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# HeLa cells apoptosis induced by 1,7-dimethyl-1,4,7,10-tetraazacyclododecane

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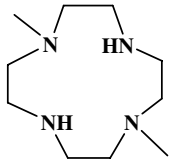
**Abstract**—Preliminary pharmacological tests showed that 1,7-dimethyl-1,4,7,10-tetraazacyclododecane (DMC) had antitumor activity against HeLa and A549 cell lines in vitro. The HeLa cells apoptosis induced by DMC was examined by flow cytometric meter, and further confirmed by observing the morphological changes and DNA fragmentation. No observation of A549 cells induced apoptosis was observed by DMC.


Macrocyclic polyamines are a family of heterocycles that could bind cations and anions, and form metal complexes. Their metal complexes have already attracted great interest because of their novel properties[1] and significant applications in biology and medicine.[2,3] Recently, many macrocyclic polyamines have been found to be greatly useful in medical application. For example, bicyclams (cyclam = 1,4,8,11-tetraazacyclotetradecane)[4] and cyclotriazadisulfonamide (CADA)[5] have already exhibited anti-HIV virus activity. Some functionalized macrocyclic polyamines showed their antitumor activities.[6] However, macrocyclic polyamines that could induce tumor cells apoptosis have not been reported yet. Apparently, detailed investigation on their mechanism will be very useful for drug design.

Anticancer agents that target DNA are among the most effective in clinical application and have produced significant increases in the survival of patients with cancer.[7] In previous work, our group found that 1,7-dimethyl-1,4,7,10-tetraazacyclododecane (DMC, Scheme 1) could hydrolyze double-strand DNA under physiological conditions.[8] Concerning the potential cytotoxic properties of DMC, its effect on human cancer cell lines HeLa (cervical cancer) and A549 (lung cancer) was studied in our laboratory. The results showed that DMC could only induce HeLa cells apoptosis although it was effective inhibitor to both cells growth. This is to our knowledge the first cell apoptosis induced by macrocyclic polyamines. Herein, we report our preliminary results of this investigation.




**Scheme 1.** 1,7-Dimethyl-1,4,7,10-tetraazacyclododecane (DMC).

DMC was synthesized according to the literature.[9] HeLa and A549 cell lines were obtained from China Center for Type Culture Collection (CCTCC) and grown in MEM supplemented with 10% FBS (Fetal Bovine Serum), incubated at 37 °C in a 5% $CO_2$, 95% air atmosphere, and subcultured every two to three days as necessary. The effect of DMC on growth of two cancer cell lines was determined using the standard MTT assay. Results in Table 1a show that DMC was effective inhibitor to HeLa and A549 cancer cells growth. DMC

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**Table 1.** The percentage of inhibition to tumor cell lines (%)

| Cell lines | Concentration (mol $L^{-1}$) | | | |
|---|---|---|---|---|
| | $10^{-4}$ | $10^{-5}$ | $10^{-6}$ | $10^{-7}$ |
| *(a) HeLa and A549 cells treated with DMC* | | | | |
| HeLa | 63.1 | 17.7 | 9.8 | 9.0 |
| A549 | 53.6 | 22.4 | 13.7 | 11.0 |
| *(b) A549 cells treated with M–DMC systems* | | | | |
| M–DMC | | | | |
| Co(II)–DMC | 35.1 | 11.0 | 4.5 | 3.7 |
| Co(II) | 8.6 | −2.9 | −6.0 | −5.6 |
| Cu(II)–DMC | 2.6 | 3.5 | 5.8 | 4.8 |
| Cu(II) | −8.1 | −3.8 | −2.1 | −0.8 |
| Ni(II)–DMC | 43.7 | 11.1 | 9.3 | 6.1 |
| Ni(II) | −3.1 | −15.1 | −5.8 | −9.2 |
| Zn(II)–DMC | 7.5 | 1.9 | 1.5 | −0.6 |
| Zn(II) | 0.1 | −10.6 | −6.3 | −2.4 |

