molecular pharmaceutics

pubs.acs.org/molecularpharmaceutics

Article

Development of Edaravone Ionic Liquids and Their Application for the Treatment of Cerebral Ischemia/Reperfusion Injury

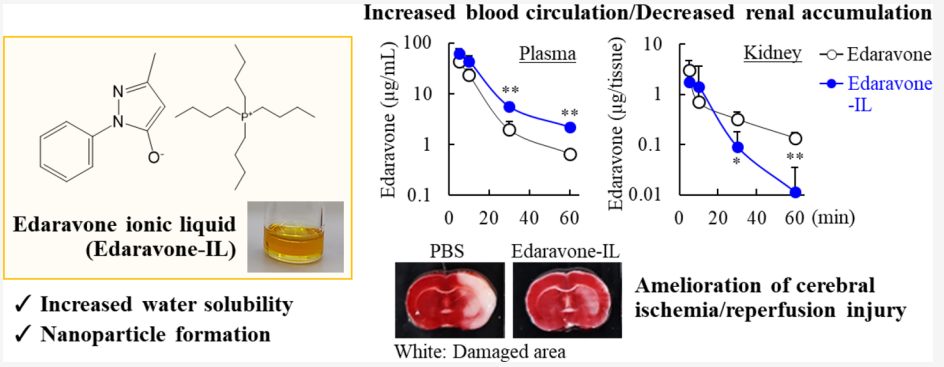
Published as part of the Molecular Pharmaceutics virtual special issue "Advances in Molecular Pharmaceutical Research from Asia".

Tatsuya Fukuta,* Mayumi Ikeda-Imafuku, and Yasunori Iwao*

Cite This: https://doi.org/10.1021/acs.molpharmaceut.3c00103

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information



ABSTRACT: Preparation of the ionic liquid (IL) form of active pharmaceutical ingredients (APIs), termed API-IL, has attracted attention because it can improve upon certain disadvantages of APIs, such as poor water solubility and low stability. Edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one) is a clinically approved cerebroprotective agent against ischemic stroke and amyotrophic lateral sclerosis, while new formulations that enable improvement of its physicochemical properties and biodistribution are desired. Herein, we report a newly developed API-IL of edaravone (edaravone-IL), in which edaravone is used as an anionic molecule. We investigated the physicochemical properties of edaravone-IL and its therapeutic effect against cerebral ischemia/reperfusion (I/R) injury, a secondary injury after an ischemic stroke. Among the cationic molecules used for edaravone-IL preparation, the IL prepared with tetrabutylphosphonium cation existed as a liquid at room temperature, and significantly increased the water solubility of edaravone without decreasing its antioxidative activity. Importantly, edaravone-IL formed negatively charged nanoparticles upon suspension in water. Intravenous administration of edaravone-IL showed significantly higher blood circulation time and lower distribution in the kidney compared with edaravone solution. Moreover, edaravone-IL significantly suppressed brain cell damage and motor functional deficits in model rats of cerebral I/R injury and showed comparable cerebroprotective effect to edaravone. Taken together, these results suggest that edaravone-IL could be a new form of edaravone with superior physicochemical properties and could be useful for the treatment of cerebral I/R injury.

KEYWORDS: *ionic liquid, edaravone, antioxidant, cerebroprotective agent, nanoparticles, cerebral ischemia/reperfusion injury*

INTRODUCTION

Ionic liquids (ILs) are commonly defined as a fluid material composed of certain combinations of organic or inorganic anions and cations.[1] They possess distinctive physicochemical properties, such as low melting point (<100 °C), high thermal stability, and low volatility, and such properties can be variously designed by the choice of anions and cations.[2] ILs have thus been used in the field of material engineering including electrochemistry and organic chemistry as the third liquid, next to water and organic solvents.[3,4]

The recent application of ILs in the pharmaceutical field has attracted attention, since ILs allow for the solubilization of poorly water-soluble active pharmaceutical ingredients (APIs),[5] transdermal permeation of small-molecular/macromolecular drugs,[6,7] and stabilization of proteins,[8] among other benefits.[1] We previously reported that an IL composed of choline and geranic acid (CAGE) increased the solubility of a

Received: February 2, 2023
Revised: March 25, 2023
Accepted: March 27, 2023

poorly water-soluble flavonoid nobiletin by 450-fold and promoted its transdermal permeation.[9] We have also demonstrated the transdermal delivery of insulin and sialidase isozyme Neu2 and resultant exertion of their biological activities *in vivo*,[10,11] indicating potential usefulness of ILs in pharmaceutical applications. As another approach to drug delivery with ILs, Kim et al. reported that a lipoidal IL composed of choline and oleate efficiently solvates verteporfin, a water-insoluble anticancer drug, and subsequently forms stable nanocomplexes <100 nm.[12] Moreover, the intravenous administration of the nanocomplexes significantly increased the tumor accumulation of verteporfin compared with verteporfin solution dissolved in dimethyl sulfoxide, resulting in enhancement of the anticancer effect in tumor-bearing mice.[12] ILs have also therefore been expected to become a promising material for drug delivery vehicles that increase the effectiveness of APIs by improving their solubility and bioavailability, among other benefits.

Edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one) is a free radical scavenger that shows potent antioxidative effects, mainly via elimination of hydroxyl radicals and suppression of lipid peroxidation.[13] Edaravone protects neuronal cells from oxidative damage and inhibits cerebral edema, so it was first approved as a cerebroprotective agent for the treatment of acute ischemic stroke in a small number of countries including Japan, China, and India.[14,15] The indication of edaravone has also been expanded in several countries as a drug to suppress the pathological progression of amyotrophic lateral sclerosis (ALS), a fatal neurodegenerative disease.[16,17] Since edaravone is a powerful neuroprotective agent against ischemic stroke and ALS, further clinical research has been in progress in several countries to broaden its application.[18,19] However, its clinical approval still remains limited, especially for ischemic stroke therapy, because of the risk of acute renal failure as a severe adverse effect following edaravone treatment.[20] Additionally, the solubility of edaravone in water is also known to be slightly poor, and aqueous edaravone is unstable since the anionic form of edaravone (pK_a = 7.0) at physiological pH 7.0 easily becomes an edaravone radical, resulting in formation of precipitates mainly composed of edaravone trimer.[21] To improve solubility and stability of edaravone in water, napadisylate salts of edaravone and edaravone nanoparticles composed of biopolymers have been developed.[22,23] Development of new edaravone formulations to further increase the usefulness of edaravone as a cerebroprotective agent has therefore been desired.

The technologies of ILs have been employed not only for drug delivery vehicles as mentioned above but also for converting drug molecules themselves into ILs to improve properties such as poor solubility and bioavailability; the latter ILs have been referred to as "API-ILs".[24] Moshikur et al. converted methotrexate (MTX), a poorly water-soluble anticancer drug, into API-ILs by using a variety of cationic molecules, which increased solubility of MTX and its anticancer activity against HeLa cells *in vitro*.[25] Berton et al. prepared two API-IL forms of lidocaine using different anionic molecules and increased its transdermal permeation, resulting in improvement of bioavailability of lidocaine compared with the crystalline salt lidocaine chloride.[26] Edaravone becomes an anionic form under the physiological conditions and exhibits its antioxidant activity by donating electrons to free radicals.[13] Based on these findings, we hypothesized that an IL form of edaravone (edaravone-IL) could be prepared by mixing edaravone with proper cationic molecules, and that it might be a new antioxidant and cerebroprotective formulation of edaravone.

In the present study, we first prepared edaravone-IL by using four kinds of cationic molecules. The physicochemical properties and antioxidative activity of the candidates of edaravone-IL were also evaluated. The pharmacokinetic profile of edaravone-IL was then evaluated *in vivo* and compared with that of edaravone solution. Finally, the cerebroprotective effect of edaravone-IL was evaluated in transient middle cerebral artery occlusion (t-MCAO) rats, an *in vivo* model of cerebral ischemia/reperfusion (I/R) injury following an ischemic stroke. The present study is the first to report development of edaravone-IL and its application for the treatment of cerebral I/R injury.

EXPERIMENTAL SECTION

Materials. Edaravone (3-methyl-1-phenyl-5-pyrazolone), 1-decyl-3-methylimidazolium chloride, 1-butyl-1-methylpyrrolidinium chloride, tetrabutylphosphonium chloride (80% in water), 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical, phenacetin, and 2,3,5-triphenyltetrazolium chloride (TTC) were purchased from Tokyo Chemical Industry (Tokyo, Japan). Choline bicarbonate (~80% in water) was bought from Sigma-Aldrich (St. Louis, MO, USA). Chloroform-*d* was purchased from Fujifilm Wako Pure Chemical (Osaka, Japan). Sodium hydrogen sulfite and L-cysteine were bought from Nacalai Tesque (Kyoto, Japan). All other chemicals were of the highest commercially available grade.

Preparation of Ionic Liquid Forms of Edaravone. Edaravone-ILs composed of edaravone and each cation were prepared as follows. Edaravone (354 mg, 2.032 mmol) was measured into a 50 mL eggplant flask and dissolved in ethanol. One equivalent of choline bicarbonate (~80% in water; 419.6 mg, 2.032 mmol), 1-decyl-3-methylimidazolim chloride (525.9 mg, 2.032 mmol), 1-butyl-1-methylpyrrolidinium chloride (361.1 mg, 2.032 mmol), or tetrabutylphosphonium chloride (80% in water; 749 mg, 2.032 mmol) was added to the 50 mL eggplant flask, and the mixture was stirred at room temperature for 1 h. The solvent was removed with a rotary evaporator at 60 °C for 4 h, and each product was further dried in a desiccator at 60 °C for 48 h. Formation of edaravone-ILs was visually confirmed, and the obtained ILs were used in the following studies.

