A Novel Potent Radical Scavenger, 8-(4-Fluorophenyl)-2-((2E)-3-phenyl-2-propenoyl)-1,2,3,4-tetrahydropyrazolo[5,1-c][1,2,4]triazine (FR210575), Prevents Neuronal Cell Death in Cultured Primary Neurons and Attenuates Brain Injury after Focal Ischemia in Rats

AKINORI IWASHITA, TAKUYA MAEMOTO, HIROHISA NAKADA, ICHIRO SHIMA, NOBUYA MATSUOKA, and HIROSHI HISAJIMA
Exploratory Research Laboratories, Fujisawa Pharmaceutical Co., Ltd., Ibaraki, Japan
Received July 3, 2003; accepted August 21, 2003

ABSTRACT
Reactive oxygen species (ROS) play a vital role in brain damage after cerebral ischemia-reperfusion injury, and ROS scavengers have been shown to exert neuroprotective effects against ischemic brain injury. We have recently identified 8-(4-fluorophenyl)-2-((2E)-3-phenyl-2-propenoyl)-1,2,3,4-tetrahydropyrazolo[5,1-c][1,2,4]triazine (FR210575) as a novel, powerful free-radical scavenger. In the present study, the neuroprotective efficacy of FR210575 was evaluated in two neuronal death models in vitro as well as rat focal cerebral ischemia models in vivo. In the first model, primary cortical cultures were exposed to a high oxygen atmosphere (50% O2) for 48 h to induce cell death with apoptotic features. Treatment with FR210575 (10⁻⁷–10⁻⁵M) significantly inhibited neuronal death. The second model used a growth-factor withdrawal paradigm. Withdrawal of TIP (transferrin, insulin, putrescine and progesterone)-supplemented medium induced apoptotic cell death after 2 days, but treatment with FR210575 exhibited dramatic protection against neuronal death. In two models of cerebral ischemia [photothrombotic occlusion of middle cerebral artery (MCA) for transient model and by permanent MCA occlusion for permanent model], rats received 3-h intravenous infusion (1–10 mg/kg/3 h) of FR210575, with brain damage determined 24 h later. FR210575 (3.2 mg/kg/3 h) significantly reduced the volume of focal damage in the cortex by 36% in the transient model and also reduced the size of ischemic brain damage in the permanent model. These findings indicate that the powerful radical scavenger FR210575 has potent neuroprotective activity and that FR210575 could be an attractive candidate for the treatment of stroke or other neurodegenerative disorders.

Oxidative stress is important in neuronal degeneration, including in cerebrovascular injuries such as stroke, neuro-pathology such as Alzheimer’s disease, Parkinson’s disease, and amyotrophic lateral sclerosis, as well as in a normal aging (Facchinetti et al., 1998). Reactive oxygen species (ROS) are critical factors for inducing neuronal damage in vitro and in vivo. For example, deprivation of survival factors results in an increase in the cellular levels of ROS (Lieberthal et al., 1998) and subsequent cellular apoptosis, both of which can be blocked by antioxidants or ROS scavengers (Atabay et al., 1996). It has been shown that normal mammalian cells are dependent upon the presence of specific growth factors for maintenance of viability. In the absence of those survival factors, these cells die by apoptosis (Raff, 1992). High-oxygen-induced neuronal death of embryonic neurons is a useful in vitro model of apoptosis (Enokido and Hatanaka, 1993; Ratan et al., 1994). Increases in ROS levels occur in this model, and specific nitric oxide radical scavengers block apoptosis (Satoh et al., 1998; Ishikawa et al., 1999).

