CHAPTER I

INTRODUCTION

Problem Statement

Arsenic, the 50th most abundant element of the earth crust, is an ubiquitously distributed contaminant of the environment including air, water, and soil. Some areas in Argentina, Chile, Taiwan, India, Bangladesh and Thailand are strongly contaminated leading to chronic poisoning in the population.

Contamination of drinking water with arsenic, at relatively high concentration and/or over a long period, has created health problems (WHO, 2001).

Arsenic-induced skin pathology includes hyperkeratosis, pigmentation changes, Bowen's disease, squamous cell carcinoma, and basal cell carcinomas. A unique spectrum of skin lesions, known as arsenical keratosis, is rather characteristic of chronic arseniasis. Bowen's disease, or squamous cell carcinoma in situ of the skin, has been well documented as a consequence of arsenical exposure (Centeno *et al.*, 2002).

Ronphibun Subdistrict in Southern Thailand, formerly a major tin-mining location, was found to be heavily contaminated with arsenic, when a governmental survey on the causes of the high prevalence of *kai dam* (literally "black fever" among its population was conducted in 1987 (Oshikawa *et al.*, 2001)

Cell signaling modification in keratinocytes may play an important role in arsenic keratosis. Actin cytoskeleton reorganization related to cell signaling modification induced by arsenite is our interest.

The toxicity effects induced by arsenic related to morphological alterations in cells exposed to arsenic often suggest underlying disruption of cytoskeletal structure elements responsible for cellular integrity, shape, and locomotion. However, specifics of the ultrastructural changes produced by arsenic remain poorly understood (Bernstam and Niagru, 2000). The current research on molecular aspects of arsenic emphasize tissue specific events in humans. It deals with current progress on the understanding of the signal transduction pathway and mechanisms underlying the sensitivity of various species, organ, and tissues to arsenic. There are many effects reported from arsenic

toxicity which relate to signal transduction disturbance. Li and Chou (1992) reported that exposure of Swiss 3T3 cells to low dose of As^{3+} (2.5 μm) results in apparent cell retraction and loss of thick cables of actin filaments. At high doses (≥ 20 μm), As^{3+} treatment caused a severe loss of microtubules. Treatment of cells with As^{3+} also induced a dose-dependent inhibition of cytoskeletal protein synthesis.

Porter *et al.* (1999) also reported that arsenate and arsenite activate MAP kinase JNK, however, the mechanism by which this occurs was not known. They showed that both arsenate and arsenite activation of JNK required Rho GTPases Rac and Rho. Arsenite stimulation of JNK required Rac/Cdc42 effector protein PAK, whereas arsenate-mediated activation of JNK was unaffected by inhibitory mutant PAK.

Smith *et al.* (2001) reported that low-level arsenite (5 μM) treatment of porcine aortic endothelial cells (PAEC) stimulated superoxide accumulation via the NADPH oxidase activation system associated with translocation of Rho GTPase Racl from cytosol to the plasma membrane.

Recently, Simeonova *et al.* (2002) reported that arsenic activates receptor tyrosine kinase EGFR and MAP kinase ERK in a human uroepithelial cell line. Arsenic-induced EGFR phosphorylation was independent of autocrine EGF, was sensitive to N-acetyl-cysteine (NAC), and did not involve the major autophosphorylation site, Tyr1173 and the inhibitor of Src activity, PP1, inhibited arsenic-induced EGFR and ERK phosphorylation.

Actin cytoskeleton disruption is induced by arsenite through cell signaling modification that are still not clear. The mechanistic study of arsenite-induced toxicity can provide information that has implications for specific antidote therapy for arsenite.

The actin cytoskeleton is a one member of cytoskeletal proteins that is vulnerable to arsenite-induced toxicity. In this study, we investigated the toxicity of sodium arsenite on cell signalings that correlate with the actin cytoskeleton disruptions especially protein tyrosine kinase, phosphotidylinositol-3 kinase (PI3K) and Rho GTPases. We used immunofluorescence microscopy coupled with laser scanning confocal microscopy to study the toxic effects of sodium arsenite on the actin cytoskeleton, focal adhesions, and mitochondrial localization in mouse fibroblasts (Balb/c 3T3). We used non-specific inhibitors such as protein tyrosine kinase inhibitor or serine/threonine kinase inhibitor

and specific inhibitors such as epidermal growth factor receptor (EGFR) inhibitor or phosphatidylinositol-3 kinase (PI3K) inhibitor in order to provide more information on the mechanisms of sodium arsenite-induced toxicity. We also used the immunoblotting technique in order to confirm the results from the immunofluorescence microscopy. Finally, we used flavone apigenin, PI3K and MAP kinase inhibitors, as an antidote for arsenite in animal experiment model.

Objectives

1. To determine as far as possible the mechanisms of toxicity of sodium arsenite (protein tyrosine kinase, phosphatidylinositol-3 kinase (PI3K) and Rho GTPase signaling pathways as well as the actin cytoskeleton disruption) in relation to cell morphology alteration and the involved signal transduction-mediated cytoskeleton disruption.
2. To assess apigenin as antidote against a range of doses of target poisons

Hypothesis

Sodium arsenite-induced toxicity through protein tyrosine kinase and phosphatidylinositol-3 kinase (PI3K) may influence Rho GTPase-mediated cell signaling and result in toxicity which can be overcome by apigenin.

Contributions of the Study

1. Better understanding of the toxicity of sodium arsenite related to protein tyrosine kinase, phosphatidylinositol-3 kinase (PI3K) and Rho GTPases signaling pathways that lead to the actin cytoskeleton disruption and toxicity.
2. Better understanding of the roles of arsenite-induced toxicity and possibly antidote therapy.