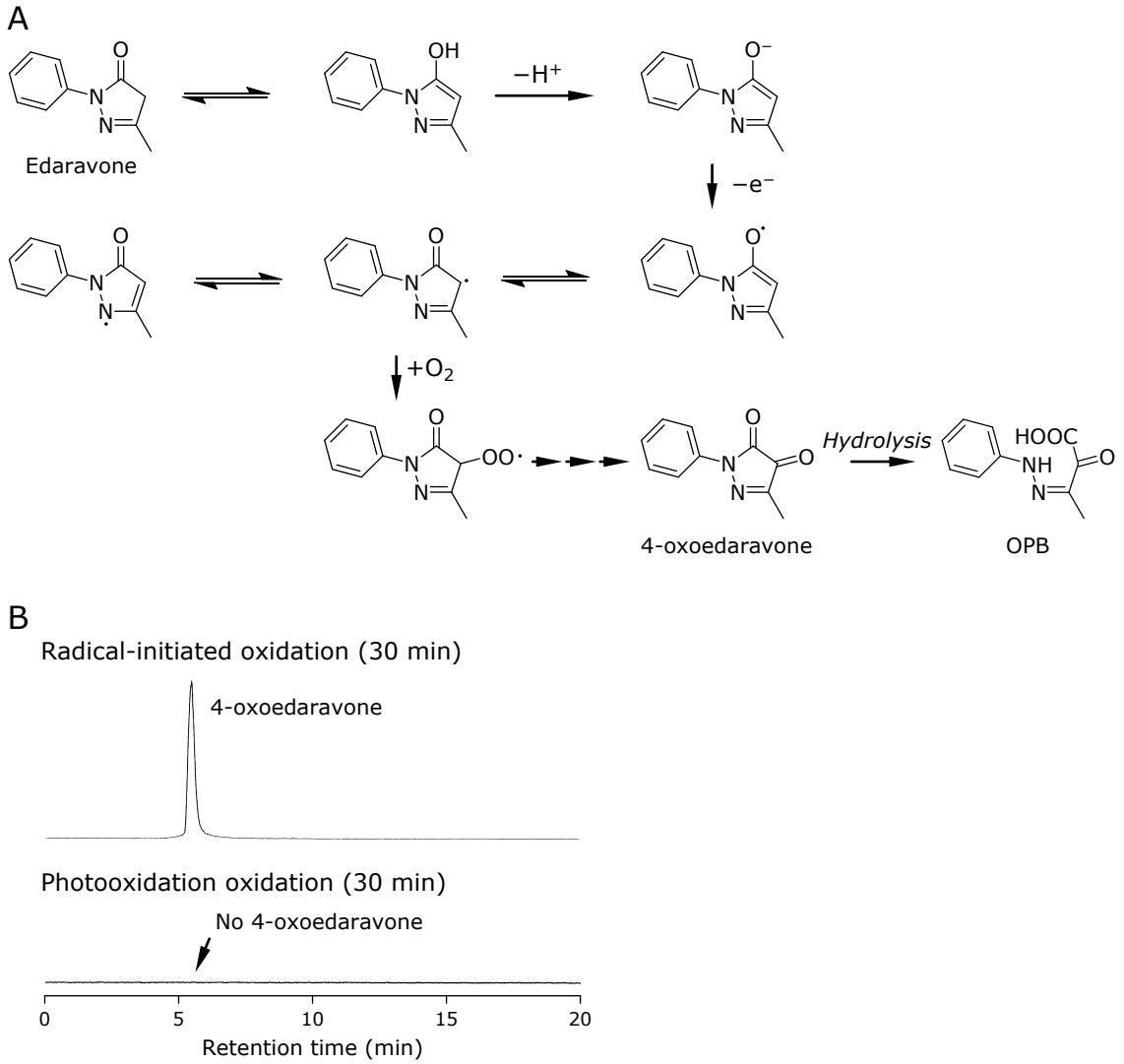

Fig. 5. (A) OPB formation mechanism in radical-induced edaravone oxidation. (B) MS chromatograms by TOFMS with positive ESI monitoring of 4-oxoedaravone sodium adduct ions (*m/z* 211); radical-initiated oxidation for 30 min (upper) and photooxidation for 30 min (lower).