The brain is particularly susceptible to radical-mediated neuronal damage because of high levels of oxygen consumption unsaturated fatty acids and iron stores, combined with low antioxidant resources. Lipid peroxidation is an important cause of neuronal damage, for example, in neurodegenerative disease such as stroke (Coyle and Puttfarcken, 1993). ROS

ABBR EV IENT S: ROS, reactive oxygen species; SOD, superoxide dismutase; FR210575, 8-(4-fluorophenyl)-2-((2E)-3-phenyl-2-propenoyl)-1,2,3,4-tetrahydropyrazolo[5,1-c][1,2,4]triazine; TBARS, thiobarbituric acid reactive substances; DCF, dichlorodihydrofluorescein; MCA, middle cerebral artery; MCI-186, 3-methyl-1-phenyl-2-pyrazolin-5-one; TIP, transferrin, insulin, putrescine, and progesterone; ANOVA, analysis of variance; rCBF, regional cerebral blood flow.
formation is increased after permanent and reversible middle cerebral artery occlusion in rats (Nelson et al., 1992; Peters et al., 1998). Infarct volume after focal cerebral ischemia is reported to be reduced by overexpressing extracellular SOD in mice (Sheng et al., 1998) and by the administration of free radical scavengers (Yamamoto et al., 1983). Thus, free radicals contribute to brain damage, especially after focal cerebral ischemia, and brain-penetrating antioxidants have potential to attenuate this cellular damage. Free radical scavengers such as α-phenyl-N-tert-butyl nitrate have been used to reduce infarct volumes in rats subjected to either permanent or transient focal cerebral ischemia (Yang et al., 2000).

We have recently identified 8-(4-fluorophenyl)-2-((2E)-3-phenyl-2-propenoyl)-1,2,3,4-tetrahydropyrazolo[5,1-c][1,2,4]triazine (FR210575) as a novel potent radical scavenger. The purpose of the present study was, first, to evaluate radical scavenging potency of FR210575 and several antioxidant compounds such as edaravone, idebenone, 2-seleno, and vitamin E in thiobarbituric acid reactive substances (TBARS) and dichlorodihydrofluorescein (DCF) assays. The second purpose of the present study was to determine whether FR210575 and other antioxidants reduced cell damage induced by ROS in established in vitro experimental neuronal cell death models. To determine the neuroprotective properties of FR210575, two protocols involving ROS generation followed by apoptosis-like cell death were designed: a high-oxygen atmosphere model and a survival factor deprivation model. Finally, the neuroprotective property of FR210575 was evaluated in two models of focal ischemia in rats: photochemically induced transient focal cerebral ischemia, a model that has characteristics of both permanent ischemia and ischemia-reperfusion (Takamatsu et al., 1998) and a permanent model of focal cerebral ischemia.

Materials and Methods

Chemicals

FR210575 (chemical structure shown in Fig. 1), MCI-186 (edaravone; 3-methyl-1-phenyl-2-pyrazolin-5-one), idebenone, and ebselen were synthesized at Fujisawa Pharmaceutical Co. Ltd. (Osaka, Japan). Vitamin E was purchased from Nakarai Tesque (Kyoto, Japan). Tissue culture medium and fetal bovine serum were purchased from Invitrogen (Carlsbad, CA), and tissue culture dishes and black plates for the DCF assay were from NUNC AS (Aastrup, Denmark). 3-(4,5-Demethyl-2-thiazolyl)-2,5-diphenyl-2H tetrazolium bromide (MTT) was from Wako Pure Chemicals (Tokyo, Japan). 2′,7′-Dichlorodihydrofluorescein diacetate (DCFH-DA) was from Molecular Probes (Eugene, OR). Unless otherwise stated, all other chemicals were purchased from Sigma-Aldrich (St. Louis, MO).

Radical Scavenging Activity

TBARS Assay. For measurement of lipid peroxidation, TBARS were estimated using the modified method of Buege and Aust (1978) and Callaway et al. (1998). Briefly, rat brain synaptosomes were prepared from Sprague-Dawley rats (Japan SLC, Shizuoka, Japan). To evaluate the inhibitory potency of FR210575, MCI-186, idebenone, ebselen, and vitamin E, different concentrations of the tested compounds were dissolved in 50% dimethyl sulfoxide, and then 5 μM was added to each rat brain synaptosome and incubated with ammonium ferric sulfate (100 μM) at 37°C for 30 min. The reaction was stopped with addition of 20% trichloroacetic acid, and the precipitated proteins were removed by centrifugation at 10,000 g for 15 min. The aliquots of supernatant were then added to an equal volume of thiobarbituric acid. The samples were heated at 95°C for 30 min, and then cooled on ice before reading absorbance at 532 nm. Concentrations of TBARS were calculated using standard curve obtained with malondialdehyde (MDA). Percentage of inhibition of TBARS production was calculated as follows: % inhibition = [(Max − Drug)/(Max − Base)] × 100, where Max is the value in the presence of ammonium ferric sulfate, Base is the value in the absence of ammonium ferric sulfate, and Drug is the value of test compounds.

