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# Reduction of hemorrhagic transformation by PJ34, a poly(ADP-ribose)polymerase inhibitor, after permanent focal cerebral ischemia in mice

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ABSTRACT

Hemorrhagic transformation is an aggravating event that occurs in 15 to 43% of patients suffering from ischemic stroke. This phenomenon due to blood-brain barrier breakdown appears to be mediated in part by matrix metalloproteinases (MMPs) among which MMP-2 and MMP-9 could be particularly involved. Recent experimental studies demonstrated that post-ischemic MMP-9 overexpression is regulated by poly(ADP-ribose)polymerase (PARP). In this context, our study aimed to evaluate the effect of PJ34 (*N*-(6-oxo-5,6-dihydrophenanthridin-2-yl)-2-(*N*,*N*-dimethylamino)acetamide), a potent PARP inhibitor, on MMP-2 and MMP-9 levels and on hemorrhagic transformations in a model of permanent focal cerebral ischemia in mice. PJ34 (6.25–12.5 mg/kg, i.p.) was given at the time of ischemia onset and 4 h later. Hemorrhagic transformations, divided into microscopic and macroscopic hemorrhages, were counted 48 h after ischemia on 12 coronal brain slices. Microscopic and macroscopic hemorrhages were respectively reduced by 38% and 69% with 6.25 mg/kg PJ34. The anti-hemorrhagic effect of PJ34 was associated with a 57% decrease in MMP-9 overexpression assessed by gelatin zymography. No increase in MMP-2 activity was observed after ischemia in our model. The vascular protection achieved by PJ34 was associated with a reduction in the motor deficit ($P<0.05$) and in infarct volume (−31%, $P<0.01$). In conclusion, our study demonstrates for the first time that PJ34 reduces hemorrhagic transformations after cerebral ischemia. Thus this PARP inhibitor exhibits both anti-hemorrhagic and neuroprotective effects that may be of valuable interest for the treatment of stroke.



## 1. Introduction

Hemorrhagic transformation occurs in 15 to 43% of patients suffering from ischemic stroke (Lyden and Zivin, 1993; Montaner et al., 2001; Okada et al., 1989). At present, no pharmacological treatment for intracerebral hemorrhage is available in clinical practice. Hemorrhage results from a loss of microvascular integrity (Hamann et al., 1996) mediated by several factors including the activation of matrix metalloproteinases (MMPs) (Heo et al., 1999).

MMPs are a family of zinc-binding proteolytic enzymes capable of degrading components of the extracellular matrix (Mun-Bryce and Rosenberg, 1998). Experimental studies have mainly focused on the role of MMP-2 (gelatinase A) and MMP-9 (gelatinase B) in cerebral ischemia because these enzymes degrade fibronectin, laminin and collagen type IV, the major components of the basal lamina of cerebral blood vessels (Del Zoppo and Mabuchi, 2003; Mun-Bryce and Rosenberg, 1998). Post-ischemic activation of MMP-2 and MMP-9 was involved in blood-brain barrier breakdown following cerebral ischemia in mice and rats (Asahi et al., 2001; Rosenberg et al., 1998) and MMP-9 was associated with hemorrhagic transformation in non human primates (Heo et al., 1999). In clinical studies, increased expression of MMP-2 and MMP-9 protein has also been reported in post mortem brain of stroke patients (Clark et al., 1997; Rosell et al., 2006). Increased blood MMP-9 was related to hemorrhagic transformation after human cardioembolic stroke (Montaner et al., 2001). Taken together, these studies pointed out the potential role of MMP-2 and MMP-9 in hemorrhagic transformation.

Recently, two studies reported that brain MMP-9 expression is regulated, at least in part, by the enzyme poly(ADP-ribose) polymerase (PARP). Kauppinen and Swanson (2005) showed in vitro that the release of MMP-9 from microglia cultures stimulated by TNF-α requires PARP activation. The study by Koh et al. (2005) is to date the only one to demonstrate in vivo that the PARP inhibitor 3-aminobenzamide (3-AB) reduced the upregulation of brain MMP-9 in a model of cerebral ischemia in rats. PARP is a constitutive enzyme initially described as a DNA repairing enzyme but recently involved in

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a variety of pathophysiological events (Nguewa et al., 2005). Hyperactivation of PARP may participate to cell death by contributing to NAD depletion, inflammation and mitochondrial release of apoptosis-inducing factor (Chiarugi, 2005; Yu et al., 2003). Accordingly, in cerebral ischemia PARP inhibitors exhibit protective activities including a reduction in brain lesion size, neurological deficit and inflammation (Chiarugi, 2005; Haddad et al., 2006). Genetic suppression of either PARP-1 or PARP-2 shows that both isoforms of PARP can participate to ischemic damage (Endres et al., 1997; Kofler et al., 2006). PJ34 (*N*-(6-oxo-5,6-dihydrophenanthridin-2-yl)-2-(*N*,*N*-dimethylamino)acetamide) is a potent non selective PARP-1 and PARP-2 inhibitors (Iwashita et al., 2004). Interestingly, in a recent study, PJ34 reduced blood-brain barrier damage and brain edema due to global cerebral ischemia in rats (Lenzser et al., 2007). However, there is to our knowledge no data showing the implication of PARP in the hemorrhagic transformations subsequent to cerebral ischemia.

