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Full paper

DJ-1-dependent protective activity of DJ-1-binding compound no. 23 against neuronal cell death in MPTP-treated mouse model of Parkinson's disease

Kazuko Takahashi-Niki [a], Ayako Inafune [b], Naruyuki Michitani [b], Yoshitaka Hatakeyama [b], Kotaro Suzuki [b], Mai Sasaki [b], Yoshihisa Kitamura [c], Takeshi Niki [d], Sanae M.M. Iguchi-Ariga [d], Hiroyoshi Ariga [a, *]

[a] Faculty of Pharmaceutical Sciences, Hokkaido University, Kita-ku, Sapporo 060-0812, Japan
[b] Central Research Laboratory, New Drug Research Center, Inc., Toiso, Eniwa 061-1405, Japan
[c] Department of Clinical and Translational Physiology, Kyoto Pharmaceutical University, 5 Nakauchi-cho, Misasagi, Yamashina-ku, Kyoto 607-8414, Japan
[d] Faculty of Agriculture, Hokkaido University, Kita-ku, Sapporo 060-8589, Japan

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ABSTRACT

Parkinson's disease (PD) is caused by dopaminergic cell death in the substantia nigra, leading to a reduced level of dopamine in the striatum. Oxidative stress is one of the causes of PD. Since symptomatic PD therapies are used, identification of compounds or proteins that inhibit oxidative stress-induced neuronal cell death is necessary. DJ-1 is a causative gene product of familial PD and plays a role in anti-oxidative stress reaction. We have identified various DJ-1-binding compounds, including compound-23, that restored neuronal cell death and locomotion defects observed in neurotoxin-induced PD models. In this study, wild-type and DJ-1-knockout mice were injected intraperitoneally with 1 mg/kg of compound-23 and then with 30 mg/kg of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) at 1 h after injection. Five days after administration, the effects of compound-23 on MPTP-induced locomotion deficits, on dopaminergic cell death and on brain dopamine levels were analyzed by rotor rod tests, by staining cells with an anti-TH antibody and by an HPLC, respectively. The results showed that compound-23 inhibited MPTP-induced reduction of retention time on the rotor rod bar, neuronal cell death in the substantia nigra and striatum and dopamine content in wild-type mice but not in DJ-1-knockout mice, indicating a DJ-1-dependent effect of compound-23.

1. Introduction

Parkinson's disease (PD) is a chronic neurodegenerative disease caused by dopaminergic cell death, and genetic and environmental factors are thought to affect the onset of PD. Onset of PD is thought be induced, to some degree, by oxidative stress, but the precise mechanisms are still not known. Although a precursor of dopamine, inhibitors of dopamine degradation and dopamine releasers have been used for PD therapy, cell death progresses during treatment. Identification of compounds or proteins that inhibit oxidative stress-induced neuronal cell death is necessary.

DJ-1 was first identified by our group as a novel oncogene product (1) and later found to be a causative gene product of a familial form of PD, PARK7 (2). DJ-1 plays various roles, including transcriptional regulation (3–9) and anti-oxidative stress reaction (10–13), and loss of its function is thought to result in the onset of PD. DJ-1 has three cysteines at amino acid numbers 46, 53, and 106 (C46, C53, and C106, respectively). Although oxidation of C106 is necessary for DJ-1 to exert its activity (12–15), excessive oxidation of C106 is thought to render DJ-1 inactive (16,17), and such oxidized DJ-1 has been observed in patients with the sporadic form of PD and Alzheimer disease (18,19).

We have shown that administration of DJ-1 protein dramatically reduced dopaminergic cell death and inhibited locomotion deficit in a PD model in rats where 6-hydroxydopamine (6-OHDA) had been injected (20), suggesting that DJ-1 is a pharmaceutical target for PD. Furthermore, we identified compounds that bind to the

* Corresponding author. Tel.: +81 11 706 3745; fax: +81 11 706 4988.
E-mail address: hiro@pharm.hokudai.ac.jp (H. Ariga).
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C106 region of DJ-1 and prevent excessive oxidation of C106 of DJ-1, and these compounds including compounds A and B, like DJ-1 protein, prevented oxidative stress-induced dopaminergic cell death and restored locomotion deficit in a PD model in rats (21–23). These compounds were found by screening the University Compound library, which contains approximately 30,000 compounds. We further screened DJ-1-binding compounds from the Zinc compound library that contains approximately 2,500,000 compounds. Of the compounds identified, N-[4-(8-methyl(4-hydroimidazo[1,2-a]pyridin-2-yl))phenyl](3,4,5-trimethoxyphenyl)carboxamide, which is DJ-1-binding compound-23 (comp-23) (Fig. 1A), protected oxidative stress-induced cell death both in cultured cells and in PD models in rats and mice, and the protective activity of comp-23 seemed to be stronger than that of compound B (24). Specificity of compound-23 toward DJ-1 was suggested by experiments using DJ-1-knockdown cells (24).