To evaluate the neuroprotective efficacy in the primary culture of neurons, the radical scavenging activity was tested, as based on the methods of LeBel et al. (1999) with minor modifications. Briefly, rat brain homogenates were diluted in HEPES-based Locke’s buffer to obtain a concentration of 5 mg of tissue/ml. The brain homogenates were pipetted into NUNC 96-well black plate and allowed to warm to room temperature for 5 min. Various concentrations of drugs were added to each well and incubated for 10 min. Then, 5 μM of DCFH-DA (10 μM final concentration) was added to each well and preincubated for 15 min at room temperature to allow the DCFH-DA to be incorporated into any membrane-bound vesicles and the diacetate group cleaved by esterases. Five microliters of 10 μM Fe2+ was administered to the wells and then incubated for 20 min. The conversion of DCF to the fluorescent product DCF was measured using the ARVO (PerkinElmer Life Sciences, Yokohama, Japan) with excitation at 485 nm and emission at 530 nm. ROS production was quantified using a DCF standard curve. Percentage of inhibition of ROS production was calculated as follows: % inhibition = [(Max − Drug)/(Max − Base)] × 100, where Max is the value in the presence of Fe2+, Base is the value in the absence of Fe2+, and Drug is the value of test compounds.

Neuroprotective Efficacy in Primary Cultured Neurons

Primary Neuronal Cultures. Primary cortical cultures were prepared from the cerebral cortex of embryonic day 17 Wistar rats. Removed cortices were dissected free of meninges and dissociated at 0.025% (w/v) trypsin. Dissociated cells were plated onto 96-well microplates at a seeding density of 2 × 10^4/well. The primary cortical cultures were maintained in Neurobasal medium with supplements of transferrin, insulin, putrescine, and progesterone (TIP), glutamic acid, glutamine, selenium, and antibiotics in an atmosphere of 95% air and 5% CO2 at 37°C for 24 h.

Drug Treatment. All drugs were dissolved in 100% dimethyl sulfoxide at 10−4 M and then diluted in neurobasal medium.

In Vitro Apoptosis Model. To evaluate the neuroprotective efficacy of FR210575, two protocols were designed as shown in Fig. 2. In the first model, cultured cells were exposed to a high oxygen atmosphere (50% O2) for 48 h to induce apoptotic cell death. In the second model, a growth factors starvation paradigm was used. Removal of TIP (transferrin, insulin, putrescine, and progesterone) from the medium induced apoptotic cell death after 2 days. FR210575 was pretreated for 24 h before the start of high oxygen stress or TIP withdrawal stress, and cell viability was determined using the MTT assay 48 h after stress was initiated.

Cell Viability Assay. To evaluate cell survival, MTT assay was performed according to a minor modification of the original method (Mosmann, 1983). Briefly, MTT was added to the cultures at a final concentration of 2 mg/ml and then incubated at 37°C for 5 h. Then, 100 μl of DMSO was added to each well, and the absorbance of the formazan blue was measured at 570 nm with a plate reader.
concentration of 2 mg/ml. After incubation at 37°C for 2 h, the reaction was stopped by addition of isopropanol containing 0.04 N HCl, and absorbance was measured at 590 nm.

Pharmacokinetic Study

Measurement of FR210575 concentration in plasma and brain were performed in rats after intravenous infusion for 3 h at 3.2 mg/kg. The plasma and brain levels of FR210575 were measured using high-performance liquid chromatography. FR210575 was dissolved and diluted in 50% polyethylene glycol and 50% ethanol solution. The injection volume was adjusted to 2 ml/kg.