In this context, the present study investigated the effect of PJ34 on hemorrhagic transformations using a mouse model of permanent focal cerebral ischemia.

## 2. Materials and methods

### 2.1. Animals

All animal experiments were performed in compliance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and the French regulations (D2001-486) regarding the protection of animals used for experimental and other scientific purposes. Male Swiss mice (27–32 g, Janvier, Le genest-St-Isle, France) were housed under standard conditions with a 12 h light/dark cycle and allowed access to food and water *ad libitum*. All efforts were made to minimize both animal suffering and the number of animals used throughout the experiment.

### 2.2. Cerebral ischemia

Mice were anesthetized with intraperitoneal ketamine (50 mg/kg) and xylazine hydrochloride (6 mg/kg). Body temperature was monitored throughout surgery by a rectal probe and maintained at 37±0.5 °C with a homeothermic blanket control unit (Harvard Apparatus, Edenbridge, Kent, U.K.). Permanent focal cerebral ischemia was induced by the occlusion of the left middle cerebral artery (MCA) using an intraluminal filament technique. Through a midline neck incision, the left common carotid artery (CCA) was isolated and ligatured with a 5-0 silk suture (Ethicon, Issy-Les-Moulineaux, France) under a microscope. Blood 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; diameter, 80 µm) coated with "thermomelting" glue (4 mm long, diameter 190 µm) was introduced through the arteriotomy and advanced into the internal carotid artery. Occlusion of the MCA was controlled by monitoring blood flow in the MCA territory by laser Doppler flowmetry (Moor Instruments Ltd, Millwey, England) for 5 min after the insertion of the filament. Mice with less than a 50% drop in blood flow were excluded from the studies. After surgery, the wound was sutured and mice were returned to their cage placed at 29 °C with free access to food and water. Sham-operated mice underwent the same surgical procedure except that no filament inserted in the CCA.

### 2.3. Motor function

Motor function impairment was assessed 48 h after ischemia by an exit circle test modified from Leinhase et al. (2006) as follows: mice were put in the centre of concentric circles of 15, 30 and 45 cm diameter in an open field. An exit circle score was assigned by a blinded observer according to the ability and rapidity of mice to exit circles for a maximum of 30 s.

Scores 1–2–3 = the mouse exits the 45 cm diameter circle within the first 10 s, 20 s and 30 s respectively;
Scores 4–5–6 = the mouse exits the 30 cm diameter circle within the first 10 s, 20 s and 30 s respectively;
Scores 7–8–9 = the mouse exits the 15 cm diameter circle within the first 10 s, 20 s and 30 s respectively;
Score 10 = the mouse remains in the smallest circle;
Score 11 = the mouse has no spontaneous motor activity.

Naïve (non operated) and sham-operated animals were also included in this test.

### 2.4. Hemorrhagic transformations and infarct volume

Immediately after the exit circle test, mice were anesthetized with sodium pentobarbitone (60 mg/kg; i.p.) and transcardially perfused with isotonic saline (NaCl, 0.9%). Brains were rapidly removed and stored at −40 °C for further evaluation of intracerebral hemorrhage and infarction.

Brains were coronally sectioned into 50 µm-thick slices at 12 levels from 6.5 to 0.5 mm anterior to the interaural line (each 500 µm interval) according to a stereotaxic brain atlas (Paxinos and Franklin, 2001), using a cryostat (Bright®, Instrument Company Ltd, Angleterre) at −15 °C.

An observer blinded to the treatment group identified on each brain level hemorrhagic transformations defined as (modified from Zhang et al., 2003):

1) Microscopic hemorrhage evident to the eye aided by a magnifying glass (×2).
2) Macroscopic hemorrhage evident to the unaided eye.

Microscopic hemorrhages were attributed a 1 point score. The sum of all microscopic hemorrhages of all brain levels gave the microscopic hemorrhage score.

Macroscopic hemorrhages were given a score of 3–4 or 5 according to their size (little, middle and gross respectively). The score of macroscopic hemorrhages at each brain level was calculated as follows: [(number of little hemorrhage × 3) + (number of middle hemorrhage × 4) + (number of gross hemorrhage × 5)]. The sum of all these brain level scores gave the macroscopic hemorrhage score. The total hemorrhagic score of the brain was then calculated by adding the microscopic and the macroscopic hemorrhage scores.

Sections used for hemorrhage evaluation were stained with cresyl violet to blindly quantify infarct volumes. The area of damaged parenchyma (unstained tissue) was measured using a computer image analysis system (Imstar, Paris, France). Each infarct area was then multiplied by the ratio of the surface of the infarcted (ipsilateral) to the intact (contralateral) hemispheres at the same level to correct the lesion for brain swelling (Golanov and Reis, 1995). The total volume of damaged tissue (in cubic millimeters) was then calculated by linear integration of the corrected lesion areas.

