Chronic Anti-ataxic Actions of the Novel Thyrotropin-Releasing Hormone (TRH) Analog, TA-0910, during and after Repeated Administration in Rolling Mouse Nagoya: Behavioral and Pharmacokinetic Studies

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Anti-ataxic effects of TA-0910, a novel thyrotropin-releasing hormone analog, in mice of the ataxic mutant mouse strain Rolling mouse Nagoya (RMN) are sustained beyond its 2-week oral administration period (Kinoshita *et al.*, *Eur. J. Pharmacol.*, 274, 65–72, 1995). We examined the concentration of TA-0910 in the central nervous system (CNS) of RMN after repeated administration in an attempt to clarify the mechanism of the sustained effect of the drug. Repeated administration of TA-0910 (3 mg/kg/d, i.p.) for 2 weeks produced a long-lasting ameliorating effect on ataxia in RMN, and this effect was maintained until 3 weeks after drug withdrawal. The concentrations of TA-0910 in the cerebrum and brain stem 24 h after the final administration were twice the concentration observed 24 h after single administration. The cerebellum concentration of TA-0910 was more than 4 times that observed 24 h after final administration. After repeated administrations, the drug concentrations in the brain tissues gradually decreased, but the drug was still detectable in the cerebrum and brain stem 3 weeks after withdrawal. However, these concentrations of TA-0910 3 weeks after withdrawal were as low as those observed 24 h after single administration when there were no anti-ataxic effects. These observations suggest that the long-lasting ameliorating effect on the ataxia during and after repeated administration of TA-0910 is not ascribable to the drug remaining in the CNS of RMN.

Key words TA-0910 (TRH analog); Rolling mouse Nagoya (RMN); chronic anti-ataxic effect; drug concentration

Thyrotropin-releasing hormone (TRH: L-pyroglutamyl-L-histidyl-L-prolineamide) exerts several central nervous system (CNS) activities, such as stimulation of locomotor activity and antagonism to pentobarbital-induced sleep,^{1,2)} apart from its thyroid stimulating hormone releasing action. Pharmacological studies have demonstrated amelioration of ataxia by TRH in cytosine arabinoside-treated mice and spinal traumatic rats.^{3,4)} These reports suggest that TRH may have a therapeutic potential in ataxia, and clinical application of this peptide as an anti-ataxic drug has been made.^{5,6)}

In Japan, TRH has been reported to ameliorate the motor deficits of patients with spinocerebellar degeneration⁷⁾; however, its therapeutic effect is very short-lived, because exogenously administered TRH is rapidly metabolized.⁸⁾ Furthermore, TRH exerts its endocrine action at doses much lower than those producing pharmacological actions on the CNS.⁹⁾

TA-0910 ((–)-N-[(S)-hexahydro-1-methyl-2,6-dioxo-4-pyrimidinylcarbonyl]-L-histidyl-L-prolineamide tetrahydrate) is a novel TRH analog which being clinically studied for use in treating spinocerebellar degeneration by oral administration. This analog has various CNS actions and its activity is 10 and 100 times stronger by intravenous and oral administration, respectively, and about 8 times longer-lasting than that of TRH.^{10–12)} Moreover, the thyrotropin-releasing activity of TA-0910 is 10 times weaker than that of TRH.¹³⁾ Both TA-0910 and TRH ameliorate the gait of the ataxic mutant mouse, Rolling mouse Nagoya (RMN), by repeated oral administration.¹⁴⁾ Although the effect of TRH on ataxia disappeared immediately after the repeated administration, the effect of TA-0910 was sustained for 2 weeks after its 2-week administration period ended.¹⁴⁾ The mechanism of the

sustained amelioration by TA-0910 after repeated administration has not here to fore been known.

The aim of the present study was to clarify the mechanism of the sustained anti-ataxic action of TA-0910 in relation to the pharmacokinetics of this drug in the CNS of RMN. We first confirmed that both TA-0910 and TRH showed the sustained anti-ataxic effect by repeated intraperitoneal administration. We next measured the concentration of TA-0910 in the blood and cerebral structures during the period when the sustained anti-ataxic effect of this analog was observed.