Focal Cerebral Ischemia Models in Rats

Animals. For transient focal ischemia, 9- to 10-week-old male Wistar rats weighing 274 to 380 g from Charles River (Hino, Japan) were used. For permanent focal ischemia, 8- to 10-week-old male Sprague-Dawley rats weighing 282 to 375 g from Japan SLC (Shizuoka, Japan) were used. All animals were housed in a room maintained at 23 ± 2°C with 55 ± 5% humidity, and with a 12-h light/dark cycle (light on at 7:00 AM). The minimum quarantine period was at least 1 week before the experiment. Animals were housed five per cage and allowed free access to food and water. All experiments in the present study were performed under the guidelines of the Experimental Laboratory Animal Committee of Fujisawa Pharmaceutical Co., Ltd. and were in strict accordance with the principles and guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize both the number of animals used and stress to the animals during experimental procedures.

Administration of FR210575. FR210575 was dissolved and diluted in 50% polyethylene glycol and 50% ethanol solution. The injection volume was adjusted to 2 ml/kg in all experiments.

Transient Focal Ischemia in Rats. Photochemically induced transient focal cerebral ischemia was performed according to the method of Matsuno et al. (1993). Rats were anesthetized with halothane (4% for induction and 1.5% for maintenance) in room air, and the right femoral vein was cannulated with a polyethylene-50 tube for administration of rose bengal or test drugs. The scalp and the left temporalis muscle were cut and, under an operating microscope, a subtemporal craniotomy was performed using a dental drill. A window (about 4 mm in diameter) was opened just anterior to the

![Fig. 3. Comparison of radical scavenging activities among FR210575, vitamin E, MCI-186, idebenone, and ebselen in the TBARS assay (A) and in the ROS assay (B). Values are means of n = 2 to 3 determinations tested in triplicate.](image-url)
foramen ovale of the mandibular nerve at the skull base and the main trunk of the left MCA was identified through the dura matter. Photoillumination with green light (wavelength 540 nm) was accomplished by using a xenon lamp (L-4887; Hamamatsu Photonics, Hamamatsu, Japan) with a heat absorbing filter and a green filter. Photoillumination was directed onto the MCA for 10 min by an optic fiber (3 mm in diameter) mounted on the window at the skull base during rose bengal injection (10 mg/kg over 1 min). Complete photothrombotic occlusion of MCA was visually confirmed through the operating microscope and was ascertained by the decrease in regional cerebral blood flow (rCBF) in the main trunk of the MCA monitored by laser-Doppler flowmetry (Omegaflow, FLO-N1; Neurosciences, Osaka, Japan). After occlusion of the MCA, the temporalis muscle and skin were closed in layers and anesthesia was discontinued. During surgery, rectal temperature was maintained at 37.4 to 38.6°C with a heating pad (TR-100, PS-100; Fine Science Tools, North Vancouver, Canada).

Permanent Focal Ischemia in Rats. The procedure for permanent MCA occlusion of rats was described previously (Furuichi et al., 2003). Briefly, rats were anesthetized with a mixture of 4% halothane and oxygen-nitrogen (30% oxygen and 70% nitrogen) and maintained with 1.5% halothane delivered through a close fitting face mask during surgery. A vertical incision was made between the left orbit and the left external auditory canal. Under an operating microscope, the temporalis muscle was sectioned, and subtemporal craniotomy was performed without removing the zygomatic arch. The main trunk of the MCA and the olfactory tract were identified according to the trapezoid method (Rosen and Harry, 1990). The IC50 with 95% confidence limits were calculated using GraphPad Prism 3.3 software (GraphPad Software, Inc., San Diego, CA).

