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Enhancement of 3-hydroxyanthranilic acid-induced T cell apoptosis through cinnabarinic acid generation☆

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Abstract. Induction of apoptosis by 100 μM of 3-hydroxyanthranilic acid (3HAA) was enhanced by SOD as well as $MnCl_2$ and further promoted in the presence of catalase. Corresponding to apoptosis induction, the generation of cinnabarinic acid (CA) through the oxidation of 3HAA was enhanced by SOD or $MnCl_2$ in the presence of catalase. Synthesized CA possessed more than 10 times higher apoptosis-inducing activity than 3HAA. CA generated from 3HAA possesses strong apoptosis-inducing activity in thymocytes through reactive oxygen species (ROS) generation, loss of mitochondrial membrane potential and caspase activation. © 2007 Published by Elsevier B.V.

Keywords: Cinnabarinic acid; 3-Hydroxyanthranilic acid; Apoptosis; Thymocytes; Reactive oxygen species

1. Introduction

3-Hydroxyanthranilic acid (3HAA) is one of the tryptophan metabolites along the kynurenine pathway and induces apoptosis in T cells. Autoxidation of 3HAA generates superoxide anion and an anthranilyl radical which, in turn, forms the quinone imine,

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followed by further oxidation and condensation between the quinone imine and 3HAA to generate cinnabarinic acid (CA) and hydrogen peroxide [1–4]. CA production is enhanced by superoxide dismutase (SOD), manganese ions or catalase [1–6]. 3HAA induces apoptosis in thymocytes through mitochondrial cytochrome *c* release and the activation of caspase-8 [7]. However, so far as we know, there is no report showing that CA possesses apoptosis-inducing activity. We show the mechanism of CA-induced apoptosis in thymocytes.

2. Materials and methods

2.1. Reagents

3HAA, catalase, SOD and 3,3-dihexyloxacarbocyanine iodide (DiOC6(3)) were purchased from Sigma-Aldrich (St. Louis, MO). Hydroethidine (HE)™ fluorescent stain was from Polysciences, Inc (Warrington, PA). Anti-cleaved caspase-3 (Asp 175) antibody was from Cell Signaling Technology Inc. (Beverly, MA).

2.2. Cell cultures

BALB/c mouse thymocytes were cultured in RPMI 1640 medium supplemented with 10% FCS, 0.3 mg/ml glutamine 100 μg/ml streptomycin, and 100 U/ml penicillin. Cells were cultured at 37 °C in a humidified atmosphere containing 5% CO_2. Cells were cultured for 18 h in apoptosis assay, for 4 h in reactive oxygen species (ROS) generation and mitochondrial membrane potential assay, and for 6 h in CA assay.

2.3. Flow cytometry

DNA fragmentation was assayed as described previously [8]. Stimulated cells were harvested and resuspended in propidium iodide. Nuclei to the “left” of the G1 peak were considered apoptosis. ROS generation was assessed using HE as described previously [8]. Loss of mitochondrial membrane potential was assessed using DiOC6(3) [9].

2.4. Assay of CA

Cells were cultured in culture medium without phenol red (Sigma-Aldrich, St. Louis, MO). Culture supernatants were collected and UV–visible spectra were measured by a UV–vis-NIR recording spectrophotometer. Culture supernatants were collected and stopped reaction by treatment with 5% trichloroacetic acid, and the absorption at 450 nm was assayed by HPLC.

2.5. Western blot

Western blot was carried out as described previously [8]. Caspase activation was assayed by western blot using anti-caspase-3 antibody.