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Effect of AMPA receptor antagonist YM872 on cerebral hematoma size and neurological recovery in the intracerebral hemorrhage rat model

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Abstract

[2,3-dioxo-7-(1*H*-imidazol-1-yl)-6-nitro-1,2,3,4-tetrahydro-1-quinoxalinyl]-acetic acid monohydrate (YM872 or zonampanel), an AMPA receptor antagonist, is in clinical development for acute ischemic cerebral infarction. Stroke patients are prone to have subsequent intracerebral hemorrhages. In order to predict potential adverse effects, YM872 was tested in a rat model with collagenase-induced intracerebral hemorrhage. The morphologically determined hematoma volumes after 24 h were compared between animal groups intravenously infused with 3600 U/kg/h heparin for 30 min, or with 20 or 40 mg/kg/h of YM872, or placebo for 4 h. Heparin enlarged hematoma volume, but neither dose of YM872 affected hematoma size. In a separate study, neurological deficits were scored at various days after intracerebral hemorrhage induction in animals with intravenous infusion for 24 h of 10 or 20 mg/kg/h YM872, or saline. The YM872 groups scored significantly better than the saline group at 14 days. These data suggest that YM872 does not exacerbate intracerebral hemorrhage and might accelerate recovery.

Keywords: Intracerebral, hemorrhage; Hematoma; Neurological deficit; AMPA receptor antagonist; YM872

1. Introduction

A superabundance of extracellular glutamate and hyperactivation of glutamate receptors are considered to be crucial events for neuronal damage in stroke. Antagonists of both the NMDA and AMPA subtypes of glutamate receptors have been shown to have neuroprotective effects in animal models of cerebral ischemia. The usefulness of NMDA receptor antagonists as therapeutic agents may, however, be limited by their adverse effects, including psychomimetic effects (Koek et al., 1988), cognitive impairment (Morris et al., 1986) and neurotoxicity (Olney et al., 1991). AMPA receptor antagonists seem not to share these unfavorable properties of NMDA receptor antagonists (Graham et al., 1996). Significant reductions of infarct volume in ischemic animal models have been found after treatment with the AMPA receptor antagonists 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(*F*)quinoxaline (NBQX) (Buchan et al., 1993; Graham et al., 1996; Xue et al., 1994), 1-(4-aminophenyl)-4-methyl-7,8-methylene-dioxy-5*H*-2,3-benzodiazepine hydrochloride (GYKI 52466) (Buchan et al., 1993; Smith and Meldrum, 1992; Xue et al., 1994), (3*S*,4a*R*,6*R*,8a*R*)-6-[2-(1(2)*H*-tetrazole-5-yl)ethyl]decahydroisoquinoline-3-carboxylic acid (LY-293558) (Bullock et al., 1994) and 6-(1*H*-imidazol-1-yl)-7-nitro-2,3-(1*H*,4*H*)-quinoxalinedione hydrochloride (YM90K) (Yao et al., 1997; Yatsugi et al., 1996). However, clinical investigations have been limited because the first generation compounds had the major drawback of being poorly soluble in water and thus nephrotoxic (Graham et al., 1996; Xue et al., 1994). A promising novel AMPA receptor antagonist, [2,3-dioxo-7-(1*H*-imidazol-1-yl)-6-nitro-1,2,3,4-tetrahydro-1-quinoxalinyl]-acetic acid monohydrate (YM872, generic name zonampanel), which is a highly water-soluble agent that retains selectivity and potency for AMPA receptors (Kohara et al., 1998; Shimizu-Sasamata et al., 1998; Takahashi et al., 1998a,b), is presently in clinical development for acute ischemic stroke.

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A subset of stroke patients (11–32%) is known to have a primary intracerebral hemorrhage (Anderson et al., 1994; Burchfiel et al., 1994; Lo et al., 1994), and 15–43% of ischemic stroke patients have a subsequent intracerebral hemorrhage which dramatically worsens clinical symptoms and may cause death (Lyden and Zivin, 1993). These epidemiological findings suggest that aggravated effects on intracerebral hemorrhage are a crucial issue in stroke treatment. Current management of intracerebral hemorrhage includes control of systemic hypertension and treatment or prevention of raised intracranial pressure, but this might result in expansion of the infarction size by decreasing blood flow in the brain. On the other hand, anticoagulants or thrombolytic drugs, which may be used to treat the cerebral infarction, have the potential of causing intracerebral hemorrhage. In the interest of safety, drugs used for stroke patients with cerebral infarction and/or intracerebral hemorrhage should be carefully investigated in animal models for their effects on intracerebral hemorrhage.

