S9-8

Cell Cycle Arrest and Apoptotic Induction by MCS-C2 in Human Leukemia HL-60 Cells

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Abstract

The purpose of the present study was to investigate the anti-proliferative and apoptotic effects of MCS-C2, a novel analogue of toyocamycin and sangivamycin, in human promyelocytic leukemia (HL-60) cells. When treated with MCS-C2, inhibited proliferation associated with cell cycle arrest and apoptotic induction was found in the HL-60 cells in a concentration-dependent and time-dependent manner. This apoptotic induction was associated with the cleavage of Bid and a release of cytochrome *c* from mitochondria into the cytosol, followed by the activation of caspase-3 and inactivation of poly (ADP-ribose) polymerase (PARP). However, there was no significant change in any other mitochondrial membrane proteins, such as Bcl-2 and Bax. Consequently, the current findings suggest that the mitochondrial pathway was primarily involved in the MCS-C2-induced apoptosis in the human promyelocytic leukemia HL-60 cells.

Introduction

Recently, considerable attention has been focused on the sequence of events referred to as apoptosis and its role in mediating the anti-neoplastic effects of diverse small chemicals in leukemia cells [2].

In the course of screening for a novel inhibitor of CDK2 and CDC2, the current authors isolated toyocamycin and sangivamycin from a culture both of *Streptomyces* sp. LPL931 [9]. Toyocamycin was first reported as an antibiotic in 1966 [1], and later shown to inhibit RNA synthesis in mammalian cells [4]. Yet, when toyocamycin was tested as a possible cancer treatment, a high toxicity was reported [10]. Accordingly, many toyocamycin analogues have been synthesized and evaluated for anti-tumor and antiviral activities since toyocamycin was first isolated four decades ago [3,6,7,8].

Therefore, in an attempt to search for a specific inhibitor that can inhibit cyclin-dependent kinase (CDK) with minimal side effects on other Ser/Thr protein kinase activity, the current authors previously synthesized an analogue of toyocamycin, MCS-C2 (Fig. 1), and evaluated its CDK inhibitory activity [5]. As such, the present study investigated the anti-neoplastic potential and mode of action of apoptosis by MCS-C2 in human leukemia HL-60 cells.

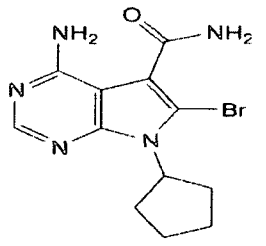

Fig. 1. Structure of MCS-C2.

Result and Discussion

1. Anti-proliferative effect of MCS-C2 in HL-60 cells

To determine the MCS-C2-inducing cell growth inhibition in a human cancer cell line, the effect of MCS-C2 on the viability of the human leukemia HL-60 cell line was assessed using an MTT assay. As shown in Fig. 2, MCS-C2 inhibited the growth of HL-60 cells treated with 0.5 to 5 mM of MCS- C2 in a time-dependent and dose-dependent manner (IC_{50}: 0.21 mM). A concentration of 3 and 5 mM of MCS-C2 significantly inhibited the growth of HL-60 cells after 12 ($P<0.05$), 24, and 48 h ($P<0.01$), when compared to the control group.

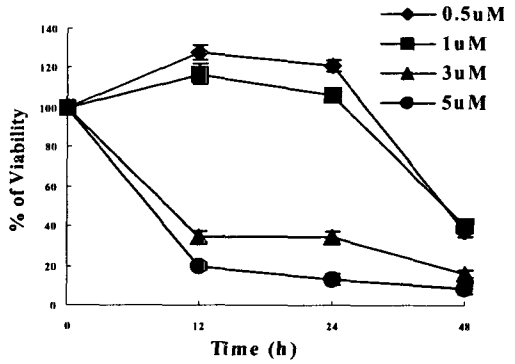

Fig. 2. Cell growth inhibition of MCS-C2 in HL-60cells.

Based on previous reports showing that toyocamycin and its analogues are highly toxic to mammalian cells due to its non-specific inhibition of various protein kinases, such as PKC and PKA, the ability of MCS-C2 to selectively inhibit the cell cycle regulator kinase, namely CDC2, was tested. As shown in Fig. 3, MCS-C2 consistently produced a dose-dependent decrease in CDC2 activity (IC_{50}: 0.38 mM). However, MCS-C2 showed no significant inhibition of PKC and PKA even at a high concentration (500 mM). Accordingly, these results suggest that MCS-C2 could selectively inhibit CDC2 kinase at 0.38 mM without affecting other protein kinases.

