A NEW METHOD TO INDUCE APOPTOSIS OF LEUKEMIA CELLS USING HEAT AND MAGNETIZABLE BEADS

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ABSTRACT

In this study, a new leukemia treatment to induce apoptosis using magnetizable beads and heat was developed. TCC-S leukemia cells were combined with magnetizable beads by an antigen-antibody reaction. The cells were circulated in a tube in which small magnets were placed underneath to aggregate the cells. A disposable hot sheet was placed beneath the magnets. After circulation, the cells were collected, incubated, and the percentage of fragmented DNA was measured. The percentage of fragmented DNA of the heated group was significantly higher. This result suggests that aggregating and heating leukemia cells can effectively induce apoptosis.

INTRODUCTION

The eradication of leukemia cells in the blood by chemical or radiation treatment often produces severe side effects. Many studies have investigated the effectiveness of a less distressful treatment, hyperthermia on tumors by electromagnetic waves, hot sheets, or infrared radiation. It was reported that apoptosis was induced in cells by heating (42~44℃) [1]. Leukemia cells, however, are distributed throughout the whole body, which poses a potential danger for patients who receive whole body hyperthermia. To address this concern, a new leukemia treatment to induce apoptosis using magnetizable beads and heat was developed. In this study, a vessel model was constructed and the effectiveness of this new treatment was assessed *in vitro*.

MATERIALS AND METHODS

TCC-S leukemia cells from a patient with chronic myelogenous leukemia in myeloid crisis were used [2]. The cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium containing 10% Fetal Bovine Serum (FBS) and 1% antibiotic-antimycotic solution. The optimal temperature to induce apoptosis to TCC-S cells was initially verified. It is reported that DNA fragmentation occurs during the early stages of apoptosis [3]. When TCC-S cells were exposed to 41℃ for 60 min, fragmented DNA was not observed; on the other hand, fragmented DNA was observed when TCC-S cells were exposed to 42~43℃ for 60 min (Fig. 1). From these results, the optimal temperature to induce apoptosis of TCC-S cells was established at 42~43℃ for 60 min.

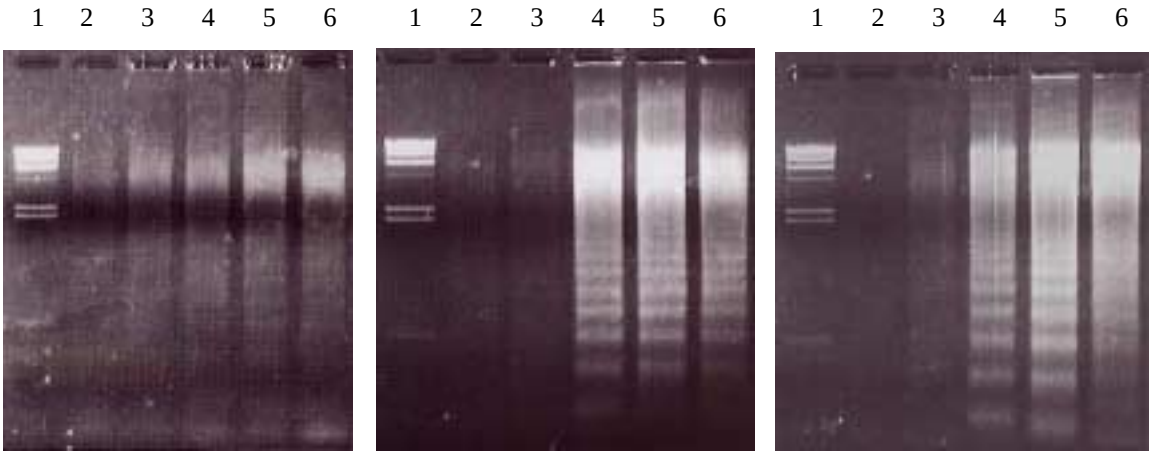

Fig. 1. Electrophoresis of DNA in TCC-S cells. When TCC-S cells were exposed to 41℃ for 60min, fragmented DNA was not observed (left). When TCC-S cells were exposed to 42, 43℃ for 60 min, fragmented DNA was observed (middle and right respectively). Lane 1: DNA marker (λ HindIII), lane 2: control, lane 3: 0 h after heated, lane 4: 6 h after heated, lane 5: 12 h after heated, lane 6: 24 h after heated.