Several animal models of intracerebral hemorrhage have been developed (Bullock et al., 1984; Rosenberg et al., 1990; Wagner et al., 1996). Intracerebral hemorrhage has been studied experimentally using infusion of autologous blood or implantation of inflatable balloons (Bullock et al., 1984; Kaufman et al., 1985; Ropper and Zervas, 1982). To achieve a more reproducible hematoma, Rosenberg et al. (1990) developed a rat model in which an intrastriatal injection of collagenase was used to disrupt the basal lamina of cerebral capillaries and cause bleeding in the brain parenchyma. The disruption and bleeding commenced 30 min after the injection and a hematoma was produced by 4 h (Del Bigio et al., 1996). Previously, we studied the relationship between the amount of bleeding and hematoma size induced by various dosages of collagenase and established a sensitive intracerebral hemorrhage rat model to detect exacerbation effects on intracerebral hemorrhage (Terai et al., 2003). The data indicated that a model with a small intracerebral hemorrhage induced with a low dose of collagenase should be used for the evaluation of drugs, which may affect intracerebral hemorrhage. In the present experiments, the effects of YM872 on intracerebral hemorrhage and neurological deficits induced by the intracerebral hemorrhage were investigated using this model.

2. Materials and methods

2.1. Animal preparation of intracerebral hemorrhage model

All experimental procedures were carried out with strict adherence to the guidelines set forth in the National Institutes of Health Guide for the Care and Use of Laboratory Animals (publication 80-23). Male Sprague–Dawley IGS rats were purchased from Nippon Charles River (Tokyo, Japan) and were used for intracerebral hemorrhage model preparation at a weight from 285 to 365 g. The animals were anesthetized with 1% halothane delivered in a 70% nitrous oxide and 30% oxygen mixture by facemask and the intracerebral hemorrhage model was prepared according to the previous method (Rosenberg et al., 1990). The head was placed in a stereotaxic frame (David Kopf Instruments, CA, USA). After a midline scalp incision, a hole was drilled through the skull and a 30-gauge needle attached to a 50-μl Hamilton microsyringe was inserted into the striatum (3 mm lateral to midline, 0.2 mm anterior to coronal suture of the bregma, depth 6.0 mm below the surface of the skull and then withdrawn 0.3 mm). Collagenase (10 U/ml, type IV, C-5138, Sigma, USA) dissolved in saline was infused through the needle at a dosage of 0.014 U in 1.4 μl for 7 min (0.2 μl/min) using a microsyringe pump (NS-100, Muromachi, Tokyo, Japan). As a control experiment, 1.4 μl of saline ($n = 4$) was infused in the same way. After infusion, the needle was left in place for 1 min and then was removed slowly (over 1 min). The bone hole was sealed with Spongel (Yamanouchi Pharmaceutical, Tokyo, Japan) and a cyanoacrylate adhesive (Alonalpha A, Toagousei, Tokyo, Japan). The scalp wound was sutured, and the animal was placed in a warm cage with free access to food and water. The total anesthetic period during the surgical operation was always less than 20 min. Cerebral bleeding and hematoma formation began slowly over the ensuing 0.5–4 h (Del Bigio et al., 1996). All animals were catheterized in the jugular vein 24 h before the intracerebral hemorrhage induction to allow intravenous infusion of agents. The femoral artery was additionally catheterized for monitoring physiological parameters. Briefly, the animals were anesthetized with the same method described above, and a polyethylene tube 50 (PE-50) was inserted into the vein or artery. The venous and arterial catheters were subcutaneously externalized through the dorsal neck region.

