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Journal of Chromatography A, 894 (2000) 165–170

JOURNAL OF
CHROMATOGRAPHY A

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Capillary electrophoretic determination of apoptosis of HeLa cells induced by trichosanthin

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Abstract

Apoptosis is a distinct mode of cell death that is responsible for deletion of cells in normal tissues; it also occurs in specific pathologic contexts. The observation of apoptosis is very important in the research of cancer and cancer therapy. The traditional observation method of apoptosis was agarose gel electrophoresis, which is depending on the determination of ladder-liking DNA fragments extracted from apoptotic cells. It is time-consuming and low-sensitive. Recently, the sieving capillary electrophoresis has been used to detect apoptosis too. However, the problem of DNA fragments contamination is still existing. Here, we have developed a capillary electrophoresis method that could detect apoptosis of whole cell directly and do not need to extract DNA fragments from cells. Apoptosis of adherent cell HeLa cell of carcinoma induced by cyclophosphamide was used as the model to establish the method. The effluence of medicine concentration on apoptosis of cells was studied in detail. It was also found that the method could detect the change of cells in the early period of apoptosis. The induction of apoptosis of HeLa cell by trichosanthin was determined with the method, and the result of flow cytometry was also proved that trichosanthin could result in apoptosis of HeLa cells. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Cell; Trichosanthin

1. Introduction

Apoptosis is a distinct mode of cell death that is responsible for deletion of cells in normal tissues, it also occurs in specific pathologic context [1]. The study of apoptosis of cancer cell has been a hotspot in the cancer therapy for a long time [2–4]. Therefore, the observation of apoptosis is very important. Till now, there are several methods developed to do the determination of apoptosis such as agarose gel electrophoresis [1], flow cytometry [1] and in situ end labeling [5,6]. Among them, flow cytometry could detect apoptosis of whole cell, but the instrument is very expensive and it could only detect the dyed cell. Other methods all depend on the detection of DNA fragments extracted from apoptotic cells, and the disadvantages of these methods include complicated producers, easy contaminated extraction steps and low sensitivity. Capillary electrophoresis has been used to detect apoptosis recently [7–10]. However, till now, all of the reports are focused on the detection of DNA fragments extracted from apoptotic cells. Therefore, the disadvantages of the traditional methods are still existing in these reports.

Here, we have developed a capillary electrophoresis method that could detect apoptotic whole cells directly and without doing any further extraction.

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PII: S0021-9673(00)00559-8

Apoptosis of HeLa cells induced by cyclophosphamide was used as the model to establish the method. The effluence of medicine concentration on apoptosis was studied in detail. In the mean time, the change of whole cell in the early period of apoptosis also could be detected by the method, which is very useful to the study of apoptosis and cancer therapy. It was found that trichosanthin could result in apoptosis of HeLa cell, and the result of flow cytometry and slab electrophoresis also proved this finding. This observation is valuable to the therapy of carcinoma.

2. Experimental

2.1. Treatment of adherent cells

Human carcinoma HeLa cells was first handled by zymine solution (0.25% in 1×phosphate buffered saline (PBS)) for a few minutes, and then diluted in DMEM (dulbeccos modified eagle medium) containing 10% (v/v) fetal bovine serum (Sigma, St. Louis, MO, USA). The cell concentration was kept at 1×10^6 cells/ml. Incubations were carried out for 24 h at 37°C under an atmosphere of air–CO_2 (95:5) in the presence of 20–80 μg/ml cyclophosphamide or 10–100 μg/ml trichosanthin. After the incubation, HeLa cells were handled by zymine solution again and diluted in the required volume of DMEM. The cell suspension was removed to Eppendorf tube and pelleted at 3000 *g* for 4 min. The supernatant was removed and HeLa cells were resuspended in 50 μl of 1×PBS and stored at 4°C for up to 24 h prior to analysis.