### 2.5. MMP zymography

In a separate set of experiments 24 h after ischemia, mice were anesthetized with sodium pentobarbitone (60 mg/kg; i.p.) and transcardially perfused with isotonic saline (NaCl 0.9%). Brains were removed and a 2 mm-thick coronal slice cut in the infarcted area was used to determine brain MMP-2 and MMP-9 levels by gelatin zymography (Zhang and Gottschall, 1997). Briefly, brain tissues were homogenized in 500 µl of lysis buffer (50 mM Tris–HCl, pH 7.6, 150 mM NaCl, 5 mM $CaCl_2$, 2 mM $NaN_3$, 0.05% BRIJ-35 and 1% Triton ®X-100). Homogenate was centrifuged at 4 °C for 10 min at 10,000 ×g

and the supernatant was recovered. Aliquots (10 µl) of the supernatant were used for the determination of total protein concentration (Bradford, 1976). The supernatants were diluted with lysis buffer to have a final protein concentration of 2 mg/ml and a final volume of 1 ml. These samples were then incubated for 60 min at 4 °C with 50 µl of gelatin-sepharose 4B (Amersham 17-0956-01), with constant shaking. After incubation, the samples were centrifuged at 4 °C for 1 min at 10,000 ×g. The pellets were washed with a working buffer (50 mM Tris–HCl, pH 7.6, 150 mM NaCl, 5 mM $CaCl_2$, 2 mM $NaN_3$ and 0.05% BRIJ-35). After a second centrifugation, the pellets were resuspended in elution buffer consisting of working buffer plus 10% dimethylsulfoxide (DMSO) for 30 min. Samples were then loaded and separated by 8% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) with 1 mg/ml swin skin gelatin (Sigma, G2500). After separation by electrophoresis, the gel was renatured and then incubated with a developing buffer (100 mM Tris–HCl, pH 7.4, 100 mM NaCl, 5 mM $CaCl_2$, 5 µM $ZnCl_2$ and 0.02% BRIJ-35) at 37 °C for 36 h. After development, the gel was stained with 0.5% Coomassie blue G-250 for 1 h and then destained appropriately with a solution of acetic acid/ethanol/water (1/2/7). MMP activity was quantified with an image analyser (Gel Doc 2000, Bio-Rad) and expressed as arbitrary units of optical density.

### 2.6. Experimental protocols

PJ34 (6.25 or 12.5 mg/kg) or its vehicle (NaCl, 0.9%) were administered intraperitoneally at the time of MCA occlusion and again 4 h after the onset of ischemia.

In the first experiment, infarct volumes and hemorrhagic transformations were evaluated at 48 h after ischemia in sham animals ($n$=6) and ischemic animals treated either with PJ34 (6.25 mg/kg or 12.5 mg/kg) or its vehicle ($n$=10–12 per group). A group of naïve animals was added when motor function was assessed ($n$=13). In the second experiment, brain MMP levels were determined 24 h after ischemia in naïve animals, sham animals and ischemic animals treated either with PJ34 (6.25 mg/kg) or its vehicle ($n$=5–6 per group).

### 2.7. Statistical analysis

Data are expressed as means±S.E.M. For infarct volume and MMP-zymogram, differences between groups were evaluated using one way analysis of variance (ANOVA) followed by a Bonferroni test. For the exit circle test and hemorrhagic scores, non parametric Kruskal–Wallis and Mann–Whitney tests were used. A $P$-value of less than 0.05 was considered significant. All tests were performed with Statview 5.0 (Abacus Concepts, Berkeley, CA, USA).

### 2.8. Drugs and reagents

All chemicals and reagents were purchased from Sigma Chemical Co. (Saint-Quentin Fallavier, France), except as specified.

## 3. Results

### 3.1. Effect of PJ34 on motor function and infarct volume 48 h after ischemia

The drops in cerebral blood flow produced by MCA occlusion were not statistically different among the three groups (79±2% of preischemic value in the vehicle-treated mice; 78±3% in mice treated with 6.25 mg/kg PJ34 and 83±2% in mice given 12.5 mg/kg PJ34).

The score in the exit circle test for naïve and sham-operated mice 48 h after surgery was 1.0±0 for both groups (Fig. 1A). Ischemia significantly increased the exit circle score (7.4±1.1, $P$<0.01) indicating motor function impairment. The highest dose of PJ34 did not improve significantly the exit circle test score but ischemic mice treated with

