J Anesth (2004) 18:113–117
DOI 10.1007/s00540-004-0230-z

Journal of
Anesthesia



# Spinal neurotoxicity and tolerance after repeated intrathecal administration of YM 872, an AMPA receptor antagonist, in rats

Tomoki Nishiyama[1], Sachiko Kawasaki-Yatsugi[2], Tokio Yamaguchi[2], and Kazuo Hanaoka[1]

[1]Department of Anesthesiology, The University of Tokyo, Tokyo, Japan
[2]Insitute for Drug Discovery Research, Yamanouchi Pharmaceutical Co. Ltd., Tsukuba, Japan

**Abstract**
*Purpose.* Although the α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor antagonist, YM 872, has been considered to be useful in analgesia for both acute and chronic pain, there are no studies of its neurotoxicity and tolerance. We examined the spinal neurotoxicity and tolerance of YM 872 analgesia by repeated intrathecal administration in rats.
*Methods.* Male Sprague-Dawley rats with lumbar intrathecal catheters received YM 872 at $1\,\mu g \cdot 10\,\mu l^{-1}$ (eight rats; YM group) or normal saline 10 μl (eight rats; C group) intrathecally once a day for 30 days. We evaluated the analgesic effects every 3 days, by tail-flick test and behavioral side effects. On the 31st day, the lumbar spinal cord was removed from four randomly selected rats in each group for histological examination.
*Results.* The YM group showed significantly longer tail-flick latency when subjected to a high-intensity light beam than the C group at each measurement time point, although no significant changes in the latency according to the time course of the study were observed for the entire study period of 30 days in either group. No rats showed any side effects. Histologically, only slight lymphocytic cell infiltration and degeneration of myelinated fibers occurred, similarly in both groups. No changes were observed in the spinal cord in either group.
*Conclusion.* Administration of YM 872 (1 μg) once a day for 30 days did not induce any tolerance and caused no histological changes in the spinal cord.

**Key words** Analgesia · α-Amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor antagonist · Tolerance · Toxicity · Spinal cord

*Address correspondence to*: T. Nishiyama, 3-2-6-603 Kawaguchi, Kawaguchi 332-0015, Japan
Received: November 11, 2003 / Accepted: January 29, 2004

## Introduction

Glutamate receptors, mainly N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors, have an important role in mediating pain in the spinal cord. NMDA receptors are involved in the hyperalgesic state underlying neuropathic pain but are not involved in acute pain [1]. NMDA receptor antagonists except for ketamine lack clinical value because of their side effects, such as a psychotomimetic action [2], learning impairment [3], and neurotoxicity [4]. On the other hand, it has been suggested that AMPA receptors have a role in both acute and persistent inflammatory pain in the spinal cord [5,6]. In our previous studies, a new AMPA receptor antagonist, YM 872 {[2,3-dioxo-7-(1H-imidazol-1-yl)-6-nitro-1,2,3,4-tetrahydro-1-quinoxalinyl] acetic acid; Yamanouchi Pharmaceutical, Tsukuba, Japan} showed analgesic effects on both acute thermal pain (50% effective dose; $ED_{50} = 1\,\mu g$) and formalin-induced inflammatory pain ($ED_{50} = 0.24\,\mu g$ in phase 1 and 0.21 μg in phase 2) in rats [6]. However, YM 872 induced transient motor disturbance and flaccidity at doses of more than 10 μg [6]. Although YM 872 had no neurotoxicity in cat brain [7], its toxicity in other organs should be elucidated before its clinical application. Thus, the first purpose of this study was to investigate any histological changes of the spinal cord caused by repeated intrathecal administration of YM 872.

For chronic pain, long-term administration of an analgesic is often required. Although morphine and clonidine are used for the treatment of chronic pain, continuous exposure of μ opioid receptors or $\alpha_2$ adrenoceptors to an agonist will produce tolerance, caused by an unknown mechanism [8]. However, there are no studies on the tolerance of AMPA receptor antagonists. The second purpose of this study was to investigate whether tolerance would occur to the analgesic effects of YM 872 given by intrathecal administration.

## Materials and methods

The protocol was approved by the Research Committee of the University. Male Sprague-Dawley rats (280–300 g; Nippon Bio-Supply, Tokyo, Japan) were implanted with chronic lumbar intrathecal catheters, under halothane (2%) anesthesia. An 8.5-cm polyethylene catheter (PE-10; Clay Adams, Parsippany, NJ, USA) was advanced caudally through an incision in the atlanto-occipital membrane to the thoracolumbar level of the spinal cord. The external part of the catheter was tunneled subcutaneously to exit on the top of the skull and plugged with a 28-G stainless-steel wire. Sixteen rats with normal motor function and behavior 7 days after surgery were used. The position of the catheter was checked by the aspiration of cerebrospinal fluid at implantation and was verified directly after the rat was killed.

