Neuroprotective Effect of PEGylated Liposomes Encapsulating FK506 on Cerebral Ischemia-reperfusion Injury

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ABSTRACT SUMMARY

Cerebral ischemia-reperfusion (I/R) injury induces significant functional deficits after stroke therapy. We have previously reported that drug delivery using liposomes is a potential therapeutic strategy for this injury. In the present study, we investigated the therapeutic effect of liposomal FK506 (tacrolimus), which show neuroprotective effect, in cerebral I/R model rats. To evaluate efficacy of the liposomal formulation, we measured infarct volume, behavioral disorder and cerebral blood flow of the model rats. As a result, the treatment with PEGylated liposomes encapsulating FK506 (FK-Lip) immediately after reperfusion significantly reduced damaged region, and improved motor function deficit and cerebral blood flow disorder. In conclusion, this research indicated that a single injection of FK-Lip improved long-term outcome in stroke patients.

INTRODUCTION

Cerebral I/R injury is a complex disorder caused by inflammation, oxidative stress and so on after recovery from ischemic stroke. This injury greatly worsens the prognosis of stroke patients; thereby, it is an important clinical issue to overcome this injury. However, few neuroprotective agents have been commercialized. Therefore, it is awaited the development of novel neuroprotectants.

After cerebral I/R events, blood–brain barrier is disrupted, and the vascular permeability is increased. We have revealed that liposomes accumulate in I/R regions by vascular hyperpermiability¹. Therefore, liposomal DDS technology is applicable to treat the cerebral I/R injury by selective drug delivery.

FK506, an immunosuppressant, is known to have neuroprotective effect². However, the frequent administration of FK506, that is required to achieve a good outcome, has the risk of side effects. Therefore, we developed FK-Lip to decrease the injected dose and the risk of side effects.

In this study, we examined the therapeutic effect of FK-Lip in cerebral I/R model rats. Moreover, we sequentially assessed functional behavior and cerebral blood flow of the rats treated with FK-Lip.

EXPERIMENTAL METHODS

Male Wistar rats weighting 170-210 g were used to make transient-middle cerebral artery occlusion (t-MCAO) model. Anesthesia was introduced 3% isoflurane, and maintained 1.5% isoflurane. MCA of rats was occluded by inserting a filament into the internal carotid artery. The success of operation was judged by hemiparesis and hyperthermia 1 h after surgery. Reperfusion was induced by withdrawal of the filament 1 h after the occlusion³. The rectal temperature was monitored, and maintained 37.0°C during surgery.

t-MCAO rats were intravenously injected with FK-Lip (30 or 100 µg/kg as FK506 dosage), FK506 (30, 100 or 300 µg/kg), vehicle (200 mg/mL 10% ethanol in PBS) or PBS immediately after reperfusion. The brain was sliced into 2.0 mm thickness 24 h after injection, and stained with 2, 3, 5-triphenyltetrazolium chloride (TTC) to visualize brain damaged area. Damaged area was calculated by Image J.

We measured regional cerebral blood flow (rCBF) at the surface of cortex in t-MCAO rats by a laser-doppler flow meter. rCBF was monitored at 1, 2, 3, 4, 5, and 6 days after reperfusion. The brain was sliced into 2.0 mm thickness 24 h after injection, and stained with 2, 3, 5-triphenyltetrazolium chloride (TTC) to visualize brain damaged area. Damaged area was calculated by Image J.

We measured regional cerebral blood flow (rCBF) at the surface of cortex in t-MCAO rats by a laser-doppler flow meter. rCBF was monitored at 1, 2, 3, 4, 5, and 6 days after reperfusion. The rats were anesthetized with isoflurane during the measurement. Changes in rCBF were expressed as percentage of the non-ischemic side. The rats were intravenously injected with each sample indicated in Fig 2 immediately after reperfusion.

We evaluated motor function of t-MCAO rats at 1, 2, 3, 5, and 7 days after reperfusion. The rats underwent a 21-point neurological score analysis, as described previously⁴. Normal rats received 21 points in this test. t-MCAO rats were treated each sample indicated in Fig 3.
RESULTS AND DISCUSSION

Brain cell death of t-MCAO rats was reduced by the treatment with FK-Lip compared with same dosage of FK506, and PBS. Additionally, the effect was observed in a dose-dependent manner, and FK-Lip (100 µg/kg) shows comparable therapeutic effect of FK506 (300 µg/kg). These results suggest that FK506 was delivered efficiently to I/R region by liposomalization.

Secondary cerebral blood flow disorder was significantly reduced by the treatment with FK-Lip. FK-Lip could suppress the inflammation and oxidative stress of t-MCAO rats after reperfusion, resulting in vascular protection. Contrary to FK-Lip-treated group, the half number of rats in PBS-treated group was died at 6 days after reperfusion in this experiment.

CONCLUSION
The present study demonstrated that FK-Lip significantly suppressed cerebral cell death induced by I/R in t-MCAO rats. Moreover, FK-Lip treatment improved motor function and blood flow of t-MCAO rats in sub acute phase after I/R. Our data provides FK-Lip have a clear potential to be a neuroprotectant if administered quickly after a cerebral stroke.

REFERENCES

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