The mechanism of the program cell death and cell cycle control of suspension-cultured tobacco cells after exposure to Cadmium stress

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Abstract

Cadmium (Cd) is a toxic metallic element that induces oxidative stress and program cell death. In this study, the apoptosis level, ROS content, and caspase 3/7 activity in the presence of Cd and MAPKs inhibitor, were investigated at 3, 6, 12 and 24 h after treatment. The results indicated that Cd induced the apoptosis, ROS and caspase 3/7 activity at 6 h and 12 h after the Cd treatment, but in Cd & inhibitor treatments, there was a time shift in the peak of apoptosis, consistent with ROS and caspase 3/7 activity. The interpretation of results suggests that Cd, by interfering with the SIPK signalling pathway leads to increasing the ROS content, caspase 3/7 activity and ultimately inducing program cell death.

Keywords: SIPK signalling pathway, Cd, Apoptosis.

Introduction

Mitogen-activated protein kinases (MAPKs) are a major group of protein kinases, playing the main role in signal transduction [1]. One of this MAPKs is salicylate-induced protein kinase (SIPK), reported during some stress signalling pathway such as heavy metals [2]. Cd as a toxic metallic element leads to severe effect such as increased ROS level and oxidative stress, membrane leakage, DNA breakdown, protein cross-linking, protein cleavage, perturbation in cell proliferation, programmed cell death (PCD) [3]. PCD, the highly regulated destruction of cells, is a functional term, used to distinguish between the controlled and organized cell death from the unregulated form of necrosis [4]. This study was designed to investigate the role of SIPK signalling pathway on apoptosis induction by Cd treatment in the tobacco cells.

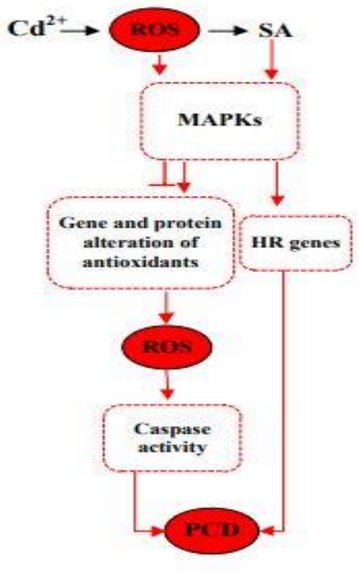
Materials and Methods

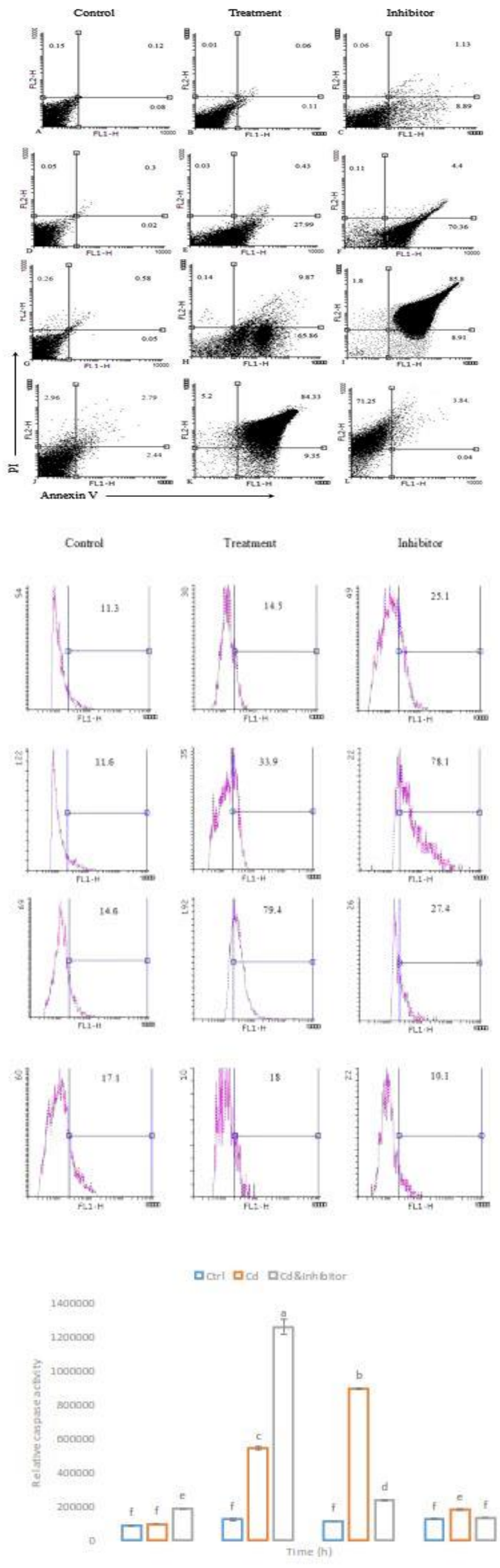
To investigate the role of Salicylate-induced protein kinase (SIPK) on Cd-induced cell death, cells were pre-treated with or without 40 μM PD98059 (Sigma, USA) as a non-competitive inhibitor of activation of MAPKK for 1h and then co-treated with 50 μM Cd for 3, 6, 12 and 24 h. After the desired periods of treatment, the cells were harvested, washed thoroughly with medium, frozen in liquid N2 and kept at -80˚C for further analysis.

Annexin V-FITC and DCFDA, the fluorogenic dye, were used to measure apoptosis and ROS level which can be detected by flow cytometry as described by the manufacturer (Abcam). Caspase activity was measured by luminescent assay system after preparation of cell lysate as described by the manufacturer (Promega).

Result and Discussion

In this study was shown that Cd treatment specifically induces ROS production, caspase activity and apoptosis induction, in a time-dependent manner. Our results at the presence of protein kinase inhibitor suggest that the SIPK signalling pathway is the mediator for effect on induction of program cell death. Liu et al. [5] have reported that Cd activates MPK3 and MPK6 in Arabidopsis via the accumulation of ROS, In addition to, it has been reported that a MAPK cascade is involved in Cd-induced cell death [6]. Based on the results of this study, the following pattern can be presented;





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