YM 872 (Yamanouchi Pharmaceutical), 10 mg, was dissolved in 0.97 ml distilled water with 30 μl 1 N NaOH to adjust the pH to 7.3–7.5. Solutions of 1 μg (2.86 nmol; $ED_{50}$ for the tail-flick test, as used in our previous study [6]) per 10 μl were made, using normal saline, before each injection. Normal saline 10 μl was used as the control. After each intrathecal drug injection, the catheter was flushed with a subsequent injection of 10 μl of normal saline to clear the dead space of the catheter (8 ± 0.9 μl; mean ± SD). Microinjector syringes were used for all injections.

Starting on the seventh day after the catheter insertion (day 0), YM 872, 1 μg·10 $\mu l^{-1}$ (eight rats; YM group) or saline 10 μl (eight rats; C group) was administered intrathecally at 7 A.M. every day for 30 days. Every 3 days, the analgesic effect and behavioral side effects were evaluated 15 min after the intrathecal drug injection. The analgesic effect was tested by the tail-flick test. We measured tail-flick latency and checked the side effects 15 min after the drug injection, because both the analgesic and side effects were greatest at that time in our previous study [6]. On the 31st day, the rats were killed with an overdose of halothane. The location of the catheter was verified in every rat. Four rats in each group were selected at random for the histological study.

For the experiment, the rats were placed in a clear plastic cylindrical cage with their tails extending through a slot provided in the rear of the tube. Noxious stimulation was provided by a beam of high-intensity light (Tail-flick Analgesia Meter MK-330A; Muromachi Kikai, Tokyo, Japan) focusing on the tail, 2 to 3 cm proximal to the end. The focus was in almost, but not exactly, the same place at every measurement. The response time was measured, and defined as the interval between the onset of the thermal stimulation and an abrupt flick of the tail. From our experience, the cutoff time in the absence of a response was set at 14 s to prevent tissue-burn injury.

The behavior (including agitation and allodynia), motor function, flaccidity, pinna reflex, and corneal reflex were examined by a blinded investigator after the tail-flick measurement. The behaviors were judged as present or absent. Agitation was judged as spontaneous irritable movement and/or vocalization. The presence of allodynia was examined by observation for agitation (escape and/or vocalization) evoked by lightly stroking the flank of the rat with a small probe. Motor function was evaluated by the placing/stepping reflex and the righting reflex. The former reflex was evoked by drawing the dorsum of either hind paw across the edge of a table. Normally rats try to place the paw ahead in a position to walk. The latter reflex was assessed by placing the rat horizontally with its back on the table, a placement which normally gives rise to an immediate, coordinated twisting of the body to an upright position. Disturbance of the righting reflex also shows impairment of the function of the central nervous system. Flaccidity was judged as a muscle weakness in raising the forepaw to a place 3–5 cm higher than the hind paw. When a 3- to 5-cm higher place is placed in front of a rat, normally, the rat will walk up to the higher place. Lack of a walking-up movement was judged as flaccidity. Pinna and corneal reflexes were examined with a paper string. When a paper string is inserted into the ear canal or touches the cornea, rats normally shake their head or blink, respectively.

Just after being killed with halothane, four randomly selected rats in each group were perfused with 10% formalin through the ascending aorta. The lumbar spinal cord was removed with the ventral and dorsal roots at the lumbar enlargement where the tip of the catheter was located, fixed in 15% formalin for 24 h, decalcified for 48 h, and then embedded in paraffin. Four slices selected randomly by an animal pathologist were examined using light microscopy after hematoxylin-eosin (H&E) staining and luxor fast blue-H&E staining. The animal pathologist, at Hatano Research Institute (Kanagawa, Japan) was blinded to the treatment. Histology was indicated as negative, very slight, slight, moderate, or severe changes according to the usual judgment by the pathologist.

Tail-flick response latency was converted to percent maximum possible effect (%MPE), according to the following formula: %MPE = [(postdrug latency − baseline latency) / (cutoff time − baseline latency)] ×100.