MATERIALS AND METHODS

Animals Forty-eight male rolling mice (RMN, 6–8 months old) and 52 male RMN (6–10 months old) were used for the behavioral and pharmacokinetic studies, respectively. They were bred by Marugo Research Service Co., Ltd. (Saitama, Japan) and were housed in groups of 5–10 in plastic cages (42W × 26D × 15H cm). The animals were kept in an air-conditioned room with controlled temperature (23 ± 1 °C), humidity (55 ± 5%), and 12 h lighting (lights on 6:30 through 18:30) and were allowed free access to a standard pellet diet (CE-1, Oriental Yeast Co., Ltd.) and tap water.

Drugs and Treatment TA-0910 and TRH tartrate were synthesized by Tanabe Seiyaku Co., Ltd.; their chemical structures are shown in Fig. 1. TRH (3, 30 mg/kg/d, i.p.) and TA-0910 (0.3, 3 mg/kg/d, i.p.) were dissolved in physiological saline (0.9% NaCl sol., Otsuka Pharmaceutical Co., Ltd.) immediately before use, and administered in a volume of 10 ml/kg body weight. ¹²⁵I-TA-0910 (spec. act., 52.4 mCi/mmol) and anti-TA-0910 antibody were prepared by S. Chishima in our laboratory.¹⁵⁾ Other drugs

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Fig. 1. Chemical Structures of TA-0910 and TRH

used were 0.1 N acetic acid (Junsei Chemical), methanol (Kanto Chemical), 0.2% bovine γ -globulin (BGG, Miles Inc.), ammonium sulfate (Nacalai Tesque) and phosphate buffered saline (PBS, Nissui Chemical).

Behavioral Study Each of the rolling mice was transferred singly to a plastic cage (42W $\times$ 26D $\times$ 15H cm) on a locomotion counter with a multiple photo-cell sensor system (SCANETT: sensitivity; MV2=10, Toyo Sangyo Co., Ltd). The number of falls was counted visually and spontaneous motor activity was recorded on a digital counter for 10 min immediately after transfer from the home cage. The index of ataxia (the fall index) was defined as the number of falls/spontaneous motor activity for 10 min.

The animals were divided into 5 similar groups of 9 or 10 mice based on the mean fall index of individual animals, which was obtained from three measurements made during 72–24 h before drug administration. The fall index was determined 24 h after the final dosage of 7- or 14-d repeated administration (once daily) and 1, 2, 3, 4 and 5 weeks after the end of the 14-d administration period.

Pharmacokinetic Study Plasma samples were obtained from the blood samples which were taken from the abdominal aorta in TA-0910-treated animals under ether anesthesia and centrifuged (Model 5900, Kubota; 3000 rpm for 15 min at 4 °C). These samples were diluted with the plasma obtained from normal animals and used for radioimmunoassay (RIA) experiment. Immediately after euthanasia by blood-letting, the brain was removed from the skull, rinsed with ice-cold physiological saline and divided into the cerebrum, cerebellum and brain stem. Thereafter, the brain structures were weighed and homogenized in 5 volumes of 0.1 N acetic acid-methanol solution of the tissue weight. The homogenate was mixed with distilled water of 2 times the tissue weight and again homogenized. The supernatant was obtained by centrifugation (Model 5900, Kubota; 3000 rpm for 20 min at 4 °C). These samples were kept at –20 °C until analysis.

The supernatant (50–500 μ l) was concentrated *in vacuo*, and 200 μ l of 0.2% bovine γ -globulin-phosphate buffered saline was added to the residue. The residue of this solution

Fig. 2. Effect of Repeated Intraperitoneal Administration of TA-0910 and TRH on the Fall Index (Number of Falls/Spontaneous Motor Activity for 10 min) in Rolling Mouse Nagoya ($n=9-10$)

(○) saline, (△) TA-0910 0.3 mg/kg/d, (▲) TA-0910 3 mg/kg/d, (□) TRH 3 mg/kg/d, (■) TRH 30 mg/kg/d. * $p<0.05$, ** $p<0.01$ compared with saline (ANOVA followed by Duncan's multiple range test).

after concentration was used as samples of the RIA. Blood samples were taken 10, 30, 60, 120 and 180 min and 24 and 48 h after single administration of TA-0910 (3 mg/kg). Samples were also obtained 24 h after the final dosage of 1- and 2-week repeated administrations, and 1, 2 and 3 weeks after drug withdrawal.

