Long-Lasting Muscle Relaxant Activity of Eperisone Hydrochloride after Percutaneous Administration in Rats

Manabu Matsunaga1, Yuji Uemura1, Yuri Yonemoto1, Kazumi Kanai1, Hironori Etoh1, Shigeru Tanaka1, Yuji Atsuta2, Yukio Nishizawa1 and Yoshiharu Yamanishi1

1Eisai Tsukuba Research Laboratories, 5-1-3 Tokodai, Tsukuba, Ibaraki 300-26, Japan
2Department of Orthopedic Surgery, Asahikawa Medical College, 4-5-3-11 Nishikagura, Asahikawa, Hokkaido 078, Japan

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ABSTRACT—Potency and duration of muscle relaxant activity of eperisone hydrochloride were examined after percutaneous administration in the intercollicular decerebrated rat rigidity model and compared to those of eperisone after intravenous injection. A continuous movement was loaded on the hindlimb of the rat model to maintain stable rigidity. The tonus of the hindlimb was recorded by EMG from the triceps surae and was quantified by using the public domain NIH Image program. Eperisone ointment administered percutaneously showed significant muscle relaxant activity at 8.4 cm² (4.2 mg of eperisone)/rat. The effect was dose-dependent and lasted over 60 min. Intravenously injected eperisone showed significant activity at 1.25 mg/kg, but the decrease of tone was lost within 30 min after injection. Plasma eperisone levels were monitored in the same model, and they were well correlated to the dosage. These results suggest that percutaneously administered eperisone is absorbed efficiently and shows potent and long-lasting muscle relaxant activity.

Keywords: Percutaneous administration, Eperisone hydrochloride, Intercollicular decerebrated rat rigidity model, NIH Image program, Muscle relaxant activity

Spasticity is a state defined as a motor disorder characterized by a velocity-dependent increase in muscle tonus and appears following lesions of the central nervous system such as ischemic stroke, trauma and several types of neuronal degeneration. Such lesions interfere with descending interneuronal control in the spinal cord and cause an increment of muscle tonus with hyperactive reflexes. Since neurotransmitters, neuromodulators, receptors and related ion-channels participate in the interneuronal control of muscle tonus, pharmacotherapy with centrally acting muscle relaxants is employed for medical treatment in parallel with physiotherapy and rehabilitation.

The centrally acting muscle relaxants reduce the increased muscle tonus and inhibit the hyperactive reflexes by antagonizing the receptor activation coupled to the excitation of motor functions or by acting on the receptors related to inhibitory functions. It has been pointed that the avoidance of drug-induced central depression, e.g., dizziness, drowsiness and excessive loss of muscle strength, is important in the medical care of spastic patients because weakness of motor functions is often observed in such patients and such depressive effects might interfere with rehabilitation.

Eperisone hydrochloride (4'-ethyl-2-methyl-3-piperidinopropiophenone hydrochloride, Myonal®), a centrally acting muscle relaxant with a low incidence of central depression, is widely used for the therapeutic treatment of spastic patients to relieve muscle stiffness and back pain. Eperisone decreases the muscle tonus in an experimental rat rigidity model and causes inhibition of both monosynaptic and polysynaptic reflexes in cats (1). However, oral administration of eperisone produces a relatively low and variable plasma level in humans (2), probably because of a first pass effect, as found in rats (3). Percutaneous application of eperisone has recently been developed, and clinical studies are now underway to improve the efficacy and compliance of the drug by avoiding rapid degradation during the absorption and distribution processes.

Evaluation of muscle relaxants in preclinical study has usually been performed in rat or cat rigidity models by intravenous, intragastric or intraduodenal administration. In this study, the potency and duration of muscle
relaxant activity were examined after percutaneous administration of eperisone in rats and compared with those after intravenous injection. However, it is generally difficult to maintain steady rigidity levels throughout an experiment. Therefore, we devised a new method in the rat intercollicular decerebration model (4) to obtain more steady rigidity, so that the muscle relaxant activity of eperisone could be properly evaluated by percutaneous administration.

