Neurotoxicity of Paclitaxel and Rapamycin in a Rat Model with Transient Blood-Brain Barrier Opening

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Objective: Drug-eluting stents and balloons are occasionally used to reduce restenosis in medically intractable intracranial atherosclerotic stenosis. The authors aimed to determine whether such drugs can cause neurotoxicity due to local effects in a rat model.

Methods: Intra-arterial catheters were placed in the right common carotid artery of rats. Mannitol was injected to transiently open the brain-blood barrier (BBB), followed by high-dose drug (paclitaxel and rapamycin) injection. The optimal time interval of transient BBB opening for maximal drug penetration was determined to be 10 minutes. Paclitaxel and rapamycin were intra-arterially administered in various doses. All the rats were neurologically evaluated, and their brain tissues were histologically examined.

Results: Neither neurological deficits nor histological abnormalities were observed in all the rats.

Conclusion: Paclitaxel and rapamycin did not cause neurotoxicity in a rat model with transient BBB opening.

Key Words: Intracranial atherosclerotic stenosis · Neurotoxicity · Paclitaxel · Rapamycin · Restenosis.

INTRODUCTION

Intracranial atherosclerotic stenosis (ICAS) is one of the causes of ischemic stroke. Recurrent stroke is known to occur in approximately 17% of patients with ICAS in 1 year, even with the best medical management2,4. Based on technical and clinical experience in the field of coronary artery diseases, endovascular treatment with stents has been attempted for patients with ICAS. Stenting in ICAS patients raises two major concerns: peri-procedural complications and high restenosis rates2,4,8. Regarding peri-procedural complications, recent studies have shown better outcomes than previous studies due to advancements in medical management, endovascular techniques and devices2,4,8. Regarding the issue of high restenosis rates after cerebral arterial stenting, a proper solution has not yet been identified. Drug-eluting stents (DESs) and drug-eluting balloons (DEBs) in the coronary system, which release drugs that inhibit intimal hyperplasia, have also been used for ICAS with good preliminary results in reducing restenosis1,2,6,7,9,10,12,13,16. However, applying these drugs to cerebral ar-
teries may provoke neurotoxicity. To our knowledge, no report has yet been published on this issue. The authors performed this study to evaluate neurotoxicity due to the local effects of high-dose paclitaxel and rapamycin using a rat model with transient blood-brain barrier (BBB) opening.

MATERIALS AND METHODS

The procedures for handling and caring for animals adhered to the guidelines that are in compliance with current international laws and policies (National Institute of Health Guide for the Care and Use of Laboratory Animals, Publication No. 85-23, 1985, revised 1996), and they were approved by the Institutional Animal Care and Use Committee of Kangwon National University (KIACUC-12-0014).

Animal model, BBB opening and drug administration

Male, specific pathogen-free, Sprague Dawley rats were obtained from Bio-Genomics (Seoul, Korea). The rats were 7–8 months old and weighed 250–350 g. An intra-arterial infusion catheter was placed in the right common carotid artery.

To model the condition of a chronically ischemic brain with a partially disrupted BBB, temporary BBB opening was attempted via intra-arterial infusion of mannitol. A 20% mannitol solution (CJ Health Care, Daeso, Korea) was administered at a rate of 0.25 mL/kg/sec for 30 seconds with an infusion pump via the intra-arterial catheter. After mannitol infusion, a 2% solution of Evans Blue (EB; Sigma, St. Louis, MO, USA) in normal saline (4 mL/kg of body weight) was injected via the intra-arterial catheter to confirm the degree of BBB opening in predetermined time intervals. Twenty-four hours after EB infusion, the whole rat brain was harvested, and the degree of EB extravasation was evaluated. A total of 25 rats (five rats per predetermined interval) were used for this purpose. Based on the degree of EB extravasation, the optimal time interval for transient BBB opening and maximal drug

Fig. 1. A rat model for drug infusion via the right internal carotid artery with intra-arterial catheter indwelling.