2.2. Capillary electrophoresis

Prior to the usage, the fused-silica capillary was rinsed with 1 *M* HCl, distilled water, 1 *M* NaOH, distilled water by 25 p.s.i. (1 p.s.i.=6894.76 Pa) pressure for 5 min in order. Prior to each run, the capillary was rinsed with running buffer for 2 min at 20 p.s.i. The cells were injected in to the capillary with 0.3 p.s.i. pressure for 2 s, and then separated at 30 kV voltage. After the separation, the capillary was rinsed with 0.1 *M* NaOH and distilled water for 2 min at 20 p.s.i.. The running buffer contained 0.3% sodium borate and 0.4% boric acid (pH 8.0). The wavelength of UV/Vis detector was 260 nm, and the scanning range was from 200 to 300 nm. The fused-silica capillary was 50 cm×100 μm I.D. with the detection length of 40 cm. The experiment was performed on P/ACE MDQ (Beckman Instrument, CA, USA).

3. Results and discussion

3.1. Study of apoptosis of HeLa cells induced by cyclophosphamide

3.1.1. Determination of apoptosis of HeLa cells induced by cyclophosphamide

There are two problems must be solved in the determination of apoptosis of whole cells by capillary electrophoresis. First, the method could separate the whole cells; second, the method could detect apoptosis of whole cells, that is, it should tell the difference between the normal cells and the apoptotic cells. Through the optimum experiments, the separation conditions were chosen and the separation of whole cells by capillary electrophoresis was realized (Fig. 1A). Because apoptosis of HeLa cells induced by cyclophosphamide was a traditional example of apoptosis, it was used to test whether the capillary electrophoresis method could detect apoptosis of whole cells. Fig. 1A was the electropherogram of normal HeLa cells and Fig. 1B was that of HeLa cells induced by cyclophosphamide. Through the comparison, peak 1 was suspected as the peak of apoptotic cells. The dying experiment proved the suspicion to be correct. Fig. 1C and Fig. 1D was the electropherogram of normal and apoptotic HeLa cells dyed by azacyanine orange (AO). Because AO like to combine with normal cells and the number of apoptotic cell in the normal cells is very few, peak 1 disappeared in Fig. 1C and became lower in Fig. 1D. Peak 2 and peak 3 were further defined as the peak of normal cells and the peak of cells adhering together. In general, Fig. 1 indicated that capillary

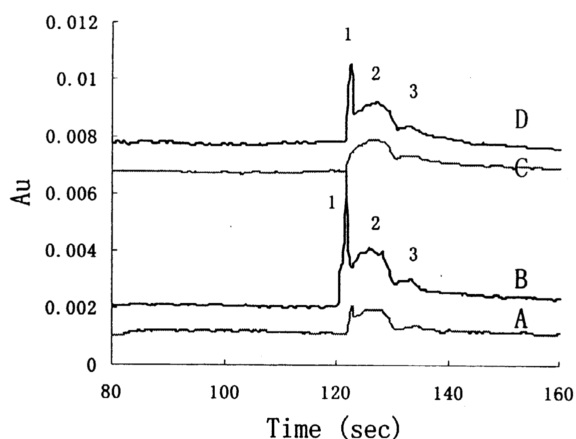

Fig. 1. The determination of apoptosis of HeLa cells induced by cyclophosphamide (A) normal HeLa cells, (B) apoptotic HeLa cells, (C) normal HeLa cells dyed with AO, (D) apoptotic HeLa cells dyed with AO. Peaks: 1, apoptotic cells; 2, normal cells; 3, normal cells adhering together. The cell samples were injected into the capillary by 0.3 p.s.i. pressure for 2 s. The fused-silica capillary (50 cm ×100 μm I.D.) and 30 kV voltage were used to do the separation. The UV/Vis detector wavelength was 260 nm. The concentration of HeLa cells was 1×10^6 cells/ml, and the concentration of cyclophosphamide was 60 μg/ml. Other conditions were the same as shown in Section 2.1.

electrophoresis could separate the whole cell as well as detect the apoptosis of HeLa cells.