Differences in the %MPE for tail-flick latency were analyzed with repeated measures analysis of variance (ANOVA), followed by the Student Newman-Keuls test. Histological findings were compared using the $\chi^2$ test. A $P$ value of less than 0.05 was considered statistically significant.

**Table 1.** Tail flick-latency before drug administration

| Days | 0 | 3 | 6 | 9 | 12 | 15 | 18 | 21 | 24 | 27 | 30 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| YM group | 3.5 ± 0.3 | 3.4 ± 0.3 | 3.3 ± 0.5 | 3.3 ± 0.6 | 3.3 ± 0.5 | 3.5 ± 0.5 | 3.4 ± 0.6 | 3.4 ± 0.5 | 3.5 ± 0.3 | 3.4 ± 0.4 | 3.5 ± 0.5 |
| C group | 3.4 ± 0.6 | 3.5 ± 0.3 | 3.5 ± 0.5 | 3.5 ± 0.5 | 3.3 ± 0.6 | 3.5 ± 0.6 | 3.5 ± 0.3 | 3.6 ± 0.5 | 3.6 ± 0.6 | 3.6 ± 0.5 | 3.5 ± 0.5 |

Values are means ± SD (s)
YM 872, 1 μg · 10 μl$^{-1}$ (YM group) or saline 10 μl (C group) was intrathecally administered once a day for 30 days

**Table 2.** Histopathological changes

| | YM group ($n$ = 4) | C group ($n$ = 4) |
|---|---|---|
| Spinal cord: parenchyma | | |
| Abnormality | None | None |
| Subarachnoid space | | |
| Lymphocytic cell infiltration | Very slight (3) | Very slight (4) |
| Fresh hemorrhage | Very slight (1) | Very slight (1) |
| Subdural space | | |
| Lymphocytic cell infiltration | Very slight (2) | Very slight (2) |
| Infiltration of macrophages | Very slight (1) | Very slight (1) |
| Fibrinous material | Very slight (1) | Very slight (1) |
| Fresh hemorrhage | Very slight (1) | Very slight (1) |
| Spinal nerve root: parenchyma | | |
| Degeneration of myelinated fibers | Very slight (1) | Very slight (2) |
| Lymphocytic cell infiltration | None | Very slight (1) |
| Proliferation of Schwann cells | Very slight (1) | Very slight (1) |
| : perineurium | | |
| Lymphocytic cell infiltration | Very slight (3) | Very slight (3) |
| Posterior root ganglion | | |
| Abnormality | None | None |

Histology was judged as negative, very slight, slight, moderate, or severe change by an animal pathologist blinded to the treatment. None, all specimens showed negative changes. Only very slight changes were seen in this study. Figures in parentheses are numbers of rats that showed the change. YM 872 1 μg · 10 μl$^{-1}$ (YM group) or saline 10 μl (C group) was intrathecally administered once a day for 30 days. Lumbar spinal cord was removed at the lumbar enlargement and four slices were examined

## Results

The tail-flick latencies before the administration of YM 872 or saline were not different between the groups and did not change during the study period (Table 1). The YM group showed significantly greater tail-flick latency than the C group at each time point measured during the study, but no significant changes were observed in tail-flick latency according to the time course of the study in either group (Fig. 1). No rats showed any observable motor disturbances or behavioral abnormalities with the dose used.

In the histological study, there were no significant differences between the two groups. No histological changes were observed in the spinal cord in either group (Table 2).

## Discussion

Intrathecal administration of YM 872, 1 μg ($ED_{50}$ dose), once a day for 30 days, provided analgesia for thermally induced pain without inducing any tolerance, and showed no apparent histological changes of the spinal cord on examination by light microscopy.

It is well known that the chronic administration of various receptor agonists may lead to the development of tolerance to the effects, in conjunction with a decrease in the number of membrane receptor molecules which specifically bind the agonist [9]. No tolerance to motor disturbance has been observed with long-term treatment with the 2,3-benzodiazepines, which are AMPA receptor antagonists [10]. In the present study, we did not detect any side effects, including motor disturbance, with YM 872.