Fig. 2. Degree of extravasation of Evans Blue in the right hemispheres of rat brains. A: Grade 0 when there is no evidence of extravasation. B: Grade 1 when the degree of extravasation is pale. C: Grade 2 when the degree is moderate. D: Grade 3 when the degree is strong.
delivery was determined to be 10 minutes (Figs. 1-3).

The doses of paclitaxel and rapamycin (Sigma) were translated from humans into animals as equivalent doses using a formula based on body surface area: animal equivalent dose (mg/kg) = human dose (mg/kg) × human $K_m$ / animal $K_m$. The drugs were injected as an intra-arterial bolus together with EB 10 minutes after mannitol administration. In the sham group, EB was infused without drugs. The brain tissues were obtained after 24 hours and 14 days. A total of 70 rats were used. Considering that the doses of the drugs coated on DESs and DEBs are 70–314 µg and 300–600 µg, respectively, a human dose of 600 µg was determined to be the maximum. In this study, 1- to 4-times higher equivalent doses were tested.

**Neurological and histological examinations**

Neurological status was evaluated using Bederson et al.'s grading. Frozen rat brains fixed with paraformaldehyde were serially sectioned on a cryostat into 30-µm coronal sections. Histologic examination was performed with cresyl violet staining (Sigma) to assess neuronal death and Fluoro-Jade C (Histochem, Jefferson, AR, USA) histofluorescence staining to localize neuronal degeneration. Immunohistochemistry was performed with anti-glial fibrillary acidic protein (Biogenesis, San Ramon, CA, USA) for astrocytes and anti-ionized calcium-binding adapter molecule 1 (Wako, Osaka, Japan) for microglia. Detailed information is provided in the Supplement Method 1.

**RESULTS**

Most of rat brains were stained with EB, with a various range of grades from 1 to 3 (Supplementary Table 1). There were no histological abnormalities in the sham group (Supplementary Table 2, Supplementary Figs. 1 and 2). No neurotoxicity of Paclitaxel and Rapamycin | Cho WS, et al. | J Korean Neurosurg Soc 2022 Feb 17. [Epub ahead of Print]
icity was observed (Bederson’s grade 0 in all cases) in any rats regardless of drug type, concentration or time period (Fig. 4 and Supplementary Figs. 3-14). Moderate degrees of inflammatory cell aggregation and neuronal damage were identified in 1 of 15 sections of just one rat brain (No. 4, day 14 after injection of 600 µg human paclitaxel dose; Supplementary Figs. 15 and 16). No rat showed neurological abnormalities.

**DISCUSSION**

The restenosis rate after treating symptomatic ICAS with bare-metal stents is known to be as high as approximately 30%\(^6,8,12\). Moreover, DESs and DEBs for ICAS caused lower rates of restenosis, ranging from 0% to 5% and from 0% to 13%, respectively\(^2,12\). Using DESs and DEBs in cerebral arteries could provoke neurotoxicity. Fortunately, chronic exposure of the brain to low doses of the drugs released from DEBs and DESs appears safe, based on the lack of reports about neurotoxicity due to systemic effects of the drugs in interventional cardiology over 15 years. And, not a few preliminary preclinical and clinical studies showed no local and systemic complications, yet\(^1,\phantom{0}2,5,7,8,10,12,13,16\). In the early period after DES and DEB placement, high amounts of the drugs are released from the devices within the cerebral arteries. These high amounts could have harmful local effects on the brain. For example, 75–80% of the drugs are released into the bloodstream after DEB inflation, and only 10–15% are attached to the target vessel wall\(^10\). This study is the first animal experiment examining neurotoxicity due to the local effects of intra-arterial injection of high-dose paclitaxel and rapamycin. Our experiment showed no neurotoxicity, even though the drugs more easily penetrated into the brain tissues due to mannitol-induced BBB opening and higher doses, up to 4-times higher than the general dose on devices, were administered.