3.1.2. Effluence of cyclophosphamide concentration on apoptosis of HeLa cells

Fig. 2 was the effluence of medicine concentration on the relative content of various cells. The relative content of the apoptotic cells (peak 1) increased while that of the normal cells (peak 2) decreased with the increasing of cyclophosphamide concentration, and the relative content of normal cells adhering together did not change largely. It could be observed that 65 μg/ml cyclophosphamide could lead to the saturation of apoptosis. Fig. 3 showed another phenomenon that the migration time of various cells increased with the increase of cyclophosphamide concentration. It is well known that adherent cells tend to bind together, and the migration of cells with big mass would naturally be slower. Therefore, it was suggested that medicine of higher concentration could accelerate the adhering of cells.

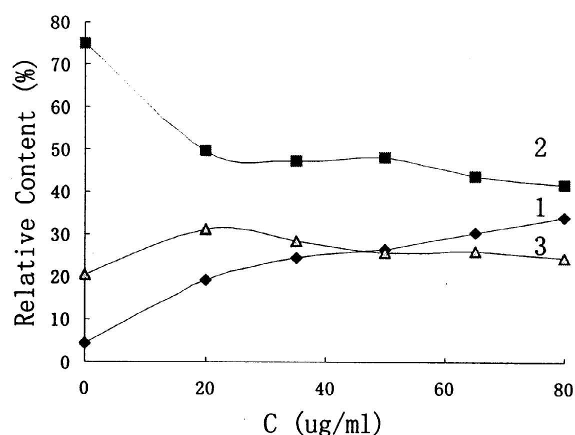

Fig. 2. The effluence of cyclophosphamide concentration on apoptosis of HeLa cell. HeLa cells induced by 20 μg/ml, 35 μg/ml, 50 μg/ml, 65 μg/ml and 80 μg/ml cyclophosphamide. Relative content of each peak was the ratio of each peak area to the total peak area. Peaks 1, apoptotic cells; 2, normal cells; 3, normal cells adhering together. The separation voltage was 20 kV, and the other conditions were the same as shown in Fig. 1.

3.1.3. Early observation of apoptosis of HeLa cells induced by cyclophosphamide

Fig. 4 was the electropherogram of HeLa cells induced by 40 μg/ml cyclophosphamide in the different period of apoptosis. In the early hood of apoptosis, the peak of apoptotic HeLa cells (peak 1) can not be detected (Fig. 4A). Peak 1 could be detected after the incubation occurred at 37°C for 5 h

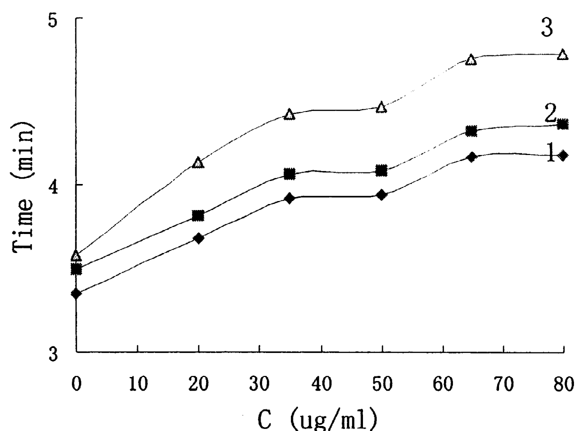

Fig. 3. The effluence of cyclophosphamideconcentration on the migration time of HeLa cell. Peaks: 1, apoptotic cells; 2, normal cells; 3, binding normal cells. Other conditions were the same as shown in Fig. 2.

