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Research Report

Neuroprotective effects of a novel water-soluble poly(ADP-ribose) polymerase-1 inhibitor, MP-124, in in vitro and in vivo models of cerebral ischemia

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ABSTRACT

Cerebral ischemia induces excessive activation of poly(ADP-ribose) polymerase-1 (PARP-1), leading to neuronal cell death and the development of post-ischemic dysfunction. Blockade of PARP-related signals during cerebral ischemia has become a focus of interest as a new therapeutic approach for acute stroke treatment. The purpose of the present study was to examine the pharmacological profiles of MP-124, a novel water-soluble PARP-1 inhibitor, and its neuroprotective effects on ischemic injury in vitro and in vivo. MP-124 demonstrated competitive inhibition of the PARP-1 activity of human recombinant PARP-1 enzyme (Ki = 16.5 nmol/L). In P388D_1 cells, MP-124 inhibited the LDH leakage induced by H_2O_2 in a concentration-dependent manner. (IC_{50} = 20.8 nmol/L). In rat primary cortical neurons, MP-124 also inhibited the NAD depletion and polymerized ADP-ribose formation induced by H_2O_2 exposure. Moreover, we investigated the neuroprotective effects of MP-124 in rat permanent and transient stroke models. In the rat permanent middle cerebral artery occlusion (MCAO) model, MP-124 was administered intravenously for 24 h from 5 min after the onset of MCAO. MP-124 (1, 3 and 10 mg/kg/h) significantly inhibited the cerebral infarction in a dose-dependent manner (18, 42 and 48%). In rat transient MCAO model, MP-124 was administered intravenously from 30 min after the onset of MCAO. MP-124 (3 and 10 mg/kg/h) significantly reduced the infarct volume (53% and 50%). The present findings suggest that MP-124 acts as a potent neuroprotective agent in focal ischemia and its actions can be attributed to a reduction in NAD depletion and PAR formation.

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Abbreviations: ADP, adenine dinucleotide phosphate; LDH, lactate dehydrogenase; MCA, middle cerebral artery; NAD, nicotinamide adenine dinucleotide; PAR, polymerized ADP-ribose; PARP, poly(ADP-ribose) polymerase

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1. Introduction

Stroke is the second leading cause of death and the first major cause of disability in developed countries. Following the onset of stroke, low oxygen and poor nutrient supply lead to excessive release of excitatory amino acids, calcium overload and the generation of reactive oxygen species (ROS) in the ischemic regions. ROS induce massive DNA damage in the neurovascular units, and poly(ADP-ribose) polymerase-1 (PARP-1) overactivation causes energy failure similar to nicotinamide adenine dinucleotide (NAD) rundown and ATP consumption resulting in cell death (Chan, 2001; Chiarugi, 2002; Chiarugi, 2005). Moreover, PARP-1 activation is essential for the activation of NF-κB and indispensable for the transfer of apoptosis-inducing factor from the mitochondria into the nucleus during ischemia (Moroni, 2008), suggesting that PARP-1 is involved in apoptosis and inflammation as well as ischemic cell death in the pathology of stroke. Focus has therefore been directed towards the importance of effectively controlling PARP-1-related signals in stroke therapy.

PARP-1 enjoys an important role as a key player in cellular defense mechanisms. In this context, PARP-1 is activated by DNA strand breakage due to several biological processes and catalyzes the poly(ADP-ribosyl)ation of nuclear proteins and PARP-1 itself. This reaction is an energetically expensive process, causing rapid depletion of cellular NAD. Under physiological conditions, it contributes to maintenance of the genomic integrity of the cells such as DNA replication and gene rearrangement (D'Amours et al., 1999; Burkle, 2001; Koh et al., 2005a, 2005b). Under pathophysiological conditions such as ischemia, the more the DNA is damaged, the more actively this repair mechanism operates. One of the consequences is that the cells markedly run out of NAD tissue stores resulting in decreases in glycolysis, mitochondrial electron transport and ATP formation (Pacher and Szabo, 2008). Finally, the situation threatens cell survival at the cost of energy synthesis for DNA repair.

