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Comparative Study of the Free Radical Scavenging Activities of Original and Generic Edaravone Determined by Electron Spin Resonance

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Abstract

Edaravone, a powerful free radical scavenger, is the drug available in the clinical practice for the treatment of cerebral infarction and amyotrophic lateral sclerosis. Recently, many generic Edaravone injections have been commercialized. Substitution of additives in generic injections has the potential effect of changing the antioxidant ability of the original Edaravone injection. We investigated the dissimilarity between original and generic Edaravone injections focusing on their free radical scavenging activities determined by electron spin resonance spectroscopy. There were no significant differences between the original and generic Edaravone regarding the antioxidant abilities toward the hydroxyl radical. However, the generics in which the additive L-cysteine was substituted with glycerin or citric acid showed significant reduction in their antioxidant activity toward superoxide (p <0.01). Our in vitro findings suggest that the antioxidant ability of generic Edaravone against the hydroxyl radical is equivalent to that of the original Edaravone and that substitution of additives in the generic Edaravone might change its antioxidant activity toward the superoxide.

Keywords: Edaravone; Antioxidant activity; Brain ischemia; Neuroprotectant

Introduction

Oxygen free radical species may play detrimental roles in brain ischemia and edema [1-3], and onset and progression of amyotrophic lateral sclerosis (ALS) [4]. Edaravone (3-methyl-1 phenyl-2-pyrazolin-5-one, MCI-186, Radicut; developed by Mitsubishi Tanabe pharma corporation, Osaka, Japan), a powerful free radical scavenger, is currently used for the treatment of cerebral infarction and ALS [5-8]. It was shown that Edaravone scavenged radicals such as hydroxyl radical ($HO^{\cdot}$), peroxyl radicals ($LO2^{\cdot}$), DPPH (1,1-diphenyl-2-picrylhydrazyl) radicals, and nitric oxide ($NO^{\cdot}$) directly [5-7,9]. It was demonstrated that the inhibition of lipid peroxidation by Edaravone was probably due to the scavenging of the chain carrying $LO2^{\cdot}$ and that, in combination with vitamin E or C, the antioxidant activity of Edaravone may be enhanced [7]. In clinical studies, Edaravone improved the core neurological deficits, the activities of daily life, and the functional outcome of stroke patients [10,11].

Recently, over 20 generic Edaravone injections have been put on the market in Japan. The generic injections should keep the same active ingredient, however, this rule cannot be applied to the additives. The additive L-cysteine present in the original Edaravone injections is substituted with citric acid, alpha thioglycerin, or glycine in some generic injections. The additives were changed in 10 generic Edaravone injections among 21 generics that have been approved in Japan in 2011 (Table 1). L-cysteine, citric acid, alpha thioglycerin, and glycine possess free radical scavenging activities, but their antioxidant potency is different. Hence, substitution of additives has the potential of changing the antioxidant ability of the original Edaravone injections.

In the present study, we investigated the dissimilarity between the original and generic Edaravone injections by focusing on their free radical scavenging activities determined by electron spin resonance (ESR) spectroscopy.

Materials and Methods

Reagents

The original Edaravone injection and 6 generic Edaravone injections are analyzed in this study. All the generics have the same active ingredient; however, the additives are different. Two generics employ glycine (A) and citric acid (E) instead of L-cysteine, while the other 4 generics have the same additives (Table 1). Solvents and other reagents are of the highest grade commercially available.

Assay for radical intensity

Superoxide ($O_2^{\cdot -}$) was generated by titanium dioxide (TiO_2) photocatalysis, as described previously [12]. $HO^{\cdot}$ was generated by the Fenton's reaction ($H_2O_2/FeSO_4$), as described previously [13,14]. Alternatively, $HO^{\cdot}$ could be generated by irradiating H_2O_2 with ultraviolet light (365 nm UV; 5s ; 40 mW; SUPERCURE-203S, Radical Research, Tokyo, Japan), as described previously [15,16]. All the solutions were prepared in 0.1 M phosphate -buffered saline (PBS) at pH 7.2. ESR spin trapping was conducted using a reactive oxygen species (ROS)-generating system containing 5-(2, 2-dimethyl-1,3-propoxycyclophosphoryl)-5-methyl-1-pyrroline-N-oxide (CYMPO; Radical Research, Tokyo, Japan). ESR measurements were performed with a JES-RE1X system (JEOL, Tokyo, Japan) connected to the WIN-RAD ESR Data Analyzer program (Radical Research , Tokyo, Japan), under the following instrument settings: microwave power, 8.00 mW; magnetic field 335.6 ± 7.5 mT; field modulation width, 0.079 mT; sweep time, 1 min; and time constant, 0.03 s. All the experiments were repeated for a minimum of 3 times. Distilled water was used as the control. For each experimental group, the antioxidant activity was calculated as 100% with a mean value of control.

