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Prevention of rt-PA induced blood–brain barrier component degradation by the poly(ADP-ribose)polymerase inhibitor PJ34 after ischemic stroke in mice

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ABSTRACT

Recombinant tissue plasminogen activator (rt-PA) is the only pharmacological treatment approved for thrombolysis in patients suffering from ischemic stroke, but its administration aggravates the risk of hemorrhagic transformations. Experimental data demonstrated that rt-PA increases the activity of poly(ADP-ribose)polymerase (PARP). The aim of the present study was to investigate whether PJ34, a potent (PARP) inhibitor, protects the blood–brain barrier components from rt-PA toxicity. In our mouse model of cerebral ischemia, administration of rt-PA (10 mg/kg, i.v.) 6 h after ischemia aggravated the post-ischemic degradation of ZO-1, claudin-5 and VE-cadherin, increased the hemorrhagic transformations (assessed by brain hemoglobin content and magnetic resonance imaging). Furthermore, rt-PA also aggravated ischemia-induced functional deficits. Combining PJ34 with rt-PA preserved the expression of ZO-1, claudin-5 and VE-cadherin, reduced the hemorrhagic transformations and improved the sensorimotor performances. *In vitro* studies also demonstrated that PJ34 crosses the blood–brain barrier and may thus exert its protective effect by acting on endothelial and/or parenchymal cells. Thus, co-treatment with a PARP inhibitor seems to be a promising strategy to reduce rt-PA-induced vascular toxicity after stroke.

Introduction

Stroke is the third cause of mortality and the first cause of acquired disability in developed countries. The only pharmacological treatment approved for acute ischemic stroke, the major type of stroke, consists in thrombolysis with recombinant tissue plasminogen activator (rt-PA). However, less than 5% of patients receive rt-PA therapy (Cronin, 2010). Indeed, the therapeutic window of rt-PA is very short: 4.5 h after the onset of symptoms (Hacke et al., 2008). Furthermore, rt-PA aggravates post-ischemic intracerebral hemorrhage, also called hemorrhagic transformations (HT) (Derex and Nighoghossian, 2008; Hacke et al., 2004). In order to increase the number of rt-PA treated patients, the objectives are to enlarge the time window for rt-PA administration and to reduce the risk of HT. This may be achieved by combining another drug treatment with rt-PA (Gutiérrez et al., 2006; Ishrat et al., 2012; Steiner and Hacke, 1998).

The poly(ADP-ribose)polymerases (PARPs) are a family of nuclear enzymes. PARP-1 accounts for more than 80% of PARP activity and has been identified as a key regulator of nuclear processes such as DNA repair, replication and transcription (Moroni, 2008). Paradoxically, PARP-1 pharmacological inhibition (Chiarugi, 2005; Komjati et al., 2005; Strosznajder et al., 2010) and gene deletion (Eliasson et al., 1997; Endres et al., 1997) showed a deleterious role of PARP in rodent models of ischemic stroke. Recently, the PARP inhibitor MP-124 showed to be also neuroprotective in a non-human primate cerebral ischemia model (Matsuura et al., 2011). Aggravation of energetic depletion and inflammation are among the mechanisms that explain the detrimental effects of PARP activation (Moroni and Chiarugi, 2009). Moreover, PARP leads to the translocation of the apoptosis inducing

Abbreviations: PARP, Poly(ADP-ribose)polymerase; PJ34, N-(6-Oxo-5,6-dihydrophenanthridin-2-yl)-(N,N-dimethylamino)acetamide hydrochloride; rt-PA, recombinant tissue plasminogen activator.

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factor (AIF) from the mitochondria to the nucleus and induces a caspase-independent programmed cell death named parthanatos (Wang et al., 2009).

Besides, it has been demonstrated that rt-PA administration after cerebral ischemia enhanced PARP-1 activation (Crome et al., 2007). Moreover, in our laboratory, we recently demonstrated that PJ34 (N-(6-oxo-5,6-dihydrophenanthridin-2-yl)-2-(N,N-dimethylamino) acetamide), a potent PARP inhibitor, attenuates HT induced by rt-PA (Haddad et al., 2013). Interestingly, minocycline, recently described as a potent PARP inhibitor (Alano et al., 2006), has also been reported to reduce rt-PA induced HT (Murata et al., 2008).

In this context, the aim of the present study was to investigate the molecular targets of PARP involved in rt-PA-induced HT. For this purpose, we examined in a model of middle cerebral artery occlusion (MCAO) in mice the effect of PJ34 on rt-PA-induced degradation of tight junction, adherens junction and basement membrane proteins of the blood–brain barrier (BBB). We also studied post-ischemic cerebral edema, HT and functional deficits. Furthermore, to determine whether PJ34 targeted endothelial cells exclusively or may act through a modulation of brain parenchymal cells of the neurovascular unit, the BBB permeability to PJ34 was examined in a validated *in vitro* model (Prieto et al., 2004).

Material and methods

Animals

Animal care and all experiments were carried out with the ethical approval of the Paris Descartes University Animal Ethics Committee (registered number P2.CM.149.10), and in accordance with the French regulations regarding the protection of animals used for experimental and other scientific purposes (D2001-486), and with the European Communities Council Directive of November 24, 1986 (86/609/EEC). Male Swiss albino mice (27–32 g, Janvier, Le Genest-St-Isle, France) were housed under standard conditions with a 12:12 hour light/dark cycle and provided with food and water *ad libitum*.