4-oxoedaravone (*m/z* 211) (Fig. 5B, upper). Then it increased and reached a plateau at 60 min (data not shown). In contrast, 4-oxoedaravone was not observed during photooxidation of edaravone (Fig. 5B, lower). This suggested that the formation of OPB upon edaravone photooxidation was not due to 4-oxoedaravone hydrolysis, which is different from radical-induced oxidation.

A plausible mechanism for the reaction of edaravone and $^1O_2$. Based on the above results, we propose a plausible mechanism for OPB production from the reaction of edaravone and $^1O_2$ (Fig. 6). Edaravone shows a tautomerism of keto- and enol-forms (**1**). At a higher pH than 7.0, which is the pK_a value of edaravone, deprotonation from the edaravone enol occurs to form an edaravone anion (**2**). An $^1O_2$ adds to the C4-C5 double bond of the edaravone anion, increasing its electron density by anionization, to produce 4,5-dioxetane (**3**). 4,5-Dioxetane gives an intermediate (**4**) having a carbocation on the C5 position with heterocleavage of the C-O bond. Then, nucleophilic addition of a water molecule to the C5-carbocation leads to subsequent C5-N1 cleavage and elimination of HO^- from the C4 position to produce OPB. This process is assumed to be the rate-determining step of the reaction, and the elimination of HO^- is thought to be suppressed under higher pH. In fact, the rate of edaravone photooxidation decreased at a pH higher than 9.5, which is inconsistent with theoretical curve of the percent ionization (Fig. 3B).

Edaravone oxidation induced by MPO. MPO is released from activated neutrophils during inflammation and forms ClO^- by catalyzing a reaction of Cl^- and H_2O_2. Since the generated ClO^- subsequently reacts with H_2O_2 to produce $^1O_2$ (Scheme 1), MPO acts as an $^1O_2$ generator as with ClO^- producer *in vivo* as discussed in the Introduction. Therefore, MPO-induced edaravone oxidation was studied. As expected, OPB was significantly formed in addition to 4-Cl-edaravone and CPB, which are specific oxidation products of ClO^-,[9] accompanied with edaravone decomposition (Fig. 7A). Next UA, a well-known biological $^1O_2$ quencher, was added to the reaction system instead of NaN_3 to avoid denaturation of MPO. We found that only OPB formation was significantly inhibited by the addition of 100 μM UA, whereas edaravone degradation and 4-Cl-edaravone formation were not affected (Fig. 7B). These results suggested that edaravone reacted with $^1O_2$ generated from the MPO system to form OPB.

In the current study, we demonstrated that edaravone reacts

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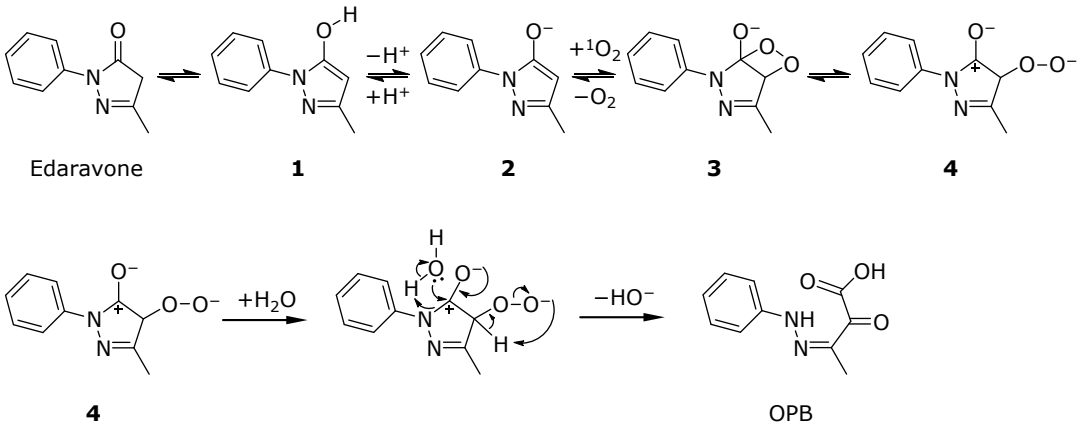

