麩醯胺添加對砷曝露小鼠細胞黏著分子表現及體內抗氧化系統之影響

Effects of glutamine on the expression of cellular adhesion molecule and antioxidant system in mice with arsenic exposure

Ya-Ling Luo*, Wan-Chun Chiu and Sung-Ling Yeh#
Graduate Institute of Nutrition and Health Sciences, Taipei Medical University

羅雅玲* 邱琬淳 葉松鈴#
臺北醫學大學 保健營養研究所

Introduction

流行病學上的調查顯示慢性砷暴露會造成心血管及高血壓等病變，而造成此種傷害的原因，可能源自於砷曝露引致活性氧物種(ROS)之產生，因而增加身體的氧化壓力，引起發炎反應並造成細胞組織之傷害。但砷所誘發的ROS在血管疾病形成過程中所扮演的角色及機轉目前並不清楚。麩醯胺(glutamine, GLN)是細胞間質中含量最多的游離胺基酸，也是免疫細胞及腸黏膜細胞之重要能量來源，在免疫功能的調節上扮演非常重要的功能。GLN在代謝壓力或疾病狀況下，被認為是一種必須胺基酸。最近的研究顯示在全靜脈營養液中添加GLN，可減少因使用靜脈營養所造成腸道中黏著分子的過度表現，並可減少腸道發炎大鼠腸道血管中白血球黏著及遷移反應。由於氧化壓力及發炎反應引致細胞黏著分子之表現是造成血管病變的主要原因之一。故本研究以GLN介入之方式探討GLN添加對於砷曝露小鼠細胞黏著分子表現及抗氧化系統之影響。

Materials and Methods

1. 將BALB/c mice，隨機分為六組：

Groups	Water	Diet
CC	Distilled H_2O	Normal
CG	Distilled H_2O	GLN
20AC	20 ppm $NaAsO_2$	Normal
20AG	20 ppm $NaAsO_2$	GLN
50AC	50 ppm $NaAsO_2$	Normal
50AG	50 ppm $NaAsO_2$	GLN

2. 5週後將小鼠犧牲，收集新鮮血液以及肝臟做分析。以流式細胞儀測量血液中白血球上黏著分子 Lymphocyte Function-associated antigen (LFA) -1及 macrophage antigen (Mac) –1的表現。肝臟組織均質後離心，收集上清液，分析GSH (glutathione), GSSG (oxidized glutathione)之濃度及 GSH-Px (glutathione peroxidase), GSH-RD (glutathione reductase)之活性。
3. 以SAS software (version 8.2)中 two-way ANOVA 進行統計分析，並以Fisher's test 比較組間差異，以$p < 0.05$表示具有統計上之差異。

Results

小鼠之體重在5週實驗期滿後顯示，添加砷之組別均顯著較未添加砷組低， GLN 添加與否則對體重無影響。

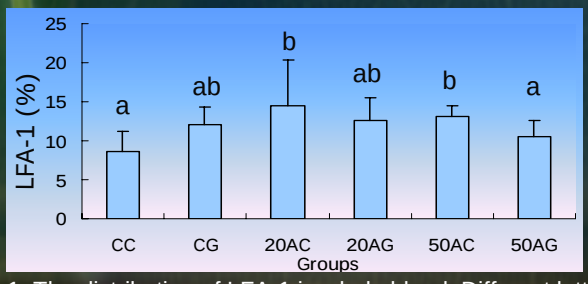

Fig1. The distribution of LFA-1 in whole blood. Different letters indicate significant difference among the groups.

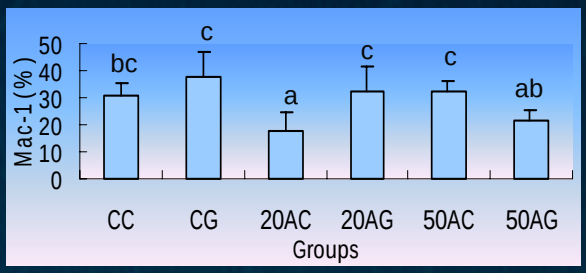

Fig 2. The distribution of Mac-1 in whole blood. Different letters indicate significant difference among the groups.