The RIA for TA-0910 was performed according to Chishima's method.¹⁵⁾ After mixing the sample previously described (100 μ l) or TA-0910 standard (100 μ l) with 200 μ l of 1/15 M PBS (pH 7.4), diluted anti-TA-0910 antiserum (100 μ l) and 125 I-TA-0910 (10000 dpm; 100 μ l), the mixture was allowed to stand at 4 °C overnight. It was stirred with saturated ammonium sulfate (pH 7.0; 500 μ l) in ice-cold for 30 min and was centrifuged (1700 $\times g$, RL-131, Tomy) for 30 min at 4 °C. The precipitate was mixed with 50% saturated ammonium sulfate (500 μ l) and centrifuged (1700 $\times g$, 30 min, 4 °C) again. After discarding the supernatant, the precipitate was counted for its radioactivity of 125 I-TA-0910 by γ -counter (Cobra 2, Packard). TA-0910 standard solutions (0.98–125 pg/100 μ l) were prepared by dilution of TA-0910 with pure water (Milli Q).

Statistical Analysis All data were expressed as the mean $\pm$ S.E. The data from the behavioral study were analyzed by analysis of variance (ANOVA) for repeated measurements, followed by Duncan's multiple range test. The data from the pharmacokinetic study were not analyzed statistically.

RESULTS

Behavioral Study Twenty-four hours after the final administration of the 1- or 2- week administration period, TA-0910 at doses of 0.3 and 3 mg/kg/d significantly decreased the fall index (Fig. 2). The fall index at the higher dose remained significantly depressed until 3 weeks after drug withdrawal. TRH at a dose of 30 mg/kg/d also depressed the fall index, and the effect was significant 24 h

Fig. 3. Concentrations of TA-0910 in Plasma, Cerebrum, Cerebellum and Brain Stem after Single Intraperitoneal Administration of TA-0910 (3 mg/kg) to Rolling Mouse Nagoya ($n=3-5$)

The cerebellum concentrations in 3, 24 and 48 h after the administration were at an undetectable level (<15 pg/g). (□) plasma, (◇) cerebrum, (○) cerebellum, (△) brain stem.

Fig. 4. Concentrations of TA-0910 in Plasma, Cerebrum, Cerebellum and Brain Stem during and after Repeated Intraperitoneal Administration of TA-0910 (3 mg/kg) to Rolling Mouse Nagoya ($n=2-5$)

The samples in the administration period were obtained 24 h after dosing. The cerebellum concentrations in 1 d, the cerebellum and plasma concentrations in 5 weeks are undetectable level (<15 pg/g). (□) plasma, (◇) cerebrum, (○) cerebellum, (△) brain stem.

after the final administration of the 2 week medication period (Fig. 2). Although not statistically significant, there was a tendency of amelioration one week after TRH withdrawal.

Pharmacokinetic Study After single administration of TA-0910 at the dose of 3 mg/kg, the plasma concentration showed a maximum ($8.3 \mu\text{g/ml}$) at 10 min and declined with a half-life of 11 min (Fig. 3). The concentrations of TA-0910 in the brain tissues, cerebrum, cerebellum and brain stem also showed maxima at 10 min after administration, and declined gradually (Fig. 3). There were no remarkable differences in peak concentrations among the tissues. The cerebellum concentration of TA-0910 3 h after the administration was an undetectable level (<10 pg/g), while the plasma and brain tissue concentrations 48 h after administration were 14 pg/ml and 16–23 pg/g, respectively.

The plasma concentrations of TA-0910 at the dose of 3 mg/kg/d 24 h after the final dosage of the 7- and 14-d administration periods were 139 and 269 pg/ml, respectively (Fig. 4), 1.5 and 3 times, respectively, the concentration 24 h after single administration. Plasma con-

centrations 1 and 2 weeks after withdrawal were 90 and 19 pg/ml, respectively, and the concentration was undetectable 3 weeks after withdrawal. The concentrations understood by now in the cerebellum and brain stem also gradually increased with repeated drug administration, and 24 h after the final dose of 14-d administration reached levels twice those 24 h after single administration (Fig. 4). However, the cerebellum concentration of TA-0910 24 h after the 7-d administration was higher than that 24 h after 14-d administration. Following drug withdrawal, concentrations in the cerebrum, cerebellum and brain stem gradually decreased, and were 42, 170 and 49 pg/g after 1 week, and 32, 67 and 43 pg/g after 2 weeks, respectively. Three weeks after withdrawal, drug concentrations in the cerebrum and brain stem were 18 and 20 pg/g, respectively, but the concentration in the cerebellum was undetectable.

