PROPOFOL INHIBITS LPS-INDUCED BV-2 MICROGLIA CELLS ACTIVATION VIA INHIBITION OF TLR4: POSSIBLE INVOLVEMENT OF GSK-3B

Bo Gui, MD, Mingyan Su, MD, Yanning Qian, MD, First Affiliated Hospital, Nanjing Medical University Nanjing, CHINA

Background: Microglia is as an immune cell in CNS and to be able to produce many inflammatory mediators in response to stressors ,so that play a critical role in the neuroinflammatory processes. Inflammation is a crossover in the pathogenesis of the chronic neurodegenerative diseases, such as Alzheimer's Disease, Parkinson's disease. Toll-like receptors (TLRs), especially TLR4 in the microglia is a important signaling pathway for inflammation response. Lipopolysaccharide (LPS) can activate microglia, which via toll-like receptor 4 (TLR4), and stimulate the expression of inflammatory cytokines. Also, glycogen synthase kinase-3β (GSK-3β) can modulate the inflammatory responses and tilt the balance in favor of pro-versus anti-inflammatory cytokines. Propofol, a well-known anesthetic, has been reported to inhibit LPS-induced inflammation response,such as in macrophagocyte. The aim of our study was to investigate the effect of propofol on LPS-induced inflammation in BV-2 microglia cells, which has been widely used in vitro experiments, and explore whether this effect is related to TLR4 and GSK-3β.

Methods: Cells were randomly divided into four groups by using random number table: C group(Control group), LPS group, Propofol group and LPS+ Propofol group. The concentration of LPS and propofol were 1μg/mL and 30μM, respectively. Cell viability was measured using 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. Interleukin (IL)-1β and tumors necrosis factor-α (TNF-α) released in culture medium were examined by enzyme-linked immunosorbent assay (ELISA). TLR4 mRNA expression was analyzed by reverse-transcription (RT) and real-time polymerase chain reaction (PCR). Protein expression of TLR4, p-GSK-3β and total GSK-3β were analyzed by western blotting. Statistical analysis was performed with GraphPad Prism statistical procedures. One-way analysis of variance was used followed by Dennett's t multiple comparison test. A P-value <0.05 was considered statistically significant.

Results: Compared with group C, the production of IL-1β, TNF-α, TLR4 and phosphorylation of GSK-3β in the LPS group were significantly increased (P<0.05). Meantime, content of IL-1β, TNF-α and TLR4 expression were significantly decreased, whereas phosphorylation of GSK-3β increased in LPS+Propofol group compared with LPS group (See: Table 1/Figure 1). No significant differences were detected in the content of above targets between group C and group Propofol (P>0.05).

Conclusions: Our results demonstrate that 30 μM propofol pretreatment reduces the release of inflammatory cytokines induced by LPS in BV-2 microglia cells. Propofol not only increase phosphorylation of GSK-3β,but also inhibits TLR4 expression. It suggests that TLR4 and GSK-3β may be the important cellular mediators involved in the anti-inflammation effects of propofol on LPS-induced neuroinflammation in microglia.

Key words: Glycogen synthase kinase 3 beta; Inflammation; Lipopolysaccharide; Propofol; Toll-like receptor

Table 1. The expression level of IL-1β and TNF-α in BV-2 microglia cells. [n=8, M(P25~P75), pg/mL]

target	C	LPS	propofol	LPS+propofol
IL-1β	200(151~368.5)	1600(1314~2210)*	230(152~397.5)	708(536~828)*#
TNF-α	215(148~300.5)	1180(946~1202)*	310(176.5~317.5)	500(319.5~554)*#

*Statistically significant from C group (*P<0.05). #Statistically significant from LPS group (#P< 0.05).

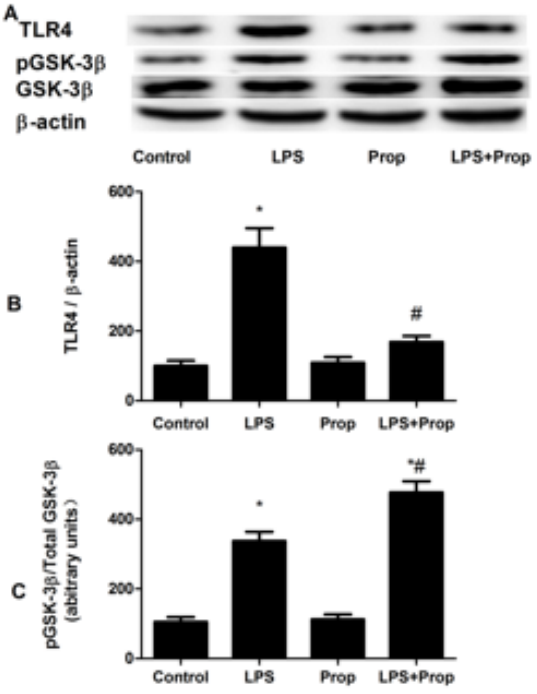

Figure 1. Effects of propofol (Prop) on TLR4 and p-GSK-3β protein expression induced by LPS in BV-2 microglia cells.*Statistically significant from C group (*P<0.05). #Statistically significant from LPS group (#P< 0.05).