Index No. 55/18/Life Sc./25

Title: STUDIES ON THE SIGNALING CASCADE, LEADING TO APOPTOSIS, UNDER OXIDATIVE STRESS IN *Giardia lamblia*

Abstract

The causative agent of giardiasis in human and animals is the amitochondriate *Giardia lamblia* which posses a unique pathway for apoptosis under oxidative stress condition. We observed that exposing *Giardia* trophozoites to H_2O_2 led to an increase in lipid peroxidation compared to the control group, which was expressed in terms of menadione production as it is the marker for lipoperoxidation. Oxidative stress and peroxidation of membrane phospholipids are positively correlated with the enhanced PLA2 activity in several organisms to produce arachidonic acid (AA). Despite of producing PLA2, our data suggested *Giardia* produces a unique 56kDa dimeric enzyme called Phospholipase B (PLB) in contrast to higher eukaryotes. The enzyme activity existed in a broad pH and temperature range but it is highly active at pH7.5 and 35°C.The enzyme is produced upon induction with oxidative (H2O2) stress, thus leading to prostaglandin E2 (PGE2) production. We also analyzed the expression of PLB protein in *G. lamblia,* which was significantly induced under increased oxidative stress condition. This specific enzyme was responsible for the production of intracellular free AA. Now, this free AA either reacylates to the cell membrane or deacylates to further produce prostaglandins. In normal un-induced controlled trophozoites the membrane reacylation process was dominant due the higher level of acyle CoA synthase (ACS) expression over the time. However, under the oxidative stressed condition the intracellular ACS expression was down regulated. This led to the increase in deacylation process. When AA deacylation becomes dominant over AA reacylation in cells, the free AA accumulates intracellularly. The free AA is an important bioactive molecule as it can be metabolized by three distinct enzyme systems – cyclooxygenases (COXs), lipoxygenases (LOXs) and cytochrome P450 (CYP) enzymes and generates a diverse spectrum of biologically active fatty acid mediators. Our data indicated that metabolism of the free AA in *Giardia* trophozoites occurred by the presence of prostaglandin specific synthase (COX). One of the lipid autacoids, derived from AA is prostaglandinE2 (PGE2). Oxidative stress in trophozoites increased the PGE2 production over the time with respect to the controlled one. After 9 hours of incubation, the PGE2 concentration in treated cells reached approximately 90 pg/ml,indicating that oxidative stress can stimulate PGE2 production. The control cells, on the other hand, maintained a relatively stable PGE2 production level throughout the incubation period. The oxidative stress and high intracellular PGE2 concentration decreased the intracellular K+ concentration over the time. *Giardia* trophozoites maintain a constant K+ concentration in un induced controlled condition (approx. 140 mM). But in case of high PGE2 concentration the intracellular K+ concentration decreased. This decreased K+ concentration enhanced cell shrinkage and externalization of phosphatidyl serine. Phosphatidyl serine exposure is the marker for apoptotic cells and it was measured by the fluorescence of the FITC.

DR. SANDIPAN GANGULY
Senior Deputy Director (Scientist F) & Head
Division of Parasitology
National Institute of Cholera & Enteric Diseases
Indian Council of Medical Research
Department of Health Research
Ministry of Health and Family Welfare
Govt. of India
P-33 CIT Road, Scheme XM, Beliaghata
Kolkata 700 010, West Bengal, India

24/01/2024

Ritaparna Sarkar
24-01-2024