TABLE 1

<table>
<thead>
<tr>
<th>Antioxidants</th>
<th>Radical Scavenging Activity</th>
<th>Neuroprotective Potency</th>
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<tr>
<td></td>
<td>TBARS Assay IC50</td>
<td>ROS Assay IC50</td>
</tr>
<tr>
<td>FR210575</td>
<td>1.7 × 10⁻⁷ M (1.5–1.9 × 10⁻⁷ M)</td>
<td>3.4 × 10⁻⁸ M (2.6–4.5 × 10⁻⁸ M)</td>
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<tr>
<td>Vitamin E</td>
<td>6.8 × 10⁻⁷ M (6.2–7.2 × 10⁻⁷ M)</td>
<td>2.7 × 10⁻⁷ M (2.4–3.1 × 10⁻⁷ M)</td>
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<tr>
<td>MCI-186</td>
<td>&gt;10⁻⁷ M</td>
<td>&gt;10⁻⁴ M</td>
</tr>
<tr>
<td>Idebenone</td>
<td>&gt;10⁻⁷ M</td>
<td>&gt;10⁻⁴ M</td>
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<tr>
<td>Ebselen</td>
<td>&gt;10⁻⁷ M</td>
<td>&gt;10⁻⁴ M</td>
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All values are expressed as mean ± S.E.M. Significance of difference between groups for was tested using one-way analysis of variance (ANOVA) followed by post hoc Dunnett’s multiple comparison test. P values less than 0.05 were considered to be significant.

Results

Radical Scavenging Activity of FR210575

We compared the radical scavenging potency of FR210575 with several other antioxidants such as MCI-186, idebenone, ebselen, and vitamin E. FR210575 was the most potent compound among compounds tested in the TBARS assay (IC50 value of 170 nM; Fig. 3 and Table 1). FR210575 was even more potent in the DCF assay (34 nM; Fig. 3 and Table 1) and again demonstrated higher potency than reference compounds. These results suggest that FR210575 possess superior radical scavenging properties compared with other antioxidants.

Neuroprotective Action in Rat Primary Cortical Cultures

Primary cultured cells exposed to a high-oxygen atmosphere (50% O2) for 48 h induced marked cell damage as shown in Fig. 4. Treatment with FR210575 (10⁻⁷–10⁻⁵ M) 24 h before exposure to high-oxygen atmosphere significantly prevented the neuronal cell death. Treatment with vitamin E (10⁻⁵–10⁻⁵ M) significantly attenuated neuronal death, whereas other antioxidants such as MCI-186, idebenone, and ebselen had no neuroprotective activity.

Similarly, TIP deprivation from culture media resulted in marked cell damage almost same as high-oxygen atmosphere conditions as shown in Fig. 5. Treatment of FR210575 (10⁻⁷–10⁻⁵ M) 24 h before deprivation of TIP significantly prevented the cell death, whereas, with the exception of vitamin E, other antioxidants (MCI-186, idebenone, and ebselen) were not effective even up to 10⁻⁵ M, consistent with the results of the high-oxygen atmosphere assay. We have characterized the type of cell death in both models as apoptosis-like cell death using microscopic observation (data not shown). Consistent with this characterization, neuronal degeneration was characterized by cell shrinkage, not by cell swelling.

Pharmacokinetic Study

The plasma and brain concentrations of FR210575 were determined at 3 h after intravenous infusion at 3.2 mg/kg/3 h. Mean plasma and brain concentration was 1.02 ± 0.28 µg/ml (n = 3) and 6.08 ± 0.87 µg/g wet tissue (n = 3), respectively. The concentration of FR210575 in the brain was not effective even up to 10⁻⁵ M, consistent with the results of the high-oxygen atmosphere assay. We have characterized the type of cell death in both models as apoptosis-like cell death using microscopic observation (data not shown). Consistent with this characterization, neuronal degeneration was characterized by cell shrinkage, not by cell swelling.
significantly higher than that in the blood and the brain/plasma concentration ratio was 5.96 at 3 h after dosing.