Statistical analysis

Statistical comparisons were made using non-repeated measures

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	Additives										
	sodium hydrogen sulfate	L-cysteine	sodium chloride	sodium hydroxide	phosphoric acid	pH regulating agent	sodium bicarbonate	glycine	citric acid	sodium citrate hydrate	alpha thioglycerin
Original	o	o	o	o	o						
Generics											
A	o		o	o	o			o			
B	o	o	o	o	o						
C	o	o	o	o	o						
D	o	o	o	o	o						
E	o			o		o			o	o	
F	o	o	o	o	o						
H	o		o	o	o						o
I	o		o	o	o				o	o	
J	o	o	o	o	o						
K	o	o	o	o	o						
L	o		o	o	o				o	o	
M	o	o	o	o	o						
N	o		o	o	o						o
O	o		o	o	o		o				
P	o	o	o	o	o						
Q	o		o	o	o				o		
R	o	o	o	o	o						
S	o	o	o	o		o					
T	o	o	o			o					
U	o		o			o					o
V	o		o	o		o			o	o	

Table 1: Changes of additives in generic Edaravone injections which have been approved in Japan at 2011.

analysis of variance (ANOVA). Data are expressed as mean ± standard deviation (SD). The statistical significance was set at $p < 0.05$.

Results

Dissimilarity between the original and generic Edaravone on $O_2^{\cdot -}$

We investigated the antioxidant effect of the original and generic Edaravone on $O_2^{\cdot -}$ generated by TiO_2 photocatalysis using the ESR spin trapping technique with CYMPO. The significant reduction in the antioxidant activity toward $O_2^{\cdot -}$ in the case of 2 generic Edaravone injections compared with that of the original drug was measured ($p < 0.01$). There were no significant differences in the generics in which the additives were equivalent to those of the original injection (generic Edaravone B, C, D and F); however, the generics A and E in which the additive L-cysteine was substituted with glycerin or citric acid respectively showed reduction in their antioxidant activity toward $O_2^{\cdot -}$ (Figure 1).

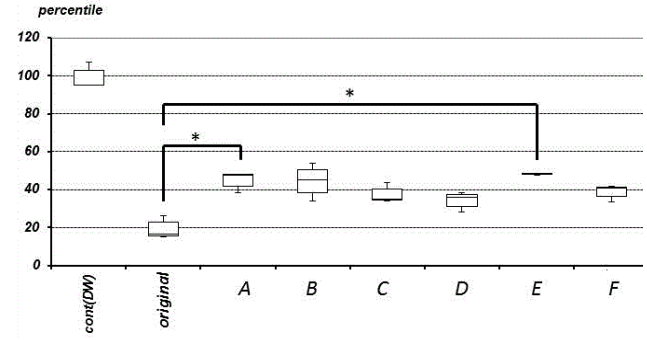

Figure 1: Antioxidant activity of the original and generic Edaravone injections toward superoxide ($O_2^{\cdot -}$) generated by the TiO_2 photocatalysis reaction calculated using the relative ESR signal intensities. The data are expressed as mean ± standard deviation (SD, n = 9) calculated as 100% with the mean value of distilled water as the control. The additives were substituted from L-cysteine to glycine in A, and to citric acid in E (Table 1). The significant differences compared to the original injection were recognized in A and E. A-F: Generic Edaravone injections which have been made in each pharmaceutical company, DW: distilled water, cont: control, *: Significantly different (p<0.01) compared to the original injection

Dissimilarity between the original and generic Edaravone on $HO^{\cdot}$

We investigated the antioxidant activity of the original and generic Edaravone on $HO^{\cdot}$ generated by Fenton's reaction or ultraviolet irradiation of H_2O_2, using ESR spin trapping with CYMPO. There were no significant differences between the original and generic Edaravone injections (Figures 2 and 3).

Discussion

The relationship between ischemic brain injury and lipid peroxidation disorder by free radicals was first described by Flamm et al. in 1978 [2]. Increasing evidence suggests that oxygen free radical species may play detrimental roles in ischemic diseases [1,3,17-19] and ALS [4] and selective free radical scavengers as brain protective agents are considered to be potential drugs against brain ischemia [20] and ALS [8]. Although the world's first clinical use of Edaravone, which has been developed as a neuroprotectant, was approved in Japan in 2001 [11], it remains under clinical investigation in various other countries [21] and its use has not been approved yet in Western countries. However, Edaravone is recommended by the American Heart Association in the guidelines for the early management of adults with acute ischemic stroke [22]. The administration of Edaravone during Alteprase infusion is likely to enhance the recanalization in patients with acute ischemic stroke [23]. The generic Edaravone injections have been approved in