The major adverse event related to paclitaxel is peripheral neuropathy\(^17\). It has been reported that peripheral neuropathy of any grade can occur with 100 to 300 mg/m\(^2\) paclitaxel. The cumulative dose that causes severe neuropathy is known to be approximately 1000 mg/m\(^2\)\(^2,17\). For example, considering the surface dose (3 µg/mm\(^2\)) and specifications of SeQuent\(^8\) Please NEO (B. Braun Medical, Melsungen AG, Berlin, Germany), a novel paclitaxel-coated balloon ranging from 2.0×10 mm to 4.0×40 mm, the total surface dose is approximately 380 µg–3 mg. As the total surface dose ranges between 1/180–1/60 of the dose that causes peripheral neuropathy, paclitaxel-related neurotoxicity hardly seems to occur, even if a full dose is released from the highest specification of the balloon. Moreover, paclitaxel cannot normally cross the BBB, and only two cases of grand mal seizure, leading to suspicion of central nervous system toxicity, have been reported\(^3\). Each ovarian cancer patient experienced seizures after intravenous injection of more than 250 mg/m\(^2\) paclitaxel in early clinical trials. However, the direct causality of paclitaxel is not clear because one patient had a brain metastasis and sub-therapeutic blood levels of phenytoin, and paclitaxel was not detected in the cerebrospinal fluid of the other patient.

Rapamycin is an immunosuppressant used for solid organ transplantation and a major drug coated on DESs. Rapamycin-related complications mainly include myelosuppression and hyperlipidemia. Although rapamycin easily crosses the BBB, neurotoxicity has not been reported except in five cases of posterior reversible encephalopathy, which was suspected to be caused by rapamycin-related neurotoxicity simply based on recovery after rapamycin discontinuation\(^5,10\). Therefore, even though our experiment did not show high-dose rapamycin-induced neurotoxicity in a rat model, using rapamycin-coated devices needs to be avoided until the adverse effects in humans are clearly shown.

There are a few limitations in this study. First, only two prototype of drugs for the DESs and DEBs were used. However, these data would be applicable because newer drugs are derivatives of rapamycin and paclitaxel. Second, it is an animal experiment. Third, the degree of BBB opening in rat models was a little inhomogeneous, however, there was no tendency of the degree of BBB opening according to some factors. The authors did not attempt additional experiments, sacrificing more animals. Further experiments in vivo or in vitro under the control of the degree of BBB opening can be necessary. Fourth, rats’ neurological states were evaluated simply with a triage test which cannot detect minor changes.

**CONCLUSION**

There were no histological or neurological evidences of neurotoxicity by intra-arterial injection of paclitaxel and rapamycin in a rat model with transient BBB opening. This
findings are expected to be helpful in consideration of the safety of drugs to reduce in-stent and post-procedural restenosis.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

INFORMED CONSENT

This type of study does not require informed consent.

AUTHOR CONTRIBUTIONS

Conceptualization: OKK
Data curation: WSC, JHC
Formal analysis: WSC, JHC
Funding acquisition: OKK, WSC, JHC
Methodology: WSC, JHC
Project administration: WSC, OKK
Visualization: WSC, JHC
Writing - original draft: WSC, JHC, OKK
Writing - review & editing: WSC, OKK

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Supplementary materials

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References


**SUPPLEMENTARY METHOD 1**

**Animal model**

Male, specific pathogen-free, Sprague Dawley rats were obtained from Bio-Genomics (Seoul, Korea). The rats were 7–8 months old and weighed 250–350 g. The animals were housed in a conventional state under adequately controlled temperature (23°C) and humidity (60%) with a 12-hour light/12-hour dark cycle for 1 week and provided with free access to food and water. The procedures for handling and caring for animals adhered to the guidelines that are in compliance with current international laws and policies (NIH Guide for the Care and Use of Laboratory Animals, NIH Publication No. 85-23, 1985, revised 1996), and they were approved by the Institutional Animal Care and Use Committee of Kangwon National University. All of the experiments were designed to minimize the number of animals used and the suffering caused by the procedures used in the present study.

