Correlation between Neurotoxic Events and Intracerebral Concentration of Tacrolimus in Rats

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The neurotoxicity associated with tacrolimus is one of the major limitations for its administration after organ transplantation. This study investigated the correlation between neurotoxicity and the intracerebral concentration of tacrolimus. Rats were given one of three doses of tacrolimus (5, 10, and 20 mg/kg/d) orally twice a day for 2 weeks and neurotoxic events were observed. The rats were sacrificed on either day 7 or 14. The trough values of the whole blood and the corresponding intracerebral concentrations were then measured. None of the rats receiving dosage of 5 mg/kg/d showed any neurotoxic symptoms throughout the two-week test period. In rats receiving a dosage of 10 mg/kg/d, however, all seven surviving rats presented tremors or seizures during the second week. In rats receiving a dosage of 20 mg/kg/d, 40% of the rats presented tremors or seizures during the first week. The threshold concentration of tacrolimus in the brain resulting in neurotoxic events was therefore estimated as approximately 700 ng/g. At concentrations over this threshold value, the intensity of the neurological event increases with the concentrations of tacrolimus in the brain. Using a linear correlation between the whole blood and intracerebral concentrations ($r=0.967$) of tacrolimus, the pharmacological threshold for the whole blood trough level was estimated as approximately 20 ng/ml, which falls into the same value reported for the incidental threshold of neurotoxicity in renal transplant recipients [Böttiger *et al.*, *Br. J. Clin. Pharmacol.*, 48, 445—448 (1999)]. Therefore, it is suggested that the rat is a good animal model to quantitatively evaluate the risk of neurotoxicity associated with tacrolimus in human, and that frequent measurement of whole blood tacrolimus concentrations is important for predicting and preventing neurotoxic events.

Key words tacrolimus; immunosuppressive agent; brain; neurotoxicity; pharmacological threshold; rats

Tacrolimus (FK506) has become an indispensable immunosuppressant that is used after organ transplantation. However, neurotoxic events associated with tacrolimus often necessitate reductions in dosage or conversions to another immunosuppressant.^{1–4)} Major neurotoxic events, such as seizure, encephalopathy and movement disorder during the early postoperative period is reported to be observed in 21.3% of all patients who underwent liver transplantation.¹⁾ It has been reported that higher whole blood trough concentration of tacrolimus is associated with higher incidence of neurotoxicity.^{4,5)} The incidence of neurotoxicity and other side effects was reported to be 41% of all patients with a concentration of 20–30 ng/ml in whole blood. This incidence increases to 76% at concentrations above 30 ng/ml after renal transplantations.⁶⁾ However, the neurotoxicity associated with tacrolimus would be better predicted by the intracerebral concentration of the drug than by the whole blood concentration. Nevertheless this value has never been measured in humans and the correlation between the neurotoxic events and the intracerebral concentration of tacrolimus has never been investigated to the best of our knowledge.

This brief report describes how useful the rat model is, to evaluate the neurotoxicity of tacrolimus after repeated oral administrations of tacrolimus, by examining 1) the relationship between the presentation of neurotoxic events and intracerebral concentrations of tacrolimus, and 2) whether neurotoxic events and intracerebral concentrations of tacrolimus can be predicted by measuring the whole blood trough concentration.

MATERIALS AND METHODS

Animals and Treatment The tacrolimus powder used in this study was provided by Fujisawa Pharmaceutical Co., Ltd. (Osaka, Japan). Solid dispersion formulations of tacrolimus (0.3, 0.6, and 1.2 mg/ml in 5% glucose) were used for oral administration. Male Wistar rats (270–300 g) were randomly assigned to three groups. Each group received a different dose of tacrolimus (5, 10, and 20 mg/kg/d. $n=12$, 15, and 15, respectively) in a volume of 2.5 ml *via* a stomach tube. The doses were administered twice a day (11 a.m./11 p.m.) for two weeks. The rats were otherwise allowed free access to food and water. Six rats from each group were anesthetized with diethylether and sacrificed by exsanguination from the abdominal aorta at 11 p.m. on day 7. Whole blood samples and the brain were obtained and stored at -20°C .

Neurotoxicity Scoring The intensity of the neurotoxic events was scored as follows: 0 for no signs of neural disorders; 1 for tremors, slight seizures, or irritability; 2 for definite tremors, seizures, or extreme irritability; and 3 for severe seizures or violent behaviors.

