麩醯胺添加對砷暴露小鼠體內抗氧化系統及發炎反應之影響

Effects of glutamine on antioxidant system and inflammation in mice with arsenic exposure

中文摘要

本研究主要在探討 GLN 添加對砷暴露小鼠血中細胞黏著分子表現及體內抗氧化系統之影響。將雄性 BALB/c 小鼠，隨機分爲 6 組，分別爲 CC 組-蒸餾水+正常飲食；CG 組-蒸餾水+ GLN 飲食；20AC 組-含 20ppm NaAsO2 砷飲水+正常飲食；20AG 組-含 20ppm 砷飲水+GLN 飲食；50AC 組-含 50ppm NaAsO2 砷飲水+正常飲食；50AG 組-含 50ppm 砷飲水+GLN 飲食。GLN 飲食爲將 GLN 取代飲食中總氮量之 20%。5 週後將小鼠犧牲，收集血液、脾臟以及肝臟作分析。新鮮血液以流式細胞儀測量白血球上黏著分子 LFA-1 (lymphocyte function-associate antigen-1)、Mac-1 (macrophage antigen-1)的表現以及 CD4+和 CD8+ T 淋巴球的分佈，取血漿分析血中胺基酸濃度。收集脾臟細胞，給予裂質原 PHA (phytohemagglutinin)或是 LPS (lipopolysaccharides)的刺激後，收集上清液以測定細胞激素 interleukin (IL)-2 IL-4、IL-6、inteferon (IFN)-g 以及 tumor necrosis factor (TNF)- a 的濃度。肝臟均質後，分析 total GSH (glutathione), GSSG (oxidized form glutathione)濃度, GSH-Px (glutathione peroxidase)以及 GSH-RD (glutathione reductase)活性。以 two-way ANOVA 進行統計分析，Fisher’ s test 比較組間差異，$p < 0.05$ 表示具有統計差異。結果顯示，砷添加組體重明顯較未添加組低。在黏著分子表現方面，20AC、20AG、50AC 三組之 LFA-1 表現明顯高於 CC 組；CG、20AG、50AC 三組之 Mac-1 表現明顯高於 50AG 組。血中胺基酸濃度分面，20AC、50AC 組的 GLN 濃度低於其他四組。在脾臟細胞激素分泌方面，CG 組的 IL-4 濃度明顯低於 20AG 和 50AG 組；CG 組的 IL-6 明顯低於其他各組；20AG 組之 IFN-g 較 CC 組爲高；而 TNF-a 之濃度則各組間均無差異。肝臟抗氧化系統方面，20AG 組的 total GSH 較 CC、CG、50AG 三組高；GSH 濃度則是六組間沒有差異；20AG 組的 GSSG 高於 20AC 組；20AC、20AG、50AC 三組的 GSH-Px 活性高於 CC 組；肝臟中 GSH-RD 活性六組間沒有統計差異。本研究結果顯示砷暴露會使血中 GLN 濃度降低，並增加肝中 GSH-Px 活性及氧化態 GSSG 之濃度，造成細胞黏著分子表現增加；而 GLN 添加可使血中 GLN 降低的情形獲得回復，在砷濃度較高時可降低血中細胞黏著分子 LFA-1 以及 Mac-1 之表現，可能可因而降低因砷暴露引致之發炎反應，並有助於砷代謝反應之進行。GLN 添加對正常小鼠可減少發炎性細胞激素 IL-6 之分泌，但在砷暴露下 GLN 添加，並無調節細胞激素分泌之作用。

關鍵字：砷暴露、glutamine、glutathione、細胞黏著分子、細胞激素

英文摘要

This study investigated the effect of glutamine (GLN) on antioxidant system and adhesion molecule expression in mice with arsenic exposure. Mice were randomly assigned to 6 groups, 2 normal groups with or without GLN supplementation were provided with distilled water, 4 experimental groups were divided by 2 levels of arsenic in drinking water, mice in each level of arsenic group were also divided to 2 groups with or without GLN supplementation. GLN replaced part of casein and provided 20% of total nitrogen in the diet. After feeding the mice for 5 weeks, all mice were sacrificed and blood, spleen and liver were collected for further analysis. Blood samples were used for measuring cellular adhesion molecule expression and amino acid concentrations. Liver specimens were used for antioxidant-related substance and enzyme activities analysis. In vitro splenocyte cytokine secretion after mitogen stimulation was also measured. The results demonstrated that arsenic exposure resulted in significant weight loss, lowered plasma GLN levels, enhanced the expression of lymphocyte function-associated antigen (LAF)-1 and macrophage antigen (Mac)-1. GLN supplementation repleted plasma GLN concentration and significantly reduced the expression of LAF-1 and Mac-1 in high arsenic group. Compared with arsenic exposure groups without GLN, oxidized from glutathione levels in liver were increased in GLN supplemented group. Antioxidant enzyme activities did not differ among the groups. The in vitro study showed that GLN supplementation reduced interleukin—6 production in non-arsenic exposure mice. The concentrations of interleukin-4, interferon-g and tumor necrosis factor-a did not differ in arsenic exposure groups despite the mice were supplemented with GLN or not. These results suggest that GLN supplementation decreased leukocyte adhesion molecule expression induced by arsenic, and enhanced glutathione metabolite production in liver which may consequently promote arsenic metabolism. However, GLN seemed to have no effect on cytokine modulation when arsenic was administered.
Keywords: Arsenic, Glutamine, Glutathione, Cellular adhesion molecule, Cytokine