Rats were anesthetized with an intraperitoneal injection of 30 mg/kg Zoletil® (Virbac, Carros, France). Additionally, 4 mg/kg gentamicin was subcutaneously injected before skin incision to prevent infection, and 3 mg/kg ketoprofen was administered after the procedures to relieve pain. Perioperatively, routine check-ups of body weight, diet, behavior and neurological status was performed daily for 4 days after procedures and every 2 days after procedures. When an abrupt loss of body weight of more than 20% of the preoperative weight or abnormal behavior was observed, the rat was euthanized.

After shaving and sterilization of the cervical skin area with betadine solution, the right common, external and internal carotid arteries were exposed. The right external carotid artery was tied with a thread to permanently occlude it, and the proximal and distal parts of the right common carotid artery were temporarily clamped with clips. After puncturing the temporarily occluded vessel segment with a 24-gauge intravenous cannula, an intra-arterial infusion catheter was placed in the right common carotid artery. The head of the catheter was plugged with a heparin cap and fixed at the surrounding soft tissues. Then, the catheter was irrigated with heparinized saline to remove the air and blood clots via the heparin cap, and the temporary clips were finally removed. When intra-arterial drug injection via the intra-arterial catheter was completed, the catheter was removed, and the puncture site was sutured with 10-0 nylon to control bleeding (Fig. 1).

**Blood-brain barrier (BBB) opening**

To model the condition of a chronically ischemic brain with a partially disrupted BBB, temporary BBB opening was attempted via intra-arterial infusion of mannitol. The rat model was designed to determine the optimal time interval between mannitol administration and drug infusion, in which temporary BBB opening was maximized and the drug was able to pass through the BBB into the brain parenchyma.

A 20% mannitol solution (CJ Health Care, Daeso, Korea) was administered at a rate of 0.25 mL/kg/sec for 30 seconds with an infusion pump via the intra-arterial catheter. Subsequently, at predetermined time intervals of 0, 5, 10, 15 and 20 minutes after mannitol administration, a 2% solution of Evans Blue (EB; Sigma, St. Louis, MO, USA) in normal saline (4 mL/kg of body weight) was injected via the intra-arterial catheter. Twenty-four hours after EB infusion, the rats were transcardially perfused with 0.1 M phosphate-buffered saline (PBS; pH 7.4) followed by 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Then, the whole brain was harvested, and the degree of EB extravasation was evaluated in the right hemisphere. A total of 25 rats (five rats per predetermined interval) were used. The degrees of EB extravasation were scored as follows: grade 0, no EB extravasation; grade 1, weak extravasation; grade 2, moderate extravasation; and grade 3, strong extravasation (Fig. 2).

**Drug administration**

A 1-mL stock solution of 5 mg/mL paclitaxel (Sigma) was made by mixing 5 mg paclitaxel powder with 1 mL 25 mg/mL dimethyl sulfoxide (Sigma). Then, 2-mL substocks of 0.05 µg/µL paclitaxel were finally made by mixing 2 µL of the paclitaxel stock solution with 2 mL of normal saline. Similarly, a 200-µL stock solution of 5 mg/mL rapamycin (Sigma) was made by dissolving 1 mg rapamycin powder with 200 µL of 25 mg/mL DMSO. Then, 2-mL substocks of 0.05 µg/µL rapamycin were finally prepared by mixing 20 µL of the rapamycin stock solution with 2 mL of normal saline.

The doses of the drugs (paclitaxel and rapamycin) were translated from humans into animal equivalent doses using a formula based on body surface area: animal equivalent dose (mg/kg) = human dose (mg/kg) × human Km / animal Km. The 60 kg-weighted human and 250 g-weighted rat Kms were 37 and 6, respectively. For example, 250 g-weighted rat equivalent doses of 600, 1200 and 2400 µg doses in a 60 kg-weighted...
human are 15.4, 30.8, and 61.7, respectively. As a dose of 600 µg is the highest dose contained on the surface of commercial DEBs, comparative doses were determined as previous doses. Each rat equivalent dose was calculated using the formula based on the body weight of each rat and injected.