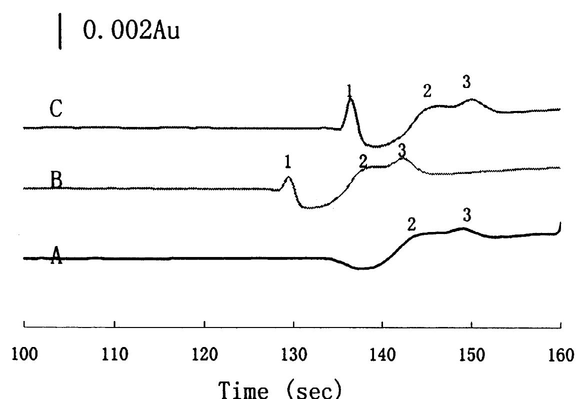

Fig. 4. Early observation of apoptosis of HeLa cells induced by cyclophosphamide. HeLa cells were induced by 40 μg/ml cyclophosphamide and incubated at 37°C for 3 h (A), for 5 h (B), for 7 h (C). Peaks: 1, apoptotic cells; 2, normal cells; 3, binding normal cells. Other conditions were the same as shown in Fig. 1.

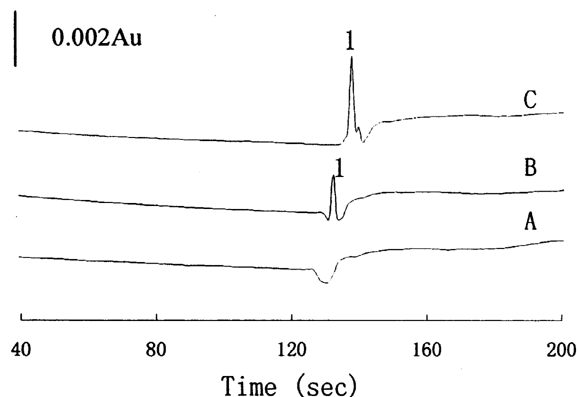

Fig. 5. The determination of apoptosis of HeLa cells induced by trichosanthin. (A) normal HeLa cells, (B) HeLa cells induced by 40 μg/ml trichosanthin, (C) HeLa cells induced by 80 μg/ml trichosanthin. Peak: 1, apoptotic HeLa cells. Other conditions were the same as shown in Fig. 1.

(Fig. 4B). With the increasing of the incubation time, the height and the area of peak 1 increased too (Fig. 4C). Because during the early period of apoptosis, the migration time of cells show a little difference from that of apoptotic ones. Fig. 4 indicated that capillary electrophoresis could detect the change of apoptotic HeLa cells during the early period of apoptosis. This is very valuable to the further research of cancer therapy. The saturation time of apoptosis of cancer cell could be determined exactly with the method, and then the optimum time of medicine injection could be chosen.

3.2. Study of apoptosis of HeLa cells induced by trichosanthin

3.2.1. Determination of apoptosis of HeLa cells induced by trichosanthin

Trichosanthin is an *N*-glycoside that attacks the 28 S rRNA of the ribosome at a highly conserved adenine residue, and it is reported that it could be used in the cancer therapy [11]. In our work, it was added in to the culture solution of HeLa cells, after 24 h incubation, apoptosis of HeLa cells was found with several detection methods. Fig. 5 was the electropherogram of HeLa cells induced by trichosanthin with different concentration. It was obvious that the peak of apoptotic cells (peak 1 in Fig. 5) appeared and increased with the increasing of trichosanthin concentration. According to the result of flow cytometry spectrum, apoptosis percentage of normal HeLa cells was 1.8%, and that of HeLa cells induced by 40 μg/ml cyclophosphamide and trichosanthin was 70.7 and 39.9%, respectively. Therefore, trichosanthin could lead to apoptosis of HeLa cells. Fig. 6 was the electropherogram of DNA fragments extracted from HeLa cells induced by cyclophosphamide (A) and trichosanthin (B), the ladder-liking bands of DNA fragments could be seen but not very clear.

3.2.2. Effluence of trichosanthin concentration on apoptosis of HeLa cells

The peaks of HeLa cells induced by trichosanthin were a little different from that of HeLa cells induced by cyclophosphamide. Besides the peak of apoptotic HeLa cells, the peaks of normal cells and cells adhering together were not very obvious. However, it is clear that with the increasing of trichosanthin concentration the peak area of apoptotic HeLa cells increased (Fig. 7A), which meant the content of apoptotic cells increased too. The result is same as that of HeLa cells induced by cyclophosphamide, and to trichosanthin, 60 μg/ml is the saturation value.