Analysis of Edaravone−Cation Interaction by ^{1}H NMR. Each edaravone-IL was dissolved in chloroform-*d* and transferred into NMR tubes. Each sample was then analyzed by ^{1}H NMR with a JEOL 400YH instrument (Tokyo, Japan).

FT-IR Measurement of the Edaravone-ILs. Interaction between edaravone and each cation was confirmed by Fourier transform infrared (FT-IR) spectroscopy using a IRPrestige-21 spectrophotometer (Shimadzu, Kyoto, Japan) equipped with an attenuated total reflection accessory (DuraSamplIR II; ST Japan, Tokyo, Japan). FT-IR spectra of edaravone, edaravone-ILs, and each cation were obtained and analyzed with LabSolutions IR software (Shimadzu, Japan).

Preparation of Edaravone Solution and Edaravone-IL Suspension. An appropriate amount of edaravone was dissolved in 0.5 mL of 1 N NaOH and ultrapure water was added until the desired concentration was obtained. The pH of the final edaravone solution was adjusted to 7.4 by addition of 1 N HCl. For the edaravone-IL suspension, an adequate amount of edaravone-IL was weighed and mixed with ultrapure

https://doi.org/10.1021/acs.molpharmaceut.3c00103
Mol. Pharmaceutics XXXX, XXX, XXX−XXX

water, followed by vortexing for 10 s to completely disperse the samples. The pH of the edaravone-IL suspension was then adjusted to 7.4 by addition of 1 N NaOH. Each sample was prepared as described above, unless otherwise stated.

Evaluation of Antioxidative Activity. The antioxidative activity of edaravone-IL was evaluated by using the DPPH radical assay. In a 96-well plate, 120 μL of 125 μM DPPH solution in ethanol (final concn 50 μL) was mixed with 156 μL of 10 mM Tris-HCl buffer (pH 7.5). Then, 24 μL of each concentration of edaravone (125, 250, 500 μM) or edaravone-IL suspension (125, 250, 500 μM as edaravone) was added, followed by incubation at 25 °C for 20 min with shaking. The final concentration of each sample was 0, 10, 20, and 40 μM as edaravone. The absorbance of the DPPH radicals was detected at 540 nm with a microplate reader (Tecan Infinite M Plex, Tecan Japan, Kanagawa, Japan). The decreased rate of the absorbance of DPPH radicals in each group was calculated as the radical scavenging activity (%) by setting the control group (0 μM edaravone) as 100%.

Assessment of Storage Stability of Edaravone-IL. Edaravone solution and edaravone-IL suspension containing antioxidative stabilizers were prepared as follows in accordance with the drug interview form of edaravone (Radicut; Mitsubishi Tanabe Pharma, Osaka, Japan). Briefly, each solution/suspension (final concn 8.61 mM as edaravone) was mixed with sodium hydrogen sulfite (final concn 9.61 mM) and L-cysteine (final concn 2.85 mM), which were dissolved in ultrapure water and used as antioxidative stabilizers, and the pH was adjusted to 4.0 by adding 1 N HCl and NaOH. The prepared samples (20 mL) were incubated at 25 °C in the dark, and 500 μL aliquots were collected after 0, 1, 3, 7, 14, 21, and 28 days. The antioxidative activity of the aliquots was evaluated by the DPPH radical assay. Also, residual edaravone concentration in the samples was measured by high-performance liquid chromatography (HPLC; Waters Alliance 2695, Tokyo, Japan). The HPLC conditions were as follows: mobile phase, 0.5% acetic acid in water/methanol = 1/1; column, CAPCELL PAK C18 MGIII (5 μm, 4.6 mm × 150 mm) (Shiseido, Tokyo, Japan); injection volume, 20 μL; flow rate, 0.95 mL/min; column temperature, 40 °C; UV detector wavelength, 240 nm.

Water Solubility of the Edaravone-IL. An excess amount of edaravone-IL or edaravone was added to ultrapure water in test tubes, and the tubes were agitated at 25 °C for 24 h. The supernatants were collected and filtered with 0.22 μm syringe filters (MilliporeSigma, MA, USA), and the edaravone concentration in the samples was measured by HPLC as described above.

Viscosity Measurement of Edaravone-IL. Viscosity of edaravone-IL was measured by using a viscometer microVISC (Rheosense, San Ramon, CA, USA) under atmospheric pressure at a shear rate 27.3 s^{-1}. Approximately 300 μL of edaravone-IL was filled into the syringe and placed into the viscometer. All measurements were performed at room temperature (20.7 °C), and three measurements with independent samples were recorded.

Measurement of Melting Point of Edaravone-IL. Melting point of edaravone-IL was determined by differential scanning calorimeter (DSC) measurement with Discovery a Hitachi DSC7000X instrument (Hitachi High-Tech Science Corporation, Tokyo, Japan). The sample was added to an aluminum pan and covered with a lid. The melting behaviors were analyzed by setting the heating and cooling rate as 5 °C/min. The DSC instrument was calibrated using an indium standard.

Particle Size and ζ-Potential of the Edaravone-IL Suspended in Water. Edaravone-IL was suspended in ultrapure water at a concentration of 3.24 mg/mL (1.2 mg/mL as edaravone) as described above. Particle size and ζ-potential of the suspension were then determined with a Zetasizer Pro (Malvern Instruments, Worcestershire, UK).

Animals. Five-week-old male BALB/c mice and 8-week-old male Wistar rats were purchased from Japan SLC, Inc. (Shizuoka, Japan). Experimental protocols using animals were reviewed and approved by the Animal and Ethics Review Committee of Wakayama Medical University. The mice and rats were bred under 12 h light/12 h dark cycles with free access to water and food.

Pharmacokinetics of Edaravone-IL. Edaravone-IL (810 μg/mL) or edaravone solution (300 μg/mL) was intravenously injected into BALB/c mice (3 mg/kg as edaravone dose). At 5, 10, 30, and 60 min after the injection, blood samples were collected using heparinized syringes under isoflurane anesthesia, followed by dissection of kidneys from euthanized mice. The plasma samples (100 μL) obtained after centrifugation of the blood samples were deproteinized with methanol/acetonitrile = 1/1 mixture (200 μL). The samples were vortexed and incubated for 30 min at room temperature, followed by centrifugation (20,000 × *g*, 10 min, 4 °C). The 50 μL supernatants were mixed with 100 μL of 0.1 μM phenacetin (internal standard) dissolved in methanol and 10 μL of 50% methanol in ultrapure water containing 0.5% formic acid. The mixtures were vortex and centrifuged (10,000 × *g*, 10 min, 4 °C), and the edaravone concentration in the obtained supernatants was measured by liquid chromatography−tandem mass spectrometry (LC-MS/MS; Triple Quad5500+, SCIEX, Tokyo, Japan) equipped with an electrospray ionization (ESI) interface. The LC-MS/MS conditions were described as below. The mobile phases consisted of solvent A (0.5% formic acid containing water) and solvent B (0.5% formic acid containing methanol) and gradients were carried out; solvent A/solvent B = 90/10 → 20/80 (0−5 min), solvent A/solvent B = 20/80 (5−7 min), and solvent A/solvent B = 20/80 → 90/10 (7−10 min). A column YMC Triart C18 (TA12S05-0502WT; 5 μm, 2.0 mm × 50 mm) (YMC, Kyoto, Japan) was employed, and the column temperature was 40 °C. The sample injection volume was 5 μL, and measurement time was 10 min. The ESI interface was applied in positive ESI (edaravone *m*/*z* 175.0 → *m*/*z* 65.1 and phenacetin *m*/*z* 180.0 → *m*/*z* 110.1), and the data acquisition was conducted using multiple reaction monitoring mode.

In case of the kidney samples, 50% methanol in ultrapure water was added in a volume three times the weight of the kidneys, and the kidney samples were homogenized, followed by centrifugation (5,000 × g, 20 min, 4 °C). The supernatants of the homogenized samples were collected, and the 50 μL supernatants were processed by the same procedures as the plasma, as described above. Thereafter, the edaravone concentrations in the kidney samples were determined by LC-MS/MS in accordance with the same experimental conditions described above.

Preparation of Transient Middle Cerebral Artery Occlusion (t-MCAO) Rats. To evaluate the neuroprotective effect of edaravone-IL, t-MCAO rats were prepared as previously reported.[27] In brief, anesthesia was induced with 3% isoflurane and maintained with 1.5% isoflurane to perform

https://doi.org/10.1021/acs.molpharmaceut.3c00103
Mol. Pharmaceutics XXXX, XXX, XXX−XXX