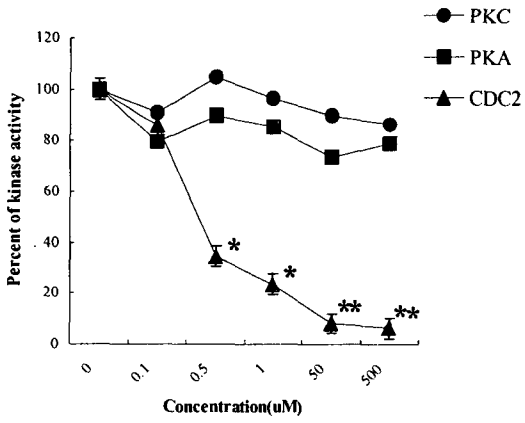

Fig. 3. Kinase activity of MCS-C2.

2. Effect of MCS-C2 on Cell Cycle Progression

To elucidate the mechanism of the MCS-C2-induced inhibition of human leukemia cell growth, the cell cycle progression was analyzed using flow cytometry. The HL-60 cells were cultured with MCS-C2 at the indicated time, washed, and stained with Propidium iodide (PI), then the cell cycle was analyzed. As shown in Fig. 4, the flow cytometric analysis revealed an appreciable arrest of cells in the G2/M phase of the cell cycle after treatment with 2 mM MCS-C2. The HL-60 cell population increased gradually from 13 % at 0 h, to 28 % after 12 h in the G2/M phase, after exposure to 2 mM of MCS-C2. Furthermore, an eventual progression to apoptosis was observed after 12 h (18 %). The percentage of S phase cells was not profoundly affected. Accordingly, treatment with MCS-C2 induced a G2/M phase arrest of the cell cycle progression of HL-60 cells.

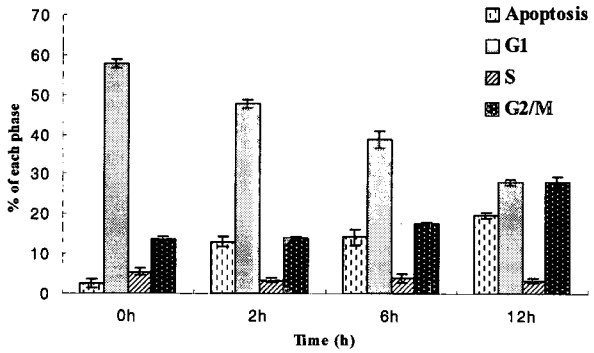

Fig. 4. Flow cytometry analysis of cell cycle and apoptosis in HL-60 cells treated with MCS-C2.

3. Induction of apoptosis by MCS-C2 in HL-60 cells

To verify whether the growth inhibitory effect of MCS-C2 was due to apoptosis, nuclear DAPI staining was used to examine any morphologic changes in the nuclei of the HL-60 cells treated with MCS-C2, and the nuclear change found in the treated HL-60 cells was typical of apoptosis: a fragmented apoptotic body after DAPI staining (Fig. 5). Apoptotic bodies were observed with 5 μM MCS-C2 after incubation for 6 h, and the number of apoptotic cells increased as the concentration of MCS-C2 increased.

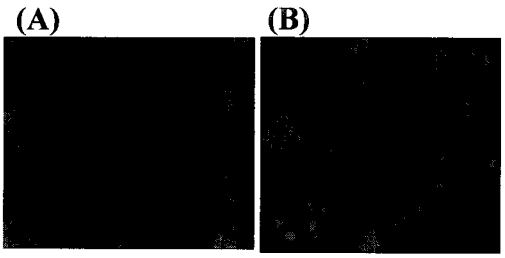

Fig. 5. Morphorogical change of HL-60 cells treated with MCS-C2. (A) : Control, (B): treated with 5 μM MCS-C2.

4. Effect of MCS-C2 on activation of caspase

To identify the mechanism of the MCS-C2-induced apoptosis, an examination was made of the changes in the intracellular proteins related to apoptosis, such as caspase-3, -8, -9, and PARP. As shown in Fig. 6B,

MCS-C2 induced the proteolytic cleavage of inactive pro-caspase-8, -9, and -3 into active caspase-8, -9, and -3, respectively.

