論 文 要 旨

N-acetyl-L-cysteine prevents arsenite-induced cytotoxicity mainly through chelation in U937 monocytes and macrophages

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【序論および目的】

Arsenic is recognized as a big threat to public health in many Asian countries since chronic exposure to inorganic arsenite induces various biological effects including cancers in various sites and immunosuppressive status. N-acetyl-L-cysteine (NAC), a widely-used antioxidant, acts as a precursor of L-cysteine and reduced glutathione (GSH) and is known to attenuate the arsenic-induced toxicity. To clarify the preventive mechanism of NAC on arsenite-induced apoptosis in U937 cells, the cytotoxicity was examined in U937 monocytes and 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-treated U937 macrophages.

【材料および方法】

The cytotoxicity was examined for the treatments of arsenite and/or NAC. U937 monocyte and macrophage were incubated with NAC (10 and 20mM) for 6hrs, followed by the addition of arsenite (50uM) for 24hrs. In the next experiment, NAC (20mM) was washed out after 6hrs pre-treatment, followed by arsenite (50uM) addition. The cytotoxicity was also measured for time course dependence of NAC post-treatment. Immunocytochemistry was used to detect the expression of anti-apoptotic protein like Bcl-2. Apoptosis was examined in U937 macrophages with and without arsenite by TUNEL assay.

【結　果】

In order to find out the preventive effects of NAC on the arsenite-induced apoptosis, the cells were incubated with non-toxic doses of NAC (10 and 20M) 1 h before arsenite administration (50μM). In the presence of 20mM NAC, the cell death of U937 monocytes was reduced from 95% to 20% while the treatment with 20mM NAC

completely prevented arsenite-induced cell death in U937 macrophages. The preventive effect of NAC was not observed when cells were pretreated with NAC for 6 hrs followed by washing the cells with regular medium before 50μM arsenite administration in both U937 monocytes and macrophages indicating that NAC in the medium can protect arsenite-induced apoptosis.

NAC was added to the culture medium 0, 1, 3, 6, and 12 h after arsenite administration. In both U937 monocytes and macrophages, the arsenite-induced apoptosis was almost completely blocked by 20 mM NAC treatment within 1 h of arsenite administration. This result showed that NAC chelate the arsenite in the medium outside the cells.

The NAC effect on arsenite-induced apoptosis in macrophages was larger than that in monocytes ($P < 0.001$). To examine the mechanism of resistance to arsenite treatment in U937 macrophages, the expression of Bcl-2, an anti-apoptotic protein, was examined by immunocytochemical assay. Bcl-2 expression was activated in TPA-treated U937 macrophages, but no activation of Bcl-2 expression in U937 monocytes was observed.

【結論及び考察】

In conclusion, the present study clearly showed that the preventive effects of NAC on arsenite-induced apoptosis in U937 monocytes and U937 macrophages are mainly through chelating arsenite in culture medium. TPA-treated U937 cells (macrophage) were more resistant to cell death as compared to monocytes. Bcl-2 activation in macrophage might be the reason of this sensitivity to arsenite exposure between U937 moncytes and macrophages.