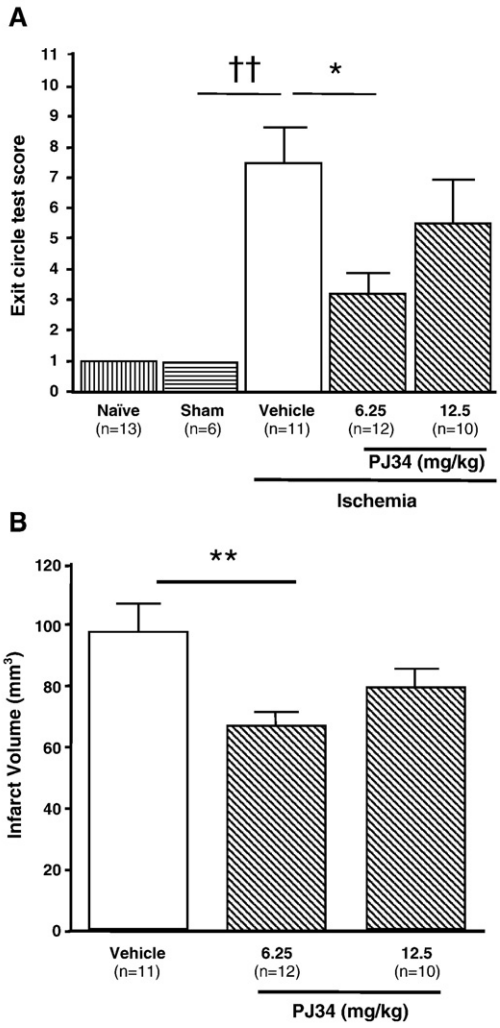


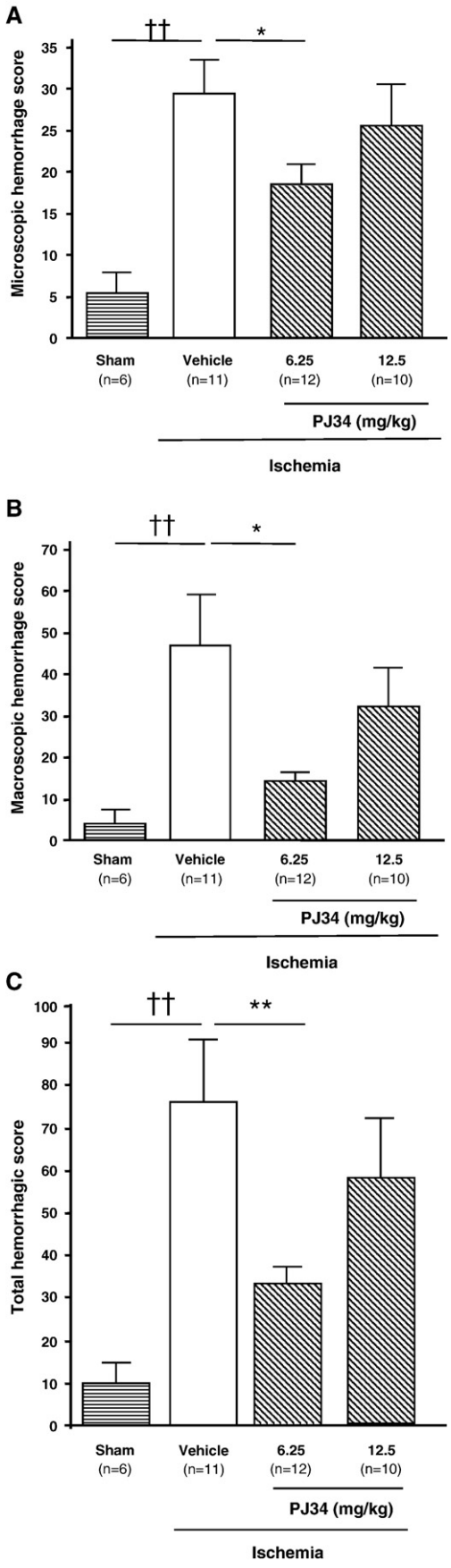

**Fig. 1.** Effect of PJ34 on the motor deficit (A) and the infarct volume (B) evaluated 48 h after the onset of ischemia. PJ34 (6.25–12.5 mg/kg, i.p.) was administered at the time of MCA occlusion and again 4 h after the onset of ischemia. ††$P$<0.01 *versus* sham-operated mice; *$P$<0.05, **$P$<0.01 *versus* vehicle-treated ischemic mice (A: Kruskal–Wallis and Mann–Whitney tests; B: ANOVA followed by Bonferroni *post hoc* test).

6.25 mg/kg PJ34 had an exit circle score of 3.1±0.7, significantly lower than that of vehicle-treated mice ($P$<0.05).

Infarct volume was not significantly reduced with the highest dose of PJ34 ($P$<0.07), but a 31% reduction was obtained with PJ34 at 6.25 mg/kg (67±4 $mm^3$ *versus* 97±9 $mm^3$ in vehicle-treated mice, $P$<0.01) (Fig. 1B).

### 3.2. Effect of PJ34 on hemorrhagic transformation 48 h after ischemia

In vehicle-treated ischemic mice, the score of microscopic and macroscopic hemorrhages (29±4 and 46±12 respectively) was significantly increased compared to sham-operated mice (5±2 and 4±3 respectively, Fig. 2A, B). Accordingly the total hemorrhagic score in vehicle-treated ischemic mice was 8.5 fold higher than in sham-operated mice ($P$<0.01). The highest dose of PJ34 had no significant effect on the hemorrhagic scores but 6.25 mg/kg PJ34 significantly reduced by 57% ($P$<0.01) the total hemorrhagic score of ischemic animals compared with that of vehicle-treated ischemic mice (Fig. 2C). This reduction in the total hemorrhagic score by 6.25 mg/kg PJ34 was due to a 38% reduction in the score of microscopic hemorrhage ($P$<0.05) and a 69% reduction in the score of macroscopic hemorrhage ($P$<0.05, Fig. 2A, B).




### 3.3. Effect of PJ34 on MMP activity 24 h after ischemia

Brains of naïve and sham-operated mice exhibit no detectable level of MMP-9 (Fig. 3). Ischemia induced a drastic increase in MMP-9 (2004±386 U.A., $P<0.001$ *versus* sham mice) predominantly expressed as the higher molecular weight latent form (105-kDa). PJ34 at 6.25 mg/kg significantly reduced by 57% the MMP-9 brain level (867±204 U.A., $P<0.01$ *versus* vehicle-treated mice, Fig. 3A, B). MMP-2 was hardly detectable in naïve and sham-operated mice and no increase was observed in ischemic mice (Fig. 3A).