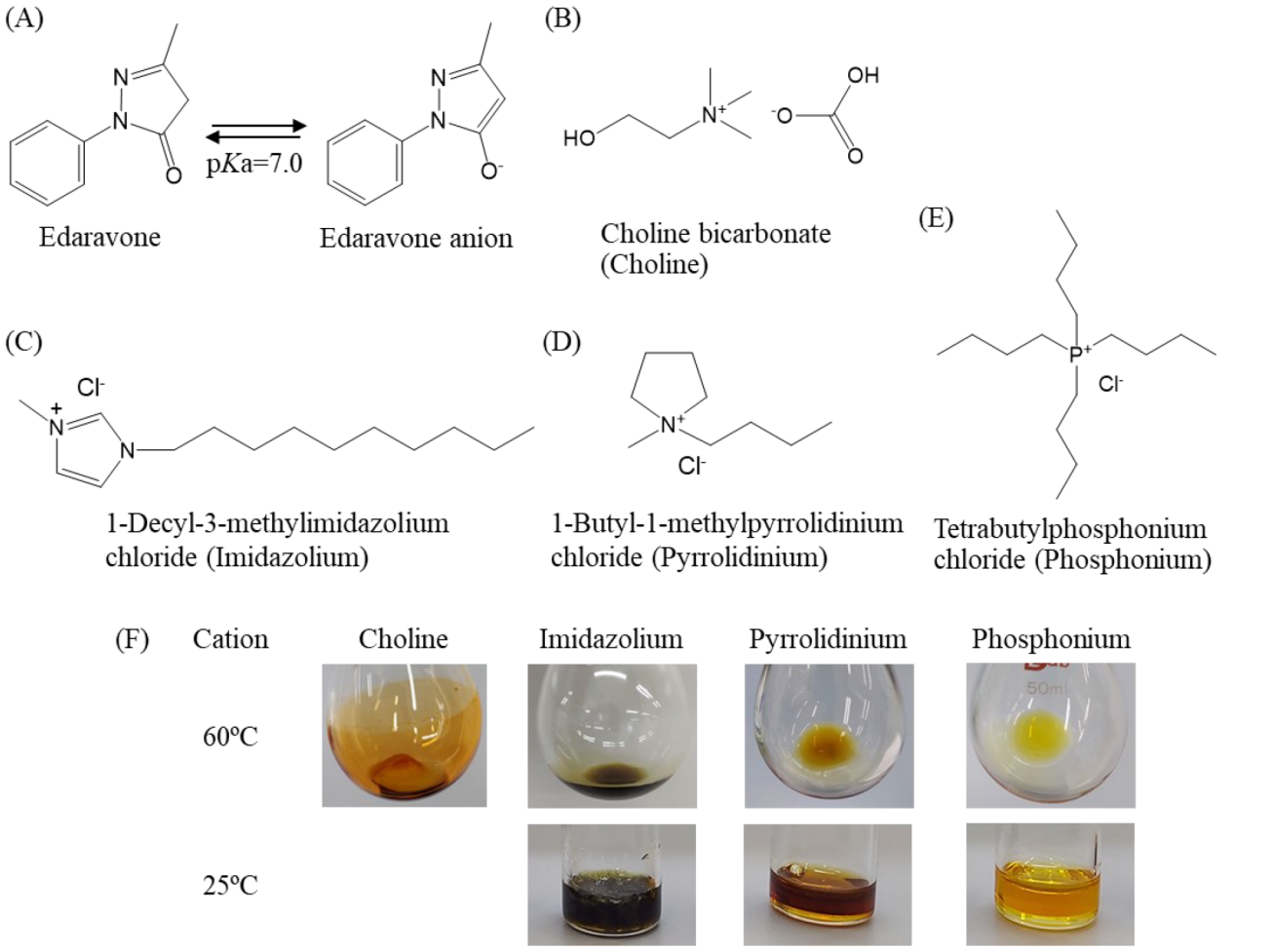


Figure 1. Preparation of edaravone ionic liquids (edaravone-ILs). (A–E) Chemical structures of edaravone, edaravone anion, and cationic molecules used to prepare edaravone-ILs. (F) Images of ILs prepared using each cationic molecule at 60 °C (immediately after 48 h drying) and 25 °C.

surgery in rats with a small animal anesthesia instrument (KN-1071; Natsume Seisakusyo Co., Tokyo, Japan). Rectal temperature of the rats was monitored and maintained at 37 °C using a small animal temperature maintenance device (BWT-100A; Bio Research Center Co., Nagoya, Japan) during the surgery. After an incision was made in the cervical skin, the right internal carotid artery (ICA) was carefully exposed. A silicon-coated nylon filament having 18 mm length (Keisei Medical Industrial, Tokyo, Japan) was inserted into the ICA and gently advanced into the MCA origin to occlude the blood flow. Isoflurane anesthesia was discontinued after the neck skin incision was sutured. At 1 h after the onset of occlusion, reperfusion of the blood was carried out by withdrawing the nylon filament under isoflurane anesthesia.

Cerebroprotective Effect of Edaravone-IL in t-MCAO Rats. t-MCAO rats were intravenously injected with edaravone-IL (3.24 mg/mL as edaravone-IL, 1.2 mg/mL as edaravone), edaravone solution (1.2 mg/mL in phosphate-buffered saline [PBS]), or PBS immediately after reperfusion following 1 h occlusion. The edaravone injection dosage was set at 3.0 mg/kg rat. At 24 h after the reperfusion, motor function of the rats was evaluated using a 21-point neurological test, as previously reported.[28] Thereafter, the brains of the t-MCAO rats were dissected after euthanization, followed by preparation of 2 mm brain slices with a rat brain slicer (Muromachi Kikai, Tokyo, Japan). The slices were then incubated at 37 °C for 20 min in 2% TTC solution in PBS to stain viable brain cells. The volume of damaged brain was determined using an image-analysis system, ImageJ.

Statistical Analysis. Statistical differences among the groups were analyzed by one-way analysis of variance with Tukey post-hoc test. Differences between two groups were determined by Student's *t* test. Data are shown as the mean ± standard deviation (SD).

RESULTS AND DISCUSSION

Preparation and Characterization of Edaravone-ILs. API-IL forms of edaravone were prepared by using edaravone as an anion (Figure 1A). Four species of cations, choline bicarbonate (choline), 1-decyl-3-methylimidazolium chloride (imidazolium), 1-butyl-1-methylpyrrolidinium chloride (pyrrolidinium), and tetrabutylphosphonium chloride (phosphonium) were used as the counterions and mixed with edaravone at a 1:1 molar ratio to prepare edaravone-ILs (Figures 1B–E). Those cationic molecules were previously used as the component of ILs for drug delivery vehicles or API-ILs.[10,29−31] The formation of edaravone-ILs was first visually judged at 60 °C following 48-h drying in a desiccator and at room temperature. As shown in the Figure 1F, the mixture of edaravone anion and choline partially formed a liquid

https://doi.org/10.1021/acs.molpharmaceut.3c00103
Mol. Pharmaceutics XXXX, XXX, XXX−XXX

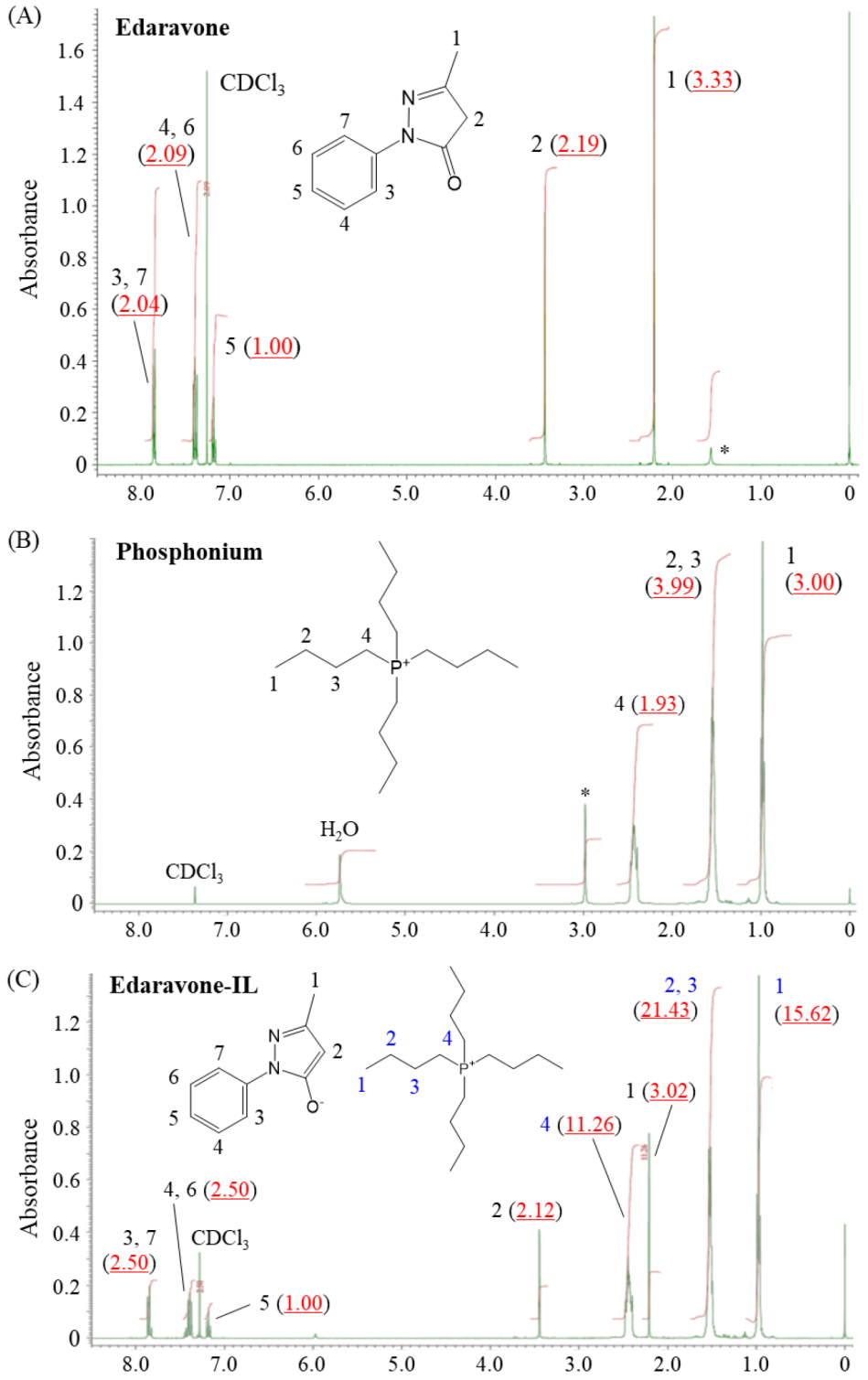


Figure 2. ^{1}H NMR spectra of edaravone, phosphonium, and edaravone-IL. Each sample (A, edaravone; B, phosphonium; C, edaravone-IL) was dissolved in chloroform-*d* and analyzed using ^{1}H NMR. Each assigned number in the NMR spectra corresponds to each number in the structural formulas. $CDCl_3$, chloroform-*d*; H_2O, 80% H_2O in tetrabutylphosphonium chloride; *unsure peak from tetrabutylphosphonium chloride. High-magnification images can be seen in Supplementary Figure 1.

