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Research report

Beneficial effects of PJ34 and INO-1001, two novel water-soluble poly(ADP-ribose) polymerase inhibitors, on the consequences of traumatic brain injury in rat

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Abstract

Traumatic brain injury produces peroxynitrite, a powerful oxidant which triggers DNA strand breaks, leading to the activation of poly(ADP-ribose)polymerase-1 (PARP-1). We previously demonstrated that 3-aminobenzamide, a PARP inhibitor, is neuroprotective in a model of traumatic brain injury induced by fluid percussion in rat, suggesting that PARP-1 could be a therapeutic target. In order to confirm this hypothesis, we investigated the effects of PJ34 and INO-1001, two PARP inhibitors from structural classes other than benzamide, on the post-traumatic consequences. Pre- and post-treatments with PJ34 (30 mg/kg/day) and INO-1001 (10 mg/kg/day) decrease the neurological deficit at 3 days post-injury and this deficit is still reduced at 7 days. These neurological recovery-promoting effects are associated with the inhibition of PARP-1 activation caused by trauma, as demonstrated by abolishment of immunostaining of poly(ADP-ribose). Thus, the present work strengthens strongly the concept that PARP-1 inhibition may be a suitable approach for the treatment of brain trauma.

Theme: Disorders of the nervous system
Topic: Trauma

Keywords: Inhibitors; Neurological deficit; Neuroprotection; Poly(ADP-ribose) polymerase; Rat; Traumatic brain injury

1. Introduction

Poly(ADP-ribose)polymerase 1 (PARP-1, EC 2.4.2.30) is an abundant constitutive nuclear enzyme which is implicated in such physiological processes as DNA repair [9], genomic stability [7], and apoptosis [16]. Moreover, PARP-1 has been shown to mediate necrotic cell death in response to excessive DNA damage under pathological conditions [16]. PARP, when activated in response to oxidative stress-induced DNA strand breaks, initiates an energy-consuming cycle by transferring ADP-ribose units from NAD to a set of nuclear proteins including histones, several chromatin-binding proteins, and PARP-1 itself [9]. This process results in rapid depletion of intracellular NAD, in a loss of ATP as it is used to synthesize new NAD, and finally to cell death [30]. Activation of PARP-1 represents an important mechanism of cerebral tissue damage in various pathological conditions associated with increased oxidative stress, including cerebral ischemia [10,11], experimental allergic encephalomyelitis [35], parkinsonism induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine [8,26], and spinal cord injury [34]. Moreover, the PARP inhibitors, such as benzamide analogues

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and isoquinoline derivatives, have been used previously to investigate the role of this nuclear enzyme in traumatic brain injury [5,24,33]. Most commercially available PARP inhibitors (e.g., 3-aminobenzamide or nicotinamide) have low potency and short cellular residence time [4]. The aim of this study was to strengthen the hypothesis that pharmacological inhibition of PARP-1 might be a suitable approach for the treatment of traumatic brain injury. Indeed, we investigated the effects of two novel potent water-soluble PARP inhibitors, the phenanthridinone-based PJ34 (*N*-(6-oxo-5,6-dihydro-phenanthridin-2-yl)-*N*,*N*-dimethylacetamide) and the isoindolinone-based INO-1001 (confidential structure), on the post-traumatic consequences induced by lateral fluid percussion in rat.

2. Materials and methods

Animal care complied with the French regulations covering the protection of animals used for experimental and other scientific purposes (D2001-486), with the European Community regulations (Official Journal of European Community L358 12/18/1986), and it conformed to the *Guide for the Care and Use of Laboratory Animals* published by U.S. National Institutes of Health (NIH Publication n° 85–23, revised 1996).

