EFFECTS OF CURCUMIN ON PROLIFERATION AND APOPTOSIS IN ACUTE MYELOID LEUKEMIA CELLS HL-60

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ABSTRACT

To investigate the curcumin killing leukemia cells *in vitro*,. Methods: The myeloid leukemic cell line HL-60 was studied by using cell culture, flow cytometrydetermining DNA content and TUNEL method measuring apoptotic cell percentage. Results: The data showed that curcumin selectively inhibited proliferation of acute myeloid leukemia (AML) HL-60 cell lines in a dose- and time-dependent manner. The growth inhibition rate was gradually increased and reached the peak at concentration of 25 μmol/L curcumin at 24h. The sub-G_1 peak appeared after 12h treatment and was increased to 34.4% at 24h. The TUNEL method further certified that apoptotic cells reached 41% at the same phase. Conclusion: curcumin possesses obvious potent of anti-leukemia cell proliferation, which is contributed to the induction of HL-60 cells apoptosis. The concentration and action time of curcumin *in vitro* provide some reference for clinical use.

Key Words: curcumin, proliferation, apoptosis, HL-60

Curcumin is the major ingredient of extracts from curry family widely used as a yellow spice and food additive. It has a variety of pharmacological effects including anti-inflammation, antioxidant, decreasing blood lipid, enhancing phagocaryosis of monocyte-macrophage system and regulating human immunity function. It has been shown to have anti-carcinogenic properties in animal models including cutaneous, gastrointestinal tract and breast cancer caused by many kinds of carcinogenesis agents [1]. *In vitro* studies have shown that curcumin selectively inhibit growth of some permanent tumor cell lines as well as induction of some kind of cell apoptosis in a dose-dependent manner[2,3]. It is suggested that curcumin has a very developing prospects in anti-tumor activities. However, reports of whether or not and in which manner curcumin has anti-proliferate impact on myeloid leukemia cells are rare at present.

Received December 25, 1999, accepted March 10, 2000
This work was supported by the National Natural Science Foundation of China (No.39770934).
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MATERIALS AND METHODS

Agents

HL-60 cell lines were purchased from England ECACC (England Cancer Association Cell Center). Curcumin (Quality Check Product) was purchased from Drug Inspection Institute of Chinese Pharmacological University, its concentration was diluted to 5×10^3 μmol/L, aliquot, and kept at -20℃, thawed prior to incubation. In sites end labeling (TUNEL) Detection Kit was purchased from Boehringer Mannheim Co.

Treatment and Methods

HL-60 cell was cultured in RPMI 1640 medium supplemented with 150ml/L fetal calf serum, passed once for 2~3 days. The doubling time of cell is approximately 24 h. After passed several generations and growing exuberantly, exchange solution in semi-quantity 24 h prior to the experiment. The viable cells were more than 98% of the total cells identified by the 0.4% trypan blue dye exclusion test. Then, the cell were treated by different concentrations of curcumin at a density of 4×10^5 cell/ml for 6~24 h, all the details of experiments must be testified repeatedly at least for two times.

Flow Cytometry Assay: HL-60 cells treated by curcumin were fixed in 700 ml/L ethanol at -20℃ for 24 h, washed fixed solution with PBS, incubated in PC buffer at 37℃ for 60 min in order to extract low-

molecular weight DNA, added propidium iodide staining solution, dyied avoiding from light at 4℃ for 20 min, then measured using flow cytometry whose variance was corrected below 3%.

TUNEL Staining Method: The drug-treated cells were cytospined. After samples were fixed, permeated, then added 50μl reactive mixture for 60min at 37℃. Following PBS washed, 50 μl converter-POD were added on samples for 30min at RT. Cells were stained with DAB substrate solution. TUNEL positive cells percent was then calculated.

RESULTS

Influence of Survival Rate and Proliferation of HL-60 by Curcumin

As shown in Figure 1 and 2, HL-60 cells were treated by curcumin at 5, 10, 25 μmol/L for 18 h, the proliferate inhibition rates of cells were 4.7%, 18.0% and 37.5% respectively. But cells were treated up to 24h, the proliferate inhibition rate reached 6.25%, 25.0% and 43.75% respectively. The results showed that curcumin inhibited proliferation of cells in a dose- and time- dependent manner. More than 90% cells treated by curcumin at a concentration of <25 μmol/L showed negative trypan blue dye exclusion staining (it approached LD_{50} at a concentration of 25 μmol/L), otherwise, 70% cells treated by curcumin at a concentration of 50 μmol/L showed positive staining. It was suggested that cells exposed at this concentration which approached LD_{90} would die from cytotoxity.