component but precipitated even under 60 °C. The formation of edaravone-IL was observed at 60 °C in the case of imidazolium having been used as cation, while the IL became solid at room temperature. On the other hand, the reaction with edaravone and pyrrolidinium or phosphonium brought about formation of edaravone-ILs at both 60 °C and room temperature. The stoichiometric analyses between edaravone and the cations were performed by ^{1}H NMR, and the results suggested that the reaction occurred at the molar ratio of 1:1 (Figure 2 and Supplementary Figure 1). In the ^{1}H NMR signals of the edaravone-IL composed of phosphonium, the chemical shift derived from water (5.7−5.8 ppm) disappeared

https://doi.org/10.1021/acs.molpharmaceut.3c00103
Mol. Pharmaceutics XXXX, XXX, XXX−XXX

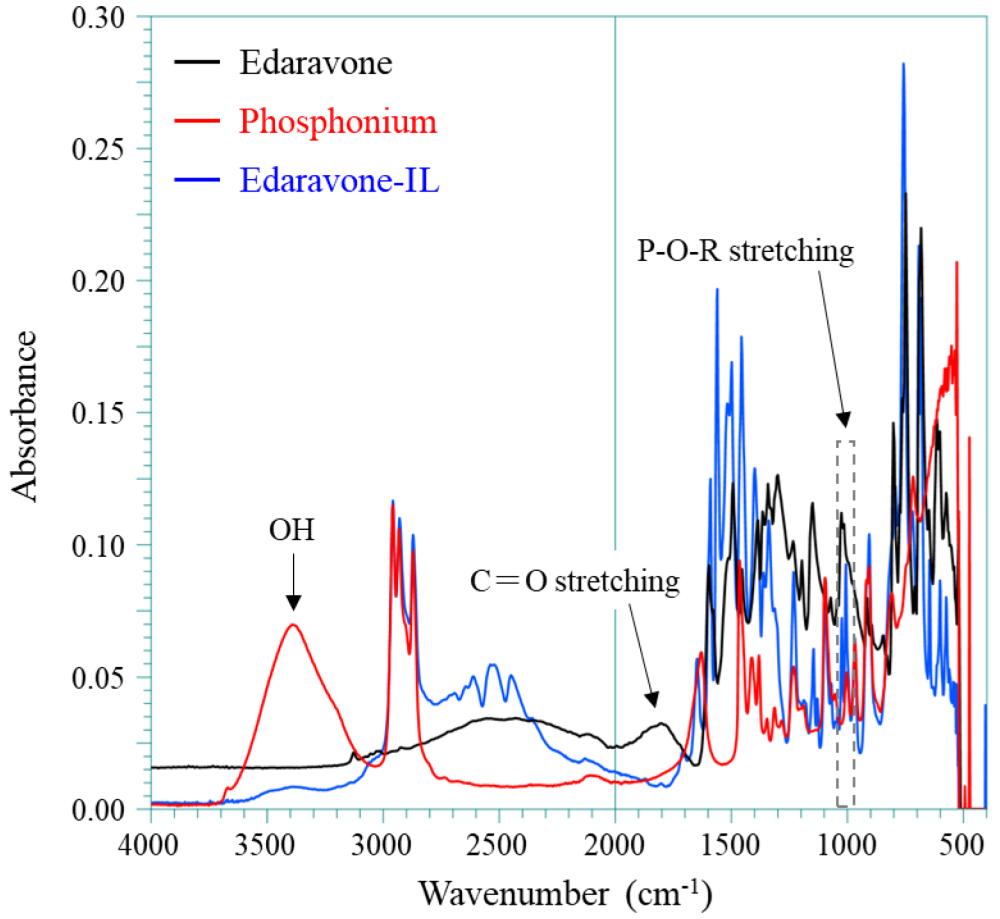


Figure 3. FT-IR spectra of edaravone, phosphonium, and edaravone-IL.

after formation of the IL, indicating that there was no water contamination (Figure 2).

The structures of edaravone-IL were further explored by FT-IR spectroscopy. The edaravone-IL prepared with pyrrolidinium precipitated in water and was considered to be unsuitable in the following *in vitro* and *in vivo* studies, so we performed FT-IR analyses for edaravone-ILs prepared with phosphonium or imidazolium. Although the detailed reason for precipitation of edaravone-IL prepared with pyrrolidinium in water is still unclear, physicochemical properties of ILs are generally known to be altered by various factors, such as combination of cation/anion species and their mixing ratio.[1] It was also previously reported that existence modes of some kinds of ILs change depending on the proportion of presenting water due to alteration in the interactions among constituent anionic/cationic molecules.[32−34] Based on these findings, we speculate that edaravone-IL prepared with phosphonium or imidazolium was compatible with water and existed stably in water, while edaravone-IL prepared with pyrrolidinium could not form stable intermolecular interactions in water and resulted in precipitate formation. The distinctive C═O stretching peak of the carbonyl group was observed at ca. 1800 cm^{-1} in the spectrum of edaravone (Figure 3). The stretching peak of the hydroxyl group at ca. 3400 cm^{-1} was observed in the spectrum of phosphonium derived from water. However, the distinctive peaks for C═O stretching and hydroxyl group, as seen in edaravone and phosphonium, respectively, were not observed after formation of edaravone-IL. Additionally, the stretching vibration derived from the P−O−R group was detected at ca. 1000−1050 cm^{-1} in the spectrum of edaravone-IL. These results indicate that edaravone-IL prepared with phosphonium could be formed via the interaction between the C═O group of edaravone and the phosphonium cation. In the case of edaravone-IL prepared with imidazolium, a peak attributed to N−O stretching at ca. 1550 cm^{-1} and a disappearance of the C═O stretching peak of the carbonyl group in edaravone were observed, indicating that edaravone-IL could also be prepared by using imidazolium (Supplementary Figure 2).

Antioxidative Properties and Stability of Edaravone-ILs. The antioxidative effect of edaravone-ILs was evaluated using the DPPH radical method.[35] As shown in Supplementary Figure 3, both edaravone-ILs prepared with phosphonium and imidazolium exhibited comparable DPPH radical scavenging activities with edaravone solution in a dose-dependent manner. These results indicate that antioxidative activity of edaravone is not decreased by API-IL formations with those cations. Considering the viewpoint of material handling that edaravone-IL prepared with phosphonium is liquid at room temperature while the IL prepared with imidazolium is solid, edaravone-IL prepared with phosphonium was regarded as edaravone-IL and used in the following experiments. Next, edaravone solution and edaravone-IL suspension were prepared at the same concentrations as the clinically approved edaravone formulation (Radicut: Mitsubishi Tanabe Pharma Co., Osaka, Japan), and temporal changes in their antioxidant activities and storage stabilities at room temperature were investigated. As cysteine and sodium hydrogen sulfite are contained in Radicut as antioxidative stabilizers of aqueous

https://doi.org/10.1021/acs.molpharmaceut.3c00103
Mol. Pharmaceutics XXXX, XXX, XXX−XXX