Paclitaxel and rapamycin were injected together with EB via the intra-arterial catheter in the optimal time interval after mannitol administration. Calculated animal doses of drugs equivalent to human doses of 600, 1200, and 2400 µg were infused as an intra-arterial bolus, and then, brain tissues were obtained 24 hours and 14 days after drug infusion. A total of 70 rats were needed; the rats were divided into six groups per drug (five rats per group), including two sham groups (five rats per group) that were examined 24 hours and 14 days after drug-free solution infusion following mannitol administration.

**Neurological and histological examination**

Neurological status was evaluated using Bederson et al.'s grading system as follows: grade 0, no observable deficit; grade 1, forelimb flexion; grade 2, decreased resistance to lateral push without circling; and grade 3, same behavior as grade 2 with circling.

For the histological examination, rats were anesthetized with sodium pentobarbital (30 mg/kg, i.p.) and transcardially perfused with 0.1 M PBS (pH 7.4) followed by 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The brains were removed and postfixed in the same fixative for 6 hours. The brain tissues were cryoprotected by infiltration with 30% sucrose overnight. Thereafter, frozen tissues were serially sectioned on a cryostat (Leica, Wetzlar, Germany) into 30-µm coronal sections, and they were then collected into six-well plates containing PBS.

To confirm neuronal death, cresyl violet staining (Sigma) was performed. The sections were mounted on gelatin-coated microscope slides. Cresyl violet acetate (Sigma) was dissolved at 1.0% (w/v) in distilled water, and glacial acetic acid was added to this solution. Before and after staining for 2 minutes at room temperature, the sections were washed twice in distilled water. After dehydration, the sections were mounted with Canada balsam (Kanto, Tokyo, Japan).

Fluoro-Jade C (FJC; Histochem, Jefferson, AR, USA) histofluorescence staining was conducted to localize neuronal degeneration. In brief, the sections were first immersed in a solution containing 1% sodium hydroxide in 80% alcohol, followed by 70% alcohol. The sections were then transferred to a 0.06% potassium permanganate solution and then transferred to a 0.0004% FJC staining solution. After washing, the sections were placed on a slide warmer (approximately 50°C) and then examined using an epifluorescence microscope (Carl Zeiss, Oberkochen, Germany) with blue (450–490 nm) excitation light and a barrier filter. With this method, neurons undergoing degeneration brightly fluoresce compared to the background.

To obtain accurate immunohistochemistry data, the sections were processed at 24 hours and 14 days after drug infusion by immunohistochemistry under the same conditions. The sections were sequentially treated with 0.3% hydrogen peroxide (H₂O₂) in PBS for 30 minutes and 10% normal goat serum in 0.01 M PBS for 30 minutes. The sections were next incubated with diluted rabbit anti-glial fibrillary acidic protein (GFAP) (diluted 1 : 1000; Biogenesis, San Ramon, CA, USA) for astrocytes and rabbit anti-ionized calcium-binding adapter molecule 1 (Iba-1) (diluted 1 : 500; Wako, Osaka, Japan) for microglia overnight at 4°C and subsequently exposed to biotinylated goat anti-rabbit IgG and streptavidin peroxidase complex (diluted 1 : 200; Vector, Burlingame, CA, USA). The sections were visualized by staining with 3,3′-diaminobenzidine in 0.1 M Tris-HCl buffer (pH 7.2) and mounted on gelatin-coated slides. After dehydration, the sections were mounted with Canada balsam (Kanto).

The degrees of neuronal damage were scored based on the cresyl violet and FJC histofluorescence staining data as follows: grade 0, no neuronal damage; grade 1, mild damage; grade 2, moderate damage; and grade 3, severe damage. The degrees of inflammation were scored based on the GFAP and Iba-1 immunohistochemistry data similar to the degrees of neural damage.