MATERIALS AND METHODS

Preparation of rat rigidity model
An intercollicular decerebrated rigidity model was prepared according to the method of Ono et al. (4). Male Wistar rats (Charles River Japan, Inc., Kanagawa), weighing 350-477 g, were anesthetized with halothane (Takeda Pharmaceutical Co., Osaka) and were fixed on a stereotaxic apparatus. Decerebration was carried out by lesioning of the midbrain with a Lesion Generator (RGF-4; Radionics Inc., Burlington, MA, USA). The lesion electrode (0.75-mm diameter) was inserted into the midbrain (AP: 0, L: ± 1.5, V: -3 mm) at the coordinates designated by Pellegrino et al. (5). The lesion was produced by maintaining the tissue temperature at approximately 80°C for 3 min on both sides (4). After lesioning, the halothane anesthesia was discontinued.

Rats that exhibited strong rigidity of the hindlimbs at 2 hr after decerebration were selected for the experiments. Each rat was placed on its back with its forelimbs fixed. The rigid hindlimb was then forced to bend about its knee with a motor-driven mechanical device. The bending of the knee was carried out not intermittently (4), but reciprocally and continuously with a constant speed at a stroke length of 4 mm once per min.

Quantification of muscle tone
Rigidity of the hindlimb in the model was evaluated in terms of muscle tone recorded by EMG from the triceps surae, by a method based on that described by Johnels and Steg (6). The EMG was amplified (AB-651J; Nihon Kohden, Tokyo), integrated (the time constant: 1 sec; EL-601G, Nihon Kohden) and recorded on a Thermal Array Recorder (RTA-1200, Nihon Kohden).

Quantification of integrated EMG was performed for the area under the curve by using an image scanner (Scanjet 3c; Hewlett Packard, Greeley, CO, USA) and the public domain NIH Image program (ver. 1.58). The data were calculated as area per min (intravenous injection) or per 5 min (percutaneous administration) by the program. The NIH Image program was written by Wayne Rasband at the U.S. National Institutes of Health and is available on the internet by anonymous ftp from zippy.nimh.nih.gov or on floppy disk part number PB93-504868 from NTIS, 5285 Port Royal Rd, Springfield, VA 22161, USA. The program used was provided from the NIFTY-serve computer network.

Prior to administration, the integrated EMG of the rigid hindlimb was recorded for 5 min, referred to pre-control integrated EMG (PRE), and was then further recorded for 30 min (in the case of intravenous injection of the drug) or 60 min (in the case of percutaneous administration). At the end of the experiments the rats were killed by injection of a lethal dose of pentobarbital sodium. Just after the death of the animals, integrated EMG was recorded, referred to non-specific integrated EMG (NSP). Relative muscle tone was calculated by using the following formula:

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\text{Muscle tone (\%)} = \left[ \frac{\text{integrated EMG} - \text{NSP}}{\text{PRE} - \text{NSP}} \right] \times 100
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Quantification of plasma level of eperisone in rat rigidity model
The concentration of eperisone in rat plasma was determined by an HPLC-UV method using an internal standard (tolperisone hydrochloride, 4'-methyl-2-methyl-3-piperidinopropiophenone hydrochloride). The HPLC system consisted of two Shimadzu LC-10AD pumps, a Shimadzu SIL-10A automatic sample injector and a Shimadzu SPD-10A UV detector (Shimadzu, Kyoto). The HPLC conditions were as follows: the column was a YMC pack A-303 (4.6 mm i.d. × 250 mm length; YMC Co., Ltd., Kyoto), the mobile phase was 5 mM sodium dodecyl sulfate (pH 2.5)/acetonitrile (1:1 v/v) at the flow rate of 1.0 ml/min, and detection was done by absorbance measurement at 256 nm. To each 0.1-ml plasma sample, 1.0 ml of distilled water, 0.1 ml of the internal standard solution (1.0 µg/ml methanol solution) and 1.0 ml of 1 M phosphate buffer (pH 7.2) were added, and extraction was performed twice with 4.0 ml of diethyl ether. To the combined organic phase, 0.2 ml of 0.1 N HCl was added for reverse extraction into the aqueous phase. After removal of the organic phase, 70 µl of the aqueous phase was subjected to HPLC. The assay limit was 2.0 ng/ml.

Drugs
The drugs used were eperisone hydrochloride (4'-ethyl-2-methyl-3-piperidinopropiophenone hydrochloride, Myonal®; Eisai, Tokyo); eperisone ointment and placebo ointment (Sansho Co., Ltd., Tokyo); and pentobarbital sodium (Nembutal Injection®; Abbott, Osaka). The composition of eperisone ointment was as follows: 10.0% eperisone hydrochloride, 5.0% cetyle lactate, 15.0% propylene glycol, 3.0% anhydrous citric acid, 1.0% polyoxyethylene hydrogenated castor oil 60, 2.0% light anhy-
drous silicic acid, 4.0% glyceryl monooleate lipophilic and 60.0% hydrocarbon gel. In the placebo ointment, eperisone hydrochloride was replaced by hydrocarbon gel.