2.2. Agent administration and grouping of animals

During intravenous infusion of agents via the catheterized jugular vein, intracerebral hemorrhage-induced animals were placed in Ballman cages (Natsume Seisakusho, Tokyo, Japan). Four administration schedules described below were used in three separate experiments (Fig. 1). A YM872 preparation (250 mg YM872 vial containing 250 mg YM872, 210 mg meglumine and 300 mg mannitol, Lot No. 0901-10-205228, Yamanouchi Pharmaceutical) and a placebo preparation (250 mg placebo vial containing 210 mg meglumine and 300 mg mannitol, Lot No. 0901-99-205229), which are currently used in clinical trials, were used in Experiments 1 and 2. In experiment 3, YM872 powder was used to profile a pharmacological efficacy of YM872 itself directly, because YM872 preparation includes other contents (e.g. meglumine and mannitol). All intravenous infusions were commenced 30 min after finishing the collagenase injection. After finishing the infusion, the animals were recovered and were individually housed in home cages.

For acute evaluation of hematoma volume (Experiment 1 in Fig. 1), animals were divided into five groups ($n = 10$

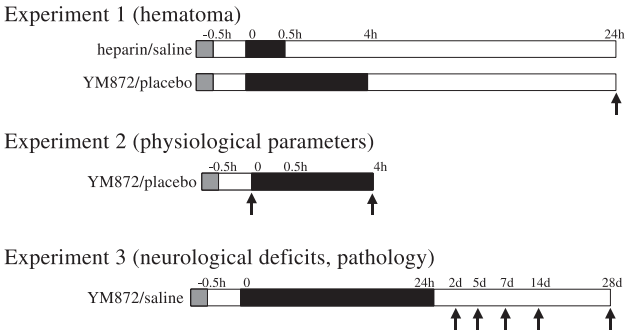

Fig. 1. Experimental protocols. Three separate experiments were performed. Intravenous infusion of agents (black bar) was commenced 30 min after 7-min injection of 10 U/ml collagenase (1.4 μl, 0.014 U, gray bar). Arrows indicate times of hematoma volume measurement in experiment 1, measurements of physiological parameters in experiment 2, and scoring of neurological deficits and pathological analyses in experiment 3.

each) according to the intravenously administered agents and the dosages (heparin, saline, 20 mg/kg YM872, 40 mg/kg YM872 and placebo). In addition to the intrastriatal collagenase injection, the heparin group was given heparin (novo heparin, Novo Nordisk Pharma, Denmark) at a dose of 3600 U/12 ml/kg/h (diluted in saline) and the saline group was given 12 ml/kg/h of saline by intravenous 30-min infusion according to a previous method (Elger et al., 1994). The other three groups were intravenously infused for 4 h by YM872 at doses of 20 mg/5 ml/kg/h (20 mg/kg YM872 group) or 40 mg/5 ml/kg/h (40 mg/kg YM872 group) or by 5 ml/kg/h of placebo (placebo group). At 24 h after collagenase injection, brain tissue was harvested immediately after rapid decapitation under pentobarbital anesthesia (50 mg/kg) for hematoma volume assessment.

Physiological parameters (Experiment 2 in Fig. 1) were monitored or measured using another three groups: 20 mg/kg YM872 group, $n = 5$; 40 mg/kg YM872 group, $n = 5$; and placebo group, $n = 5$. Each material was infused for 4 h at 5 ml/kg/h and physiological parameters were measured before and after 4 h of infusion.

In a separate experiment for evaluation of neurological deficits (Experiment 3 in Fig. 1), animals were divided into three groups: 10 mg/5 ml/kg/h YM872 group, $n = 6$; 20 mg/5 ml/kg/h YM872, $n = 9$; and 5 ml/kg/h saline, $n = 9$. Drug or saline was administered for 24 h. At various time points, after collagenase injection, the neurological deficits of each animal were observed according to the method described below. After the final observation (28 days), brain tissue was harvested immediately after rapid decapitation under pentobarbital anesthesia (50 mg/kg) for pathological assessment.