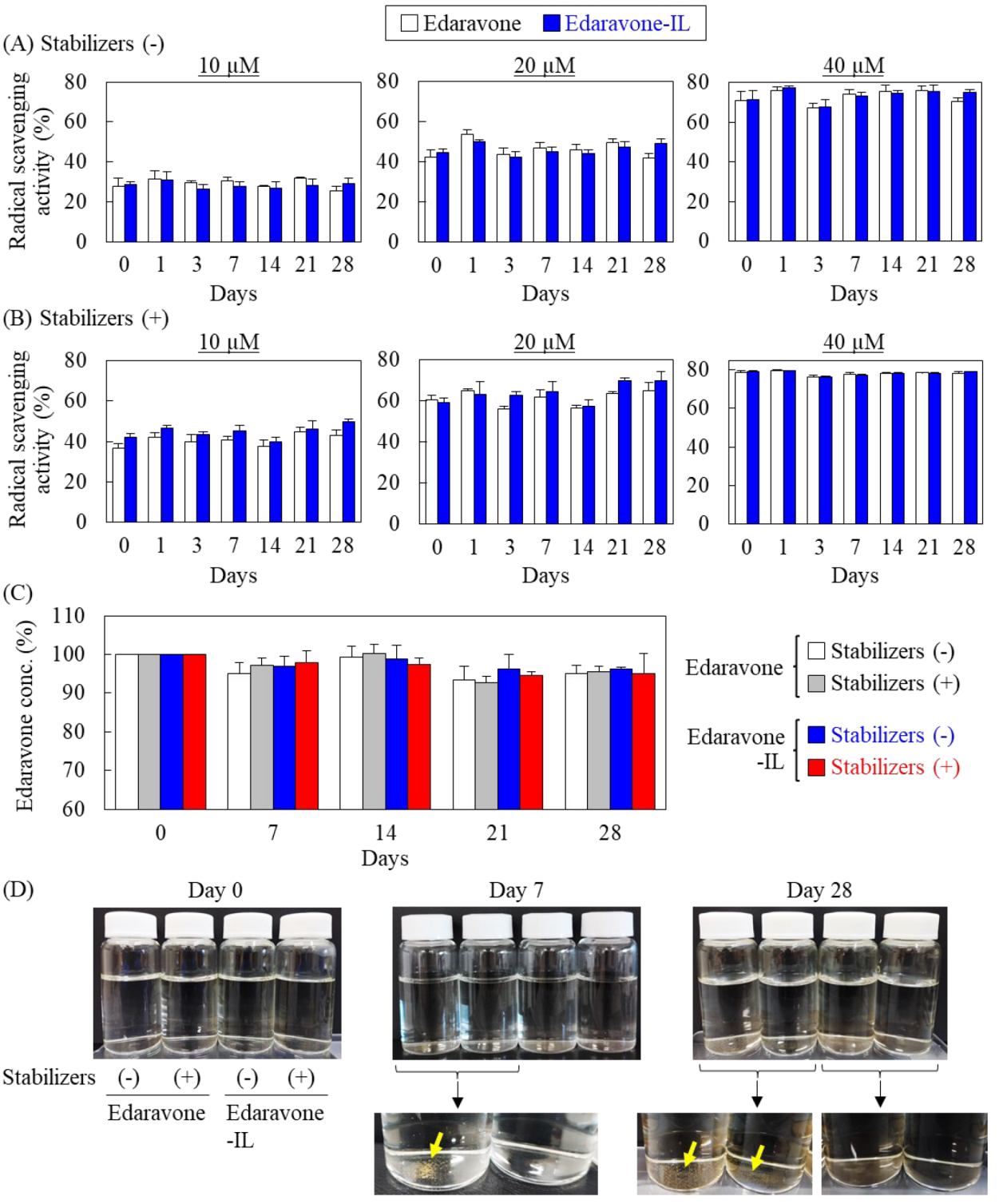


Figure 4. Antioxidative activity and stability of edaravone-IL. (A, B) After incubation of edaravone and edaravone-IL at 25 °C with/without antioxidative stabilizers (cysteine and sodium hydrogen sulfite) for 0, 1, 3, 7, 14, 21, and 28 days, radical scavenging activities of each concentration of edaravone and edaravone-IL were evaluated by the DPPH method. With the control group (water without agents) as 100%, the decreased percentage of the absorbance of DPPH in each group was calculated as the radical scavenging activity (%). (C) Residual edaravone concentration in edaravone solutions and edaravone-IL suspensions with or without the stabilizers was determined by HPLC after 0, 7, 14, 21, and 28 days of incubation at 25 °C. (D) Images of each sample obtained at 0, 7, and 28 days after incubation. Yellow arrows indicate precipitates. Data are mean ± SD (n = 3).

edaravone solution,[21] the effect of those stabilizers on edaravone-IL was also evaluated. The DPPH radical scavenging activities of edaravone solution and edaravone-IL at 0, 1, 3, 7, 14, 21, and 28 days after sample preparation were

https://doi.org/10.1021/acs.molpharmaceut.3c00103
Mol. Pharmaceutics XXXX, XXX, XXX−XXX

assessed, and the results showed no changes in radical scavenging activities in both groups until day 28 and similar antioxidative activities in a dose-dependent manner (Figure 4A). Their radical scavenging activities increased by adding the stabilizers due to their antioxidative properties, although there were almost no differences between edaravone solution and edaravone-IL, even in the presence of the stabilizers (Figure 4B). The concentrations of edaravone in each sample following incubation at room temperature were measured by HPLC. The edaravone concentration in each sample slightly decreased by ca. 5% through 28 days, but there were no differences between edaravone solution and edaravone-IL, regardless of the presence of the stabilizers (Figure 4C). On the other hand, certain precipitates were visually observed in edaravone solution without stabilizers from 7 days after the start of incubation, and those were also seen in edaravone solution with or without stabilizers at 28 days after incubation (Figure 4D). The precipitates were previously reported to mainly consist of edaravone trimer, which is formed by reaction among edaravone radicals generated in aqueous solution and small amounts of edaravone.[21] Notably, such precipitates were not observed in the suspensions of edaravone-IL with or without stabilizers, even 28 days after incubation (Figure 4D). Other researchers have reported the utility of using ILs as a vehicle to stabilize and suppress aggregation of paclitaxel, a poorly water-soluble anticancer drug, and an enzyme lysozyme in aqueous solutions.[36,37] Since the precipitates observed in edaravone solution without stabilizers were not observed in edaravone-IL suspension not containing stabilizers, the stability of edaravone in aqueous solution was considered to be improved by converting edaravone into the API-IL, similar to the previous findings on IL-mediated stabilization of paclitaxel and lysozyme in aqueous solutions. Previously, the Food and Drug Administration (FDA) pointed out the risk of allergic reactions from Radicut treatment due to sodium hydrogen sulfite being included as a stabilizer.[38] Edaravone-IL, which exists stably in water without sodium hydrogen sulfite, may therefore avoid the potential risk of allergic reactions.

Water Solubility and Physicochemical Properties of Edaravone-IL. The water solubility of edaravone-IL was evaluated by agitating the excess amount of edaravone-IL in water at 25 °C for 24 h and comparing with that of edaravone. The water solubility of edaravone was determined to be 1.96 ± 0.05 mg/mL by HPLC analysis (Table 1), similar to the data described in the drug interview form of Radicut (2 mg/mL in water) and some reports.[13] Notably, the solubility of edaravone significantly increased 1.77-fold to 3.47 ± 0.35 mg/mL (as edaravone) by formation of edaravone-IL (Table 1). Also, edaravone-IL was rapidly dispersed in water within a short mixing time (10 s), whereas edaravone did not easily dissolve in water without pH control with NaOH, as previously reported[39] and required a long time to dissolve in water alone (data not shown). These results indicate that forming the API-IL of edaravone brings about an increase in water solubility of edaravone, similar to other API-ILs previously reported.[25,40,41] Conversion of a crystalline API to API-IL form has been reported to disrupt the crystal structure of the API, allowing for increase in its water solubility.[42] Based on this finding, we consider that breakdown of the crystal structure of edaravone by API-IL formation with phosphonium and maintaining the ionic complex in water contributed to the increase in edaravone solubility. The viscosity and melting point of edaravone-IL were 7753.7 ± 61.3 mPa·s (at 20.7 °C) and −41.3 °C, respectively. Since viscosity, which is mostly mediated by intermolecular forces depending on the species of cationic and anionic molecules, and melting point below 100 °C are fundamental properties of ILs,[43,44] these results indicate that edaravone-IL definitely possesses unique properties as ILs. Interestingly, edaravone-IL was found to form nanoparticles with a diameter of 199.2 ± 68.0 nm in water with a negatively charged ζ-potential of −26.6 ± 1.2 mV (Table 2). Nanoparticulation of APIs is known to contribute to their improved solubility and stability,[45] so nanoparticle formation of edaravone-IL was suggested to be a mechanism for increasing water solubility of edaravone. Although the detailed molecular structure of the edaravone-IL nanoparticles is must be elucidated, we speculate that, after dispersing the edaravone-IL in water, repeated structures of edaravone anions and four carbon chains of tetrabutyl phosphonium cations may form nanostructures which present edaravone anions on the outermost layer, resulting in formation of edaravone-IL nanoparticles with negatively charged surfaces.

Table 1. Water Solubility of Edaravone and Edaravone-IL

	edaravone	edaravone-IL (as edaravone)
solubility (mg/mL)	1.96 ± 0.05	3.47 ± 0.35[a]

[a]**$P < 0.01$ vs edaravone.

Table 2. Physicochemical Properties of Edaravone-IL in Water

	size (d, nm)	ζ-potential (mV)
edaravone-IL	199.2 ± 68.0	−26.6 ± 1.2

Pharmacokinetics of Intravenously Injected Edaravone-IL in the Blood and the Kidneys. Next, the pharmacokinetics of intravenously injected edaravone-IL in the blood and the kidneys of mice was evaluated, and we investigated whether formation of the API-IL affected the pharmacokinetics of edaravone. The results showed that edaravone-IL exhibited significantly higher concentration in plasma at 30 and 60 min after injection than edaravone solution (Figure 5A), indicating that stability in blood circulation could be increased by formation of API-IL of edaravone with phosphonium. According to the drug interview form of edaravone (Radicat), edaravone is mainly metabolized in the liver and converted to glucuronide and sulfate conjugates, followed by rapid excretion in the urine. The distribution half-life ($t_{1/2}\alpha$)/elimination half-life ($t_{1/2}\beta$) of edaravone, edaravone glucuronide, and edaravone sulfate in male rats are 0.07/1.26, 0.07/0.63, and 0.10/1.68 h, respectively. It was previously reported that formation of an API-IL has the potential to avoid the metabolism of the API in the body.[46] Based on this finding, it is speculated that formation of API-IL of edaravone might contribute to avoidance of its metabolism, which allowed for increased blood concentration of unmetabolized edaravone. In addition, the amount of edaravone in kidneys significantly decreased in the edaravone-IL group compared with the edaravone solution group 30 and 60 min after injection (Figure 5B). Nanoparticle formation of edaravone-IL (199.2 ± 68.0 nm in diameter) is considered to be the reason for the decrease in edaravone amount in the kidney, since nanoparticles larger than 15 nm were reported be unable to distribute in the kidney due to the

https://doi.org/10.1021/acs.molpharmaceut.3c00103
Mol. Pharmaceutics XXXX, XXX, XXX−XXX