Another similar result is the changing of migration time of HeLa cells. Fig. 7B showed that the migration time of HeLa cells became a little longer with

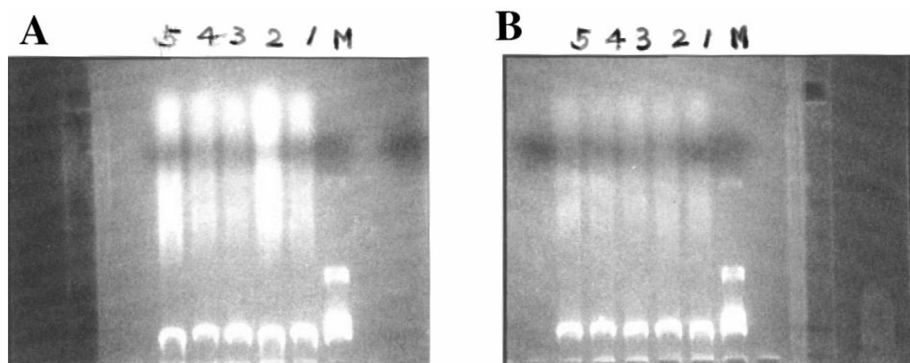

Fig. 6. The slab electrophoresis of DNA fragments extracted from HeLa. (A) HeLa cells induced by cyclophosphamide with different concentration, (B) HeLa cells induced by trichosanthin with different concentration. The other conditions were the same as shown in Section 2.1.

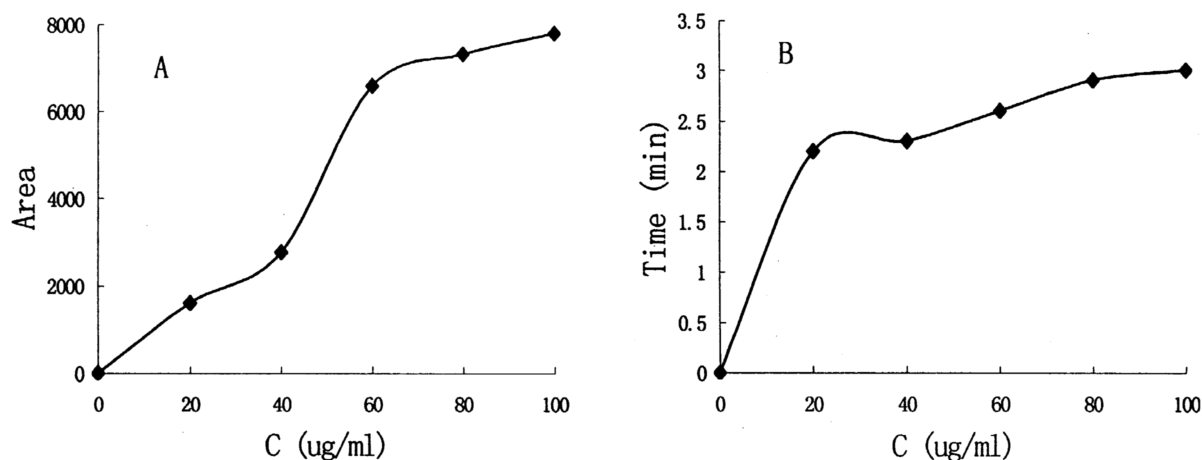

Fig. 7. The effluence of trichosanthin concentration on the peak area and the migration time of apoptotic HeLa cells. (A) the influence of trichosanthin concentration on the peak area of apoptotic HeLa cells, (B) the influence of trichosanthin concentration on the migration time of apoptotic HeLa cells. Other conditions were the same as shown in Fig. 1.

the increasing of trichosanthin concentration. This phenomenon had already been found in Fig. 3, at here, it appeared again. This provided one more evidence to the above presumption, that, the concentrated medicine could accelerate the binding of adherent cells.

Acknowledgements

This research was supported by the National Natural Science Foundation of China.

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