The speed at which tolerance develops depends greatly on the pharmacokinetic characteristics of the drug [11]. Specifically, shorter duration of action is associated with faster development of tolerance. The rate of onset of tolerance is also linked to the time during which the receptor is exposed to the ligand. It is possible that slower development of tolerance may be obtained by continuous administration of the drug rather than by intermittent exposure of the receptors to high concen-

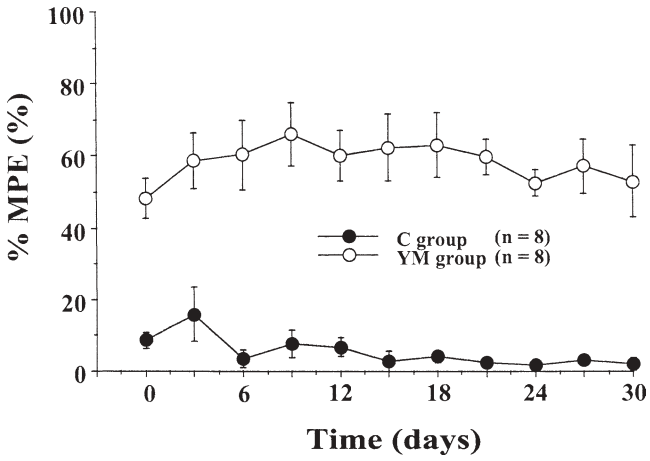


**Fig. 1.** Tail-flick latency is shown as percent maximum possible effect (*% MPE*): %MPE = [(postdrug latency − baseline latency) / (cutoff time − baseline latency)] ×100. *Bars* indicate the SD. Day *0* is the first day of the analgesic measurement. The YM group (*open circles*) received YM 872 $1\,\mu g \cdot 10\,\mu l^{-1}$, and the C group (*closed circles*) received saline 10 μl. The YM group showed significantly larger %MPE than the C group at every time point ($P < 0.05$). No significant changes according to time course were observed in either group

trations from intermittent doses. Therefore, we chose intermittent administration rather than continuous infusion. If glutamate has some roles in the development of tolerance, as shown in previous studies [9, 12], glutamate antagonists such as YM 872 should have a benefit to inhibit tolerance.

Before the clinical application of a drug, its safety and side effects should be tested. It has been reported that muscle tone was decreased by the intraperitoneal administration of an AMPA receptor antagonist, CNQX [13], while muscle tone was not affected by intraperitoneal GYKI-52466 [14]. Ataxia was induced by NBQX in rats [15]. A potent AMPA receptor antagonist, YM 90K, has no psychotomimetic action and shows no histological changes in the rat brain [16]. However, the clinical application of AMPA receptor antagonists has been limited because of poor water solubility and nephrotoxicity caused by precipitation of the drug in the kidney [17]. Thus, analogues with better water solubility have been desired. YM 872 used in our study is much more water-soluble than other formulations of AMPA receptor antagonists [18], and precipitation in the kidney was not observed in rats [7]. With most AMPA receptor antagonists, the therapeutic dose is close to that causing severe motor impairment [19]. At the doses used to reduce nociception, flaccidity was found with the competitive AMPA receptor antagonist ACEA 2085, and motor disturbance was found at higher concentrations [5]. However, YM 872 did not induce any motor disturbances with the $ED_{50}$ dose in the present study. Also, YM 872 had no neurotoxicity in cat brains in a cerebral ischemia model [7]. The present study suggests that no histological changes were produced in the spinal cord by the intermittent intrathecal administration of YM 872, although the number of rats tested was too small to reach a definite conclusion.

Clinically, LY-293558, an AMPA receptor antagonist, was intravenously administered to volunteers, without serious adverse events, except for hazy vision that was resolved in 60 min [20]. A 3-h intravenous infusion of the AMPA receptor antagonist, YM 90K, at 36 mg caused little change in blood or urine chemistry, electrocardiogram, vital signs, or in levels of insulin, glucagon, or blood glucose in healthy volunteers [21]. YM 872 should be tested, as these compounds have been, before clinical application.

In conclusion, although a limited number of rats was examined, administration of the $ED_{50}$ dose of YM 872 (1 μg) once a day for 30 days did not induce any tolerance or any histological changes of the spinal cord on examination by light microscopy. Further studies are necessary, using different doses and different animals, to confirm its safety and lack of induction of tolerance.