The cells were treated for 72 h and incubated with 5 g $L^{-1}$ MTT for 4 h. The amount of MTT formazan produced was determined by measuring absorbance at 570 nm.[11] All data are represented as means ± SD values obtained from eight separate cultures.

is a versatile chelating agent and could form stable complexes with transition metal ions,[9] so cytotoxicity of cobalt (II), copper (II), nickel(II), and zinc (II) ions in the absence and presence of DMC, respectively, was also evaluated. The M–DMC systems for our study were obtained by adding an appropriate amount of a solution of metal ion to a solution of DMC.[10] The M:DMC molar ratio was 1:1 for all tested systems. Results in Table 1b show that metal ions alone could not exhibit positive antitumor activity, and DMC was the most effective inhibitor to A549 cells growth in all tested systems, and the inhibitory activity of system sharply decreased when copper or zinc ions coordinated with DMC. These results may suggest that cytotoxicity, for M–DMC systems, could be quite safely ascribed to the presence of macrocyclic ligand.

In order to gain more insight into cell-death pathways, we have carried out the apoptosis study. Apoptosis, a type of programmed cell death, is a physiological 'cell-suicide' program that is essential for embryonic development, immune-system function, and the maintenance of tissue homeostasis in multicellular organisms.[12] Apoptotic misregulations are connected with several diseases, in particular cancer. Small molecules which could overcome the apoptosis deficiency of cancer cells are therefore of high medical significance.[13] Apoptosis determination was performed by the flow cytometry assay staining with propidium iodide (Fig. 1). Dose–response analysis indicated that the percentage of apoptotic HeLa cells was about 55% at the concentration of 300 μmol $L^{-1}$, and the percentage of apoptotic A549 cells was just about 7% at the concentration of 200 μmol $L^{-1}$ (Fig. 2a). The changes in cell cycle are shown in Figure 2b and c. DMC caused changes in the proportion of HeLa cells in S phase (from 21.6% to 33.3% to 0%) accompanied by changes in $G_2$-M phase (from 2.4% to 25.2% to 15.3%), and changes in $G_0$–$G_1$ phase (from 76.0% to 60.5% to 84.7%). While DMC exhibited slight perturbation effects on A549 cells cycle. So cytotoxic mechanisms are also different in tumor cells