## 4. Discussion

The present study shows for the first time that the PARP inhibitor PJ34 drastically reduced the hemorrhagic transformation that occurs after permanent cerebral ischemia in mice. This anti-hemorrhagic effect was obtained with the lowest dosage of PJ34 whereas the highest dosage was devoid of effect. A loss of neuroprotection (reduction in infarct size) was similarly reported when doses of PJ34 or other PARP inhibitors were increased in models of cerebral ischemia (Couturier et al., 2003; Iwashita et al., 2004; Takahashi et al., 1999). Excessive inhibition of PARP and its repairing capacities were the mechanisms put forward to explain this phenomenon. Accordingly it would be interesting to examine whether PJ34 at dosages below 6.25 mg/kg could lead to an even better effect in our model. Future studies should also examine the possibility to suppress or at least delay the first injection of the PARP inhibitor performed at the onset of ischemia. A wider therapeutic window of PJ34 would extend its potential use in clinical practice.

A better understanding of the mechanisms involved in hemorrhagic transformation is crucial for patients suffering from ischemic stroke as serial computed tomography studies showed that 15 to 43% of them exhibit this phenomenon (Lyden and Zivin, 1993; Okada et al., 1989). This percentage even increases up to 70% with more sensitive techniques able to detect small amounts of blood in brain parenchyma such as magnetic resonance imaging (Hornig et al., 1993). Despite this clinical consideration, the research has mainly focused this last decade on preventing hemorrhage induced by thrombolytic agents administered to restore blood flow after cerebral ischemia. By contrast, studies on spontaneous hemorrhagic transformation remain limited.

In our model of permanent focal cerebral ischemia, spontaneous microscopic and macroscopic hemorrhages were observed throughout the brain parenchyma 48 h after MCA occlusion. The reduction in both microscopic and macroscopic hemorrhages achieved by 6.25 mg/kg PJ34 suggests that PARP is involved in these hemorrhagic transformations. This result is in accordance with a recent study showing that PJ34 reduces the loss of the tight junction protein occludin and the blood-brain barrier opening in a model of global ischemia in rats (Lenzser et al., 2007). Among the mechanisms that may contribute to post-ischemic blood-brain barrier disruption, MMP-2 and MMP-9 activation has retained particular attention (Del Zoppo and Mabuchi, 2003). MMP activation was recently associated with alteration of tight junction proteins in a model of transient focal cerebral ischemia in rats (Yang et al., 2007). Furthermore treatments with MMP inhibitors demonstrated the implication of theses enzymes in cerebral hemorrhage and/or edema (Rosenberg and Navratil, 1997; Sumii and Lo, 2002; Wang and Tsirka, 2005). Experiments with mice deleted for the MMP-9 gene strongly suggest a role of this gelatinase in blood-brain barrier opening after cerebral ischemia (Asahi et al., 2001). Furthermore Heo et al. (1999) reported that MMP-9 was

**Fig. 2.** Effect of PJ34 on microscopic (A), macroscopic (B) and total hemorrhagic scores (C). PJ34 (6.25–12.5 mg/kg, i.p.) was administered at the time of MCA occlusion and again 4 h after the onset of ischemia. Hemorrhage scores were evaluated 48 h after ischemia. ††$P<0.01$ *versus* sham-operated mice; *$P<0.05$, **$P<0.01$ *versus* vehicle-treated ischemic mice (Kruskal–Wallis and Mann–Whitney tests).

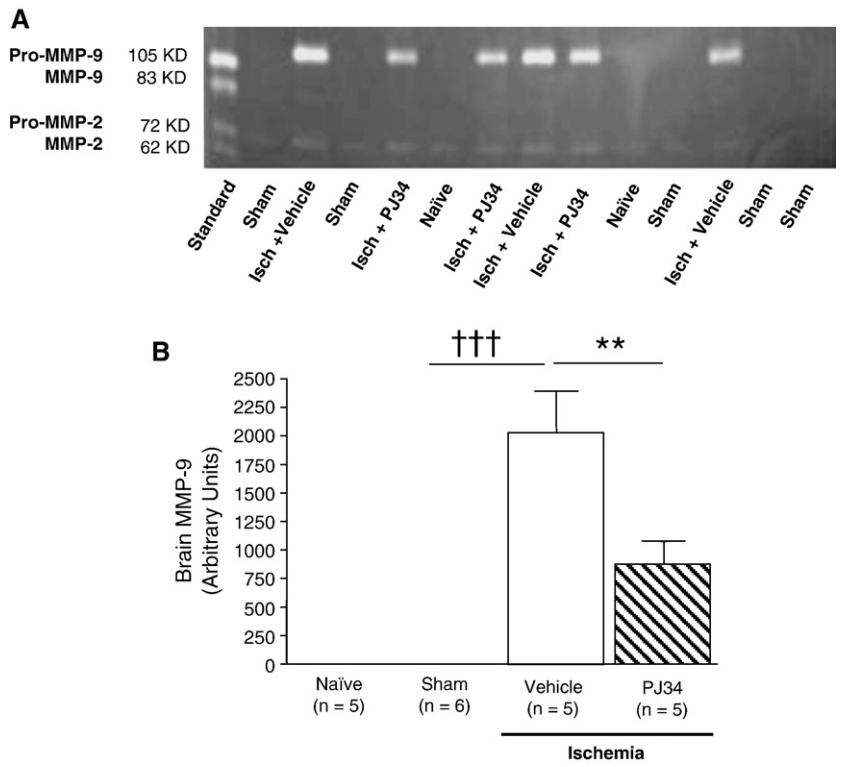



**Fig. 3.** Effects of PJ34 (6.25 mg/kg, i.p.) on MMP levels 24 h after the onset of ischemia. (A) Representative zymogram, (B) quantification of MMP-9 levels expressed as arbitrary units (Isch: Ischemic mice). †††: $P<0.001$ versus sham-operated mice; **$P<0.01$ *versus* vehicle-treated ischemic mice (ANOVA followed by Bonferroni *post hoc* test).

