Brain Permeable Iron Chelators VK-28 and M30 Rescue Degenerated Nigral Dopamine Neurons in Animal Model of PD

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INTRODUCTION

A pivotal role of iron in the pathogenesis of PD has been hypothesized since numerous studies have shown a progressive accumulation of iron and ferritin in PD patients, specifically in the SN pars compacta (1, 2). The major mechanism of iron-mediated damage in nigrostriatal neurons is related to the fact that iron is a significant generator of reactive oxygen species (ROS) (3). Recently, iron chelation has been shown to protect neurodegeneration in PD animal model induced by N-methyl-4-pheny-1, 2, 3, 6-tetrahydropyridine (MPTP). Chelation of iron, either by the oral administration of iron chelators or over-expression of the heavy subunit of the iron-binding protein ferritin, protected DA neurons, maintained DA production, and prevented the motor deficits associated with MPTP administration (4). More recently, iron has been found in the rim of Lewy body where α-synuclein, ubiquitin, and tyrosine hydroxylase (TH) are also present (5). Furthermore, there is also evidence that iron overload facilitates the fibrillation of human α-synuclein and impairs the proteasomal activity (6, 7).

In the present study, we further evaluated the neurorescue properties of two newly developed brain permeable iron chelators VK-28 [5-(4-(2-hydroxyethyl) piperazin-1-yl (methyl)-8-hydroxyquinoline] and M30 [5-(N-methyl-N-propargyaminomethyl)-8-hydroxyquinoline] in vivo, against nigrostriatal degeneration induced by lactacystin.

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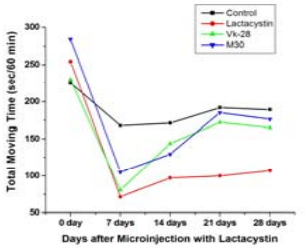
METHODS

60 C57BL/6 mice were bilateral injected with lactacystin (1.25ug in 2ul NS vs. vehicle control at equal volume) into medial forebrain bundle (MFB) using the following coordinates (in mm): (1.34 posterior, ±1.17 lateral, and 5.1 ventral from bregma). Continuous intraperitoneal administration of VK-28 (5mg/kg/day) or M30 (5mg/kg/day) was introduce 7 days after the microinjection. The animals were sacrificed 28 days after the microinjection with lactacystin. Locomotive activity and rotarod tests were performed weekly to evaluate the behavioral changes. Neurode-generarion and nigrostriatal deficiency induced by lactacystin were indicated by immunostaining of TH-positive cells and HPLC assay of DA and its metabolites. Proteasome activity assay were applied to determine the proteasomal dysfunction produced by lactacystin. Tissue iron concentrations in midbrain were determined spectrophotometrically to manifest the change of iron after lactacystin lesion. Immunostaining of CD11b-positive cells and GFAP-positive cells was introduced to indicate the microglial activation after microinjection of lactacystin.

Fig.1 Time-dependent locomotive changes



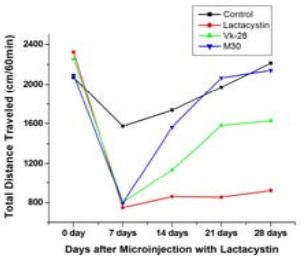


Fig.2 Time-dependent Rotarod Performance

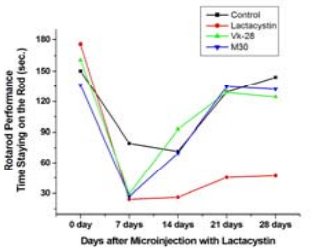


Fig.3 Capabilities of VK-28 and M30 against DA neuron lost

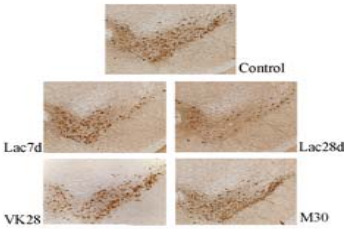


Fig.4 Capabilities of VK-28 and M30 against depletion of DA and its metabolites

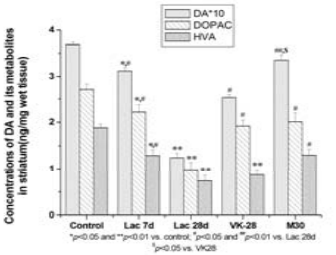


Fig.5 Proteasome activity in ventral midbrain

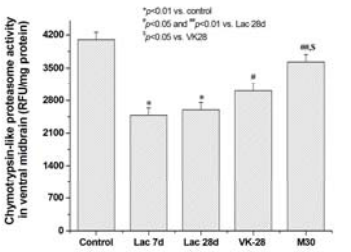


Fig.6 Iron concentration in ventral midbrain

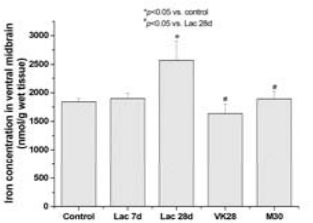
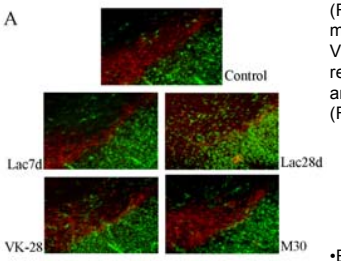
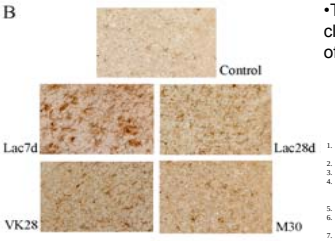


Fig.7 VK-28 and M30 against glial activation





RESULTS

7 days after bilateral microinjection with lactacystin (1.25ug/side) into MFB, significant drops of locomotive activity and rotarod performance were documented, and the defects remain litter improved at the end of the experiment(Fig.1,2). A loss of 22.5% DA neurons was traced in the SN on 7th day, which was up to 67.0% on the 28th day ($p<0.01$) (Fig.3). Accordingly, a progressive decrease of DA and its metabolites were found in striatum(Fig.4). Up to 30% inhibition of proteasomal activity ($p<0.05$) was evident in ventral midbrain since 7th day until the 28th day(Fig.5). The iron concentration was shown to be elevated by 32.7% 28 days after lactacystin lesion (Fig. 6). Remarkable activation of microglia were seen on the 7th day and still could be seen 28 days after the microinjection of lactacystin (Fig.7)..

Treatment with VK-28 or M30 could effectively alleviate the movement decline (Fig.1,2). Both VK-28 or M30 exerted significant neurorescue effects against lactacystin-induced lost of DA neuron and depletion of DA and its metabolites (Fig.3,4). The drugs attenuated DA neurons loss by 63.2% and 82.2%, respectively ($p< 0.01$) and rescued up to 68.5% and 90.8% of control striatal DA level, respectively.. Furthermore, both drugs alleviated proteasomal inhibition (Fig.5) and iron accumulation (Fig. 6), as well as inhibited microglial activation (Fig. 7). However, as a derivative of both VK-28 and rasagiline, M30 was found to be more potent in rescuing DA neurons, restoring of DA and its metabolites, and alleviating proteasomal inhibition and iron accumulation (Fig. 3-6).

CONCLUSIONS

- Both VK-28 and M30 have showed marked rescuing effects on DA neurons against neurodegeneration induced by ubiquitin-proteasome system (UPS) failure.
- Bifunctional drug M30 seems to be more potent than VK-28 in the UPS-impairment induced animal model of PD.
- The result of this study may warrant further preclinical and clinical tests both VK-28 and M30 potentials in the treatment of PD .

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