

Inhibition of harringtonine-induced apoptosis by tetradecanoylphorbol acetate in human leukemia HL-60 cells¹

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KEY WORDS HL-60 cells; apoptosis; tetradecanoylphorbol acetate; harringtonines; camptothecin; *myc* genes

AIM: To study the changes of the apoptosis induced by camptothecin (Cam) or harringtonine (Har) in human leukemia HL-60 cells after the cells were preincubated with tetradecanoylphorbol acetate (TA).

METHODS: Chromatin condensation observation, flow cytometry, DNA agarose gel electrophoresis, and Dot blot hybridization. **RESULTS:** After the HL-60 cells were preincubated with TA 200 nmol · L⁻¹ for 6 h, the apoptosis induced by Har 0.1 mg · L⁻¹ for 3 h was drastically inhibited, and the apoptosis by Cam 0.2 mg · L⁻¹ for 3 h was partly inhibited. On the other hand, the expression level of *c-myc* gene in HL-60 cells decreased apparently after the preincubation of TA. **CONCLUSION:** TA preincubation inhibited the apoptosis induced by Har obviously or by Cam partly in human leukemia HL-60 cells, and the expression of *c-myc* gene decreased drastically in the preincubated cells, which might result in the inhibition of apoptosis.

Harringtonine (Har), a domestic antitumor drug, induced apoptosis in human leukemia HL-60 cells^[1], so did camptothecin (Cam)^[2]. Inhibitors of protein kinase C (PKC) can induce apoptosis^[3], and tetradecanoylphorbol acetate (TA), a PKC activator, inhibited the induction of apoptosis by different inducers in various types of cells^[4, 5]. Yet high activity of PKC was found in the Har-resistant HL60 cells^[6]. Furthermore, apoptosis was induced by the increment of expression of *c-myc* gene^[7]. Although with a high activity of PKC, the expression level of *c-myc* decreased obviously^[8] and the resistant HL-60

cells had resistance to the tetrandrine-induced apoptosis^[9]. So in this paper the changes of apoptosis and expression of *c-myc* gene were examined during the apoptosis induced by Har and Cam, after HL-60 cells were preincubated with TA.

MATERIALS AND METHODS

Drugs and cell culture Har was purchased from Beijing Union Pharmaceutical Factory and made up into a solution of 1 g · L⁻¹ with PBS buffer. Cam from Sigma was dissolved in Me₂SO at a concentration of 1 g · L⁻¹. TA from Xichuan Pharmaceutical Factory, Henan Province was dissolved in Me₂SO to a concentration of 1 mol · L⁻¹. Human leukemia HL-60 cells were routinely cultivated in RPMI 1640 medium (Gibco), supplemented with 10 % - 12 % bovine serum at 37 °C in 95 % air + 5 % CO₂. The cells at logarithmic growth stage were used in all the experiments.

Drug treatment TA 200 nmol · L⁻¹ (ultimate concentration) was added to the culture medium with the cells at logarithmic growth stage and the cells were incubated normally for 6 h. After that, Har 0.1 mg · L⁻¹ or Cam 0.2 mg · L⁻¹ were added to the preincubated or control HL-60 cells for 3 h, respectively, to induce apoptosis of the cells.

Detection of apoptosis Chromatin condensation was observed in the cells stained with the fluorescent dyes hoechst 33342 and propidium iodide (PI) at the same time, and the cells with condensed or fragmented chromatin were considered as apoptotic cells, thus the apoptotic cell number of 200 cells/sample was counted and obtained. Cells 2 × 10⁶ were used in flow cytometry and the DNA from 3 × 10⁶ cells treated with the drugs was taken for agarose gel electrophoresis^[9].

Expression of *c-myc* gene by Dot blot hybridization

Total RNA was extracted from 5 × 10⁶ cells in absence or presence of the drugs for 1.5 h, and taken for Dot blot hybridization^[9].

RESULTS

Chromatin condensation and percent of apoptotic cells After the HL-60 cells were preincubated with TA 200 nmol · L⁻¹ for 6 h, the chromatin condensation induced by Har 0.1 mg · L⁻¹ for 3 h was apparently inhibited and the percentage of apoptotic cells decreased from 36 % to 9 %, but the

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induction of apoptosis by Cam was only partly inhibited (Tab 1). In all the samples, PI negative ratio was >95%, suggesting the integrity of cell membrane during apoptosis.

Tab 1. Percentage of apoptotic cells in HL-60 cells preincubated with TA 200 nmol·L⁻¹ for 6 h, treated with Har 0.1 mg·L⁻¹ or Cam 0.2 mg·L⁻¹ for 3 h. n = 9 samples (200 cells/sample), $\bar{x} \pm s$. *P < 0.01 vs Har; †P < 0.01 vs Cam.