2.3. Pathological analyses

For both Experiments 1 and 3, the brain was quickly removed, immersed in ice-cold saline for 1 min and set on a tissue chopper (McILWAIN Laboratory Engineering, Surrey, UK). Chopped brain sections of 1-mm thickness (coronally from anterior pole to posterior pole of the cerebral cortex) were mounted on glass slides. Eight brain sections transversing the whole striatum were observed using a Leica surgical operating microscope (M651 model, Germany) equipped with a digital camera (Fujix digital camera HC-2500 3CCD, Fujifilm, Japan). It took less than 5 min to take the digital images of the eight brain sections including the hematoma in each brain. For further estimation of infarct volume, the brain sections were incubated with 2% TTC (2,3,5-triphenyltetrazolium chloride, Tokyo Kasei, Japan) solution for 20 min at 37 °C, and the digital images were taken again. The acquired digital images were converted to TIFF files for image analysis using Adobe Photoshop 5.0 (Adobe System, USA). The color images were analyzed with a Macintosh G4 computer using MacSCOPE ver 2.59 (Mitani, Tokyo, Japan). The hematoma area (mm^2) in each brain section was determined from the red-colored area in the striatum by the image analysis. The hematoma volume (mm^3) was calculated by integration of the hematoma area (mm^2) in each coronal 1-mm-thick brain section. Areas of the dissected left side and right side of the coronal sections and TTC-negative areas surrounding the hematoma were measured and integrated as left hemisphere volume, right hemisphere volume and TTC-negative volume, respectively. The brain edema/atrophy and the infarct volume were calculated by the following formulas: edema/atrophy (%), (left hemisphere volume/right hemisphere volume) × 100; infarct volume (mm^3), TTC-negative volume (mm^3) − hematoma volume (mm^3). All data were expressed as mean ± S.E.M.

Brain tissues taken from animals for evaluation of neurological deficits at 28 days of intracerebral hemorrhage induction in Experiment 3 were further treated as follows. After TTC staining, the sections were immersed in 10% formalin solution for 30 min, washed with saline three times and incubated for 10 min with Prussian blue staining solution (1% potassium ferrocyanide and 1% hydrochloric acid) for detection of hemosiderin which is identical to hematoma of earlier stage. The hemosiderin-positive areas (blue-colored) were measured according to the same method described above, and the hematoma volume was determined by the integration of the areas.

2.4. Measurement of physiological parameters

For measurement of hematological parameters, blood was collected using a heparinized glass capillary from a catheter retaining in the femoral artery before the start of administration and immediately before the completion of administration. Hematocrit, blood pH, blood carbon dioxide partial pressure (P_{CO2}) and blood oxygen partial pressure (P_{O2}) were measured using a blood gas analyzer (ABL555, Radiometer Trading KK, USA). These data were corrected based on the rectal temperature at the time of blood collection. For measurement of blood pressure and heart rate, the catheter was connected to a pressure transducer (P23XL, Gould Electronics, OH, USA), and the measure-

Table 1
Hematoma volume and edema at 24 h after ICH induction in five groups of rats (experiment 1)

	Saline	Heparin	Placebo	YM872	YM872
Dose (mg/5 ml/kg/h)	–	–	0	20	40
(U/12 ml/kg/h)	0	3600	–	–	–
Infusion time (h)	0.5	0.5	4	4	4
N	10	10	10	10	10
Hematoma (mm^3)	28.3 ± 3.3	52.1 ± 6.0[b]	31.3 ± 1.8	26.3 ± 2.0	29.7 ± 3.0
Edema (%)	108.7 ± 1.4	114.5 ± 1.5[a]	107.5 ± 1.5	107.6 ± 0.9	109.9 ± 1.1

All data are expressed as mean ± S.E.M. Hematoma volumes and edema ratios between saline and heparin groups and between saline and placebo groups were analyzed by Student's *t*-test, and those among 20 mg/kg YM872, 40 mg/kg YM872 and placebo were done by Dunnett's test. [a]$P<0.05$ and [b]$P<0.01$ were considered statistically significant.

ment was conducted from before the start of administration to the completion of administration. Evaluation was made using mean blood pressures and heart rates obtained at the relatively stable points in time before the start of administration and before the completion of administration.

2.5. Observation and scoring of neurological deficits

Neurological deficits of each animal were observed in a regular cage for animal care at the time points of 2, 5, 8, 14 and 28 days after the end of the collagenase infusion. The observation was done in a blind manner to avoid subjective factors. The neurological deficits were scored into 5° (0–4) by behavioral patterns after pushing forward the hip of the animal according to the following criteria. Score 4, sedation or inability to move; score 3, rotates or turns right but does not go straight nor turn left; score 2, preferentially turns right and sometimes goes straight but never turns left; score 1, preferentially turns right but sometimes turns left; score 0, normal with no preference in turning direction. Each animal was pushed by the hip at least 10 times and observed for 2 min. The same animals were used at each time point and the brains were used for pathological analysis at 28 days.