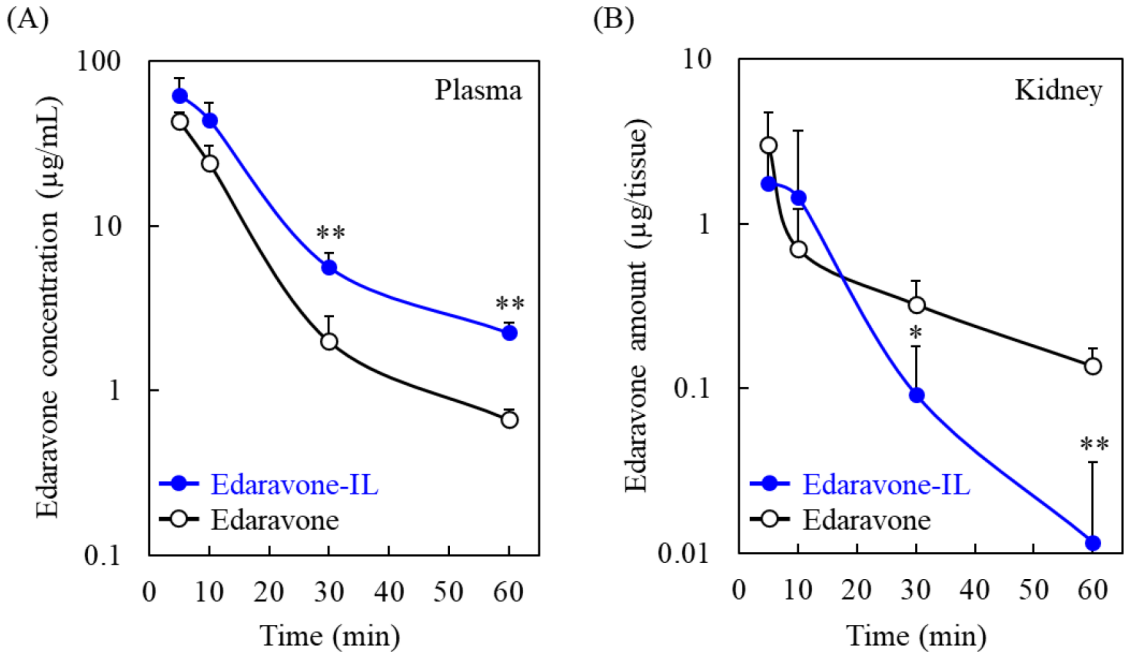


Figure 5. Pharmacokinetics of edaravone-IL in plasma and kidney after intravenous administration. (A, B) BALB/c mice were intravenously administered edaravone-IL (3.0 mg/kg as edaravone dose) or edaravone (3.0 mg/kg). At 5, 10, 30, and 60 min after the injection, plasma and kidneys were collected from the mice, and the edaravone concentration in them was measured by LC-MS/MS. Data are mean ± SD (n = 3−4). *P < 0.05 and **P < 0.01 vs edaravone.

presence of glomerulus.[47] Although acute renal disorders after edaravone treatment have been recognized as severe adverse events for ischemic stroke therapy,[20] the use of edaravone-IL might prevent the onset of the disorders in comparison to edaravone.

Therapeutic Effect of Edaravone-IL on Cerebral I/R Injury. Finally, the cerebroprotective effect of edaravone-IL for cerebral I/R injury was investigated in t-MCAO rats through the assessment of damaged brain volume and motor functional deficits 24 h after I/R. In Figure 6A, the right brain hemisphere in each brain slice indicates the ischemic hemisphere. The red area represents viable brain cells stained with TTC, while the white area represents damaged brain cells. In the PBS-treated control group, I/R-induced widespread brain damage was observed in the ischemic hemisphere (Figure 6A). Treatment with edaravone significantly suppressed the brain damage as previously reported (Figure 6A,B). Importantly, the intravenous administration of edaravone-IL showed comparable effect to that of edaravone solution (Figure 6A,B), indicating that the potent antioxidative activity of edaravone was not impaired *in vivo* by formation of edaravone-IL. Further, the edaravone-IL administration significantly ameliorated the motor function deficits of t-MCAO rats compared with those in the PBS-treated group and showed similar effect to edaravone solution (Figure 6C). These results suggest that edaravone-IL should be a novel pharmaceutical formulation of edaravone while retaining its potent cerebroprotective effect and that it could be useful for the treatment of cerebral I/R injury.

Intravenous administration of edaravone-IL was selected to evaluate its potential as a cerebroprotective agent against cerebral I/R injury and to compare with edaravone solution used in the clinical settings. As shown in Figure 6, edaravone-IL showed comparable therapeutic effect to edaravone solution in t-MCAO rats, indicating that the API-IL form of edaravone could become a promising formulation to improve its utility. As ILs have been demonstrated to function as excellent drug penetration enhancers through skin and mucosa,[10,48,49] application of edaravone-ILs for different administration routes other than intravenous injection should be an interesting approach. For instance, amelioration of diabetes mellitus-induced skin flap necrosis by topically administered edaravone was previously reported via reduction in oxidative stress.[50] Topical application of edaravone has also been expected for treatment of other inflammatory skin diseases, such as psoriasis and atopic dermatitis, because oxidative stress is involved in their pathological progression.[51] Transdermal delivery of edaravone-IL may therefore bring about increase in the therapeutic effects of edaravone on those skin disorders via the IL-mediated enhanced permeability through the skin. Another possible administration route includes the intranasal pathway, aiming for nose-to-brain delivery. Tanigawa et al. reported that intranasal administration of the API-IL form of etodolac prepared with proline ethyl ester exhibits improved retention of etodolac on the nasal mucosa and increased efficiency of the nose-to-brain delivery compared with etodolac solution.[52] Based on this finding, it is expected that edaravone-IL can also be applied to the nose-to-brain delivery for the treatment of brain diseases. Recently, an oral suspension formulation of edaravone (RADICAVA ORS; Mitsubishi Tanabe Pharma) was approved by the FDA as a therapeutic agent against ALS; the formulation allowed for noninvasive administration with decreased burden on patients with ALS.[53] By further demonstrating the usefulness of edaravone-IL, there may be a future possibility of application of the IL via other administration routes for the treatment of cerebral I/R injury and ALS.

To prepare edaravone-ILs, we used only four kinds of cations that have previously been applied to prepare API-ILs.[10,29−31] It has been reported that the properties of the

https://doi.org/10.1021/acs.molpharmaceut.3c00103
Mol. Pharmaceutics XXXX, XXX, XXX−XXX

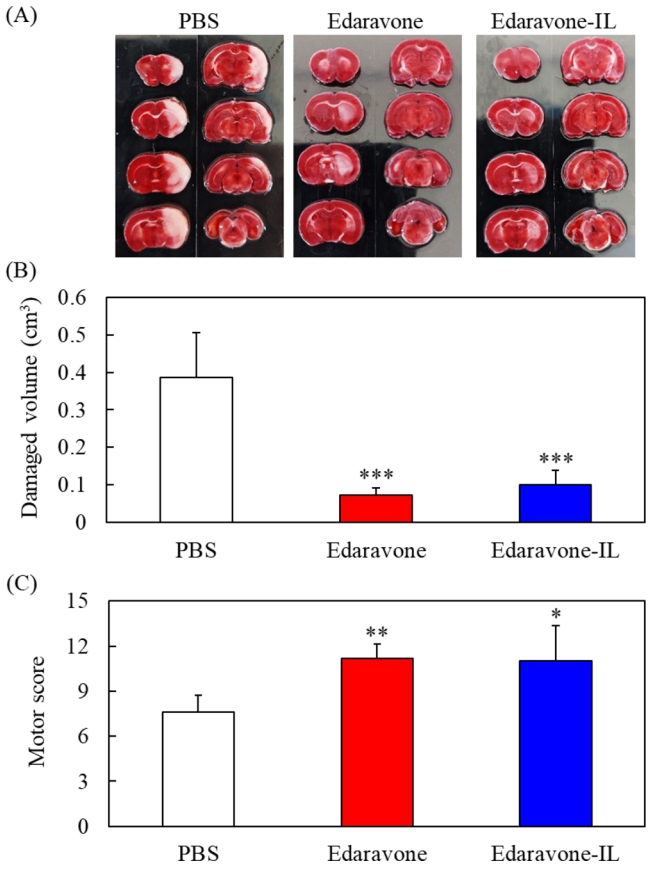


Figure 6. Therapeutic effect of edaravone-IL on cerebral I/R injury in t-MCAO rats. (A) t-MCAO rats were intravenously injected with edaravone-IL (3.0 mg/kg edaravone dose), edaravone (3.0 mg/kg), or PBS immediately after reperfusion following 1-h occlusion. At 24 h after reperfusion, coronal 2 mm brain sections were prepared and stained in TTC solution to visualize viable brain cells (red area, viable cells; white area, damaged cells). (B) Damaged brain volume was analyzed using ImageJ. (C) The motor function of the rats was assessed by the 21-point neurological test before brain dissection. Data are mean ± SD (n = 6). *P < 0.05, **P < 0.01, and ***P < 0.001 vs PBS.

prepared ILs change depending on the species of cations/anions and on their mixing ratio.[54,55] Although phosphonium cation-based edaravone-IL was selected based on the viewpoint of material handling (liquid at room temperature) among the prepared edaravone-ILs, there may be better cation pairs to improve solubility, stability, and bioavailability of edaravone and to increase its cerebroprotective effect. In addition, the mixing ratio of edaravone and cations can be optimized to enhance the performance of the edaravone-IL for its application via other administration routes as well as for the treatment of different diseases. Interestingly, edaravone-IL formed nanoparticles with approximately 200 nm diameter upon suspension in water (Table 2). The detailed mechanisms of the nanoparticle formation of edaravone-IL need to be elucidated; however, it is possible that the physicochemical properties of edaravone-IL, such as size and ζ-potential, can also be tuned by the composition of edaravone-IL. Consideration of the above-mentioned matters could lead to increase in the functionality and utility of edaravone-ILs for the treatment of the brain diseases.