Drugs	Apoptotic cells	
	Number	%
Control	4.0 ± 0.8	2.0 ± 0.4
TA	3.1 ± 1.2	1.6 ± 0.6
Har	73 ± 6	36 ± 3
Har + TA	18 ± 4	9 ± 2 ^c
Cam	103 ± 6	52 ± 3
Cam + TA	80 ± 8	40 ± 4 ^f

Detection of apoptosis by flow cytometry

The apparent "sub-G₁" peak showed in the HL-60 cells treated with Har 0.1 mg·L⁻¹ for 3 h, but disappeared in the TA-preincubated cells treated with the same concentration of Har. Although the "sub-G₁" peak was obviously observed in the HL-60 cells treated with Cam 0.2 mg·L⁻¹ for 3 h, it had no apparent changes in the TA-preincubated HL-60 cells treated with Cam (Fig 1).

Cell DNA electrophoresis No DNA "ladder" pattern was observed in the TA-preincubated HL-60 cells treated with Har 0.1 mg·L⁻¹ for 3 h, while a typical DNA "ladder" pattern showed in HL-60 cells treated with Har. The same DNA "ladder" patterns appeared in both HL-60 cells and TA-preincubated HL-60 cells treated with Cam 0.2 mg·L⁻¹ for 3 h (Fig 2).

Expression of c-myc gene In the HL-60 cells preincubated with TA 200 nmol·L⁻¹ for 6 h, or cells treated with TA 200 nmol·L⁻¹ for 6 h + Har 0.1 mg·L⁻¹ for 1.5 h or TA 200 nmol·L⁻¹ for 6 h + Cam 0.2 mg·L⁻¹ for 1.5 h, the expression of the c-myc gene decreased drastically, and the expression had no apparent changes in the cells treated with Har 0.1 mg·L⁻¹ or Cam 0.2 mg·L⁻¹ for 1.5 h (Fig 3, A).

The expression level of β-actin gene in all the treated cells had no changes vs the control, suggesting that equal amount of total cellular RNA was loaded on the hybridization filter (Fig 3, B).

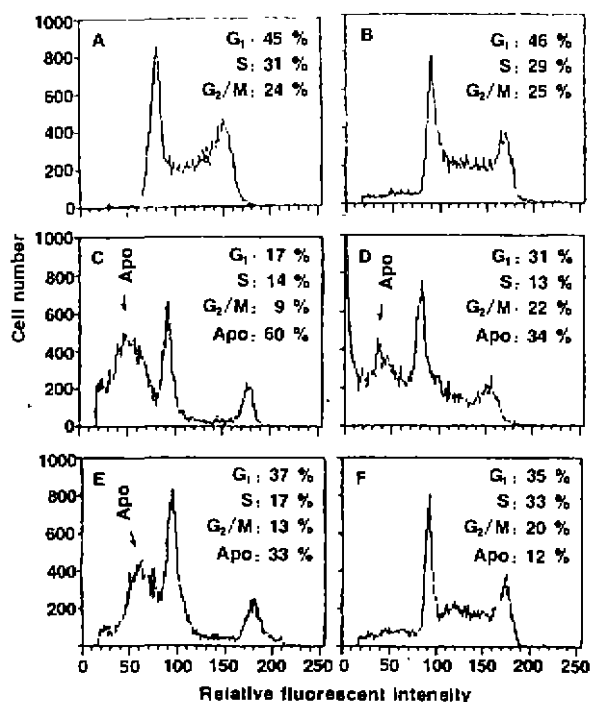


Fig 1. DNA frequency of HL-60 cells and TA-preincubated HL-60 cells treated with Cam 0.2 mg·L⁻¹ or Har 0.1 mg·L⁻¹ for 3 h. (A) HL-60 cells, control; (B) TA 200 nmol·L⁻¹ for 6 h; (C) Cam 0.2 mg·L⁻¹ for 3 h; (D) Har 0.1 mg·L⁻¹ for 3 h; (E) TA 200 nmol·L⁻¹ for 6 h + Cam 0.2 mg·L⁻¹ for 3 h; (F) TA 200 nmol·L⁻¹ for 6 h + Har 0.1 mg·L⁻¹ for 3 h.