Fig. 6. A plausible mechanism for the reaction of edaravone and $^1O_2$.

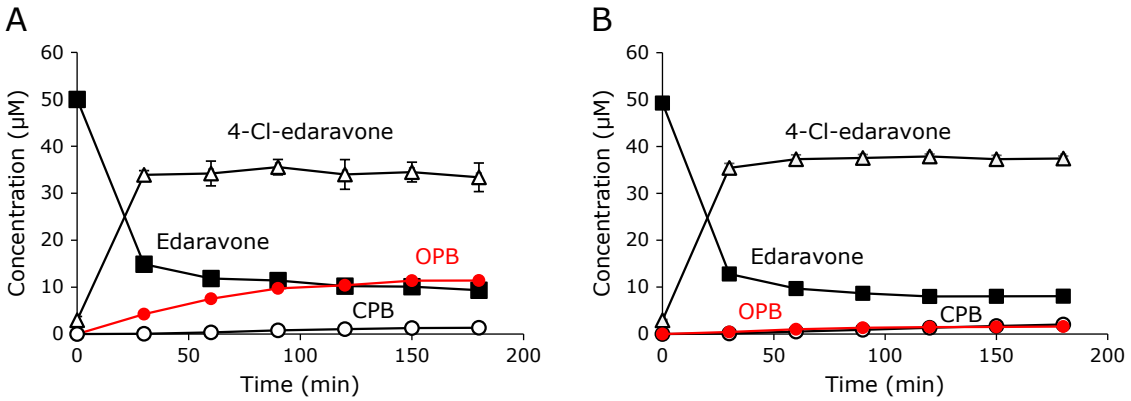

Fig. 7. Edaravone degradation and its oxidative metabolites formed by induction by ROS generated from the MPO system. (A) Changes in concentrations of edaravone (■), OPB (●), 4-Cl-edaravone (△), and CPB (○) during MPO-induced edaravone oxidation (n = 3), and the inhibitory effect by the addition of 100 μM UA (B) (n = 3).

with $^1O_2$ faster than some biological $^1O_2$ scavengers such as UA, His, Met, and Trp, and it gives OPB as an oxidative metabolite. The mechanism is different from radical-induced oxidation, and it proceeds due to edaravone ionization increasing the electron density of its pyrazoline ring, and the rate is further controlled by the addition of H_2O and the elimination of HO^-. Edaravone was also oxidized by $^1O_2$ formed from MPO, indicating that edaravone treatment may be beneficial for MPO-related injuries such as inflammation. A combination of OPB and 4-Cl-edaravone detected from patient samples treated with the drug can be a marker for MPO activation. Edaravone may be used as a drug for the treatment of injuries generally related to oxidative stress because of its function as an effective scavenger of multiple ROS.

Abbreviations

5-AE	5-acetyl-edaravone
ECD	electrochemical detector
His	histidine
Met	methionine
MPO	myeloperoxidase
OPB	2-oxo-3-(phenylhydrazono)butanoic acid
RB	rose bengal
TFA	trifluoroacetic acid
TOFMS	time-of-flight mass spectrometry
Trp	tryptophan
UA	uric acid

Conflict of Interest

We have not received any financial support or other benefits from commercial sources for the work reported in this manuscript. None of the authors have financial interests that could create potential conflicts of interest or the appearance of a conflict of interest with regard to this work.

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