CONCLUSIONS

In the present study, we prepared API-ILs of edaravone by mixing edaravone (as the anionic molecule) and cationic molecules in a 1:1 molar ratio. The edaravone-IL composed of phosphonium, the IL of which exists as a liquid at room temperature, significantly increased the water solubility of edaravone without decreasing its potent antioxidative activity. Also, edaravone-IL showed better water stability compared with edaravone solution, without forming precipitates at room temperature for 28 days. Importantly, the intravenously administered edaravone-IL exhibited significantly higher blood circulation capability and lower renal distribution than edaravone solution, probably due to the formation of nanoparticles. Finally, treatment with edaravone-IL significantly ameliorated cerebral I/R injury in t-MCAO rats and showed comparable cerebroprotective effect to edaravone solution. Based on these findings, we suggest that the API-IL form of edaravone should improve solubility, stability, and biodistribution of edaravone and that edaravone-IL could therefore be applied as a new form of edaravone for the treatment of brain diseases. Further usefulness of edaravone-IL is expected to be demonstrated with consideration of the species and the mixing ratio of cation molecules and by exploring administration routes other than the intravenous route. This is the first report that prepared edaravone-IL and demonstrated its utility for the treatment of cerebral I/R injury.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.molpharmaceut.3c00103.

^{1}H NMR spectra of imidazolium, pyrrolidinium, and edaravone-IL prepared by using those cationic molecules, FT-IR spectra of edaravone-IL prepared with imidazolium, and antioxidative activity of edaravone, edaravone-IL prepared with phosphonium, and edaravone-IL prepared with imidazolium by the DPPH method (PDF)

AUTHOR INFORMATION

Corresponding Authors

Tatsuya Fukuta − *Department of Physical Pharmaceutics, School of Pharmaceutical Sciences, Wakayama Medical University, Wakayama 640-8156, Japan*; orcid.org/0000-0001-8878-8602; Phone: +81-73-488-2661; Email: fukuta@wakayama-med.ac.jp

Yasunori Iwao − *Department of Physical Pharmaceutics, School of Pharmaceutical Sciences, Wakayama Medical University, Wakayama 640-8156, Japan*; Phone: +81-73-498-7644; Email: iwao@wakayama-med.ac.jp

Author

Mayumi Ikeda-Imafuku − *Department of Physical Pharmaceutics, School of Pharmaceutical Sciences, Wakayama Medical University, Wakayama 640-8156, Japan*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.molpharmaceut.3c00103

Notes

The authors declare no competing financial interest.

https://doi.org/10.1021/acs.molpharmaceut.3c00103
Mol. Pharmaceutics XXXX, XXX, XXX−XXX

■ ACKNOWLEDGMENTS

The authors thank Seigo Sanoh and Takuya Yamashita at School of Pharmaceutical Sciences, Wakayama Medical University, Japan, for technical help with LC-MS/MS analyses. The authors also thank Hiromasa Uchiyama, Kazunori Kadota, and Yuichi Tozuka at Faculty of Pharmacy, Osaka Medical and Pharmaceutical University, Japan, for technical help with DSC analysis. This research was funded by the Japan Society for the Promotion of Science (KAKENHI, Grant number 21K18065). We acknowledge proofreading and editing by Benjamin Phillis at Wakayama Medical University.

■ REFERENCES

(1) Curreri, A. M.; Mitragotri, S.; Tanner, E. E. L. Recent Advances in Ionic Liquids in Biomedicine. *Adv. Sci.* **2021**, *8* (17), e2004819.
(2) Egorova, K. S.; Gordeev, E. G.; Ananikov, V. P. Biological Activity of Ionic Liquids and Their Application in Pharmaceutics and Medicine. *Chem. Rev.* **2017**, *117* (10), 7132−7189.
(3) Brett, C. M. Deep eutectic solvents and applications in electrochemical sensing. *Curr. Opin. Electrochem.* **2018**, *10*, 143−148.
(4) Haumann, M.; Riisager, A. Hydroformylation in room temperature ionic liquids (RTILs): catalyst and process developments. *Chem. Rev.* **2008**, *108* (4), 1474−1497.
(5) Md Moshikur, R.; Chowdhury, M. R.; Moniruzzaman, M.; Goto, M. Biocompatible ionic liquids and their applications in pharmaceutics. *Green Chem.* **2020**, *22* (23), 8116−8139.
(6) Islam, M. R.; Chowdhury, M. R.; Wakabayashi, R.; Tahara, Y.; Kamiya, N.; Moniruzzaman, M.; Goto, M. Choline and amino acid based biocompatible ionic liquid mediated transdermal delivery of the sparingly soluble drug acyclovir. *Int. J. Pharm.* **2020**, *582*, 119335.
(7) Mandal, A.; Kumbhojkar, N.; Reilly, C.; Dharamdasani, V.; Ukidve, A.; Ingber, D. E.; Mitragotri, S. Treatment of psoriasis with NFKBIZ siRNA using topical ionic liquid formulations. *Sci. Adv.* **2020**, *6* (30), eabb6049.
(8) Reslan, M.; Kayser, V. Ionic liquids as biocompatible stabilizers of proteins. *Biophys. Rev.* **2018**, *10* (3), 781−793.
(9) Hattori, T.; Tagawa, H.; Inai, M.; Kan, T.; Kimura, S. I.; Itai, S.; Mitragotri, S.; Iwao, Y. Transdermal delivery of nobiletin using ionic liquids. *Sci. Rep.* **2019**, *9* (1), 20191.
(10) Banerjee, A.; Ibsen, K.; Iwao, Y.; Zakrewsky, M.; Mitragotri, S. Transdermal Protein Delivery Using Choline and Geranate (CAGE) Deep Eutectic Solvent. *Adv. Healthc. Mater.* **2017**, *6* (15), 1601411.
(11) Minami, A.; Fujita, Y.; Goto, J.; Iuchi, A.; Fujita, K.; Mikami, Y.; Shiratori, M.; Ishii, A.; Mitragotri, S.; Iwao, Y.; et al. Enhancement of elastin expression by transdermal administration of sialidase isozyme Neu2. *Sci. Rep.* **2021**, *11* (1), 3302.
(12) Kim, J.; Shi, Y.; Kwon, C. J.; Gao, Y.; Mitragotri, S. A Deep Eutectic Solvent-Based Approach to Intravenous Formulation. *Adv. Healthc. Mater.* **2021**, *10* (18), e2100585.
(13) Watanabe, K.; Tanaka, M.; Yuki, S.; Hirai, M.; Yamamoto, Y. How is edaravone effective against acute ischemic stroke and amyotrophic lateral sclerosis? *J. Clin. Biochem. Nutr.* **2018**, *62* (1), 20−38.
(14) Müllges, W.; Franke, D.; Reents, W.; Babin-Ebell, J.; Toyka, K. V.; Ko, N.; Johnston, S.; Young, W.; Singh, V.; Klatsky, A. Effect of a novel free radical scavenger, Edaravone (MCI-186), on acute brain infarction. *Cerebrovasc. Dis.* **2003**, *15* (3), 222−229.
(15) Fukuta, T.; Oku, N.; Kogure, K. Application and Utility of Liposomal Neuroprotective Agents and Biomimetic Nanoparticles for the Treatment of Ischemic Stroke. *Pharmaceutics* **2022**, *14* (2), 361.
(16) Houzen, H.; Kano, T.; Horiuchi, K.; Wakita, M.; Nagai, A.; Yabe, I. Improved Long-Term Survival with Edaravone Therapy in Patients with Amyotrophic Lateral Sclerosis: A Retrospective Single-Center Study in Japan. *Pharmaceuticals (Basel)* **2021**, *14* (8), 705.
(17) Rothstein, J. D. Edaravone: A new drug approved for ALS. *Cell* **2017**, *171* (4), 725.
(18) Fidalgo, M.; Ricardo Pires, J.; Viseu, I.; Magalhaes, P.; Gregorio, H.; Afreixo, V.; Gregorio, T. Edaravone for acute ischemic stroke - Systematic review with meta-analysis. *Clin. Neurol. Neurosurg.* **2022**, *219*, 107299.
(19) Xu, X.; Shen, D.; Gao, Y.; Zhou, Q.; Ni, Y.; Meng, H.; Shi, H.; Le, W.; Chen, S.; Chen, S. A perspective on therapies for amyotrophic lateral sclerosis: can disease progression be curbed? *Transl. Neurodegener.* **2021**, *10* (1), 29.
(20) Hishida, A. Determinants for the prognosis of acute renal disorders that developed during or after treatment with edaravone. *Clin. Exp. Nephrol.* **2009**, *13* (2), 118−122.
(21) Tanaka, M.; Sugimura, N.; Fujisawa, A.; Yamamoto, Y. Stabilizers of edaravone aqueous solution and their action mechanisms. 1. Sodium bisulfite. *J. Clin. Biochem. Nutr.* **2017**, *61* (3), 159−163.
(22) Casares, A. F.; Van Der Geest, R.; Moolenaar, S. H. Edaravone salt. U.S. Patent No. US11117868, 2021.
(23) Fan, Y.; Wu, W.; Lei, Y.; Gaucher, C.; Pei, S.; Zhang, J.; Xia, X. Edaravone-Loaded Alginate-Based Nanocomposite Hydrogel Accelerated Chronic Wound Healing in Diabetic Mice. *Mar. Drugs.* **2019**, *17* (5), 285.
(24) Wu, X.; Zhu, Q.; Chen, Z.; Wu, W.; Lu, Y.; Qi, J. Ionic liquids as a useful tool for tailoring active pharmaceutical ingredients. *J. Controlled Release* **2021**, *338*, 268−283.
(25) Moshikur, R. M.; Chowdhury, M. R.; Wakabayashi, R.; Tahara, Y.; Moniruzzaman, M.; Goto, M. Ionic liquids with methotrexate moieties as a potential anticancer prodrug: Synthesis, characterization and solubility evaluation. *J. Mol. Liq.* **2019**, *278*, 226−233.
(26) Berton, P.; Di Bona, K. R.; Yancey, D.; Rizvi, S. A. A.; Gray, M.; Gurau, G.; Shamshina, J. L.; Rasco, J. F.; Rogers, R. D. Transdermal Bioavailability in Rats of Lidocaine in the Forms of Ionic Liquids, Salts, and Deep Eutectic. *ACS Med. Chem. Lett.* **2017**, *8* (5), 498−503.
(27) Ishii, T.; Asai, T.; Oyama, D.; Fukuta, T.; Yasuda, N.; Shimizu, K.; Minamino, T.; Oku, N. Amelioration of cerebral ischemia−reperfusion injury based on liposomal drug delivery system with asialo-erythropoietin. *J. Controlled Release* **2012**, *160* (1), 81−87.
(28) Hunter, A.; Hatcher, J.; Virley, D.; Nelson, P.; Irving, E.; Hadingham, S.; Parsons, A. Functional assessments in mice and rats after focal stroke. *Neuropharmacology* **2000**, *39* (5), 806−816.
(29) Wu, H.; Deng, Z.; Zhou, B.; Qi, M.; Hong, M.; Ren, G. Improved transdermal permeability of ibuprofen by ionic liquid technology: Correlation between counterion structure and the physicochemical and biological properties. *J. Mol. Liq.* **2019**, *283*, 399−409.
(30) Farooq, U.; Patel, R.; Ali, A. Interaction of a surface-active ionic liquid with an antidepressant drug: Micellization and spectroscopic studies. *J. Sol. Chem.* **2018**, *47* (3), 568−585.
(31) Heyert, A. J.; Knox, S. L.; Lindberg, G. E.; Baker, J. L. Influence of an ionic liquid on the conformational sampling of Xaa-Pro dipeptides. *J. Mol. Liq.* **2017**, *227*, 66−75.
(32) Takeda, J.; Iwao, Y.; Karashima, M.; Yamamoto, K.; Ikeda, Y. Structural evaluation of the choline and geranic acid/water complex by SAXS and NMR analyses. *ACS Biomater. Sci. Eng.* **2021**, *7* (2), 595−604.
(33) Tanner, E. E.; Piston, K. M.; Ma, H.; Ibsen, K. N.; Nangia, S.; Mitragotri, S. The influence of water on choline-based ionic liquids. *ACS Biomater. Sci. Eng.* **2019**, *5* (7), 3645−3653.
(34) Baldelli, S. Influence of water on the orientation of cations at the surface of a room-temperature ionic liquid: A sum frequency generation vibrational spectroscopic study. *J. Phys. Chem. B* **2003**, *107* (25), 6148−6152.
(35) Mishra, K.; Ojha, H.; Chaudhury, N. K. Estimation of antiradical properties of antioxidants using DPPH assay: A critical review and results. *Food Chem.* **2012**, *130* (4), 1036−1043.
(36) Chowdhury, M. R.; Moshikur, R. M.; Wakabayashi, R.; Tahara, Y.; Kamiya, N.; Moniruzzaman, M.; Goto, M. Ionic-Liquid-Based Paclitaxel Preparation: A New Potential Formulation for Cancer Treatment. *Mol. Pharmaceutics* **2018**, *15* (6), 2484−2488.