Administration of eperisone

For percutaneous administration, the dosage is represented by the application area of eperisone ointment per body. Prior to application, hair at the abdominal skin was cut off with an electric hair clipper, and the stratum corneum was removed by stripping the skin 20 times with adhesive tape (No. 600; Sekisui, Osaka) (7). Eperisone ointment was spread on Parafilm (5 x 5 cm²; American National Can, Greenwich, CT, USA) to give a defined weight and area (1.3 mg of eperisone/2.5 cm², 4.2 mg/8.4 cm² and 12.5 mg/25 cm²), and the remaining area was filled with placebo ointment. The film was placed on the site until completion of the test (for 60 min).

For intravenous injection, eperisone was dissolved in saline and injected through a catheter inserted into the cervical vein at 1.25, 2.5, 5 and 10 mg/kg in a volume of 1 ml/kg.

Statistics

The data were analyzed using repeated-measures analysis of variance (ANOVA), followed by Bonferroni’s multiple t-test to examine the statistical significance of differences between control and eperisone-treated groups.

RESULTS

Muscle relaxant activity of eperisone

Potency and duration of muscle relaxant activity of eperisone were examined in the intercollicular decerebrated rat rigidity model. Rigidity was evaluated in terms of muscle tone recorded by EMG from the triceps surae.

Placebo ointment tended to increase the muscle tone after percutaneous administration (Fig. 1). Although the muscle tone was slightly decreased after administration of eperisone ointment at 2.5 cm² (1.3 mg of eperisone)/rat compared to that after placebo ointment, it was not significantly different from that before administration (Fig. 1A). Eperisone ointment significantly decreased the muscle tone in the triceps at 8.4 cm² (4.2 mg)/rat (Fig. 1B, repeated-measures ANOVA) and 25 cm² (12.5 mg)/rat.
(Fig. 1C, repeated-measures ANOVA). The effect was apparent for over 60 min after administration. The maximal inhibition of the muscle tone, which was observed 10 to 15 min after administration, and the dosage were well correlated: the muscle tone was decreased to 62.9%, 38.9% and 29.4% at 2.5, 8.4 and 25 cm²/rat, respectively (Fig. 1).

Intravenous injection of saline did not affect the muscle tone. Eperisone decreased the muscle tone in the triceps surae from 2 to 6 min after injection at 1.25 mg/kg (Fig. 2). The decrease of the muscle tone was recovered within 30 min after injection at all dosages tested. The degree and duration of inhibition correlated well to the dosage: the muscle tone was decreased to 12.1%, 23.2%, 3.4% and 0% at 1.25, 2.5, 5 and 10 mg/kg, respectively (Fig. 2).

**Plasma levels of eperisone in rat rigidity model**

Plasma eperisone levels were monitored in the rat rigidity model. The highest plasma level was observed at 5 min after percutaneous administration (Fig. 3) and 2.5 min after intravenous injection (Fig. 4), respectively. The minimum plasma level of eperisone showing a myotonic effect was estimated to be approximately 300 ng/ml from the plasma levels at 60 min after percutaneous administration of 8.4 cm²/rat and at 5 min after intravenous injection of 1.25 mg/kg. The plasma levels 5 to 30 min after percutaneous administration at 8.4 cm² (4.2 mg)/rat were approximately equal to those in the same period after intravenous injection at 5 mg/kg.

![Fig. 2](image_url)

**Fig. 2.** Muscle relaxant activity of eperisone injected intravenously in the intercollicular de cerebrated rat rigidity model. EMG was recorded from the triceps surae. Each point represents the mean ± S.E.M. of 6 separate experiments. ○: saline as vehicle (the same control group in A, B, C and D); ●: eperisone, 1.25 mg/kg (A); ▲: eperisone, 2.5 mg/kg (B); ■: eperisone, 5 mg/kg (C); ▼: eperisone, 10 mg/kg (D). *: statistically significant difference between the saline group and eperisone group, P < 0.05 (Bonferroni's multiple t-test).
DISCUSSION