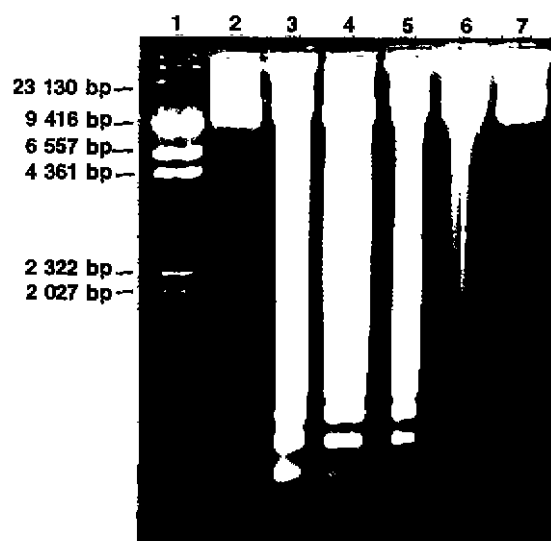


Fig 2. Agarose gel electrophoresis of DNA extracted from HL-60 cells and TA-preincubated HL-60 cells treated with Cam 0.2 mg·L⁻¹ or Har 0.1 mg·L⁻¹ for 3 h. (1) DNA Marker: λDNA/Hind III; (2) Control; (3) Cam; (4) Cam + TA; (5) Har; (6) Har + TA; (7) TA.

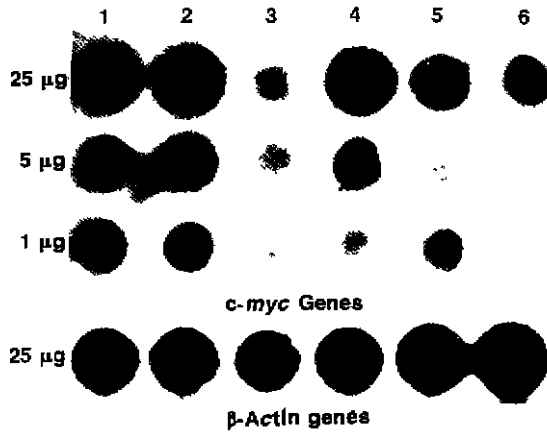


Fig 3. Expression changes of *c-myc* genes and β -actin genes detected by Dot blot hybridization using the total cell RNA extracted from HL-60 cells and TA-preincubated HL-60 cells treated with Har $0.1 \text{ mg} \cdot \text{L}^{-1}$ or Cam $0.2 \text{ mg} \cdot \text{L}^{-1}$ for 1.5 h. (1) Control, HL-60 cells; (2) Har; (3) TA + Har; (4) Cam; (5) TA + Cam; (6) TA.

DISCUSSION

The results indicated the different responses to the induction of apoptosis by Har and Cam in the TA-preincubated HL-60 cells. The inhibitory action of TA may be through PKC, for TA could arouse the total PKC activities of many types of cells. However, the PKC activity of the TA-preincubated HL-60 cells remains to be determined. The various responses to apoptosis induced by Har and Cam are due to different mechanisms, for Har was an inhibitor of protein synthesis^[10], and Cam was an inhibitor of topoisomerase I^[11]. The TA-preincubated HL-60 cells drastically decreased the expression of *c-myc* gene, and thus resulted in the inhibition of apoptosis induced by Har, implying a link among PKC, *c-myc* gene, and apoptosis in HL-60 cells, *ie*, activation of PKC led to the downregulation of *c-myc* gene, then resulted in the block of apoptosis in the cells. However, it is elusive that inhibition of apoptosis in the differentiated HL-60 cells was uninvolved in the expression of *c-myc* gene^[12,13]. So there may be a complex role for the *c-myc* gene in the induction of apoptosis. In conclusion, this paper demonstrated that in the TA-preincubated HL-60 cells, the induction of apoptosis were apparently (by Har) or partly (by Cam) inhibited. And the decrement of *c-myc* gene

expression may play a role in inhibiting apoptosis.

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十四酰佛波乙酸酯抑制三尖杉酯碱诱导的人白血病 HL-60 细胞凋亡¹

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关键词 HL-60 细胞; 细胞凋亡; 十四酰佛波乙酸酯; 三尖杉酯碱类; 喜树碱; *myc* 基因

目的: 研究十四酰佛波乙酸酯(TA)预处理后, 三尖杉酯碱(Har)和喜树碱(Cam)诱导人白血病HL-60细胞凋亡的变化。 **方法:** 染色质凝集观察, 流式细胞术, DNA 琼脂糖凝胶电泳和点杂交。 **结**

果: TA 200 nmol·L⁻¹预处理 HL-60 细胞 6 h, 明显抑制 Har 0.1 mg·L⁻¹作用 3 h 诱导的细胞凋亡, 但只部分抑制 Cam 0.2 mg·L⁻¹作用 3 h 诱导的细胞凋亡。 TA 预处理 HL-60 细胞明显降低 *c-myc* 基因的表达。 **结论:** TA 明显抑制由 Har 和部分抑制由 Cam 诱导的 HL-60 细胞凋亡, 而且 TA 预处理的细胞中 *c-myc* 基因表达明显下降, 这可能导致了诱导细胞凋亡的抑制。

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