Pro-inflammatory signalling along the P2X7R/NOX2 inflammatory axis in microglia and after experimental TBI.

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Introduction: Microglia, the resident immune cells of the central nervous system play both protective and damaging roles following traumatic brain injury (TBI). Among the key drivers of microglial pro-inflammatory signalling are purinergic P2X7 receptors (P2X7R). Activation of P2X7R by high concentrations of its endogenous ligand, ATP, can signal downstream to enhance NOX2 activity and ROS production, which can induce NLRP3 inflammasome assembly and pro-inflammatory cytokine release. Here we investigated microglial inflammatory signalling along the P2X7R/NOX2/NLRP3 inflammasome axis in vitro and after experimental TBI in mice.

Methods: Immortalised microglia and primary microglia (wild type and P2X7R knockout) were activated (LPS+ BzATP) and treated with a P2X7R antagonist, JNJ-47965567. ROS, NO, IL-1beta, TNF-alpha production, NOX2 expression, and NOX activity were measured by plate-based assays or Western immunoblotting. In addition, C57BL/6J male mice were subjected to controlled cortical impact and administered JNJ-47965567 (30mg/kg, I.P.) starting at 3h post-injury with repeat dosing through 7 days post-injury (dpi). Neurobehavioral assessments were performed and brain tissue collected for flow cytometry of microglial function related to NOX2/IL-1beta signalling.

Results: In vitro, P2X7R pharmacological inhibition (JNJ-47965567) or genetic ablation suppressed pro-inflammatory microglial activation by attenuating NOX2 signalling and NLRP3 activity, resulting in significant reductions in downstream mediators such as ROS, NO, LDH, TNF-alpha, and IL-1beta production. In vivo, TBI induced a robust neuroinflammatory response, characterised by increased numbers of pro-inflammatory microglia and infiltrating monocytes in the injured brain at 7dpi. Treatment with JNJ-47965567 markedly reduced these neuroinflammatory cell populations; however, these cellular effects did not translate into neurobehavioral improvements, as TBI-induced deficits in motor and cognitive performance were not improved by JNJ-47965567 treatment.

Conclusion: The P2X7R/NOX2 inflammatory axis plays an important role in pro-inflammatory microglial activation and may be therapeutically targeted to mitigate damaging post-traumatic neuroinflammatory responses.