https://doi.org/10.1021/acs.molpharmaceut.3c00103
Mol. Pharmaceutics XXXX, XXX, XXX−XXX

(37) Kaur, M.; Singh, G.; Kaur, A.; Sharma, P. K.; Kang, T. S. Thermally Stable Ionic Liquid-Based Microemulsions for High-Temperature Stabilization of Lysozyme at Nanointerfaces. *Langmuir* **2019**, *35* (11), 4085−4093.
(38) Tanaka, M.; Motomiya, S.; Fujisawa, A.; Yamamoto, Y. Stabilizers of edaravone aqueous solution and their action mechanisms. 2. Glutathione. *J. Clin. Biochem. Nutr.* **2017**, *61* (3), 164−168.
(39) Parikh, A.; Kathawala, K.; Tan, C. C.; Garg, S.; Zhou, X. F. Development of a novel oral delivery system of edaravone for enhancing bioavailability. *Int. J. Pharm.* **2016**, *515* (1−2), 490−500.
(40) Moshikur, R. M.; Chowdhury, M. R.; Wakabayashi, R.; Tahara, Y.; Moniruzzaman, M.; Goto, M. Characterization and cytotoxicity evaluation of biocompatible amino acid esters used to convert salicylic acid into ionic liquids. *Int. J. Pharm.* **2018**, *546* (1−2), 31−38.
(41) Shayanfar, S.; Shayanfar, A. Ionic liquid forms of carvedilol: preparation, characterization, and solubility studies. *J. Pharm. Innov.* **2019**, *14* (4), 382−390.
(42) Stoimenovski, J.; MacFarlane, D. R.; Bica, K.; Rogers, R. D. Crystalline vs. ionic liquid salt forms of active pharmaceutical ingredients: a position paper. *Pharm. Res.* **2010**, *27*, 521−526.
(43) Rogers, R. D.; Seddon, K. R. Ionic liquids–solvents of the future? *Science* **2003**, *302* (5646), 792−793.
(44) Shirota, H.; Castner, E. W. Physical properties and intermolecular dynamics of an ionic liquid compared with its isoelectronic neutral binary solution. *J. Phys. Chem. A* **2005**, *109* (42), 9388−9392.
(45) Hagedorn, M.; Liebich, L.; Bögershausen, A.; Massing, U.; Hoffmann, S.; Mende, S.; Rischer, M. Rapid development of API nano-formulations from screening to production combining dual centrifugation and wet agitator bead milling. *Int. J. Pharm.* **2019**, *565*, 187−198.
(46) Jadhav, N. R.; Bhosale, S. P.; Bhosale, S. S.; Mali, S. D.; Toraskar, P. B.; Kadam, T. S. Ionic liquids: Formulation avenues, drug delivery and therapeutic updates. *J. Drug Delivery Sci. Technol.* **2021**, *65*, 102694.
(47) Liang, X.; Wang, H.; Zhu, Y.; Zhang, R.; Cogger, V. C.; Liu, X.; Xu, Z. P.; Grice, J. E.; Roberts, M. S. Short- and Long-Term Tracking of Anionic Ultrasmall Nanoparticles in Kidney. *ACS Nano* **2016**, *10* (1), 387−395.
(48) Peng, K.; Gao, Y.; Angsantikul, P.; LaBarbiera, A.; Goetz, M.; Curreri, A. M.; Rodrigues, D.; Tanner, E. E. L.; Mitragotri, S. Modulation of Gastrointestinal Mucus Properties with Ionic Liquids for Drug Delivery. *Adv. Healthc. Mater.* **2021**, *10* (13), e2002192.
(49) Vaidya, A.; Mitragotri, S. Ionic liquid-mediated delivery of insulin to buccal mucosa. *J. Controlled Release* **2020**, *327*, 26−34.
(50) Kim, Y. S.; Lee, H. Y.; Jang, J. Y.; Lee, H. R.; Shin, Y. S.; Kim, C. H. Redox treatment ameliorates diabetes mellitus-induced skin flap necrosis via inhibiting apoptosis and promoting neoangiogenesis. *Exp. Biol. Med. (Maywood)* **2021**, *246* (6), 718−728.
(51) Sato, T.; Mizuno, K.; Ishii, F. A novel administration route for edaravone: I. Effects of metabolic inhibitors on skin permeability of edaravone. *Int. J. Pharm.* **2009**, *372* (1−2), 33−38.
(52) Tanigawa, H.; Suzuki, N.; Suzuki, T. Application of ionic liquid to enhance the nose-to-brain delivery of etodolac. *Eur. J. Pharm. Sci.* **2022**, *178*, 106290.
(53) Shimizu, H.; Nishimura, Y.; Shiide, Y.; Matsuda, H.; Akimoto, M.; Matsuda, M.; Nakamaru, Y.; Kato, Y.; Kondo, K. Evaluation of Pharmacokinetics, Safety, and Drug-Drug Interactions of an Oral Suspension of Edaravone in Healthy Adults. *Clin. Pharmacol. Drug Dev.* **2021**, *10* (10), 1174−1187.
(54) Zakrewsky, M.; Lovejoy, K. S.; Kern, T. L.; Miller, T. E.; Le, V.; Nagy, A.; Goumas, A. M.; Iyer, R. S.; Del Sesto, R. E.; Koppisch, A. T.; et al. Ionic liquids as a class of materials for transdermal delivery and pathogen neutralization. *Proc. Natl. Acad. Sci. U. S. A.* **2014**, *111* (37), 13313−13318.
(55) Tanner, E. E. L.; Curreri, A. M.; Balkaran, J. P. R.; Selig-Wober, N. C.; Yang, A. B.; Kendig, C.; Fluhr, M. P.; Kim, N.; Mitragotri, S. Design Principles of Ionic Liquids for Transdermal Drug Delivery. *Adv. Mater.* **2019**, *31* (27), e1901103.

https://doi.org/10.1021/acs.molpharmaceut.3c00103
Mol. Pharmaceutics XXXX, XXX, XXX−XXX