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

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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 µg·10 µl−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

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 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: ED50 = 1 µg) and formalin-induced inflammatory pain (ED50 = 0.24 µg 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 10µg [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 α2 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 (280–300g; 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), 10mg, was dissolved in 0.97ml distilled water with 30µl 1N NaOH to adjust the pH to 7.3–7.5. Solutions of 1µg (2.86nmol; ED₉₀ for the tail-flick test, as used in our previous study [6]) per 10µl were made, using normal saline, before each injection. Normal saline 10µl was used as the control. After each intrathecal drug injection, the catheter was flushed with a subsequent injection of 10µl of normal saline to clear the dead space of the catheter (8 ± 0.9µl; mean ± SD). Microinjector syringes were used for all injections.

Starting on the seventh day after the catheter insertion (day 0), YM 872, 1µg·10µl⁻¹ (eight rats; YM group) or saline 10µl (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 3cm 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 14s 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 3–5cm higher than the hind paw. When a 3- to 5cm 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 24h, decalcified for 48h, 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)] x 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 χ² test. A P value of less than 0.05 was considered statistically significant.