Rigidity models, such as the intercollicular decerebrated rigidity and ischemic decerebrated rigidity models, have been employed to evaluate the efficacy of muscle relaxants. In this study, the intercollicular decerebrated rat rigidity model was prepared with a radiofrequency lesion generator according to Ono et al. (4). Although the lesion causes a marked and reproducible rigidity of the hindlimb by avoiding bleeding or stress to rats (4), the rigidity level tends to fluctuate due to physical and chemical stimuli such as sound, vibration and injection of drug solutions. The intercollicular decerebration-induced rigidity is classified as r-rigidity, and the rigidity level corresponds to the afferent input levels. In order to maintain stable rigidity, the rigid hindlimb was continuously moved by a mechanical device and forelimbs were fixed in this study. The continuous load by moving the rigid hindlimb resulted in more stable rigidity of the model and avoided the influences of other stimuli, and the fixing of the forelimbs decreased the movement of rats and change of rigidity caused by body motion.

Measurements of dorsireflex tension with strain gauges or by EMG spikes have widely been used to assess the rigidity level. However, the tension tends to be affected by change of the fulcrum position, and the spikes do not reflect the EMG activity. The integrated EMG reflects the muscle tonus more directly. In this study, the tonus of the hindlimb was recorded quantitatively by integrated EMG from the triceps by the method of Johnels and Steg (6). Integrated EMG was graphically analyzed by using the public domain NIH Image program, which is a graphical application used in the analysis of electrophoresis experiments. As a result, the evaluation of muscle relaxant efficacy in this system was highly reproducible under appropriate conditions.

As mentioned in the introduction, percutaneous application of eperisone has recently been examined in clinical studies. Several advantages of percutaneous administration might be expected: for example, improving the efficacy by avoiding the first pass effect, improving the compliance of patients who can not take other forms (e.g., oral form or implanted infusion pump) of the drug for various reasons and reducing the number of drug administrations. In this study, it was confirmed that percutaneous application of eperisone ointment on stripped rat abdominal skin, through which the drug can be absorbed efficiently (7), affords a high plasma level of eperisone in a short time.

Eperisone ointment reduced the rigidity after percutaneous administration in a dose-dependent manner. The plasma eperisone levels, monitored in the same rat rigidity model, were similar after percutaneous administration at 8.4 cm² (4.2 mg)/rat and intravenous injection at 5 mg/kg, and decreased slowly in a similar manner. The myonolytic effect was significant at 8.4 cm² (4.2 mg of eperisone) and 25 cm² (12.5 mg)/rat. Although the dosage of 8.4 cm² (4.2 mg)/rat (body weight: about 420 g)
corresponds to approximately 10 mg/kg intravenous injection, the duration of muscle relaxant activity after percutaneous administration was longer than that after 10 mg/kg intravenous injection. The plasma level of eperisone decreased after percutaneous administration, while the muscle relaxant activity of eperisone was well maintained. The cause of this discrepancy is unknown. However, it is possible that the myotonolytic effect of eperisone depends not on the plasma level, but on the concentration in the target organs. These results suggest that percutaneously administered eperisone was absorbed efficiently and exhibited muscle relaxant activity for a long period.

In the preliminary tests, the absorption rate of eperisone after spreading eperisone ointment on the intact skin was too slow to examine the efficacy (data not shown). Therefore, before application, the stratum corneum at the abdominal skin was removed by stripping the skin 20 times with adhesive tape (7). The increase in the plasma eperisone levels after percutaneous administration was unexpectedly rapid. The rapid increase of the plasma level might be caused by an excessive stripping of the skin. As mentioned above, the plasma level of eperisone decreased after the initial increase, despite the presence of eperisone ointment on the skin until the end of the test. Although the cause of the decrease is unknown, it might be speculated that the excessive stripping caused damage of the skin such as inflammatory changes, and this resulted in a decline of the absorption rate of eperisone through the skin. It is expected that the stripping procedure does not elevate the total amount of absorbed drug, although it might accelerate the initial absorption rate. Therefore, the muscle relaxant activity of eperisone ointment resulted from the test under stripped skin condition likely reflects the activity of eperisone ointment in the normal skin condition.

In conclusion, eperisone was absorbed efficiently after percutaneous administration and showed potent and long-lasting muscle relaxant activity. We expect that better efficacy and compliance can be obtained in clinical practice, by the use of a percutaneous dosage form of eperisone.

REFERENCES