**Supplementary References**

effects of magnesium sulfate on blood-brain barrier disruption caused by intracarotid injection of hyperosmolar mannitol in rats. Life Sci 76 : 201-212, 2004
Supplementary Table 1. Extravasation of EB in rat brain

<table>
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<th>Day</th>
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Values are degrees of brain staining by EB. The grades of EB extravasation was scored as follows: grade 0, no extravasation; grade 1, pale; grade 2, moderate; and grade 3, strong. EB: Evans Blue.
Supplementary Table 2. Histologic findings of brain in sham group

<table>
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Values are degrees of neuronal damage and inflammation, respectively. The degrees of neuronal damage and inflammation were scored as follows: grade 0, no neuronal damage or inflammation; grade 1, mild damage or inflammation; grade 2, moderate damage or inflammation; and grade 3, severe damage or inflammation. All the positive histological findings were observed only on 1 or 2 of 15 slices of each right hemisphere.
**Supplementary Fig. 1.** Cresyl violet (A), glial fibrillary acidic protein (B), and ionized calcium-binding adapter molecule 1 (C) staining of No. 1 rat brain of sham group on the 1st day. There are no evidence of brain damage in the right hemispheres after transient blood-brain barrier opening with mannitol, compared to the normal left hemispheres. Scale bar, 2 mm.
Supplementary Fig. 2. Cresyl violet (A), glial fibrillary acidic protein (B), and ionized calcium-binding adapter molecule 1 (C) staining of No. 1 rat brain of sham group on the 14th day. There are no evidence of brain damage in the right hemispheres after transient blood-brain barrier opening with mannitol, compared to the normal left hemispheres. Scale bar, 2 mm.
Supplementary Fig. 3. Cresyl violet (A), glial fibrillary acidic protein (B), ionized calcium-binding adapter molecule 1 (C), and Fluoro-Jade C (D) staining of No. 1 rat brain on the 1st day after 600 μg human dose of paclitaxel. There are no evidence of brain damage in the right hemispheres after transient blood-brain barrier opening with mannitol, compared to the normal left hemispheres. Scale bar, 2 mm.
Supplementary Fig. 4. Cresyl violet (A), glial fibrillary acidic protein (B), ionized calcium-binding adapter molecule 1 (C), and Fluoro-Jade C (D) staining of No. 1 rat brain on the 14th day after 600 μg human dose of paclitaxel. There are no evidence of brain damage in the right hemispheres after transient blood-brain barrier opening with mannitol, compared to the normal left hemispheres. Scale bar, 2 mm.
**Supplementary Fig. 5.** Cresyl violet (A), glial fibrillary acidic protein (B), ionized calcium-binding adapter molecule 1 (C), and Fluoro-Jade C (D) staining of No. 4 rat brain on the 1st day after 1200 μg human dose of paclitaxel. There are no evidence of brain damage in the right hemispheres after transient blood-brain barrier opening with mannitol, compared to the normal left hemispheres. Scale bar, 2 mm.
Supplementary Fig. 6. Cresyl violet (A), glial fibrillary acidic protein (B), ionized calcium-binding adapter molecule 1 (C), and Fluoro-Jade C (D) staining of No. 1 rat brain on the 14th day after 1200 μg human dose of paclitaxel. There are no evidence of brain damage in the right hemispheres after transient blood-brain barrier opening with mannitol, compared to the normal left hemispheres. Scale bar, 2 mm.
Supplementary Fig. 7. Cresyl violet (A), glial fibrillary acidic protein (B), ionized calcium-binding adapter molecule 1 (C), and Fluoro-Jade C (D) staining of No. 1 rat brain on the 1st day after 2400 μg human dose of paclitaxel. There are no evidence of brain damage in the right hemispheres after transient blood-brain barrier opening with mannitol, compared to the normal left hemispheres. Scale bar, 2 mm.
