学 位 論 文 の 要 旨

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学 位 論 文 名　Upregulation of Protease-Activated Receptor-1 in Astrocytes in Parkinson Disease: Astrocyte-Mediated Neuroprotection through Increased Levels of Glutathione Peroxidase

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論 文 内 容 の 要 旨

INTRODUCTION

Parkinson disease (PD) is a neurodegenerative disorder characterized by the progressive and selective loss of dopaminergic neurons in the substantia nigra (SN). Although the neurodegeneration in SN could be induced by mitochondrial dysfunction, oxidative stress, excitotoxicity, apoptosis or inflammation, the clear mechanism of this chronic SN neuronal cell loss in PD remains elusive. Previous studies have demonstrated the proliferation of activated microglia and upregulation of immune/inflammatory cytokine expression in microglia in SN of human PD, while increase in number of astrocytes and glial fibrillary acid protein (GFAP) immunoreactivity was also found in the region. In SN pars compacta (SNpc) of PD brain, upregulation of brain-derived neurotrophic factor (BDNF) was demonstrated in astrocytes surrounding fragmented nigral neurons. It is suggested that activated microglia play a deleterious role in SN dopaminergic neurons, while it is possible that the SNpc astrocytes in patients with PD might have taken a role as a producer of neuroprotective molecules.

Thrombin has been known as one of the factors that induce glial activation, and thrombin-mediated activation of microglia induces increase of inflammatory factor and results in the degeneration of SN dopaminergic neurons. Thrombin is a mutifunctional serine protease that plays an important role in the coagulation cascade by converting fibrinogen to fibrin, and is generated from the precursor prothrombin, which is endogenously expressed in specific neurons of human, mouse and rat brain, including dopaminergic neurons in the SN. Recent studies have also demonstrated that thrombin retracts neuritis on neurons and induces morphologic change in

astrocytes *in vitro*. Generally, thrombin induces pleiotropic cellular responses through activation of protease-activated receptor (PAR)-1, -3 or -4. Thrombin converted from endogenous prothrombin can activate PARs in the disease process in CNS, and accumulation of thrombin was found in senile plaques in patients with Alzheimer disease. Activation of PARs by thrombin may occur in the disease progression process of PD. We investigated expression of PARs in SN of PD brains and cultures of human neurons, astrocytes, oligodendrocytes and microglia and then thrombin-mediated activation of human astrocytes in culture. In addition, we studied whether thrombin-activated astrocytes have neuroprotective effect for human cerebral neuron × human neuroblastoma hybrid neurons in culture.

MATERIAL AND METHODS

We investigated expression of PARs, prothrombin and thrombin in SNpc of PD (n = 10) and control subjects (n = 10) immunohistochemically, which were obtained from the autopsied brains and were fixed in formalin and embedded in paraffin, and gene expression of PARs in cultures of human neurons, astrocytes, oligodendrocytes and microglia, prepared from human fetal brain tissues (14-weeks gestation, with the approval of the University of British Columbia Clinical Screening Committee for Research involving Human Subjects) by RT-PCR. The double-immunostainings were performed for cell type-specific markers/PARs or cell type-specific markers/prothrombin.

Next we investigated thrombin-mediated activation of human astrocytes. Astrocytes were stimulated with thrombin in DMEM. We examined morphologic feature, the proliferation of astrocytes using MTT assay and the expression of glial cell line-derived growth factor (GDNF), glutathione peroxidase (GPx), nerve growth factor (NGF) and inflammatory cytokines/chemokine in astrocyte by RT-PCR after thrombin treatment. Also the production of GDNF or GPx in culture supernatants was determined by enzyme-linked immunosorbent assay (ELISA) kits specific for human GDNF or GPx.

Next we studied neuroprotective effect exerted by thrombin-activated astyrocytes. Confluent astrocyte cultures were washed twice with phosphate buffered saline, fed with fresh serum-free DMEM and grown for 48 hrs in the presence (GCM-Thr) or absence (GCM-NC) of thrombin (1 U/ml). Pooled GCMs were centrifuged at 3000 × g to remove cellular debris, sterilized by filter and aliquots were stored at −80°C until use. Human cerebral neuron × human neuroblastoma hybrid neurons (A1) were incubated with DMEM, GCM-Thr or GCM-NC containing thrombin for 48 hrs, and viability of A1 neurons was assessed by the MTT assay. A1 neurons were also incubated in GCM-Thr or GCM-Thr with GPx inhibitor mercaptosuccinic

acid (MS) containing thrombin.

RESULTS AND DISCUSSION

Immunohistochemical study revealed that expression of PAR-1 was demonstrated only in GFAP-positive astrocytes in SNpc, and the number of astrocytes expressing PAR-1 increased in SNpc of PD as compared with non-neurological control brain. Immunoreactivity for thrombin and prothrombin found in astrocytes and the vessel walls was stronger in SNpc of PD brains, which indicates that PAR-1 activation in consort with thrombin may be closely involved in initiation and progression of PD pathogenesis. *In vitro* study showed that only PAR-1 among three types of thrombin receptors was detected in purified populations of human astrocytes and neurons, but not in oligodendrocytes or microglia as determined by RT-PCR. These results suggest that the PAR-1 acts as a main thrombin receptor in human brain and that PAR-1 was chiefly expressed in astrocytes in human SNpc.

Thrombin treatment of human astrocytes in cultures induced morphologic change and marked proliferation of astrocytes. Increased gene expression of GDNF and GPx but no change in the expression of NGF and inflammatory cytokines/chemokine (IL-1β, IL-6, IL-8, MCP-1) was found in thrombin/PAR-1-activated astrocytes. ELISA study showed that GPx released from astrocytes was increased in a time- and dose-dependent manner in spite of small increase of GDNF.

Although thrombin showed neurotoxicity against A1 neurons in dose-dependent manner, the conditioned medium derived from thrombin-pretreated astrocyte cultures (GCM-Thr) promoted the survival of A1 neurons. The protective effect was completely inhibited with a GPx inhibitor, MS. GPx is known to be the primary neuronal defence against the main cytotoxic oxygen species that cause neuronal cell death, and the results indicate that GPx released from thrombin/PAR-1-activated astrocytes are responsible for neuroprotection of A1 neurons against thrombin cytotoxicity.

CONCLUSION

We demonstrated increase of PAR-1-positive astrocytes in SNpc of autopsied PD brain and neuroprotective effect of PAR-1-activated human astrocytes *in vitro*. The results indicate that the increased population of PAR-1-positive astrocytes found in SNpc of PD brain might play a role in protecting neurons from external or internal insults causing neuronal cell death and is a restorative move of the brain to delay or block the progression of PD pathology.