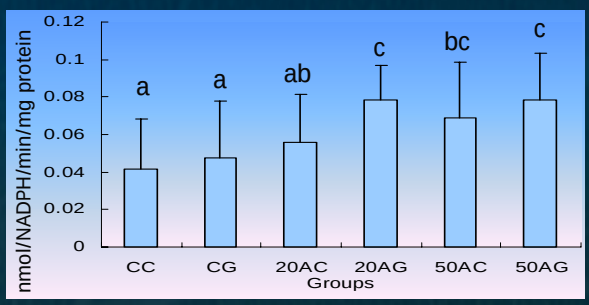

Fig 3. Oxidized form glutathione (GSSG) concentration in the liver. Different letters indicate significant difference among the groups.

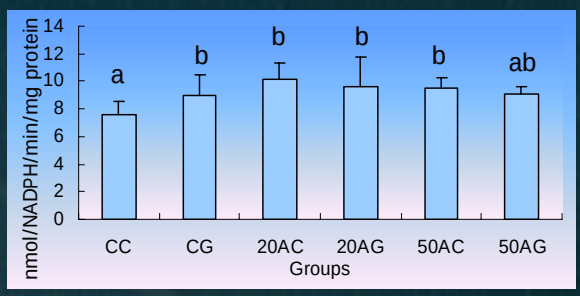

Fig 4. Glutathione peroxidase (GSH-Px) concentration in the liver. Different letters indicate significant difference among the groups.

肝臟中GSH濃度及GSH-RD活性，則在六組之間並無差異。

Conclusion

砷暴露會造成白血球上黏著分子之表現增加，而GLN添加在砷濃度較高時可降低細胞黏著分子LFA-1以及 Mac-1之表現。另外，砷暴露會增加肝中GSH-Px活性及氧化態GSSG之濃度；而GLN添加組之GSSG濃度更顯著高於未添加組，可能是GLN之添加，使肝臟GSH的合成增加，因而促進清除過氧化物代謝反應之進行， 使GSH 轉變成GSSG 的量增加。由以上的研究結果顯示砷暴露小鼠補充GLN，可能可降低因砷暴露引致之發炎反應，並有助於自由基之清除，進而減少砷暴露造成之傷害。

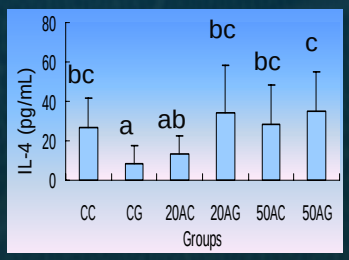

Figure4. The concentration of IL-4 released by PHA-stimulated splenocytes for 24 hours. Different letters indicate significant difference among the groups.

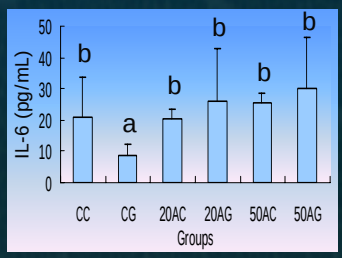

Figure5. The concentration of IL-6 released by LPS-stimulated splenocytes for 24 hours. Different letters indicate significant difference among the groups.

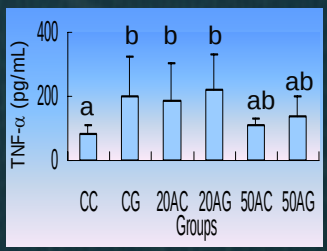

Figure6. The concentration of IFN-γ released by LPS-stimulated splenocytes for 24 hours. Different letters indicate significant difference among the groups.

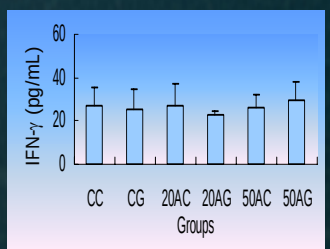

Figure7. The concentration of TNF-α released by LPS-stimulated splenocytes for 24 hours.