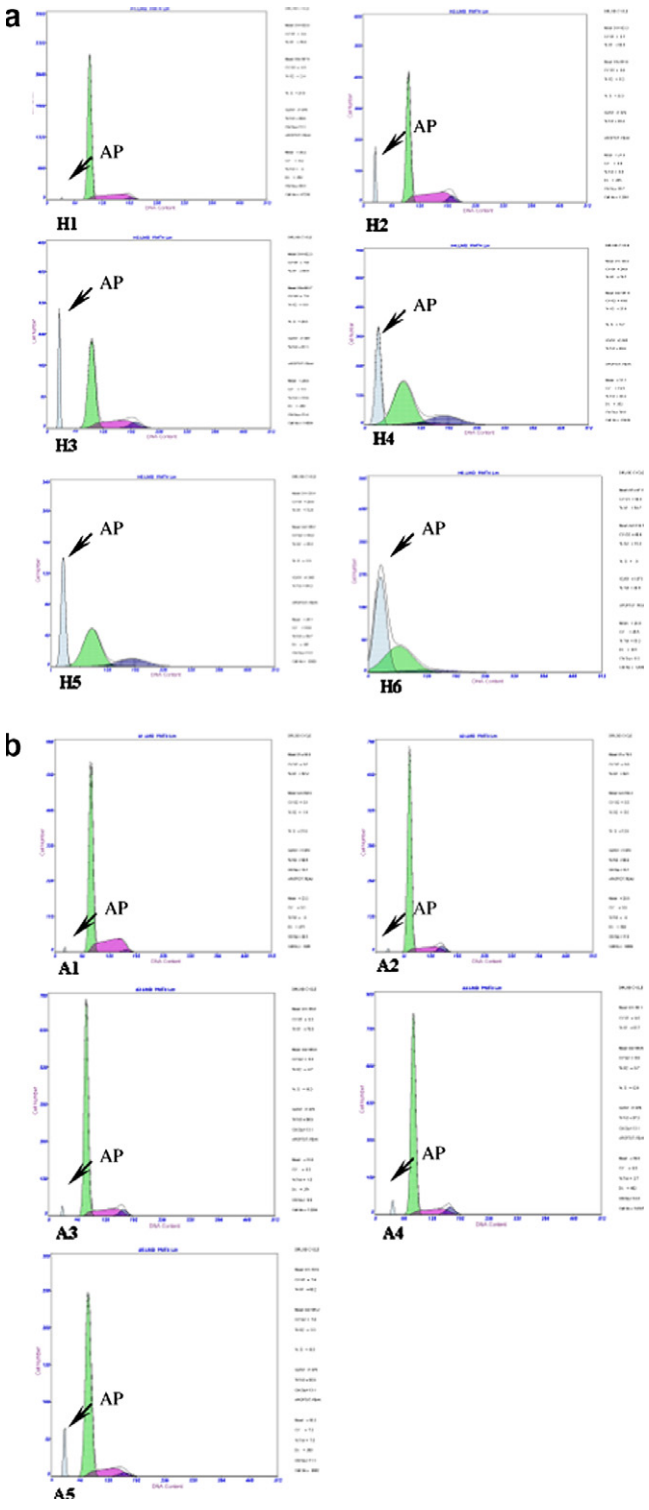


**Figure 1.** The histogram of drug-induced cell cycle arrest and apoptosis of DMC in (a) HeLa and (b) A549 cells as measured by flow cytometric analysis. The *x*-axis is DNA content and the *y*-axis is the number of cells with that fluorescence intensity. H1(A1), H2(A2), H3(A3), H4(A4), H5(A5), H6 represent the cells treated with 0, 50, 100, 150, 200, and 300 μmol $L^{-1}$ DMC and, respectively. AP means apoptosis peak. Experiments were repeated three times and the representative results are shown.

treated with DMC. The apoptosis involves many complicated processes, including initiation by death-inducing signals, integration of different signals, and