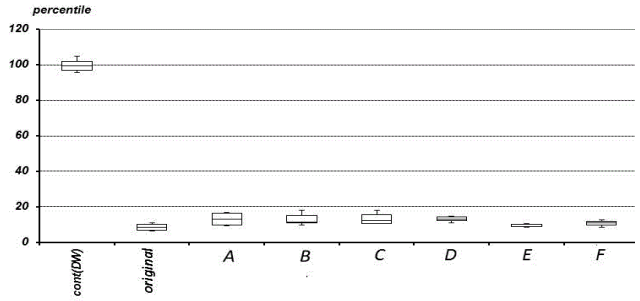

Figure 2: Antioxidant activity of the original and generic Edaravone injections towards the hydroxyl radical ($HO^{\cdot}$) generated by the Fenton reaction calculated using the ESR signal intensities. The data are expressed as mean ± standard deviation (SD, n = 9) calculated as 100% with the mean value of distilled water as the control. There were no significant differences between the original and generic injections. A-F: Generic Edaravone injections which have been made in each pharmaceutical company, and the additives were substituted from L-cysteine to glycine in A and to citric acid in E, DW: distilled water, cont: control

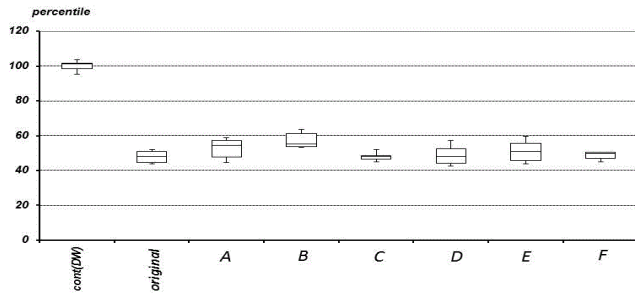

Figure 3: Antioxidant activity of the original and generic Edaravone injections toward the hydroxyl radical ($HO^{\cdot}$) generated by the ultraviolet irradiation of H_2O_2, calculated using the ESR signal intensities. The data are expressed as mean ± standard deviation (SD, n = 9) calculated as 100% with the mean value of distilled water as the control. There were no significant differences between the original and generic injections. A-F: Generic Edaravone injections which have been made in each pharmaceutical company and the additives were substituted from L-cysteine to glycine in A and to citric acid in E, DW: distilled water, cont: control

Japan in 2011 and are now produced in several countries such as Korea, India, and China.

The basic chemical structure of Edaravone (3-Methyl-1-phenyl-2-pyrazolin-5-one) was found to show promising activity as an antioxidative radical scavenger, was capable of quenching $HO^{\cdot}$, which among other oxidative free radicals has the strongest oxidative ability, and could inhibit both $HO^{\cdot}$- dependent and $HO^{\cdot}$- independent lipid peroxidation. Furthermore, additional free radical scavenging and antioxidative actions of Edaravone were identified in $LOO^{\cdot}$- induced peroxidation [7] and peroxynitrite ($ONOO^-$)-induced tyrosine nitration [6,9]. However, this basic chemical structure of Edaravone does not show antioxidant activity for $O_2^{\cdot-}$, which has very low oxidative ability [6]. In our study, the ability of the generic Edaravone as a free radical scavenger for $HO^{\cdot}$was found to be equivalent to that of the original drug, proving the safety of the basic chemical structure of the generic Edaravone. Therefore, the generic Edaravone drugs have achieved the objective of the development of original Edaravone equally.

Whether Edaravone could scavenge $O_2^{\cdot-}$ or not is still controversial [24]. It is known that the thiol group (-SH) of L-cysteine reacts with the DPPH radical and thus scavenges free radicals [25,26]. Consistently, a significant reduction in the $O_2^{\cdot-}$ scavenging activity was found only in those generics in which the additive L-cysteine was substituted with glycerin or citric acid. Even though $O_2^{\cdot-}$ has low oxidative ability, it is the key radical that initiates other free radical chain reactions. The fact that the substitution of additives affected the scavenging activity of Edaravone for $O_2^{\cdot-}$ showed that L-cysteine plays an important role in scavenging $O_2^{\cdot-}$ in Edaravone injections. There is a report mentioned that 1mM Edaravone injection suppressed the formation of $O_2^{\cdot-}$ in the xanthine oxidase-hypoxanthine system [24]. Therefore, the generic Edaravone injections in which additive L-cysteine has been substituted with another additive have the possibility to reduce its own ability as an antioxidative radical scavenger. However, our study is an *in vitro* analysis, while the additives injected into the veins are immediately diluted and spread by a large amount of blood; therefore, *in vivo*, the radical scavenging activity of L-cysteine might disappear. Further *in vivo* studies are necessary to draw the conclusion that additive changes in generic Edaravone involve the antioxidant ability in clinical use.

Conclusion

Our in vitro findings suggest that the antioxidant ability of generic Edaravone against the $HO^{\cdot}$ radical is equivalent to that of the original Edaravone and that substitution of additives in the generic Edaravone might change its antioxidant activity toward the $O_2^{\cdot-}$.

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