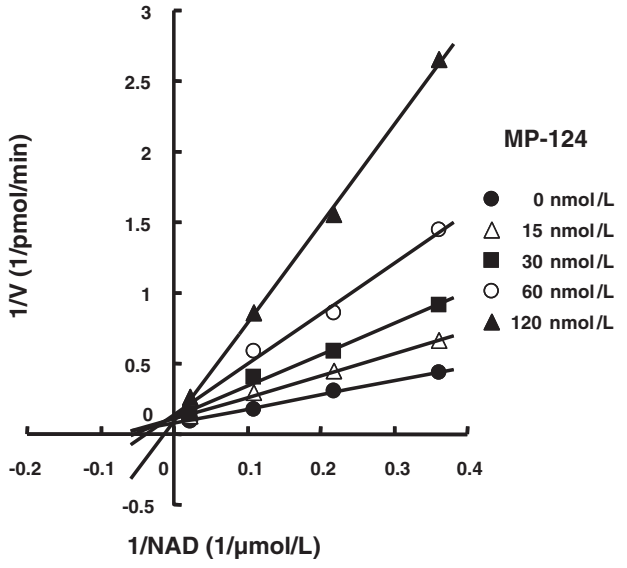

Fig. 1 – Enzyme kinetic analysis of PARP inhibition by MP-124. Lineweaver–Burk plot analysis demonstrated that the inhibitory action of MP-124 was competitive.

It has been reported using PARP-1 knockout mice that stroke-induced DNA damage overactivates PARP-1, exhausts ATP production, and abrogates infarct volume and long-term stroke recovery (Endres et al., 1997; Goto et al., 2002). 3-Animobenzamide (3-AB), a classical PARP inhibitor reduced infarct volume and ameliorated long-term stroke recovery in animal stroke models (Takahashi et al., 1999; Ding et al., 2001; Yang et al., 2002; Couturier et al., 2003; Koh et al., 2004). PJ-34 (*N*-(6-oxo-5,6-dihydrophenanthridin-2-yl)-*N*,*N*-dimethylacetamide), a selective PARP inhibitor induced neuroprotective effects in in vitro and in vivo models of stroke (Abdelkarim et al., 2001). However, such PARP inhibitors are poor in their enzyme-inhibiting activity, cell permeability, specificity, or safety. Recently, we identified and developed a novel water-soluble PARP-1 inhibitor, MP-124 (an isoquinoline derivative) (Fujio et al., 2004). To assess the pharmacological profiles of MP-124 in cerebral ischemic injury, we investigated the neuroprotective effects of MP-124 employing PARP-1 enzyme assay, H_2O_2-induced neurotoxicity, and rat permanent and transient MCAO models.

2. Results

2.1. *Inhibition of PARP-1 activity and the cell protective action in P388D$_1$ cells*

As shown in Fig. 1, Lineweaver–Burk plots of MP-124 in the enzymatic kinetic analysis revealed that MP-124 competitively inhibited PARP-1 activity. The Ki value of MP-124 was 16.5 (nmol/L). As summarized in Table 1, we evaluated the inhibition of PARP-1 activity in the case of human recombinant PARP-1 and the efficacy of MP-124 for the prevention of cell death in P388D$_1$ cells. MP-124 demonstrated more powerful inhibition of PARP-1 activity than did other well known PARP inhibitors, PJ-34 and 3-AB. The IC_{50} values were 28.0, 40.6 and 22,700 nmol/L, respectively. As an index of cell injury, the H_2O_2-induced LDH leakage was estimated in P388D$_1$ cells which do not produce endogenous O_2^-. At 1 h after treatment with 0.1 mmol/L of H_2O_2, the LDH activity induced by the H_2O_2 increased to about 50% above the level in the control. MP-124, PJ-34 and 3-AB at 15 min after exposure to H_2O_2 inhibited the LDH leakage in a concentration-dependent manner. The IC_{50} value for MP-124 was 20.8 nmol/L, which was about 3 and 1000 times more potent than that for PJ-34 and 3-AB, respectively.

2.2. *Neuroprotective action and its mechanism in rat cortical neurons*

We assessed the neuroprotective effect of MP-124 in rat cortical neurons. As shown in Fig. 2, H_2O_2 exposure increased the optical density (OD) of the LDH leakage (from 0.147±0.021 to 0.330±0.01), while MP-124 at 0.1–3 μmol/L decreased the OD of the LDH leakage in a dose-dependent manner (from 0.330±0.01 to 0.181±0.006, 0.224±0.011, 0.241±0.006 and 0.266±0.016) and demonstrated a statistically significant action at a concentration of 0.3 μmol/L or greater. The rate of inhibition by MP-124 at 0.1–3 μmol/L was 34.0%–81.3%. The IC_{50} value for MP-124 was 365 nmol/L. Since excessive NAD expenditure in injured neuronal cells has been reported to contribute to PARP-induced cell death (Cole and Perez-Polo, 2002), the H_2O_2-induced NAD reduction was measured in rat cortical neurons to assess the mechanism of the neuroprotective