## References

1. Ault B, Hildebrand LM (1993) Effects of excitatory amino acid receptor antagonists on a capsaicin-evoked nociceptive reflex: a comparison with morphine, clonidine and baclofen. Pain 52:341–349
2. Koek W, Woods JH, Winger GD (1988) MK-801, a proposed noncompetitive antagonist of excitatory amino acid neurotransmission, produces phencyclidine-like behavioral effects in pigeons, rats and rhesus monkeys. J Pharmacol Exp Ther 245:969–974
3. Morris RGM, Anderson E, Lynch GS, Baudry M (1986) Selective impairment of learning and blockade of long-term potentiation by an N-methyl-D-aspartate receptor antagonist, AP5. Nature 319:774–776
4. Olney JW, Labruyere J, Wang G, Wozniak DF, Price MT, Sesma MA (1991) NMDA antagonist neurotoxicity: mechanism and prevention. Science 254:1515–1518
5. Nishiyama T, Yaksh TL, Weber E (1998) Effects of intrathecal NMDA and non-NMDA antagonists on acute thermal nociception and their interaction with morphine. Anesthesiology 89:715–722
6. Nishiyama T, Gyermek L, Lee C, Kawasaki-Yatsugi S, Yamaguchi T (1999) The spinal antinociceptive effects of a novel competitive AMPA receptor antagonist, YM872, on thermal or formalin-induced pain in rats. Anesth Analg 89:143–147
7. Takahashi M, Ni JW, Yatsugi-Kawasaki S, Toya T, Yatsugi S, Shimizu-Sasamata M, Koshiya K, Shishikura J, Sakamoto S, Yamaguchi T (1998) YM 872, a novel selective α-amino-3-hydroxy-5-methylisoxazole-4-propinoic acid receptor antagonist, reduces brain damage after permanent focal cerebral ischemia in cats. J Pharmacol Exp Ther 284:467–473
8. Stevens CW, Monasky MS, Yaksh TL (1988) Spinal infusion of opiate and alpha-2 agonists in rats: tolerance and cross-tolerance studies. J Pharmacol Exp Ther 244:63–70

9. Overstreet DH, Yamamura HI (1979) Receptor alterations and drug tolerance. Life Sci 25:1865–1878
10. De Sarro G, Di Paola ED, Gareri P, Gallelli L, Scotto G, De Sarro A (1999) Effects of some AMPA receptor antagonists on the development of tolerance in epilepsy-prone rats and in pentylenetetrazole kindled rats. Eur J Pharmacol 368:149–159
11. Kissin I, Lee SS, Arthur GR, Bradley EL (1996) Time course characteristics of acute tolerance development to continuously infused alfentanil in rats. Anesth Analg 83:600–605
12. McLemore GL, Kest B, Inturrisi CE (1997) The effects of LY293558, an AMPA receptor antagonist, on acute and chronic morphine dependence. Brain Res 778:120–126
13. Maj J, Rogoz Z, Skuza G, Jaros T (1995) Some behavioral effects of CNQX and NBQX, AMPA receptor antagonists. Pol J Pharmacol 47:269–277
14. Maj J, Rogoz Z, Skuza G, Kolodziejczyk K (1995) Some central effects of GYKI 52466, a non-competitive AMPA receptor antagonist. Pol J Pharmacol 47:501–507
15. Filliat P, Pernot-Marino I, Baubichon D, Lallement G (1998) Behavioral effects of NBQX, a competitive antagonist of AMPA receptors. Pharmacol Biochem Behav 59:1087–1092
16. Izumisawa N, Kawakami A, Ohata T, Hanada T, Okeda R (1995) YM 90K, an AMPA antagonist, has no neurotoxic effects on cerebrocortical neurons in rats. Exp Neurol 134:199–204
17. Xue D, Huang ZG, Barnes K, Lesiuk HJ, Smith KE, Buchan AM (1994) Delayed treatment with AMPA, but not NMDA, antagonists reduces neocortical infarction. J Cereb Blood Flow Metab 14:251–261
18. Kohara A, Okada M, Tsutsumi R, Ohno K, Takahashi M, Shimizu-Sasamata M, Shishikura J, Inami H, Sakamoto S, Yamaguchi T (1998) In-vitro characterization of YM872, a selective, potent and highly water-soluble α-amino-3-hydroxy-5-methylisoxazole-4-propionate receptor antagonist. J Pharm Pharmacol 50:795–801
19. Kubova H, Vilagi I, Mikulecka A, Mares P (1997) Non-NMDA receptor antagonist GYKI 52466 suppresses cortical afterdischarges in immature rats. Eur J Pharmacol 333:17–26
20. Gilron I (2001) LY-293558 Eli Lilly & Co. Curr Opin Investig Drugs 2:1273–1278
21. Umemura K, Kondo K, Ikeda Y, Teraya Y, Yoshida H, Homma M, Uematsu T, Nakashima M (1997) Pharmacokinetics and safety of the novel amino-3-hydroxy-5-methylisoxazole-4-propionate receptor antagonist YM90K in healthy men. J Clin Pharmacol 37:719–727