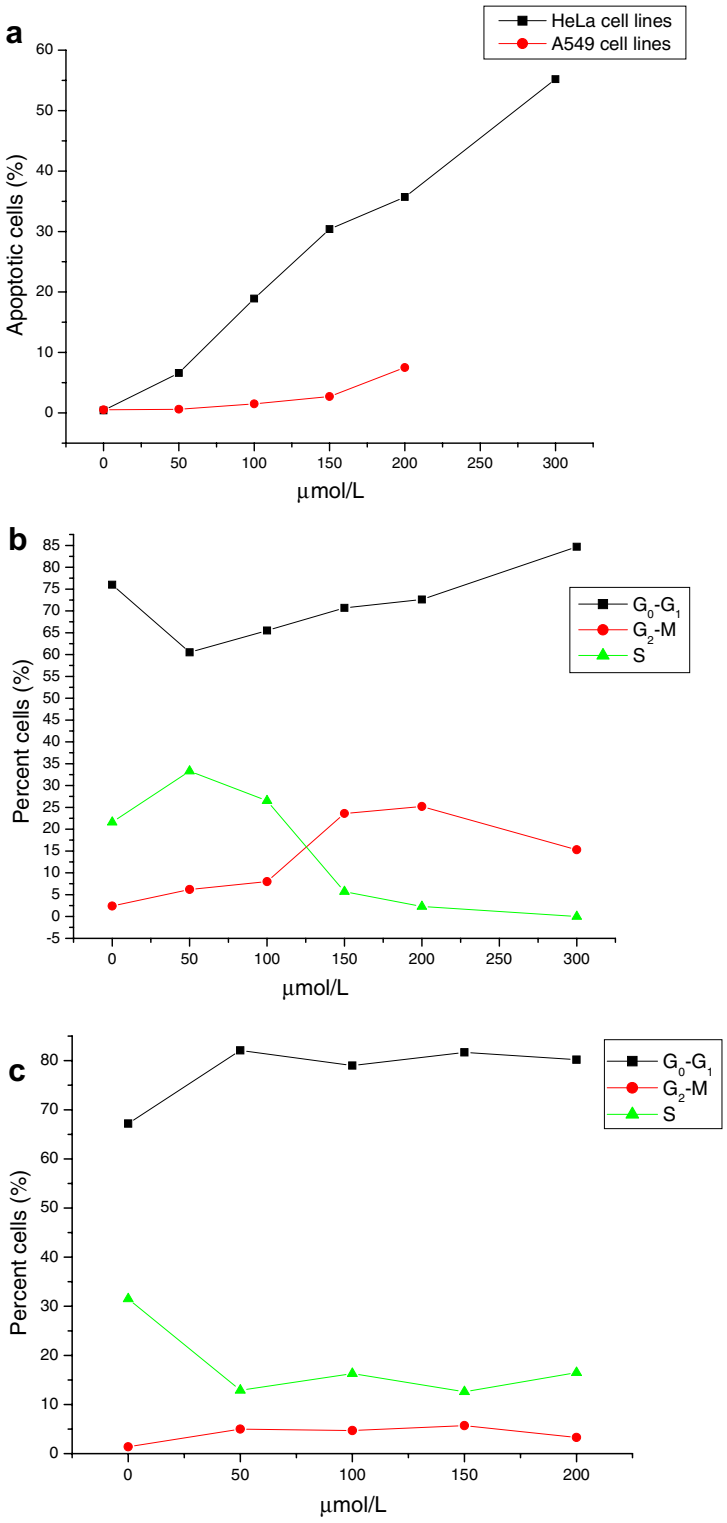


**Figure 2.** (a) Dose–response curve on DMC-induced apoptosis; (b) the perturbation of DMC to HeLa cells cycle; (c) the perturbation of DMC to A549 cells cycle. HeLa cells were treated with 50, 100, 150, 200, and 300 μmol $L^{-1}$ DMC for 72 h. A549 cells were treated with 50, 100, 150, and 200 μmol $L^{-1}$ DMC for 72 h. Cells were then fixed with 70% ethanol and stained with 0.1 g $L^{-1}$ propidium iodide solution. Cell suspensions were then incubated at 37 °C for 30 min, and stained nuclei were analyzed with a Beckmen Coulter Eltra flow cytometer.[15]

determination of whether cells live or die.[14] The exact mechanism of HeLa cells apoptosis induced by DMC needs to be further investigated.

Apoptosis is regulated by genetic mechanisms and is principally characterized by morphological and biochemical changes in the nucleus, including chromatin condensation and internucleosomal DNA fragmentation.[16] To confirm the existence of apoptosis in HeLa cells treated with DMC, morphology of apoptosis and the ordered DNA ladder detection were investigated. HeLa cells treated with 50 μmol $L^{-1}$ DMC