2.6. Statistical analyses

Morphologically determined hematoma volumes between saline and heparin groups and between saline and placebo groups were analyzed by Student's *t*-test, and those among 20 mg/kg YM872, 40 mg/kg YM872 and placebo were done by Dunnett's test. Values were expressed as the mean ± S.E.M., and $P<0.05$([a]) and $P<0.01$([b]) were considered statistically significant. Physiological parameters, brain edema, brain atrophy and infarct volume were compared in the same manner between groups. Non-parametric neurological deficit scores of the 10 mg/kg YM872, 20 mg/kg YM872 and saline groups were compared at each time point of 2, 14 and 28 days using Steel's test. All values and the medians were expressed, and $P<0.05$([a]) and $P<0.01$([b]) were considered statistically significant.

Table 2
Measured physiological parameters in three groups of ICH rat model after 4 h infusion (experiment 2)

	Placebo	YM872	YM872
Dose (mg/5 ml/kg/h)	–	20	40
N	5	5	5
Blood pressure (mm Hg)	127 ± 14	126 ± 10	125 ± 16
Heart rate (beats/min)	390 ± 14	367 ± 10	357 ± 16
pH	7.46 ± 0.03	7.44 ± 0.01	7.45 ± 0.01
Hematocrit (%)	38.1 ± 1.7	39.4 ± 1.0	37.6 ± 1.3
P_{CO2} (mm Hg)	35.8 ± 2.8	42.4 ± 1.1	40.4 ± 1.4
P_{O2} (mm Hg)	99.4 ± 4.6	100.8 ± 9.5	99.8 ± 10.5
Rectal temperature (°C)	37.7 ± 0.2	37.3 ± 0.4	36.9 ± 0.3

All data are expressed as mean ± S.E.M. Statistical analyses were performed in each parameter between placebo and YM872 groups with Dunnett's test. No significant difference were shown between placebo and YM872 groups.

3. Results

In a control experiment, neither hematoma nor neurological deficits were detected by 2 days in animals with an intrastriatal injection of saline instead of collagenase, although collagenase clearly induced hematoma in the striatum. Effects of YM872 on intracerebral hemorrhage were investigated using a collagenase-induced intracerebral hemorrhage rat model (Experiment 1, Fig. 1). Intravenous 30-min infusion of the positive control heparin (1800 U/kg) enlarged the hematoma and enhanced brain edema significantly compared with saline infusion at 24 h after collagenase injection (Table 1). There were, however, no

Table 3
Effect of 24 h infusion of YM872 on recovery of neurological deficits in ICH rat model (experiment 3)

	Saline					YM872					YM872				
Dose (mg/5 ml/kg/h)	–					10					20				
N	9					6					9				
Days	2	5	8	14	28	2	5	8	14	28	2	5	8	14	28
Median	3	2	1	1	0	2	1	0.5	0[a]	0	3	2	1	1[a]	0
Score 4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Score 3	7	1	0	0	0	1	0	0	0	0	5	0	0	0	0
Score 2	1	5	3	3	0	4	2	1	0	0	4	6	1	0	0
Score 1	1	3	6	6	3	1	4	2	2	1	0	3	5	6	1
Score 0	0	0	0	0	6	0	0	3	4	5	0	0	3	3	8

Values indicate the number of animals scored by neurological deficits degrees and median of the scores of each day examined. The neurological scores of YM872 and saline groups were compared at each time point using the Steel's test. [a] $P<0.05$ was considered statistically significant.

