AZATHIOPRINE REDUCE INFLAMMATORY CYTOKINES IN LPS-STIMULATED MURINE MACROPHAGES.

Oliveira, L.G.¹; Silva, F.C.¹; Cordeiro, C.H.T.¹; Medeiros, V.P.¹; Aguiar, J.A.K.¹; Chebli, J. M.²

¹Departamento de Bioquímica, Instituto de Ciências Biológicas, Universidade Federal de Juiz de Fora, Juiz de Fora, Minas Gerais, Brazil; ²Departamento de Gastroenterologia, Faculdade de Medicina, Universidade Federal de Juiz de Fora, Juiz de Fora, Minas Gerais, Brazil.

Inflammatory bowel disease is characterized by chronic intestinal inflammation. Azathioprine and its metabolite 6-mercaptopurine (6-MP) are immunosuppressive and anti-inflammatory drugs that are widely used in patients with inflammatory bowel disease. However, established understanding of anti-inflammatory action mechanism of azathioprine has not yet been fully clarified. In this study we investigated the effects of azathioprine in RAW 264.7 LPS stimulated cells. Murine RAW 264.7 (8 x 10⁴ cells) were stimulated with 1 μg/ml of LPS and were treated with different concentrations of azathioprine (0.2, 0.6, 1.0 μM) for 48 hours. The level of nitric oxide (NO) was determined by Griess reagent, and the other inflammatory marker, tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) were measured by ELISA. Moreover, to evaluate cytotoxicity, we performed (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (MTT) assays and measured the cell viability. Azathioprine at a dose of 1.0 μM was able to significantly reduce NO production by 46%, and TNF-α production by 47%, the reduce effect of compound were accompanied by dose dependent in LPS-stimulated cells. Additionally, this drug significantly inhibited the production of IL-6 by 35%, at a dose of 1.0μM. Cell viability, as measured by the MTT assay, showed that azathioprine had no significant cytotoxicity in Raw 264.7 cells. These data demonstrate that azathioprine affects murine RAW 264.7 cells stimulated with LPS, inhibiting IL-6, TNF-α and nitric oxide synthesis, might contribute to the anti-inflammatory activities of azathioprine.

Key Words: Azathioprine; Lipopolysaccharide; Inflammatory Bowel Disease; Anti-inflammatory