associated with hemorrhagic transformations after focal cerebral ischemia in primates. By contrast the role of MMP-2 remains more questionable. In our model, no increase in MMP-2 activation was detected 24 h after ischemia, which is not in favour of the implication of MMP-2 in hemorrhagic transformation. However an increase in MMP-2 activation was described by others at earlier (in the first hours) and more delayed (4 days) times after cerebral ischemia (Planas et al., 2001; Rosenberg et al., 1998). In contrast to MMP-2, MMP-9 expression was markedly increased at 24 h in our model. PJ34 reduced this MMP-9 increase by 57% as shown by gelatin zymography. This result is in accordance with a previous study showing that 3-AB, another PARP inhibitor, reduces plasma as well as brain MMP-9 activities in a rat model of transient focal cerebral ischemia (Koh et al., 2005). MMP-9 expression is regulated by transcription factors among them nuclear factor-κB (NF-κB) and activator protein-1 (AP-1) both known for being regulated by PARP (Ha et al., 2002; Hassa and Hottiger, 1999). Thus PJ34 may have downregulated NF-κB and AP-1 activity and subsequently reduced MMP-9 expression.

The cellular source(s) of MMPs after focal stroke are not fully identified. Immunohistochemical evidence suggests that post-ischemic MMP-9 expression increases in neurons, glial and endothelial cells (Planas et al., 2001; Rosenberg et al., 2001; Tejima et al., 2007). Recent studies have also shown the importance of neutrophil-derived MMP in post-ischemic cerebral vascular damage (Gidday et al., 2005). Because PARP inhibitors decrease post-ischemic neutrophil infiltration into brain parenchyma (Couturier et al., 2003; Koh et al., 2005; Lenzser et al., 2007), PJ34 may have reduced brain MMP-9 expression in our model by decreasing recruitment of neutrophils in brain tissue. PJ34 was also recently shown to suppress microglial activation in a model of global ischemia (Hamby et al., 2007). Whether such an effect on microglia by PJ34 also contributes to the decrease in MMP-9 in our model remains to demonstrate.

The anti-hemorrhagic effect of PJ34 in our study was associated with an improvement of the motor function and a decrease in the infarct volume. Such neuroprotective effects are already well documented for PJ34 and other PARP inhibitors in various models of cerebral ischemia (Chiarugi, 2005; Haddad et al., 2006). The decrease in MMP-9 achieved by PJ34 may be involved in its neuroprotective effect. Treatment with MMP-9 neutralizing antibody or deletion of MMP-9 gene showed that MMP-9 expressed within the first 24 h after stroke is implicated in the initial tissue destruction and contributes to the rapidly developing brain injury that occurs after stroke (Asahi et al., 2001; Romanic et al., 1998). Finally it cannot be excluded that the decrease in the brain lesion size afforded by PJ34 has contributed to reduce hemorrhagic transformations.

In summary, PJ34 reduces the spontaneous hemorrhagic transformations that occur in our model of permanent cerebral ischemia in mice and this effect may result from a reduction in MMP-9 overexpression. The PARP inhibitor also decreases the motor deficit and infarct volume. Thus PJ34 combines both anti-hemorrhagic and neuroprotective effects and could be therefore of particular interest for the treatment of stroke. Furthermore the anti-hemorrhagic effect of PJ34 demonstrated in the present study associated with the recent finding that recombinant tissue plasminogen activator increases the hyperactivation of PARP after cerebral ischemia (Crome et al., 2007) suggests that PJ34 could also be used as an adjunctive treatment to improve the safety of thrombolysis in acute ischemic stroke.