Table 4
Hematoma volume, infarction volume and atrophy from three groups of ICH rat model at 28 days after ICH induction (experiment 3)

	Saline	YM872	YM872
Dose (mg/5 ml/kg/h)	–	10	20
N	9	6	9
Hematoma (mm^3)	3.8 ± 0.5	4.0 ± 0.7	3.5 ± 0.4
Atrophy (%)	94.7 ± 0.8	93.1 ± 1.4	95.0 ± 0.7
Infarction (mm^3)	6.6 ± 0.5	7.3 ± 1.0	5.7 ± 0.2

All data are expressed as mean ± S.E.M. Hematoma volumes, atrophy ratios and infarction volumes between placebo and YM872 groups were analyzed by Dunnett's test. No significant difference were shown between placebo and YM872 groups.

differences in hematoma volume or brain edema among the animal groups with 4-h infusions of placebo, 20 mg/kg/h YM872 or 40 mg/kg/h YM872, nor between the saline infusion and placebo infusion groups (Table 1). An infarct area, which was demonstrated as the TTC-negative area, was not detectable around the hematoma at 24 h after intracerebral hemorrhage induction in the intracerebral hemorrhage animals infused with heparin or saline. Preliminary data showed that a TTC-negative area surrounding the hematoma could be seen after 3 weeks following intracerebral hemorrhage induction (data not shown).

The influence of YM872 on the physiological parameters of the intracerebral hemorrhage rat model, blood pressure, heart rate, blood pH, hematocrit, P_{CO2} and P_{O2}, was investigated in Experiment 2. The physiological parameters were not changed between before and 4 h after intracerebral hemorrhage induction (data not shown). At 4 h, there was no difference between the 20 mg/kg/h YM872, 40 mg/kg/h YM872 and placebo groups (Table 2).

Neurological deficits were assessed up to 28 days following a 24-h infusion of YM872 in Experiment 3. The deficits were most severe at 2 days and had almost disappeared by 28 days (Table 3). Although there were no differences in neurological deficit scores at 2 days, the scores of the 10 and 20 mg/kg YM872 groups were significantly better than that of the saline group at 14 days ($p = 0.012$ and $p = 0.033$, respectively). Most animals in each group had recovered neurologically at 28 days. On the 28th day, the hematoma volume, atrophy and infarct volume in the brains did not differ among the groups (Table 4).

4. Discussion

In the present study, induction of small intracerebral hemorrhage by an intrastriatal injection of a low dose of collagenase (0.014 U) and the aggravating effect of intravenously infused heparin on intracerebral hemorrhage (1.8-fold) was confirmed. The enlarging of the hematoma by heparin was somewhat lower than in the previous study (3.4-fold) (Terai et al., 2003). This may depend on a saturating tendency in hematoma size because the hematoma in the saline-infused control group (28 mm^3) was larger than that in the previous experiment (19.2–25.1 mm^3). We have reported previously that higher dosages of collagenase result in larger hematoma, but the hematoma size is saturating, indicating that smaller hematoma is necessary for detection of an aggravating effect (Terai et al., 2003). The data suggest that the collagenase-induced hematoma is reproducible and sensitive enough for evaluation of drugs which may affect intracerebral hemorrhage.

In contrast to heparin, intravenous 4-h-infusion of YM872 at 20 or 40 mg/kg/h did not influence the hematoma volume or brain edema at 24 h as well as placebo infusion. Further intravenous 24-h-infusions of YM872 at 10 or 20 mg/kg/h did not influence the hematoma volume, infarct volume or brain atrophy at 28 days compared with saline infusion. Moreover, physiological parameters in the intracerebral hemorrhage model were not affected acutely by YM872 infusion as well as placebo. The data suggest that YM872 has no aggravating effects on intracerebral hemorrhage unlike heparin. These observations imply that the relative safety of YM872 might be better than that of heparin which is currently in use clinically for thrombosis or embolism patients because an intracerebral hemorrhage once occurring is enlarged by heparin administration.

It has been shown that intravenous infusion of YM872 significantly reduces by 50% the infarct volume in middle cerebral artery occlusion rat models at 24 h and at 7 days (Kawasaki-Yatsugi et al., 1998, 2000; Shimizu-Sasamata et al., 1998; Takahashi et al., 1998a) and in a cat model (Takahashi et al., 1998b), and that the minimum effective dose is 4-h-infusion of 10 mg/kg/h in a rat middle cerebral artery occlusion model. In general, in order to predict adverse effects, higher doses are commonly used for the evaluation. The dosages of YM872 used in the present study were 2–4-fold higher than the minimum effective dose for ischemic cerebral infarction in the rat and cat.