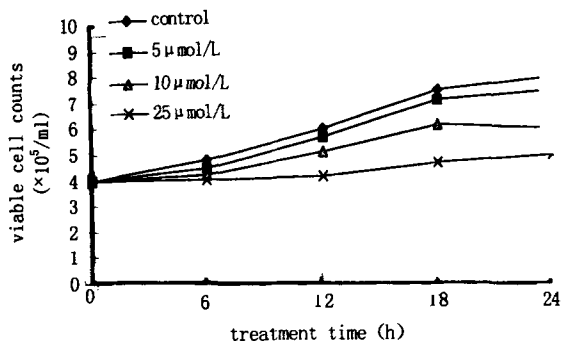

Fig. 1. The influence of curcumin on porliferation of HL-60 cells.

HL-60 Cells Apoptosis Induced by Curcumin

Dynamic changes of HL-60 cells apoptosis induced by curcumin: Cells treated by 25 μmol/L curcumin acting on variant time points were analyzed for DNA content by means of FACS, in HL-60 cells DNA contents histogram, sub-G_1 peak was gradually raised with the progress of time. The same trend was also displayed using the method of synchronous TUNEL techniques(Table 1).

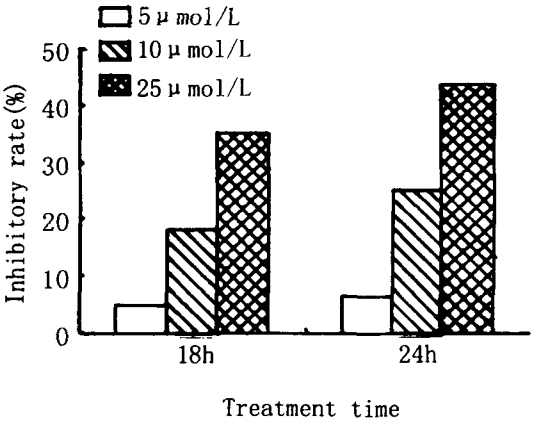

Fig. 2. The proliferate inhibitory rate of HL-60 cells caused by curcumin at different time

Table 1. The apoptotic rate of HL-60 cells caused by 25 μmol/L curcumin at different time

	12h	18h	24h
Sub-G1	8.29	10.48	34.40
TUNEL positive cell percentage	10	14	41

Relationship between the curcumin concentration and apoptosis-inducing: the analysis of DNA content histogram illustrated inducing-apoptosis rate was increased in a dose-dependent manner from 5 to 25 μmol/L, which was approved through TUNEL techniques (Table 2).

Table 2. The apoptotic rate of HL-60 cells treated by different concentration of curcumin at 24h

Concentration	Sub-G1	TUNEL positive cell rate
Control	2.01	2
5μmol/L	2.88	4
10μmol/L	8.17	9
25μmol/L	34.40	41

DISCUSSION

We documented from this investigation that curcumin had a potent of inhibiting proliferation of acute myeloid leukemia HL-60 cells. The change of proliferate inhibitory rate caused by curcumin *in vitro* appeared in a dose-and time-dependent manner and

reached peak at 24 h, which was very similar to the research results obtained from other different tumor cell lines acted by curcumin [4,5]. Sigh et al. considered that curcumin inhibited proliferation of endothelia; cell by arresting cells in early S phase that probably related to the inhibitory action on thymidine kinase enzyme [6]. By means of FACS techniques analyzing DNA content distribution of HL-60 cells, we found that sub-G_1 peak appeared after treating cells for 12 h, augmented with the increase of time and concentration and reached 34.4% at a concentration of 25 μmol/L in 24 h. It was suggested that one of the mechanisms of inhibiting HL-60 cell proliferation by curcumin probably associated with its inducing cellular apoptosis. In order to prove our guess, we did further quality and quantity analysis on HL-60 apoptotic cells treated by curcumin by means of TUNEL methods and found that TUNEL positive rates of HL-60 cells treated by 5, 10, 25 μmol/L curcumin for 24 h were 4%, 9%, 41% respectively, which were approximately similar to the trend of sub-G_1 peak change. However, controversy existed at the study of this problem. Sawai once recognized when investigating ceremid inducing HL-60 cellular apoptosis that curcumin didn't possess the capacity of inducing HL-60 cellular apoptosis [7], but Lin et al. made a reverse conclusion [8]. We supposed that this contradiction was probably related to the usage of different concentrations of curcumin among the experiments done by Sawai, Lin and us. It was discovered that the concentration of 10~25 μmol/L in our experiment was similar to that of 10~20 μmol/L used by Lin whereas the maximal using concentration by Sawai was only 2 μmol/L. So according to this discovery, we speculated that curcumin at a mini-dose probably insufficiently induced HL-60 cellular apoptosis,

In our previous study, we investigated that Ara-C induced HL-60 cellular apoptosis [9]. In HL-60 cell lines, the needed concentration of Ara-C was 1000~2000 μmol/L which was equal to the middle or maximal dose in clinical use susceptible to toxicity to induce the same cellular apoptosis rates through the methods of FACS technique. However, in this experiment, the needed concentration of curcumin approaching to induce of the same apoptotic rate was 25 μmol/L. Compared with these two drugs, it was clearly seen that curcumin had high efficiency and low toxicity anti-tumor effects those indicated that curcumin could be applicable to the clinical use and had a prosperous future.

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