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## References

Asahi, M., Wang, X., Mori, T., Sumii, T., Jung, J.C., Moskowitz, M.A., Fini, M.E., Lo, E.H., 2001. Effects of matrix metalloproteinase-9 gene knock-out on the proteolysis of blood-brain barrier and white matter components after cerebral ischemia. J. Neurosci. 21, 7724–7732.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.
Chiarugi, A., 2005. Poly(ADP-ribosyl)ation and stroke. Pharmacol. Res. 52, 15–24.
Clark, A.W., Krekoski, C.A., Bou, S.S., Chapman, K.R., Edwards, D.R., 1997. Increased gelatinase A (MMP-2) and gelatinase B (MMP-9) activities in human brain after focal ischemia. Neurosci. Lett. 238, 53–56.
Couturier, J.Y., Ding-Zhou, L., Croci, N., Plotkine, M., Margaill, I., 2003. 3-Aminobenzamide reduces brain infarction and neutrophil infiltration after transient focal cerebral ischemia in mice. Exp. Neurol. 184, 973–980.
Crome, O., Doeppner, T.R., Schwarting, S., Müller, B., Bähr, M., Weise, J., 2007. Enhanced poly(ADP-ribose) polymerase-1 activation contributes to recombinant tissue plasminogen activator-induced aggravation of ischemic brain injury in vivo. J. Neurosci. Res. 85, 1734–1743.
Del Zoppo, G.J., Mabuchi, T., 2003. Cerebral microvessel responses to focal ischemia. J. Cereb. Blood Flow Metab. 23, 879–894.
Endres, M., Wang, Z.Q., Namura, S., Waeber, C., Moskowitz, M.A., 1997. Ischemic brain injury is mediated by the activation of poly(ADP-ribose)polymerase. J. Cereb. Blood Flow Metab. 17, 1143–1151.
Gidday, J.M., Gasche, Y.G., Copin, J.C., Shah, A.R., Perez, R.S., Shapiro, S.D., Chan, P.H., Park, T.S., 2005. Leukocyte-derived matrix metalloproteinase-9 mediates blood-brain barrier breakdown and is proinflammatory after transient focal cerebral ischemia. Am. J. Physiol. Heart Circ. Physiol. 289, H558–H568.
Golanov, E.V., Reis, D.J., 1995. Contribution of cerebral edema to the neuronal salvage elicited by stimulation of cerebellar fastigial nucleus after occlusion of the middle cerebral artery in rat. J. Cereb. Blood Flow Metab. 15, 172–174.
Ha, H.C., Hester, L.D., Snyder, S.H., 2002. Poly(ADP-ribose) polymerase-1 dependence of stress-induced transcription factors and associated gene expression in glia. Proc. Natl. Acad. Sci. U. S. A. 99, 3270–3275.
Haddad, M., Rhinn, H., Bloquel, C., Coqueran, B., Szabo, C., Plotkine, M., Scherman, D., Margaill, I., 2006. Anti-inflammatory effects of PJ34, a poly(ADP-ribose) polymerase inhibitor, in transient focal cerebral ischemia in mice. Br. J. Pharmacol. 149, 23–30.
Hamann, G.F., Okada, Y., del Zoppo, G.J., 1996. Hemorrhagic transformation and microvascular integrity during focal cerebral ischemia/reperfusion. J. Cereb. Blood Flow Metab. 16, 1373–1378.
Hamby, A.M., Suh, S.W., Kauppinen, T.M., Swanson, R.A., 2007. Use of a poly(ADP-ribose) polymerase inhibitor to suppress inflammation and neuronal death after cerebral ischemia-reperfusion. Stroke 38, 632–636.
Hassa, P.O., Hottiger, M.O., 1999. A role of poly (ADP-ribose) polymerase in NF-kappaB transcriptional activation. Biol. Chem. 380, 953–959.
Heo, J.H., Lucero, J., Abumiya, T., Koziol, J.A., Copeland, B.R., del Zoppo, G.J., 1999. Matrix metalloproteinases increase very early during experimental focal cerebral ischemia. J. Cereb. Blood Flow Metab. 19, 624–633.
Hornig, C.R., Bauer, T., Simon, C., Trittmacher, S., Dorndorf, W., 1993. Hemorrhagic transformation in cardioembolic cerebral infarction. Stroke 24, 465–468.
Iwashita, A., Tojo, N., Matsuuara, S., Yamasaki, S., Kamijo, K., Ishida, J., Yamamoto, H., Hattori, K., Matsuoka, N., Mutoh, S., 2004. A novel and potent poly(ADP-ribose) polymerase-1 inhibitor, FR247304 (5-chloro-2-[3-(4-phenyl-3,6-dihydro-1(2H)-pyridinyl)propyl]-4(3H)-quinazolinone), attenuates neuronal damage in in vitro and in vivo models of cerebral ischemia. J. Pharmacol. Exp. Ther. 310, 425–436.
Kauppinen, T.M., Swanson, R.A., 2005. Poly(ADP-ribose) polymerase-1 promotes microglial activation, proliferation, and matrix metalloproteinase-9-mediated neuron death. J. Immunol. 174, 2288–2296.
Kofler, J., Otsuka, T., Zhang, Z., Noppens, R., Grafe, M.R., Koh, D.W., Dawson, V.L., de Murcia, J.M., Hurn, P.D., Traystman, R.J., 2006. Differential effect of PARP-2 deletion on brain injury after focal and global cerebral ischemia. J. Cereb. Blood Flow Metab. 26, 135–141.
Koh, S.H., Chang, D.I., Kim, H.T., Kim, J., Kim, M.H., Kim, K.S., Bae, I., Kim, H., Kim, D.W., Kim, S.H., 2005. Effect of 3-aminobenzamide, PARP inhibitor, on matrix metalloproteinase-9 level in plasma and brain of ischemic stroke model. Toxicology 214, 131–139.