Supplementary Fig. 8. Cresyl violet (A), glial fibrillary acidic protein (B), ionized calcium-binding adapter molecule 1 (C), and Fluoro-Jade C (D) staining of No. 1 rat brain on the 14th day after 2400 μg human dose of paclitaxel. There are no evidence of brain damage in the right hemispheres after transient blood-brain barrier opening with mannitol, compared to the normal left hemispheres. Scale bar, 2 mm.
Supplementary Fig. 9. Cresyl violet (A), glial fibrillary acidic protein (B), ionized calcium-binding adapter molecule 1 (C), and Fluoro-Jade C (D) staining of No. 1 rat brain on the 1st day after 600 μg human dose of rapamycin. There are no evidence of brain damage in the right hemispheres after transient blood-brain barrier opening with mannitol, compared to the normal left hemispheres. Scale bar, 2 mm.
Supplementary Fig. 10. Cresyl violet (A), glial fibrillary acidic protein (B), ionized calcium-binding adapter molecule 1 (C), and Fluoro-Jade C (D) staining of No. 1 rat brain on the 14th day after 600 μg human dose of rapamycin. There are no evidence of brain damage in the right hemispheres after transient blood-brain barrier opening with mannitol, compared to the normal left hemispheres. Scale bar, 2 mm.
Supplementary Fig. 11. Cresyl violet (A), glial fibrillary acidic protein (B), ionized calcium-binding adapter molecule 1 (C), and Fluoro-Jade C (D) staining of No. 2 rat brain on the 1st day after 1200 μg human dose of rapamycin. There are no evidence of brain damage in the right hemispheres after transient blood-brain barrier opening with mannitol, compared to the normal left hemispheres. Scale bar, 2 mm.
**Supplementary Fig. 12.** Cresyl violet (A), glial fibrillary acidic protein (B), ionized calcium-binding adapter molecule 1 (C), and Fluoro-Jade C (D) staining of No. 1 rat brain on the 14th day after 1200 μg human dose of rapamycin. There are no evidence of brain damage in the right hemispheres after transient blood-brain barrier opening with mannitol, compared to the normal left hemispheres. Scale bar, 2 mm.
**Supplementary Fig. 13.** Cresyl violet (A), glial fibrillary acidic protein (B), ionized calcium-binding adapter molecule 1 (C), and Fluoro-Jade C (D) staining of No. 1 rat brain on the 1st day after 2400 μg human dose of rapamycin. There are no evidence of brain damage in the right hemispheres after transient blood-brain barrier opening with mannitol, compared to the normal left hemispheres. Scale bar, 2 mm.
**Supplementary Fig. 14.** Cresyl violet (A), glial fibrillary acidic protein (B), ionized calcium-binding adapter molecule 1 (C), and Fluoro-Jade C (D) staining of No. 1 rat brain on the 14th day after 2400 μg human dose of rapamycin. There are no evidence of brain damage in the right hemispheres after transient blood-brain barrier opening with mannitol, compared to the normal left hemispheres. Scale bar, 2 mm.
Supplementary Fig. 15. Cresyl violet (A-F) and Fluoro-Jade C (G and H) staining of the No. 4 rat brain on 14th day after 600 μg human dose of paclitaxel. A and B (magnified photo of dotted circle in A), moderate aggregation of polymorphonuclear inflammatory cells in the striatum. C and D (magnified photo of dotted circle in C), frequent neuronal death in the cortex. E and F (magnified photo of dotted circle in E), frequent neuronal damage in the hippocampus. G and H (partly magnified photo of dotted circle in G), frequent neuronal degeneration in the striatum. Scale bar, 500 μm (E); 200 μm (A and G); 100 μm (B, C, F, and H); and 50 μm (D).
Supplementary Fig. 16. Immunohistochemistry for glial fibrillary acidic protein (A-D) and ionized calcium-binding adapter molecule 1 (E and F) in the No. 4 rat brain on 14th day after 2400 μg human dose of rapamycin. A and B (magnified photo of dotted circle in A), rarely activated astrocytes in the striatum. C and D (magnified photo of dotted circle in C), rarely activated astrocytes in the cortex. E and F (magnified photo of dotted circle in E), rarely activated microglia in the cortex. Scale bar, 500 μm (A, C, and E); 100 μm (B); and 50 μm (D and F).