Arginine is neuroprotective through suppressing HIF-1α/LDHA-mediated inflammatory response after cerebral ischemia/reperfusion injury

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Supplement Fig 1. Arginine is neuroprotective after rat cerebral ischemia/reperfusion injury. (a) TTC staining (left) shows the infarct region after 24 h following I/R insult and quantification analysis (right) of the infarct volume shows that arginine administration reduces I/R-induced infarct volume (n = 6 in each group, *p < 0.05 versus the I/R + vehicle, Student’s t-test). (Scale bar, 5 mm). (b) Arginine treated rats have lower scores in the mNSS test on days 7 and 14 after I/R injury compared to rats in the I/R + vehicle group. (n = 6 in each group, *p < 0.05 versus I/R + vehicle, two-way ANOVA test). (c) Arginine treated rats have lower scores in the beam-walking test on days 7 and 14 after I/R injury compared to rats in the I/R + vehicle group. (n = 6 in each group, *p < 0.05 versus the I/R + vehicle, two-way ANOVA test). (d) Arginine treated rats have higher a ratio in the MST test at days 7 and 14 after I/R injury compared to rats in the I/R + vehicle group (n = 6 in each group, *p < 0.05 versus the I/R + vehicle, two-way ANOVA test).
Supplement Fig 2. Arginine administration to microglia co-cultured with neuron in Transwell system is neuroprotective in OGD insult. (a, b) Cell viability test (a) and LDH release test (b) show that administration of arginine to microglia reduces neuron death after OGD insult (n = 6 in each group, *p < 0.05 versus Sham, #p < 0.05 versus Vehicle, one-way ANOVA test).
Supplement Fig 3. Regulation of LDHA by HIF-1α. (a, b) Western blotting shows that LW6 inhibits LDHA protein level in rats (a) and cultured primary microglia (b) (n = 6 in each group, *p < 0.05 versus vehicle, Student’s t-test). (c) Western blotting shows transfection of WT-HIF-1α increases LDHA protein level in BV-2 cell line (n = 6 in each group, *p < 0.05 versus EV, two-way ANOVA test).
Supplement Fig 4. Regulation of LDHA by arginine after transfection of LDHA and OGD. (a) Western blotting shows transfection of LDHA upregulates LDHA expression in BV-2 cell line (n = 6 in each group, *p < 0.05 versus EV, Student’s t-test). (b) Western blotting shows LDHA overexpression maintains the expression level of LDHA after OGD insult in BV-2 cell line administrated with arginine (n = 6 in each group, *p < 0.05 versus OGD, one-way ANOVA test).
Supplement Fig 5. Arginine-mediated inhibition of inflammatory response is blocked by transfection of LDHA in OGD BV-2 cell line. (a, b) Pro-inflammatory markers (a) and anti-inflammatory markers (b) in FX11 group showed no significance from FX11 + arginine group (n = 6 in each group, Student’s t-test).