Assay for Concentration of Tacrolimus The concentrations of tacrolimus in the whole blood and brain were measured by a two-step enzyme-linked immunoabsorption assay (ELISA), as described previously.⁷⁾

Statistical Analysis The correlation between the concentration of tacrolimus in the brain and the intensity of neurotoxic events was analyzed using Spearman's rank correlation analysis. Data were expressed as the mean $\pm$ S.E., and

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$p < 0.05$ was considered as statistically significant.

RESULTS

Survivals None of the 12 rats that had received a dose of 5 mg/kg/d dropped out from the study. Seven rats that had received a dose of 10 mg/kg/d and six rats that had received a dose of 20 mg/kg/d dropped out from this experiment due to death or general prostration. Death was caused by severe neurotoxicity and/or multiple organ failure. Four rats receiving a dose of 20 mg/kg/d were withdrawn from the experiment after the first week for humanitarian reasons.

Observation of Neurotoxicity and Tacrolimus Concentration in Whole Blood and Brain Six of the rats receiving the lowest dose of 5 mg/kg/d were sacrificed on day 7, while the remaining 6 were sacrificed on day 14. None of the rats showed any neurotoxic symptoms throughout the duration of the study (grade 0). The whole blood and intracerebral concentrations of tacrolimus on day 14 were 3.95 ± 0.72 ng/ml and 122 ± 13.4 ng/g, respectively ($n=6$).

In the group that received a dose of 10 mg/kg/d, 2 rats died in the first week and 6 rats were sacrificed on day 7. Five rats died in the second week, and only 2 rats survived to be sacrificed on day 14. One out of the 15 rats (6.7%) experienced a grade 3 seizure in the first week. When it was sacrificed on day 7, the whole blood and intracerebral concentrations of tacrolimus were 97 ng/ml and 3200 ng/g, respectively. The other 5 rats sacrificed on day 7 showed no neurotoxic symptoms (grade 0), and their whole blood and intracerebral concentrations of tacrolimus were 20.8 ± 2.6 ng/ml and 554 ± 127 ng/g, respectively. In the second week, the remaining 7 rats all showed some signs of neurotoxic events (grade 1 to 3). Two rats (grade 1 and 2) survived to be sacrificed on day 14. The whole blood concentrations of tacrolimus of the two rats were 34 and 120 ng/ml, while the intracerebral concentrations were 690 and 1700 ng/g, respectively. The mean intracerebral concentrations of tacrolimus in the dead rats (grade 1 to 3) were 2400 ± 400 ng/g ($n=7$).

In the group that received the highest dose of 20 mg/kg/d, 6 rats died in the first week and 5 rats were sacrificed on day 7. Because of this high death rate, the administration of drugs to the remaining 4 rats was stopped in the second week for humanitarian reasons. Nine of the 15 (46.7%) rats experienced neurotoxic events in the first week (grade 1 to 3). The whole blood and intracerebral concentrations on day 7 were 194 ± 67 ng/ml and 2400 ± 630 ng/g, respectively ($n=5$; grade 2 to 3). Six rats (grade 1 to 3) died within a week, and the intracerebral concentration of tacrolimus in these rats was 3630 ± 650 ng/g.

Correlation between Neurotoxic Events and the Intracerebral or Whole Blood Concentration of Tacrolimus The correlation between the intracerebral concentration of tacrolimus and the intensity of neurotoxic events is shown in Fig. 1. The threshold for neurotoxic events was estimated as 700 ng/g. At concentrations over this threshold, the intensity of neurotoxic events increases with increasing concentrations in the brain, as shown in Fig. 1. The intracerebral and whole blood concentrations of tacrolimus have a significant linear correlation ($r=0.967$) (Fig. 2). By extrapolating this correlation, the threshold value for the whole blood trough concentrations that may indicate a risk of neurotoxic events was es-

Fig. 1. Correlation between the Intensity for Neurotoxic Events and the Intracerebral Concentration of Tacrolimus

The threshold value of the intracerebral concentration of tacrolimus (▲) resulting in neurotoxic events was estimated to be 700 ng/g. At concentrations above this threshold, the intensity of neurotoxic events increases with increasing concentrations in the brain (○=5 mg/kg/d, ■=10 mg/kg/d, ◇=20 mg/kg/d).