Leinhase, I., Schmidt, O.I., Thurman, J.M., Hossini, A.M., Rozanski, M., Taha, M.E., Scheffler, A., John, T., Smith, W.R., Holers, V.M., Stahel, P.F., 2006. Pharmacological complement inhibition at the C3 convertase level promotes neuronal survival, neuroprotective intracerebral gene expression, and neurological outcome after traumatic brain injury. Exp. Neurol. 199, 454–464.
Lenzser, G., Kis, B., Snipes, J.A., Gaspar, T., Sandor, P., Komjati, K., Szabo, C., Busija, D.W., 2007. Contribution of poly(ADP-ribose) polymerase to postischemic blood-brain barrier damage in rats. J. Cereb. Blood Flow Metab. 27, 1318–1326.
Lyden, P.D., Zivin, J.A., 1993. Hemorrhagic transformation after cerebral ischemia: mechanisms and incidence. Cerebrovasc. Brain Metab. Rev. 5, 1–16.
Montaner, J., Alvarez-Sabin, J., Molina, C.A., Angles, A., Abilleira, S., Arenillas, J., Monasterio, J., 2001. Matrix metalloproteinase expression is related to hemorrhagic transformation after cardioembolic stroke. Stroke 32, 2762–2767.
Mun-Bryce, S., Rosenberg, G.A., 1998. Matrix metalloproteinases in cerebrovascular disease. J. Cereb. Blood Flow Metab. 18, 1163–1172.
Nguewa, P.A., Fuertes, M.A., Valladares, B., Alonso, C., Perez, J.M., 2005. Poly(ADP-ribose) polymerases: homology, structural domains and functions. Novel therapeutical applications. Prog. Biophys. Mol. Biol. 88, 143–172.
Okada, Y., Yamaguchi, T., Minematsu, K., Miyashita, T., Sawada, T., Sadoshima, S., Fujishima, M., Omae, T., 1989. Hemorrhagic transformation in cerebral embolism. Stroke 20, 598–603.
Paxinos, G., Franklin, K., 2001. The Mouse Brain in Stereotaxic Coordinates. Academic Press, San Diego.
Planas, A.M., Sole, S., Justicia, C., 2001. Expression and activation of matrix metalloproteinase-2 and -9 in rat brain after transient focal cerebral ischemia. Neurobiol. Dis. 8, 834–846.
Romanic, A.M., White, R.F., Arleth, A.J., Ohlstein, E.H., Barone, F.C., 1998. Matrix metalloproteinase expression increases after cerebral focal ischemia in rats: inhibition of matrix metalloproteinase-9 reduces infarct size. Stroke 29, 1020–1030.
Rosell, A., Ortega-Aznar, A., Alvarez-Sabin, J., Fernandez-Cadenas, I., Ribo, M., Molina, C.A., Lo, E.H., Montaner, J., 2006. Increased brain expression of matrix metalloproteinase-9 after ischemic and hemorrhagic human stroke. Stroke 37, 1399–1406.
Rosenberg, G.A., Navratil, M., 1997. Metalloproteinase inhibition blocks edema in intracerebral hemorrhage in the rat. Neurology 48, 921–926.
Rosenberg, G.A., Estrada, E.Y., Dencoff, J.E., 1998. Matrix metalloproteinases and TIMPs are associated with blood-brain barrier opening after reperfusion in rat brain. Stroke 29, 2189–2195.
Rosenberg, G.A., Cunningham, L.A., Wallace, J., Alexander, S., Estrada, E.Y., Grossetete, M., Razhagi, A., Miller, K., Gearing, A., 2001. Immunohistochemistry of matrix metalloproteinases in reperfusion injury to rat brain: activation of MMP-9 linked to stromelysin-1 and microglia in cell cultures. Brain Res. 893, 104–112.
Sumii, T., Lo, E.H., 2002. Involvement of matrix metalloproteinase in thrombolysis-associated hemorrhagic transformation after embolic focal ischemia in rats. Stroke 33, 831–836.
Takahashi, K., Pieper, A.A., Croul, S.E., Zhang, J., Snyder, S.H., Greenberg, J.H., 1999. Post-treatment with an inhibitor of poly(ADP-ribose) polymerase attenuates cerebral damage in focal ischemia. Brain Res. 829, 46–54.
Tejima, E., Zhao, B.Q., Tsuji, K., Rosell, A., van Leyen, K., Gonzalez, R.G., Montaner, J., Wang, X., Lo, E.H., 2007. Astrocytic induction of matrix metalloproteinase-9 and edema in brain hemorrhage. J. Cereb. Blood Flow Metab. 27, 460–468.
Wang, J., Tsirka, S.E., 2005. Neuroprotection by inhibition of matrix metalloproteinases in a mouse model of intracerebral haemorrhage. Brain 128, 1622–1633.
Yang, Y., Estrada, E.Y., Thompson, J.F., Liu, W., Rosenberg, G.A., 2007. Matrix metalloproteinase-mediated disruption of tight junction proteins in cerebral vessels is reversed by synthetic matrix metalloproteinase inhibitor in focal ischemia in rat. J. Cereb. Blood Flow Metab. 27, 697–709.
Yu, S.W., Wang, H., Dawson, T.M., Dawson, V.L., 2003. Poly(ADP-ribose) polymerase-1 and apoptosis inducing factor in neurotoxicity. Neurobiol. Dis. 14, 303–317.
Zhang, J.W., Gottschall, P.E., 1997. Zymographic measurement of gelatinase activity in brain tissue after detergent extraction and affinity-support purification. J. Neurosci. Methods 76, 15–20.
Zhang, L., Zhang, Z.G., Zhang, R.L., Lu, M., Krams, M., Chopp, M., 2003. Effects of a selective CD11b/CD18 antagonist and recombinant human tissue plasminogen activator treatment alone and in combination in a rat embolic model of stroke. Stroke 34, 1790–1795.