In this study, to further examine the specificity of compound-23 toward DJ-1, wild-type and DJ-1-knockout mice were injected with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a neurotoxin that inhibits activity of mitochondrial complex I, in the presence or absence of compound-23, and then the effects of compound-23 on a locomotion deficit, dopaminergic cell death and dopamine levels in the striatum and substantia nigra of DJ-1-knockout mice were examined. The results showed that while MPTP-induced deficit in wild-type mice were restored by administration of compound-23, DJ-1-knockout mice showed little effect,

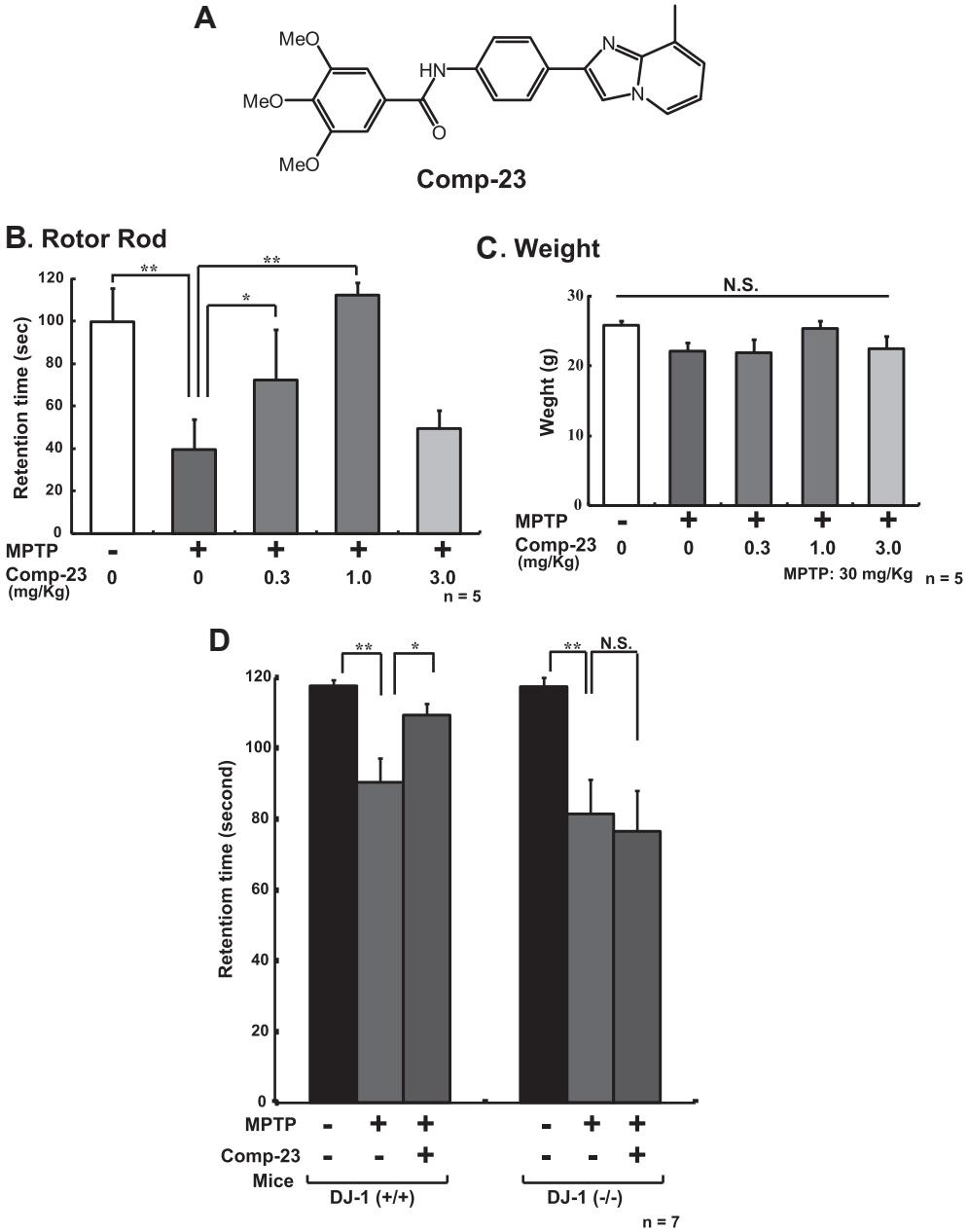

Fig. 1. Effect of compound-23 on MPTP-induced decrease in locomotion behavior of mice. A. Chemical structure of compound-23. B and C. DJ-1(+/+) C57BL/6 mice were injected by i.p. with various doses of compound-23. One hr after injection, mice were injected with 30 mg/kg MPTP. This combination of injection was carried out every day for 4 days, and rotor-rod tests of mice were carried out at 5 days after first injection (B). Mouse weight was also measured (C). The number of experiments (n) is 5. D. DJ-1(+/+) and DJ-1(−/−) mice were injected with compound-23 and MPTP as described in the legend for Fig. 1B and C, and rotor-rod tests of mice were carried out. n is 7. Significance: $^{*}p < 0.05$, $^{**}p < 0.01$. N.S.: Non-significant.