It has been demonstrated in a collagenase-induced intracerebral hemorrhage rat model that the neurological deficits are most severe from 1 to 3 days after intracerebral hemorrhage induction and almost disappear in a month (Del Bigio et al., 1996, 1999; Rosenberg et al., 1990). In the present experiment, saline-infused and YM872-infused groups showed a similar pattern in appearance and recovery of the neurological deficits. However, the deficits in the groups infused for 24 h with YM872 (10 and 20 mg/kg/h) were significantly lower than that in the saline-infused group at 14 days after intracerebral hemorrhage induction. These data suggest that YM872 can either accelerate recovery or reduce the deficits associated with intracerebral hemorrhage. In general, acute treatment of stroke patients followed by rehabilitation and earlier commencement of rehabilitation results in better recovery. Thus, YM872, which does not aggravate intracerebral hemorrhage and also may be beneficial for neurological deficits induced by intracerebral hemorrhage, could be useful for hemorrhagic stroke patients as well as ischemic cerebral infarction.

The neurological deficits are due to brain tissue destruction, generalized edema, and reduced blood flow in the surrounding tissue with subsequent neuronal loss (Del Bigio et al., 1996; Jenkins et al., 1989; Lee et al., 1996; Matz et al., 1997). In the present experiment, brain edema, atrophy and infarct volume were investigated. Heparin infusion significantly enhanced brain edema at 24 h after intracerebral hemorrhage induction but YM872 infusion did not. At the same time point, no infarcted areas in the brains were detectable by TTC staining. YM872 showed no influence on brain atrophy or infarct volume at 28 days in this intracerebral hemorrhage model. Brain edema has been demonstrated by T_2-weighted magnetic resonance imaging to be most severe from 1 to 3 days and to be transformed into atrophy around 14 days in a collagenase-induced intracerebral hemorrhage rat model (Del Bigio et al., 1996). It may be interesting to investigate the effect of YM872 on the time point of edema/atrophy transformation, although pathological differences between placebo/saline-infused and YM872 groups were not found in the present experiments. Further experiments may be necessary to find mechanisms that can explain the effect of YM872 on the neurological deficits.

It has been recently shown that AMPA receptors are expressed in oligodendrocytes as well as in neurons, and that oligodendrocytes share with neurons a high vulnerability to AMPA receptor-mediated death (Alberdi et al., 2002; Follett et al., 2000; McDonald et al., 1998). McDonald et al. (1998) have demonstrated that oligodendrocytes in primary cultures from mouse forebrain are killed by glutamate and AMPA or by deprivation of oxygen and glucose, and that the cell death is blocked by the AMPA receptor antagonist NBQX. Moreover, oligodendrocytes in rat subcortical white matter express AMPA receptors and are selectively injured by microstereotaxic injection of AMPA but not NMDA (McDonald et al., 1998). Although the TTC staining used in the present experiments could not detect ischemic changes in white matter, T_2-weighted magnetic resonance imaging has demonstrated an increase of signal intensity around the corpus callosum as well as in and around the hematoma in the collagenase-induced intracerebral hemorrhage rat model (Del Bigio et al., 1996). The increase in signal intensity may indicate plasma-derived edema fluid in the brain areas, resulting in ischemic and inflammatory events including neuronal and oligodendrocytic damage. It may be interesting to clarify oligodendrocytic damage in the corpus callosum which may lead to the neurological deficits of intracerebral hemorrhage model.

In summary, YM872, in dosages 2–4 times, the effective dose for ischemic stroke models did not show an untoward effect in the intracerebral hemorrhage model, it did not aggravate hemorrhage and neither did it had an adverse effect on the measured physiological parameters. Hence, these data suggest that the use of YM872 in ischemic stroke does not increase the risk for intracerebral hemorrhage. Determination of the effect of YM872 on the neurological deficits in intracerebral hemorrhage animals resulted in the finding of a trend towards YM872 accelerating recovery in dosages 1–2 times the effective dose for improving cerebral ischemic brain injury. The hypothesis is put forward that in the clinical correlate, in hemorrhagic stroke patients, this might provide the opportunity to use YM872 and initiate rehabilitation programs earlier, presumably benefiting overall patient outcome.

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