The experimental system was assembled as follows (Fig. 2). TCC-S cells were combined with magnetizable beads (Dynabeads Protein G, diameter = 2.8 μm, Dynal) by an antigen-antibody reaction. The cells were circulated in a silicon tube (length: 1 m, inner diameter: 1 mm, outer diameter: 3 mm) containing RPMI with 10% FBS and 1% antibiotic-antimycotic solution at a circulation speed of 1.5~2.5 ml/min. The cell density was approximately 1.0×10^6 cells/ml. Small magnets were placed under the tube to aggregate the cells containing magnetizable beads. A disposable hot sheet (43~45℃) was placed beneath the magnets. The entire experimental system was placed in a 37℃ temperature regulated case. The cells were classified into three groups, control group (TCC-S cells with beads, non-circulation), 37℃ group (TCC-S cells with beads, circulation) and heated group (TCC-S cells with beads, circulation). Except for the heating and circulation, the three groups were treated under the same conditions. After 1 hour of circulation, the cells were collected and incubated for 24 hours at 37℃ in 5% CO_2. After the incubation, the cells were collected and the percentage of fragmented DNA was measured by the diphenylamine method [4].

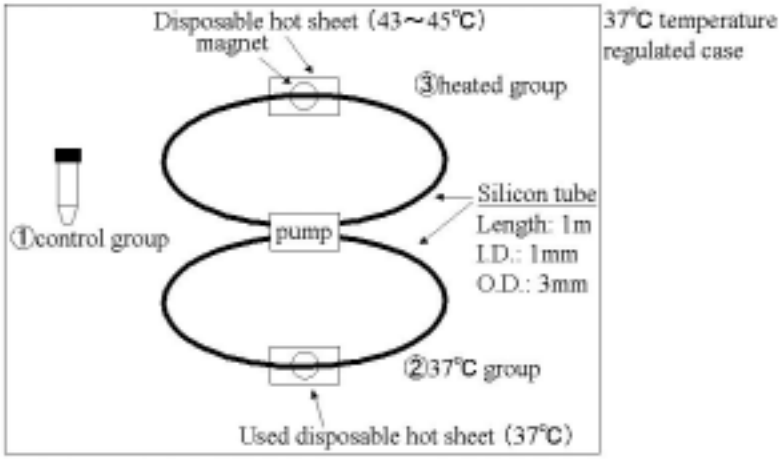

Fig. 2. Experimental system of vessel model.

RESULT AND DISCUSSION

The percentage of fragmented DNA of the heated group was significantly higher than the control and 37℃ groups (Fig. 3). This

result suggests that aggregating and heating leukemia cells can effectively induce apoptosis. Further studies on improving the experimental system and methods to efficiently induce apoptosis can possibly lead to beneficial therapeutic applications in the future.

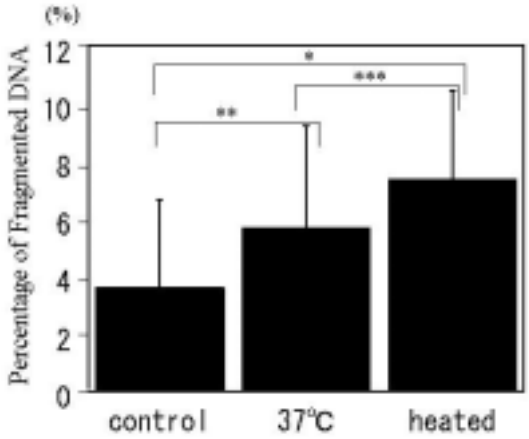

Fig. 3. The percentage of fragmented DNA. N=8. Error bar is ±1SD. *: p<0.001, **: p<0.01, ***: p<0.05.

References

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