Neuroimmunotoxic effects of aflatoxin B1 visualized by bioluminescence

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Abstract

Aflatoxin B1 (AFB1) is a poisonous substance, which is classified as group 1 carcinogen by International Agency for Research on Cancer (IARC). The impact of AFB1 on neural cells and systems has poorly been understood. Microglials and astrocytes are key accessory protective cells in brain. To assess the cellular effects of AFB1 on brain, murine pure primary astrocytes and microglia cell line (BV2) were exposed separately to environmentally relevant level (~10 ng/ml) of AFB1 for various timepoints in culture. At each timepoints, apart from assessing of cellular chemiluminescence (CL) and bioplex ELISA and multiplex qPCR-based pro/anti-inflammatory cytokines, concentration of intracellular ATP and caspase-3/7 activity was determined by the phenomenon of bioluminescence (BL) and luciferase reactions. Further, cytochrome c release from mitochondria was carried out by western blot and percentage of apoptotic cells was obtained using flow cytometry as well. Upon AFB1 exposure, CL tended to increase in microglial cells with a lesser extent in the astrocytes. AFB1 also induced secretion of pro-inflammatory cytokines (i.e. TNF-α and IL-6) on both microglial cells (more TNF-α) and astrocytes (more IL-6). mRNA expression of TLR2, TLR4, MyD88 and NF-kB were up-regulated with different timing and levels among cells. Interestingly, remarkable induction of BL-based caspases activation (apoptosis/necrosis) and ATP depletion in microglia and astrocytes-exposed AFB1 was detected. The results indicated that, biologically relevant level of AFB1 induces apoptosis in brain’s key accessory protective cells, glials and astrocytes, through ATP depletion and caspases activation. Immunotoxicologically, pro-inflammatory and pro-apoptotic properties of low level of AFB1 in vitro could potentially explain the immune dysregulation in neurodegenerative disorders in brain.

Keywords: Aflatoxin B1, Bioluminescence, Microglial cells, Astrocytes, Neurotoxicity, Neuroinflammation.