**Neuroprotective Action in Rat Focal Cerebral Ischemia**

**Effect of FR210575 on Ischemic Damage after Thrombotically Induced Transient Focal Cerebral Ischemia.** rCBF in the main trunk of MCA decreased within 4 to 9 min after the initiation of photoillumination, demonstrating rapid formation of thrombus after the photochemical reaction. In the control group, rCBF decreased to 20.5 ± 2.6% (n = 6) of the baseline level at 1 h after the MCA occlusion and remained 23.2 ± 3.1% (n = 6) of the baseline at 3 h after the occlusion. Visual inspection of MCA main trunk at 12 h after the MCA occlusion revealed that MCA was recanalized in all cases, suggesting the presence of spontaneous recanalization during 3 to 12 h after the ischemia in this model. When FR210575 was infused intravenously for 3 h at the end of the 10-min photoillumination period, FR210575 (1 and 3.2 mg/kg/3 h) did not alter rCBF; rCBF was 22.1 ± 4.8% (n = 7) of the baseline at 1 h after the MCA occlusion and was 20.9 ± 1.5% (n = 7) of the baseline at 3 h after the occlusion.

In the control group, the dorsolateral cortex and basal ganglia showed extensive damage that could be clearly differentiated from normally perfused area using 2,3,5-triphe-
nyltetrazolium chloride staining. The volume of ischemic brain infarction in the cerebral cortex and striatum in the control group was 157.93 \pm 4.46 and 66.37 \pm 2.30 \text{mm}^3, respectively. FR210575 dose dependently reduced the size of infarcted cortical area when administered immediately after MCA occlusion (Fig. 6A). However, FR210575 only minimally affected striatal infarction. Cortical damage was reduced by 17 and 36\% at the doses of 1 and 3.2 mg/kg/3 h, respectively, and the protection at the dose of 3.2 mg/kg/3 h was statistically significant compared with control group (\(P < 0.01\) by one-way ANOVA followed by Dunnett’s multiple comparison test).

To determine the time window for efficacy of FR210575, we examined ischemic volume after administration was delayed to 3 h after MCA occlusion. FR210575 (1 or 3.2 mg/kg/3 h i.v.) still significantly reduced ischemic brain damage in this paradigm, although the magnitude of protection was smaller than when administered immediately after the ischemic insult (Fig. 6B).

**Effect of FR210575 on Ischemic Damage after Permanent Focal Cerebral Ischemia.** Permanent occlusion of the left MCA resulted in ischemic brain damage within the MCA territory, i.e., the dorsolateral cortex and striatum. The volume of damage in the cortex and the striatum of vehicle-
treated control groups was 125.24 \pm 11.33 and 53.66 \pm 3.49 mm³, respectively. FR210575 at doses of 3.2 and 10 mg/kg/3 h administered intravenously immediately after MCA occlusion reduced the size of ischemic brain damage dose dependently with significant effects at doses of 10 mg/kg/3 h, although the reduction of cortical damage was smaller than that in transient models (Fig. 6C).

**Discussion**

The aim of the present study was to evaluate the neuroprotective activity of FR210575 on ROS-mediated neuronal damage both in vitro and in vivo models. FR210575 had potent antioxidant activity both in the TBARS and DCF assays and is one of the most potent antioxidants we have ever tested.

The use of defined media in neuroprotective assays for primary cultures simplifies interpretation of the experiments. We found that a combination of the growth factors TIP promoted long-term survival of our primary cultured neurons. Subsequent deprivation of these survival factors, as well as exposure to a hyperbaric atmosphere, results in an increase in the cellular levels of ROS and apoptotic-like cell death (data not shown). As expected given the potent radical scavenging activity of FR210575, this compound markedly attenuated the cell death induced by both TIP deprivation and high-oxygen exposure. These results suggest that both TIP deprivation and high-oxygen exposure generate a large amount of ROS and that these ROS might have induced apoptotic cell death. Pretreatment of FR210575 at relatively low concentration markedly prevented the apoptotic cell death, probably by means of ROS scavenging action. Vitamin E also reduced the neuronal death both in TIP deprivation and high-oxygen exposure model, and the potency was consistent with its radical scavenging activity. Other antioxidant such as MCI-186, idebenone, and ebselen showed no neuroprotective properties, being consistent with their weak radical scavenging activities. These results suggest that FR210575 possess superior neuroprotective property compared with many other antioxidants, and the neuroprotective property apparently involves its radical scavenging activity in our culture systems.