DISCUSSION

TA-0910 at a dose of 3 mg/kg improved the ataxia of RMN 10–60 min after single intraperitoneal administration, and the maximum effect was observed 10 min after administration.¹⁶⁾ The effect of the drug gradually declined and had almost disappeared 3 h after it was administered. The concentrations in the brain tissues showed peak levels 10 min after the administration, and also declined gradually afterward. These findings suggest that the anti-ataxic action of single administration of TA-0910 may depend on its concentration in the brain tissues.

Repeated administration of TA-0910 for 2 weeks produced a long-lasting ameliorating action on ataxia in RMN, and the effect of the drug at a dose of 3 mg/kg/d was maintained until 3 weeks after it was withdrawn. Repeated administration of TRH at a higher dose (30 mg/kg/d) also produced a long-lasting ameliorating action on ataxia in this model during medication, and there was an ameliorating tendency one week after withdrawal (Fig. 3). From these observations, the effects of these drugs were believed to occur through the same mode of action.

The concentrations of TA-0910 in the cerebrum and brain stem at a dose of 3 mg/kg/d gradually increased with repeated administration for 2 weeks, and 24 h after the final dosage of repeated administration were twice the level they were 24 h after single administration. The cerebellum concentrations of TA-0910 24 h after the final dosage of the 7- and 14-d administration periods were about 10- and 4-times, respectively, those in the cerebrum and brain stem. After the end of repeated administration, the concentrations in the brain tissues gradually decreased, but the drug still remained in the cerebrum and brain stem 3 weeks after the final dosage. The concentrations of TA-0910 that remained 3 weeks after withdrawal, however, were extremely low (pg order) and at the same levels as those 24 h after single administration when there was no anti-ataxic action.

The ameliorating action on ataxia in RMN was shown to be based on activation of the ventral tegmental field (VTF).¹⁶⁾ However, it is difficult to believe that most of the unchanged TA-0910 detected after the chronic treatment is locally distributed in the VTF and the nuclei terminating in the VTF. Taken together with these results,

the remaining TA-0910 in the brain tissues is not likely to play an important role in ameliorating the ataxia in this model, and the long-lasting effect of TA-0910 after repeated administration is thought to be through an unknown mechanism different from the action after single administration.

RMN shows dysfunction of the cerebellum and VTF which receive projections from the cuneiform nucleus, the so-called mesencephalic locomotor region, accompanied by a decrease in the local cerebral glucose utilization.¹⁶⁾ From these observations, it is suspected that the tissue construction, such as neural network, is abnormal in these brain regions. In fact, the cerebellum of RMN shows a lower number of granular cells, a decreased organ size and abnormal neurotransmitter levels.¹⁷⁻¹⁹⁾ Therefore, the brain stem including the VTF is thought to have similar abnormalities in the neural network.

Endogenous TRH levels are increased in the experimentally injured spinal cord.^{20,21)} Exogenously administered TRH reduces the neuronal loss induced by axotomy²²⁾ and accelerates neurite extension in cultured spinal ventral horn neurons,²³⁾ suggesting that TRH has a neurotrophic factor (NTF)-like action. TA-0910 also accelerates the neurite extension *in vitro* like TRH and accelerates recovery from the ataxia in the spinally injured rat when chronically administered.^{14,24)} From these reports, TA-0910 is thought to have a NTF-like action. In the present study, repeated administration of TA-0910 may have stimulated repeatedly the neural circuit with abnormal structures and restructured a new and normalized network. The new network may play an important role in the anti-ataxic action of TA-0910 by maintaining the structure for 3 weeks after withdrawal.

In conclusion, the long-lasting amelioration of ataxia in RMN during and after chronic administration of TA-0910 is not ascribable to the remainder of the drug in the central nervous system of RMN. To understand the mechanism of the long-lasting anti-ataxic action by TA-0910 after withdrawal, future studies will be focused on the neurotrophic factor-like action of this drug in the brain stem of RMN.

Acknowledgment The authors would like to thank Dr.

S. Takayama for reading the manuscript and offering helpful suggestions.

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