SUPPLEMENTARY DATA

Protective Role of S-Nitrosoglutathione (GSNO) Against Cognitive Impairment in Rat Model of Chronic Cerebral Hypoperfusion

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SUPPLEMENTARY DATA 1

The extraction of lipidated ApoE from astroglia culture

Formation of apolipoprotein E (ApoE) and amyloid-β (Aβ) complexes and their endocytosis by LDL-receptor related protein 1 (LRP1) have been shown to play critical roles in endothelial Aβ efflux and microglial Aβ degradation [1, 2]. In the brain, ApoE is expressed and lipidated by ATP-binding cassette sterol transporter ABCA1 principally by astroglia in response to the activation of liver X-receptors (LXRs) [3]. Therefore, the primary cultured rat astrocytes were incubated with synthetic LXR agonist TO901317 (TO; 10 μM) for 3 days and the resulted media was concentrated with Centricon® 10kDa cut-off centrifugal concentrator (Millipore). The 20X concentrated conditioned media then diluted with fresh DMEM and re-concentrated three times to minimize residual TO901317 from media. The resulted conditioned media was subjected to western blot to confirm the expression of ApoE as shown in the Supplementary Figure S1 (Supplementary Figure 1A). To confirm the lipidation of ApoE, the concentrated media obtained from control (CON) or TO901317 (TO) treated astrocyte culture were subjected to iodixanol density gradient ultracentrifuge as described previously [4]. Purified LDL and HDL (EMD Biosciences) were used as the standards for lipoprotein density. Following the fractionation, the protein levels of ApoE, ApoB, and ApoAII in the fractions of astroglial conditioned media, LDL, and HDL, respectively, were analyzed by western blot to determine the density of ApoE particles in astroglial conditioned media. The data presented in Suppl. Figure 1B-i and ii indicate that ApoE particles generated from astroglia is lipidated particles that has similar density range with HDL.

Studies have demonstrated that the lipidation of ApoE increases its binding affinity to Aβ [5], and facilitates the Aβ clearance by enhancing the activities of microglial degradation and endothelial efflux [1, 2]. To examine whether ApoE particles extracted...
Supplementary Figure 1. The extraction of lipidated ApoE from astroglia culture. A) The effect of LXR agonist TO901317 (TO; 10 μM/2 days) on ApoE expression was examined in primary cultured rat astroglia. B) The lipidation of ApoE particles from astroglial media was examined by iodixanol density gradient ultracentrifugation. i) Purified LDL (ApoB) and HDL (ApoAII) were used as the standards for lipoprotein density. ii) The physical interaction between astroglia-secreted ApoE particles and Aβ42 was analyzed by iodixanol density gradient ultracentrifugation following the incubation of green fluorescence (FAM)-labeled Aβ42 with basal media (BM; DMEM alone) or concentrated conditioned media obtained from TO-treated astroglia (CM). Following the fractionation, green fluorescence in each fraction was measured and expressed as a percent of total fluorescence intensity. The change in Aβ distribution was also expressed by subtracting fluorescence intensities of each fraction of CM from that of BM.

Supplementary Figure 2. GSNO treatment inhibits Aβ-induced inflammatory gene expression. A) The effect of GSNO pretreatment and interferon-γ (IFNγ) on NFkB-Luciferase expression was analyzed by dual luciferase assay system according to the instruction manual (Promega). For this, BV2 immortalized murine microglial cells were transfected with pNFkB-Luc which expresses luciferase in response to NFkB activation and pRL-TK plasmid as a transfection control. Next day, the cells were incubated in serum free media overnight and treated with GSNO (250 μM) and Aβ25-35 (10 μM) and IFNγ (25 ng/ml). GSNO was pretreated 3 hrs prior to Aβ25-35/IFNγ treatment. The cells were then incubated for 24 h and analyzed for luciferase activity. B) To examine the effect of GSNO treatment on microglial iNOS and COX-2 expression, BV2 microglial cells were pretreated with increasing doses of GSNO, treated with Aβ25-35/IFNγ, and analyzed for iNOS and COX-2 protein levels by western blot. The β-actin was used for internal loading control.

from astroglia culture indeed are able to interact with Aβ, the basal media (BM; fresh DMEM) which did not contain any lipoprotein particles or concentrated conditioned media (CM) which was obtained from TO901317 treated astroglia was incubated with fluorescence labeled Aβ42 for 1 h at 37°C and subjected to iodixanol density gradient ultracentrifugation. Following the fractionation, the distribution of Aβ in the gradient was analyzed by measuring fluorescence in each fraction (Supplementary Figure 1C-i). We observed that the incubation of Aβ with the astroglial conditioned media increased the Aβ-distribution in the fractions containing HDL-like ApoE particles (fraction# 6-8) (Supplementary Figure 1C-ii), thereby suggesting the existence of physical interaction between astrogial-derived HDL-like ApoE particles and Aβ peptide.
SUPPLEMENTARY DATA 2

The anti-inflammatory activity of GSNO in microglia

Anti-inflammatory effect of GSNO has been proposed by many laboratories, including ours [6–12]. To examine whether GSNO treatment is able to inhibit Aβ-induced proinflammatory gene expression in microglia, cultured BV2 murine microglia was pretreated with GSNO, treated with synthetic Aβ peptide (Aβ25-35; 10 μM) and interferon-γ (IFNγ; 25 ng/ml), and analyzed for NFκB activation and gene expression of iNOS and COX-2. The data presented in Supplementary Figure 2A and B show that the increased activities of NFκB and gene expression of iNOS and COX-2 were efficiently suppressed by GSNO pretreatment, suggesting that GSNO is a potent anti-inflammatory agent under Aβ-induced inflammatory conditions.

REFERENCES