In addition to in vitro efficacy, we also demonstrate that FR210575 provides significant cerebroprotection after transient cerebral ischemia. Cerebral ischemia/reperfusion is accompanied by enhanced production of free radicals (Nelson et al., 1992), because reoxygenation during reperfusion provides oxygen as a substrate for numerous enzymatic oxidation reactions that produce reactive oxidants (Chan, 1994, 1996). The infarct volume is reduced by the administration of free radical scavengers (Yamamoto et al., 1983), and the marked neuroprotective effect of radical scavenger indicates that reactive oxygen species play an important role in brain damage after transient ischemia. FR210575 reduced cortical infarct volume by 36% compared with vehicle treated control. The neuroprotective effect of FR210575 was more prominent in the cortex than in the striatum, consistent with previous reports concerning neuroprotectants in stroke models (Schmid-Elsaesser et al., 2000), and the observation that free radical formation in focal cerebral ischemia-reperfusion in rats occurs predominantly in the peri-infarct region (Fukuyama et al., 1998). Furthermore, our results could also be compatible with the observation that SOD activity...
is increased in the penumbra region after transient focal ischemia, whereas no significant changes in SOD activity occurred in either the core region or striatum (Toyoda and Lee 1997).

As shown by the pharmacokinetic study, the brain concentration of FR210575 at 3.2 mg/kg/3 h (i.v. infusion) was found to be 6.08 μM/g, which is estimated as more than 10−3 M, at 3 h post-dosing. This dosing regimen yielded sufficient brain levels of FR210575 that should sufficiently exert radical scavenging activity in the brain. As expected from a good pharmacokinetic profile, treatment with FR210575 immediately after the ischemic insult produced robust and significant neuroprotection, whereas delayed treatment with 1 mg/kg/3 h FR210575 still slightly, but significantly, attenuated the cortical infarct volume. These results suggest that reperfusion-induced ROS generation persists for some time, and neuroprotective effects of FR210575 in cerebral ischemia presumably are related to its free radical trapping properties.

Furthermore, the present results suggest that the therapeutic time window for FR210575 in transient focal ischemia is about 3 h.

FR210575 provided less neuroprotection after permanent ischemia than transient ischemia, possibly because free radical generation is lower when reperfusion does not occur. The marked influence of free radicals may be enhanced by reperfusion, which is obviously not a critical factor in permanent focal ischemia. However, in stroke patients, drastic re-perfusion after focal occlusion rarely occurs. Therefore, it might be beneficial and also be required to possess the potential to reduce an infarct volume both in transient and in permanent ischemia model.

Although the putative mechanism of neuroprotection by FR210575 is an ROS scavenger, infarct volume can also be reduced by improving rCBF. However, there was no enhancement of rCBF to cortical area of the infarct in 1 and 3.2 mg/kg/3 h FR210575-treated rat as assessed by rCBF monitored for at least 3 h after the MCA occlusion. Furthermore, reduction of infarct volume and neurological deficits in transgenic mice overexpressing SOD do not depend on changes in CBF but rather are correlated with reduced oxidative stress in ischemic brain tissue (Yang et al., 1994; Sheng et al., 1998). Therefore, it is unlikely that FR210575 has intrinsic vasoactive effects, and the beneficial effects of this compound do not result from improvement of rCBF.

Despite numerous strategies for inhibiting radical-mediated damage, only a small number of centrally acting synthetic antioxidants, including tert-butyl nitrate, edaravone and ebselen, have been proposed as candidates for clinical development (Dorey et al., 2000). FR210575 is even more potent than these compounds in vitro, with significant neuroprotective activity after cerebral ischemia, suggesting that this compound and/or water-soluble derivative of FR210575 would be an attractive therapeutic candidate for stroke.

Acknowledgments

We thank Dr. Akira Katayama for pharmacokinetic study and Dr. Raymond D. Price for critical review of the manuscript.

References


Address correspondence to: Akinori Iwashita, Department of Neuroscience, Medicinal Biology Research Laboratories, Fujisawa Pharmaceutical Co., Ltd., 2-1-6 Kashima, Yodogawa-ku, Osaka 532-8514, Japan. E-mail: aki.iwashita@po.fujisawa.co.jp