学 位 論 文 内 容 の 要 旨

博士（環境科学）　　氏　名　Md. Mostafizur Rahman

学 位 論 文 題 名

Study on the efficacy of dietary compounds to detoxify toxic metals *in vitro*
（試験管内で毒性金属を解毒するための食餌中化合物の有効性に関する研究）

In the era of globalization, environmental pollution has become a major human health threat. Almost every kind of pollutants in turn reaches to the food chain and creates burden for the human health with numerous diseases manifestations.

Analysis of 145 groundwater samples from central part of Bangladesh showed that most of the studied hydrochemical parameters of the samples from Gopalganj (pH, EC, TDS, Na^{+}, Cl^{-}, HCO^{3-}, CO^{3-}, As, Mn, Fe, B, NO^{3-} and so on) exceeded the limits. In addition, mean Hazard Quotient (HQ) and Hazard Index (HI) values based on As, Fe, Mn, B, NO^{3-}, and F^{-} imply that the groundwater poses substantial health risk for both adults and children. The carcinogenic risk among adults and children were also high. Similar findings were also found in Manikganj district. The groundwater could have some potential health risks from exposure to As and nitrate.

In the second part of the research, fundamental study has been conducted to understand the potential cytoprotective roles of nutritional trace elements and supplements against toxic metals in vitro cellular systems. Thus, it was aimed to investigate the effects of Zn on Cd-induced cytotoxicity and apoptosis using PC12 cells. Cell viability and DNA fragmentation assays in PC12 cells exposed to Cd and/or Zn revealed that Cd (5 and 10 μM) alone induced significant cell death, and co-exposure to Zn (5, 10, and 100 μM) for 48 h had a protective effect. Assessment of intracellular glutathione (GSH) levels and lactate dehydrogenase (LDH) activity suggested that Cd (10 μM)-induced oxidative stress and disrupted cell membrane integrity. Addition of Zn (10 and 100 μM) reduced Cd-mediated cytotoxicity. Moreover, changes in expression of the apoptotic factors, Bax, Bcl-2, Bcl-x, and cytochrome c were measured via western blotting analyses, and expression of caspase 9 was detected via reverse transcriptase polymerase chain reaction (RT-PCR). Western blots showed that Zn (10 and 100 μM) suppressed Cd-induced apoptosis (10 μM) by reducing cytochrome c release into the cytosol, and downregulating the proapoptotic protein, Bax. In addition, expression of caspase 9 was lower in Cd (5 μM)-treated PC12 cells when co-treated with Zn (2 and 5 μM). These findings suggest that the effective

inhibition of Cd-induced apoptosis in PC12 cells by Zn might be due to suppression of mitochondrial apoptosis pathway and inhibition of Cd-induced production of ROS.

After that, we hypothesized that co-exposure of Se with As may have suppressive effects on As-induced cytotoxicity in PC12 cells. Upon Se (10 μM) co-exposure with As (10 μM) increases cell viability, and suppresses As-induced oxidative stress and DNA damage. Furthermore, the western blotting analyses revealed that simultaneous exposure of both metals significantly inhibited autophagy which may further suppressed apoptosis through positively regulation of key proteins; p-mTOR, p-Akt, p-Foxo1A, p62, and expression of ubiquitin, Bax, Bcl2, NFκB, and caspase 3, although those are negatively regulated by As. In addition, RT-PCR analysis confirmed the involvement of caspase cascade in cell death process induced by As, and subsequent inhibition by co-exposure of Se with As. The cellular accumulation study of As in presence/absence of Se via inductively coupled plasma mass spectrometry confirmed that Se effectively retarded the uptake/accumulation of As in PC12 cells. Finally, these findings imply that Se is capable to modulate As-induced intrinsic apoptosis pathway via enhancement of mTOR/Akt autophagy signaling pathway through employing antioxidant potentials and through inhibiting the cellular uptake of As in PC12 cells.

Likewise, we have investigated the protective role of ALA (250 μM) and DHLA (50 μM) against As, Cd and Pb stress in PC12 and Caco-2 cells. We found that both ALA and DHLA have significant cytoprotective effects against As, Cd and Pb toxicities in PC12 as well as Caco-2 cells. Cell viability of both cells was improved upon combined exposure of ALA-metals/DHLA-metals than the only metal-treated group. ALA showed cytoprotection at 250 μM concentration where as DHLA showed a similar effect at 50 μM. We found a significant increase of GSH level and Nrf2 protein in the co-treated group which was significantly lowered by the metal-treated group alone. Subsequently, DHLA (50 μM) showed potentials for the protection of DNA from possible damage caused by the metal exposure in PC12 cells. Protein analysis reveals an upregulation of survival factor mTOR, Akt and downregulation of death marker Bax, cleaved PARP was induced by ALA/DHLA in both PC12 and Caco-2 cells to protect the cells from As and Cd induced toxicity.

Chronic ingestion of trace levels of toxic metals such as As, Cd and Pb may possess serious health hazards. And deficiency of essential trace elements could aggravate the deleterious effects. However, proper dietary intake of essential trace elements and dietary supplements may reduce the toxic effects of metals. This study suggested beneficial role of Zn, Se and ALA/DHLA against toxic metals in vitro, and recommended for further investigation using animal model.