Fig. 2. Correlation between the Intracerebral and Whole Blood Trough Concentration of Tacrolimus

A strong linear correlation was found between the whole blood (≤ 100 ng/ml) and the intracerebral concentrations ($r=0.967$). The threshold value for whole blood trough concentrations, that may indicate a risk of neurotoxic events was estimated to be 21.2 ng/ml (Δ). (▲=the threshold value of the intracerebral concentration of tacrolimus).

timated to be approximately 20 ng/ml.

DISCUSSION

In patients that have received an organ transplant, the whole blood trough concentrations of tacrolimus is monitored to control the immunosuppressive activity and/or adverse effects of the agent. A recent report has suggested that no significant relationship exists between the incidence of rejection and the whole blood tacrolimus concentrations.^{3,4} On the other hand, higher whole blood trough concentrations of tacrolimus are associated with a higher incidence of neurotoxic events, and lower doses may result in a lower toxicity or fewer side effects.^{4,5} Based on these observations, lower oral doses of tacrolimus have been clinically introduced to liver transplantation.^{8,9} Nevertheless, the basis of predicting tacrolimus neurotoxicity from whole blood trough concentrations is obscure, since the correlation between the neurotoxic events and intracerebral (*i.e.*, site of action) concentration of the agent has not yet been defined. To clarify this relationship, since it is very difficult to measure the intracerebral

concentration of tacrolimus in human, we conducted an experimental study using rats. Our animal experiment was designed to mimic the clinical setting; rats were administered with tacrolimus orally twice a day and the whole blood trough concentrations of the drug were measured. Then, we evaluated the correlation between the intracerebral and whole blood concentration at trough levels after repeated oral administration for one or two weeks, in order to provide clinically meaningful insights.

The results of this study show a direct association between neurotoxic events and intracerebral concentrations of tacrolimus in rats and this is the first report that shows this relationship to the best of our knowledge. Neurotoxic events in rats receiving oral doses of tacrolimus appeared when the intracerebral concentration of tacrolimus was higher than 700 ng/g. This value is thus estimated to be the threshold concentration of tacrolimus associated with neurotoxic events. At concentrations above this value, the intensity of neurotoxic events increases with increasing concentrations in the brain (Fig. 1). Furthermore, the intracerebral concentration of tacrolimus shows a strong linear correlation with the whole blood trough concentration ($r=0.967$) (Fig. 2). These findings highlight the importance of monitoring whole blood tacrolimus trough concentrations in order to predict the appearance of neurotoxic events. By extrapolating the linear relationship between intracerebral and whole blood concentrations, the whole blood tacrolimus threshold value for neurotoxic events was estimated to be 21.2 ng/ml, which is consistent with previous reports that indicate a correlation between adverse events and whole blood tacrolimus concentrations of over 20 ng/ml in organ transplant recipients.^{5,7)} This result suggests that the concentration-neurotoxicity correlation may be similar between rats and human, although the drug metabolism may be different. Furthermore, this relationship may be common regardless of the interindividual variability of the drug metabolism.

The ratio of intracerebral to whole blood concentration of tacrolimus may be affected by the efflux pump, P-glycoprotein, at the blood brain barrier.¹⁰⁾ In fact, Yokogawa *et al.* revealed that the intracerebral concentration of tacrolimus increased markedly in *mdr1a* knockout mice at relatively short times after single intravenous administration.¹¹⁾ On the other hand, the level of immunophilin for tacrolimus (FKBP) in the brain is 10 times greater than those in other tissues.¹²⁾ The extensive intracerebral binding of the drug to FKBP may result in high and sustained accumulation of tacrolimus in the

brain. Since the intracerebral to whole blood concentration ratio of tacrolimus appears to be linear in our study (Fig. 2), it is likely that the FKBP-tacrolimus complex may serve as a deep compartment at steady state, which will cover the contribution of P-glycoprotein-mediated nonlinear transport in the intracerebral distribution.

In conclusion, it is suggested that the rat is a good animal model to quantitatively evaluate the risk of neurotoxicity associated with tacrolimus in human, and that frequent measurement of whole blood tacrolimus concentrations is important for predicting and preventing neurotoxic events.

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