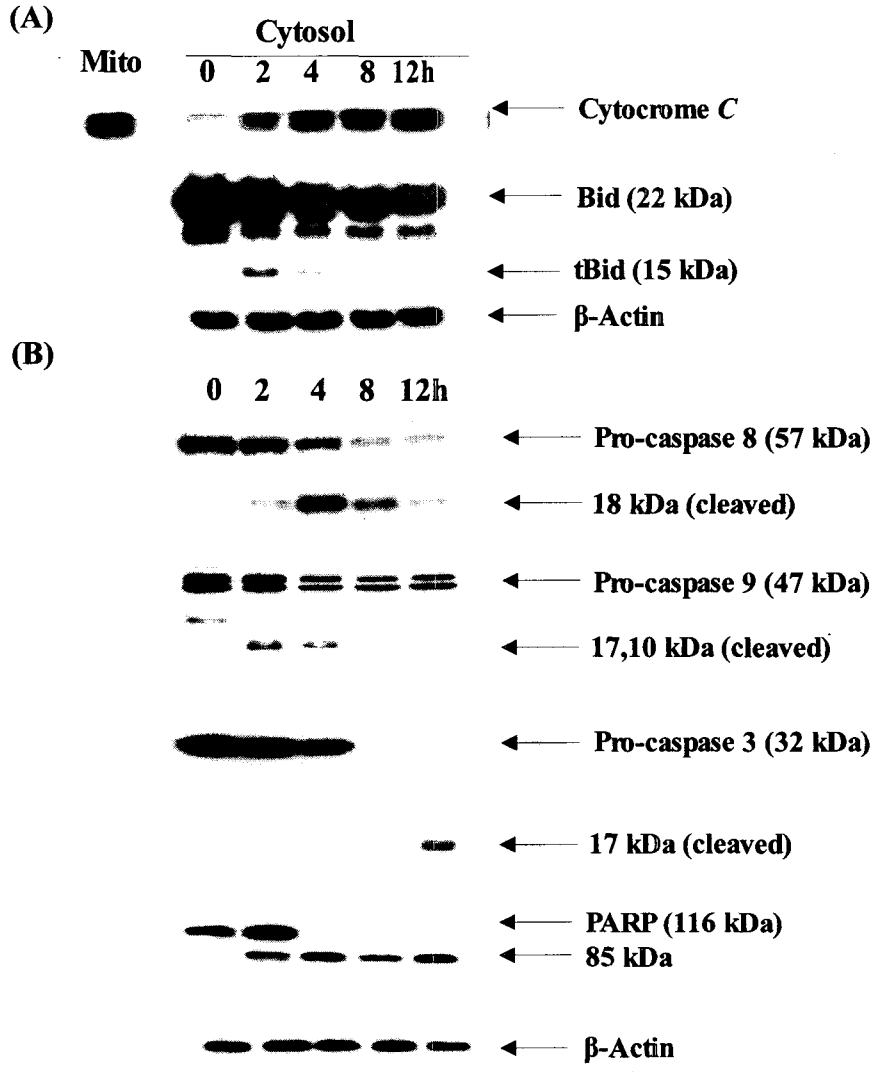

Fig. 6. Protein levels related with apoptosis regulation in HL-60 cells treated with MCS-C2.
(A): Released cytochrome c from mitochondria to cytosol and cleaveged Bid. (B): Activated caspase-9,-8,-3 and inactivated PARP.

5. Effect of MCS-C2 on cytochrome c and Bcl-2 protein

To demonstrate the involvement of mitochondrial protein in the process of MCS-C2-induced apoptosis, the cytochrome *c* released from the mitochondria into the cytosol during treatment with 5 μM MCS-C2 was analyzed by a Western blot analysis. The results showed that the amount of cytosolic cytochrome *c* gradually increased after 2 h until 12 h in contrast to the mitochondrial cytochrome *c* at 0 h (Fig. 6A). Plus, the involvement of the cleavage of Bid in the cytochrome *c* release during the treatment of the cells was also examined. As shown in Fig. 6A, Bid was detected in a 22 kDa pro-form by an immunoblot analysis and cleaved to a 15 kDa fragment after treatment with 5 μM MCS-C2.

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