Cerebral ischemia

Permanent focal cerebral ischemia was produced by endovascular occlusion of the left middle cerebral artery (MCA) (Haddad et al., 2013). The mice were anesthetized with intraperitoneal ketamine (50 mg/kg, Vetoquinol, Lure, France) and xylazine hydrochloride (6 mg/kg, Bayer Health Care, Germany) (vehicle: NaCl 0.9%, 10 ml/kg). Body temperature was monitored throughout surgery by a rectal probe and maintained at 37 ± 0.5 °C with a homoeothermic blanket control unit (Harvard Apparatus, Edenbridge, Kent, UK). After a midline neck incision, the left common carotid artery (CCA) was isolated under a microscope and ligatured with a 5-0 silk suture (Ethicon, Issy-Les-Moulineaux, France). The circulation in the external and internal carotid arteries was temporarily interrupted with a 5-0 silk suture. An arteriotomy was performed in the CCA proximal to the carotid bifurcation. A nylon monofilament (Sensas, 80 μm diameter) coated with "thermomelting" glue (4 mm long, 190 μm diameter, Jet Melt, Radiospares, Beauvais, France) was introduced through the arteriotomy and advanced into the internal carotid artery to occlude the origin of the MCA. Occlusion of the MCA was checked for 5 min after insertion of the filament with a laser Doppler flowmeter (Moor Instruments Ltd, Millwey, UK) and a probe fixed on the skull in the left MCA territory. The drop in blood flow was calculated as the percentage of the pre-ischemic value for each mouse. Mice with less than a 50% drop in blood flow were excluded from the studies. After surgery, the wound was sutured and the mice were returned to their home cages, maintained at 30 °C, with free access to food and water. In order to prevent dehydration, the mice were injected subcutaneously with 0.5 ml of NaCl 0.9%. Sham-operated mice underwent the same surgical procedure except the introduction of the filament.

Experimental protocols

Six hours after the onset of ischemia, the mice were given either rt-PA (10 mg/kg, Actilyse®, Boehringer-Ingelheim, Biberach an der Reiss, Germany) or saline via the tail vein with a 10% bolus and 90% continuous perfusion over 30 min. This protocol (notably the delayed treatment time) was chosen on the basis of previous publications (Ishiguro et al., 2010; Murata et al., 2008; Zhang et al., 2009) and of rt-PA aggravating effect on hemorrhagic transformations in our model (Haddad et al., 2013). PJ34 (1 or 3 mg/kg, i.p.) or saline was administered at the time of middle cerebral artery occlusion and again 4 h after the onset of ischemia. This protocol was based on our previous studies (Haddad et al., 2008, 2013, see discussion).

In the first experiment, the mice were randomly assigned to one of the six groups: sham (n = 12), vehicle (n = 10), rt-PA alone (n = 11), rt-PA plus PJ34 at 1 mg/kg (n = 8), rt-PA plus PJ34 at 3 mg/kg (n = 11) and PJ34 alone at 3 mg/kg (n = 9). Twenty-four hours after ischemia, behavioral tests were performed, then the mice were euthanized and their brains were rapidly removed. They were sectioned into 7 × 1-mm thick coronal slices using a chopper (Mickle Laboratory Engineering, Gomshall Surrey, UK). The third and fifth slices located within the core of the lesion were stored immediately at −80 °C for Western blotting of the BBB proteins. The remaining slices were pooled and used to evaluate brain water content.

In the second experiment, mice were divided into the same six groups (n = 10–11). Twenty-four hours after ischemia, animals were anesthetized with sodium pentobarbitone (60 mg/kg, i.p., Ceva Santé animale, Libourne, France) and perfused transcardially with 0.9% NaCl. The brains were removed and sectioned into 7 × 1-mm thick coronal slices. The third and fifth slices were used to evaluate HT by Western blotting of brain hemoglobin; the remaining slices were used to measure the infarct volume.

The third experiment focused on two proteins of the basement membrane, collagen IV and laminin. A preliminary study was performed to evaluate their expression by Western blotting in three groups of animals: sham (n = 3–6), vehicle treated-ischemic mice (n = 5–8), and rt-PA treated-ischemic mice (n = 5–7). Thereafter, the expression of both proteins was evaluated by immunohistochemistry, and the mice were randomly assigned into four groups: sham (n = 3), vehicle (n = 4), rt-PA alone (n = 4) and rt-PA plus PJ34 at 1 mg/kg (n = 4). Twenty-four hours after ischemia, mice were anesthetized with sodium pentobarbitone (60 mg/kg, i.p., Ceva Santé animale, Libourne, France) and perfused transcardially with saline. The brains were then quickly removed, frozen in isopentane (−40 °C) and stored at −40 °C. Twenty μm-thick coronal brain sections were cut on a cryostat (−18 °C, Jung CM 3000, Leica) at 6 levels from 2.2 mm anterior to the bregma to 2.8 mm posterior to the bregma (1-mm intervals) according to the stereotaxic brain atlas of Paxinos and Franklin (Paxinos and Franklin, 2001). The sections were collected on gelatin-coated slides and stored at −20 °C before immunohistochemistry.

In the fourth experiment, HT and cerebral edema were evaluated by magnetic resonance imaging (MRI) in sham (n = 4), ischemic mice (n = 4), ischemic mice treated with rt-PA (n = 8) and in ischemic mice treated with both rt-PA and PJ34 at 1 mg/kg (n = 8).

Behavioral tests

Behavioral tests were performed in a dedicated room at 20–22 °C. Because cerebral ischemia was produced on the left side of the brain, sensorimotor functions were examined in the contralateral (right) side using a battery of sensorimotor tests described below. Each test