2.1. Materials

Male Sprague–Dawley rats were supplied by Iffa-Credo (L'Arbresle, France). Diaminobenzidine, hydrogen peroxide, gelatine, and sucrose were purchased from Sigma Chemicals Corporation (Saint Louis, MO, USA). Chicken antibody against poly(ADP-ribose) antibody was a generous gift from Dr. John R. Simon (Tulip BioLabs, Inc. West Point, PA, USA). Goat serum, biotinylated goat anti-chicken immunoglobulin G and streptavidin–biotin peroxidase complex (Vectastain Elite ABC) were obtained from Vector Laboratories (Burlingame, CA, USA). The phenanthridinone-based PARP inhibitor PJ34 (*N*-(6-oxo-5,6-dihydro-phenanthridin-2-yl)-*N*,*N*-dimethylacetamide) and the isoindolinone-based PARP inhibitor INO-1001 (confidential structure) were synthesized as previously described [13,18,20,36].

2.2. Fluid percussion-induced brain injury

Male Sprague–Dawley rats (weighing 300–350 g) were anaesthetised with chloral hydrate (400 mg/kg, i.p.) and placed on a stereotaxic frame. During surgery, animals were positioned on a heating blanket (Harvard, UK) to maintain body normothermia (37.5 ± 0.5 °C).

Traumatic brain injury of moderate severity was induced by fluid percussion using the protocol initially described by Toulmond et al. [39] and modified by Wahl et al. [42]. The scalp was incised and a 3 mm craniotomy was made lateral to the right temporoparietal cortex with a dental drill (coordinates: 3.5 mm anterior and 6 mm above the interaural line, [29]), taking care to leave the dura mater intact. A 3 mm diameter polyethylene tube was placed over the dura mater, fixed securely into the craniotomy site with dental cement (Perfex, USA), and connected to a solenoid valve (Danfoss, Denmark). The opposite end of the valve was connected to a high performance liquid chromatography pump (Gilson). The system was filled with sterile water, providing a calibrated outflow pressure of 1.6–1.8 bar. A solenoid valve opening for 20 ms and controlled with a timer (Omron, Japan) triggered the percussion directly onto the dura mater. The applied cortical pressure was measured extracranially by a pressure transducer (Emka Technologies, France) connected to an oscilloscope (DSO 400, Gould, France). Immediately after fluid percussion, the tube was removed, the scalp sutured, and the animal was returned to its home cage in a room warmed at 26–28 °C to recover from the anaesthesia. Thereafter, rats were group-housed under temperature- and light-controlled conditions with food and water ad libitum.

2.3. Preparation of brain tissue for immunohistochemistry

Rats were anaesthetised with sodium pentobarbital (60 mg/kg, i.p.) and perfused transcardially with 200 ml of heparinised saline followed by 500 ml of phosphate-buffered saline (PBS, 0.1 M, pH 7.4) containing 4% paraformaldehyde. The brains were then removed, kept for 1 h in the same fixative solution, and placed in two successive 10% sucrose solutions, each for 24 h. The brains were rapidly frozen in isopentane and stored at −40 °C until used. Serial coronal sections were cut (20 μm) on a cryostat (−18 °C, Jung CM 3000, Leica), collected on gelatine-coated slides, and processed for immunohistochemistry.

2.4. Immunohistochemistry of poly(ADP-ribose)

Before starting immunostaining, sections were dried for 30 min at room temperature then fixed in 4% paraformaldehyde. All sections were incubated for 15 min in 1.5% (vol/vol) hydrogen peroxide in methanol before the primary antibody was added in order to quench the endogenous peroxidase activity. Non-specific binding sites were blocked using 2% (vol/vol) normal goat serum in PBS for 90 min at 37 °C. Sections were then incubated overnight at 4 °C with the primary antibody (chicken antibody against poly(ADP-ribose), diluted 1:250 in PBS). Specific labelling was detected by incubating the sections for 30 min at 30 °C with the secondary antibody (biotinylated goat anti-chicken IgG, 1:200 dilution) and amplified with streptavidin–biotin peroxidase complex. Diaminobenzidine was used as chromogen. Sections were counterstained with nuclear fast red, dehydrated, and mounted. Photomicrographs were taken. As negative controls, alternative sections were incubated without the primary antibody. All immunohistochemical samples were examined by an investigator in a blinded fashion.