OP2-4-4

The Neuroprotective Effect of Macrophage Migration Inhibitory Factor (MIF) in Hypoxic Neuronal Cells

Su Hwan Bae[1*], In Kyung Hong[1], Dae Yul Kim[1†]

Asan Medical Center, Department of Rehabilitation Medicine[1]

Objective
Macrophage inhibitory factor (MIF) is a member of the inflammatory cytokine and is expressed in a variety of cells, including T cells, monocytes, and endothelial cells. Brain derived neurotrophic factor (BDNF) is known to be associated with neuroplasticity. Several previous studies have reported that MIF has a protective function during an ischemic event. However, little is known about the neuroprotective function of MIF in ischemic stroke. The purpose of this study is to demonstrate MIF is associated with neuroprotection in the human neuroblastoma cells with oxygen glucose deprivation model (OGD model)

Methods
The human neuroblastoma cell line SH-SY5Y (ATCC CRL-2266) were maintained in Dulbecco's Modified Eagle's Medium (DMEM, Gibco Life Technologies, Carlsbad, CA) supplemented with 10 % fetal bovine serum (FBS), 50U/ml penicillin, 50ug/ml streptomycin (Gibco), in a humidified incubator at 37'C and 5 % CO2. Cultures were transferred to a multi-gas incubator containing a gas mixture of 1% O2. The medium was replaced with a pre-warmed (37'C) glucose-free DMEM. The solution was bubbled with an anaerobic gas mix (95 % N2, 5 % CO2) for 1 hour. Cell cultures subjected to OGD were incubated in the solution at 37'C for a 4 h to produce oxygen deprivation (OGD) and then returned to the normal aerobic environment (OGD/R). At the same time, we administered 30ug/ml MIF recombinant or 50uM ISO-1 (MIF antagonist). Experimental parameters were assayed at 24 h following re-oxygenation solution. We assigned cell cultures to one of four groups: OGD 4h, OGD 4h/reoxygenation 24h, OGD 4h/reoxygenation 24hr with MIF, OGD 4h/reoxygenation 24hr with ISO-1. Then we analyzed the differences between 4 groups about BDNF, MIF by western blot. b-actin was used to match baseline in all groups. Then histological study was performed to observe expression levels of BDNF by immunocytochemistry.

Results
Figure 1 and Table 1 represent the MIF and BDNF expression levels of neuroblastoma cells from western blot. The expression levels of MIF in OGD 4h, OGD 4h/R24h group and the expression level of BDNF in MIF-OGD 4h/R24h group were significantly increased

than control group. On the other hand, the expression levels of MIF and BDNF in ISO-1-OGD 4h/R24h group were significantly decreased. Figure 2 represent the histological finding of neuroblastoma cells with BDNF staining from immunocytochemistry. There was no significant difference between all groups.

Conclusions

MIF administration induced increasing expression levels of BDNF in OGD/R model of neuroblastoma cells. Also, MIF expression level was increased in OGD and OGD/R model. These imply that MIF is an important factor for the neuroprotection in hypoxic neuronal cells. Thus, MIF could be the novel therapeutic modality for the neuroprotection of the cerebral ischemia. For this work, further in vivo study will be necessary.

Table 1. The MIF and BDNF expression levels of neuroblastoma cells in OGD 4h, OGD 4h/R24h model

	MIF	BDNF
	%	%
Control	55.7	128.89
[a]OGD 4h	74.74	126.34
[b]OGD 4h/R24h	79.37	88.07
[c]MIF-OGD 4h/R24h	104.07	282.83
[d]ISO-1-OGD 4h/R24h	28.81	98.91

[a]OGD 4h: oxygen glucose deprivation 4hrs, [b]OGD 4h/R24h: oxygen glucose deprivation 4hrs and reoxygenation 24hrs, [c]MIF: macrophage migration inhibitory factor, [d]ISO-1: MIF inhibitor
%: band of each group / band of b-actin

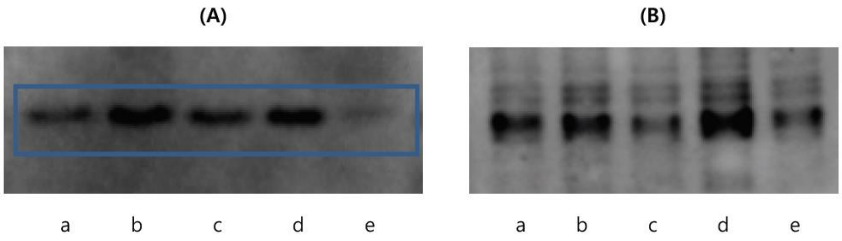

Figure 1. The band of MIF and BDNF expression levels of neuroblastoma cells in western blot. (A) The MIF expression level increased in OGD 4h, OGD 4h/R24h group and decreased ISO-1-OGD 4h/R24h group. (B) The BDNF expression level increased in MIF-OGD 4h/R24h group and decreased in ISO-1-OGD 4h/R24h group. a: Control, b: OGD 4h, c: OGD 4h/R24h, d: MIF-OGD 4h/R24h, e: ISO-1-OGD 4h/R24h

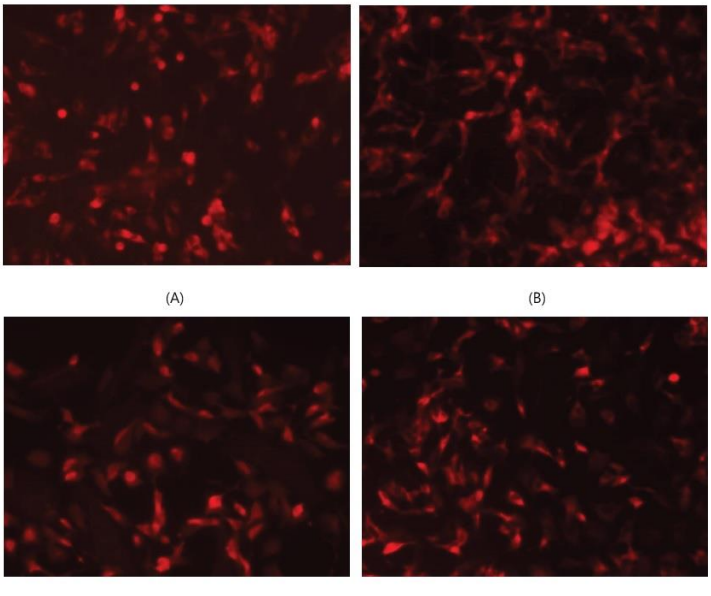

Figure 2. The histological findings of neuroblastoma cells with BDNF staining from immunocytochemistry.