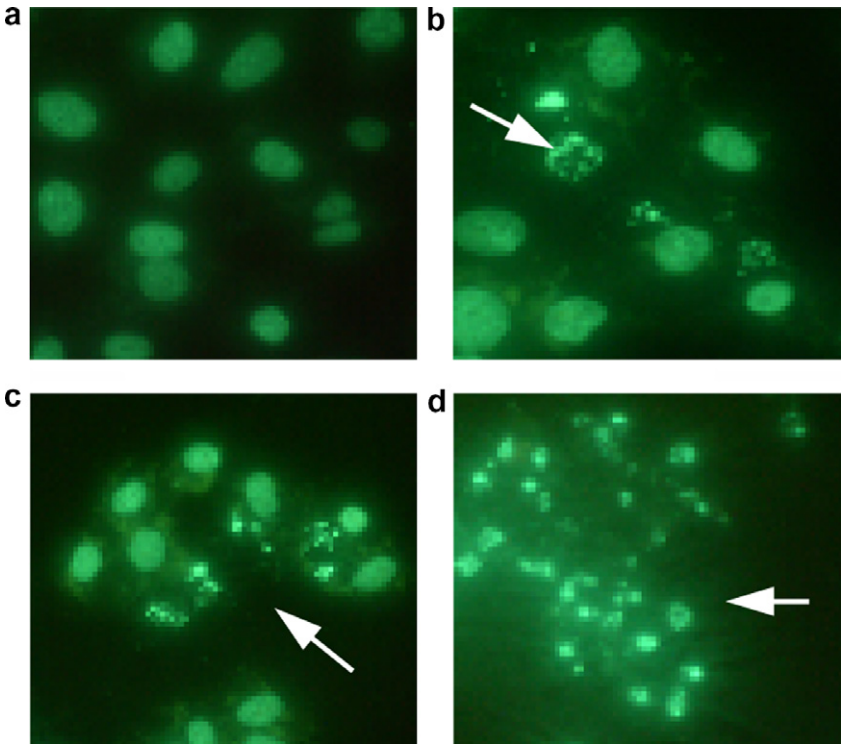


**Figure 3.** Hoechst 33258 staining of HeLa cells (400×). The cells were treated with increasing DMC concentrations after 72 h of cultivation. (a) control; (b) 50 μmol $L^{-1}$; (c) 100 μmol $L^{-1}$; (d) 200 μmol $L^{-1}$.

produced no obvious morphologic changes, but in cells treated with 100 and 200 μmol $L^{-1}$ DMC, there was an increase in the percentage of rounded cells with the characteristic morphological changes of apoptosis including membrane blebbing, chromatin condensation, and the formation of apoptotic bodies after being stained with Hoechst 33258 (Fig. 3).[17] DMC-induced apoptosis of HeLa cells in a concentration-dependent manner was revealed, that is in good accordance with the result obtained by flow-cytometric analysis. DNA of HeLa cells treated with DMC was extracted and analyzed on 2% agarose gel as Treves described.[18] As illustrated in Figure 4, agarose gel electrophoresis of DNA extracted from HeLa cells treated with 200 μmol $L^{-1}$ DMC exhibited a progressive increase in nonrandom fragmentation into a ladder of 180–200 bp with treated time increasing. Agarose gel electrophoresis revealed that DMC-induced apoptosis of HeLa cells in a time-dependent manner.

In conclusion, 1,7-dimethyl-1,4,7,10-tetraazacyclododecane (DMC) showed antitumor activity against HeLa and A549 cell lines in vitro. Apoptosis of DMC-stimulated HeLa cells was determined by flow-cytometric analysis, morphological changes, and oligonucleosomal DNA fragments. Results indicated that DMC-induced apoptosis of HeLa cells in a concentration- and time-dependent manner. However, DMC did not exhibit induction effects on A549 cells apoptosis. Further work is in progress to elucidate the detailed mechanisms of apoptosis induced by DMC and its analogues.

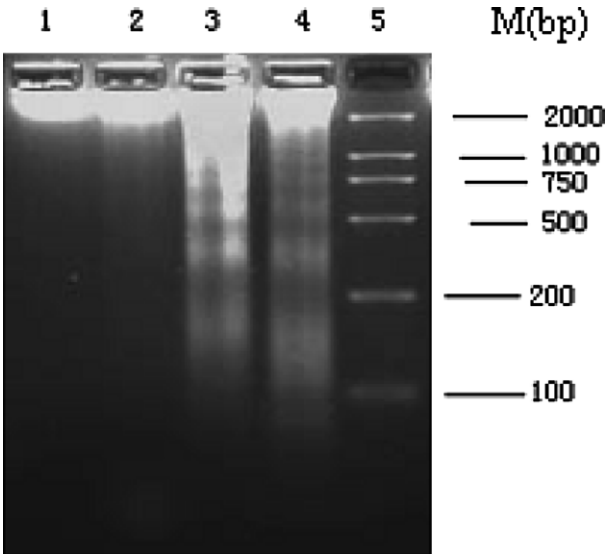


**Figure 4.** HeLa cells were incubated with or without 200 μmol $L^{-1}$ DMC. Cells were lysed and the DNA was prepared and electrophoresed on 2% agarose gels and stained with ethidium bromide for detection of DNA fragmentation. Lane 1, untreated cells; lane 2, cells treated for 24 h; lane 3, cells treated for 48 h; lane 4, cells treated for 72 h; lane 5, DNA maker.

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