Consequences of IL-1 type I receptor deficient signaling: reduced cytotoxic and vasogenic edema, suppression of inflammation and inducible nitric oxide synthase (iNOS) production

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INTRODUCTION: IL-1 cytokine family members are potent mediators of inflammation involved in multiple aspects of neurodegeneration. They are rapidly secreted in response to various insults such as cerebral ischemia, head injury, CNS infections, seizures, and their levels are elevated in neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease. IL-1 ligands can cause neurodegeneration by activating microglial cells that can produce free radicals such as NO, and glutamate, which can activate NMDA receptors resulting in microglia-induced cytotoxicity [1].
Two types of IL-1 receptors have been identified. The IL-1 type I receptor (IL-1R1) is believed to be responsible for intracellular signaling, whereas the IL-1 type II receptor (IL-1RII) binds IL-1 but is incapable of signal transduction. We used IL-1R1 knock-out mice (IL-1R1 -/-) [2] to test the hypothesis that deleting the IL-1R1 would decrease recruitment of macrophages into the CNS, lead to lower levels of iNOS, and, consequently, result in reduced infarct size following hypoxia-ischemia.

METHODS: Male IL-1R1 null and wild-type (WT) mice, 4 to 6 months old, of a C57BL/6 background were used. The animals were anesthetized with halothane (4% induction, 1.5% maintenance in air) and the right carotid artery was double ligated. To induce hypoxia, each animal was exposed to a gas mixture of 8% O_2/balance N_2 for 22 min. MR imaging was performed on a 3.0 T Bruker MRI spectrometer. At 30 min following HI, 6 mice (3 WT and 3 IL-1R1 null mice) were simultaneously imaged under isoflurane anesthesia (2%) using a hexagonal coil [3] with a diffusion-weighted imaging sequence (4 slices, 1 mm thickness, 0.5 mm slice separation, TR/TE=1500/68.8 ms, Δ=35.22, δ=20 ms, b-value=730, 625X625 μm resolution) in 3 min 12 s total imaging time. At 48 h, mice were anesthetized with 2 mg/kg of xylazine, 15 mg/kg of ketamine i.p., and imaged using a T_2-weighted RARE sequence (10 slices, 0.5 mm thickness, TR/TE=3000/69.4 ms, 78X78 μm resolution, NEX= 8, 6 min total imaging time) to determine the infarct size. To quantify T_2 changes at 48 h post HI, each mouse was imaged with a multi-echo spin echo sequence in addition to RARE. The spin echo sequence had TR/TE=3000/10.13-151.95 ms, 156X78 μm resolution and N=15 echoes, 2 averages in 13 min, with the same slice thickness and positioning as RARE imaging. Slices were segmented by setting an intensity threshold using the CCHIPS/IDL software [4]. Infarct volumes were corrected for brain edema as described previously [5], and calculated as percent of the hemisphere. T_2 maps were calculated on a pixel-by-pixel basis from the multi-echo images. RNA protection assay [6] was used to determine levels of macrophage inflammatory protein 1 alpha (MIP-1 α) and macrophage/monocyte chemotactic protein 1 (MCP-1), and Western blot was used to determine iNOS protein levels in 3 WT and 3 IL-1R1 null mice in the contralateral and ipsilateral cortex. The statistical analysis was done using Wilcoxon test for the infarct comparison, and ANOVA followed by Fisher's LSD post hoc test for chemokines and iNOS.

RESULTS: At 30 min following HI, 5 out of 7 WT and 1 out of 6 IL-1R1 null mice had elevated signal intensities on DWI, an indication of cytotoxic edema, Fig. 1. Initially, there was elevated signal intensity in the cortex and the striatum of IL-1R1 null mice. After 48 h, the elevated signal intensity was mainly restricted to the striatum (Fig. 1), and cortex appeared to have near normal T_2 values. In contrast, in some WT mice (2/7) cytotoxic edema began in the cortex, but evolved at 48 h into vasogenic edema encompassing both cortex and the striatum, matched with elevated T_2 values in both areas (Fig. 1). IL-1R1 mice exhibited significantly reduced ($p<0.05$) vasogenic edema (total and cortical) at 48 h post HI when compared to the WT mice (Fig. 2). Corresponding to reduced vasogenic edema, IL-1R1 null mice had significantly reduced levels of MIP-1 α and MCP-1 chemokines at 18 h compared to WT mice (Fig. 3A), and significantly reduced protein iNOS levels at 48 h (Fig. 3B).

DISCUSSION: The presented results demonstrate that the absence of IL1-R1 signaling will prevent early cytotoxic and delayed vasogenic edema as a consequence of HI. The reduced cytotoxic edema in IL-1R1 null mice points to preserved Na^+-K^+ ATPase function in IL-1R1 null mice and, which may be new mechanism independent of IL-1's effects on immune cells. Lower levels of MIP-1 α and MCP chemokines will result in lower number of invading mononuclear/polymorphonuclear leukocytes and reduced activation of microglia/macrophages, leading to lower iNOS levels. Reduced levels of inflammatory chemokines at 18 h followed by reduced iNOS levels and significantly reduced vasogenic edema at 48 h in IL1-R1 null mice suggest that inflammation followed by NO mediated free radical damage may be responsible for increasing the severity of brain damage after HI. The presented data indicate the application of IL-1 receptor antagonists may provide neuroprotection after a stroke.

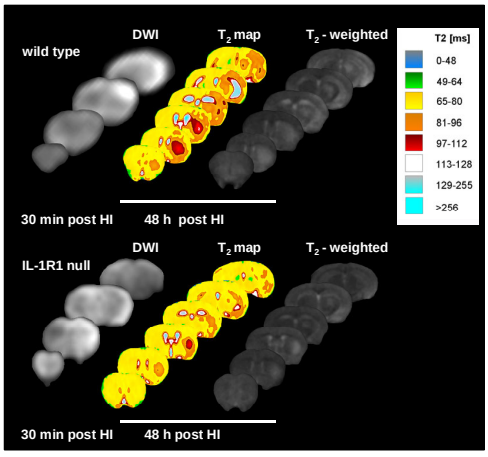

Figure 1. Evolution of brain damage in WT (A) vs. IL-1R1 null (B) mice from 30 min to 48 h post HI. Diffusion-weighted images, T_2 maps and T_2-weighted images of one WT mouse and one IL-1R1 null mouse. Color scale represents the T_2 values.

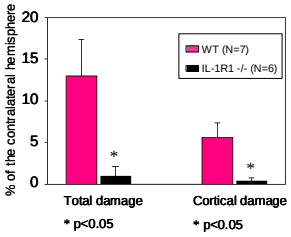

Figure 2. Total and cortical infarct volumes at 48 h after HI. (*p<0.05)

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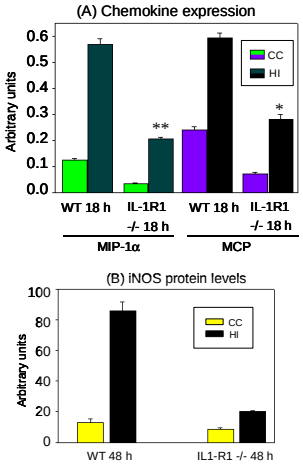

Figure 3A Chemokine expression at 18 h. **3B** iNOS protein levels in WT and IL-1R1 null mice at 48 h following HI. (*p<0.05 and **p<0.01)