Hyperthermia induces differentiation without apoptosis in permissive temperatures in human erythroleukaemia cells

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Hyperthermia induces differentiation without apoptosis in permissive temperatures in human erythroleukaemia cells

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Abstract

Purpose: The aim of the present study was to investigate whether induction of differentiation by hyperthermia is accompanied by apoptosis and necrosis to further evaluate the benefits of using hyperthermia as a differentiation inducing physical modality.

Materials and method: Differentiation was evaluated in K562 erythroleukaemia cells by measuring haemoglobin synthesis and flow cytometric measurement of glycophorin A expression. Apoptosis was measured by Annexin-V-FITC and Propidium Iodide (PI) double staining assay. Apoptosis and necrosis was also evaluated morphologically using staining with acridine orange/ethidium bromide (AO/EtBr) by fluorescence microscopy. Heat shock protein 70 (HSP70) level was measured by ELISA kit.

Results: Hyperthermia (43°C) induced differentiation as judged by increased haemoglobin synthesis and glycophorin A expression. No sign of apoptosis or necrosis could be detected at this temperature. Cell viability did not change due to heat treatment, and cellular proliferation was reduced in a dose (heating time) dependent manner. At 45°C, hyperthermia induced apoptosis and necrosis with minimal or no sign of differentiation. HSP70 level was significantly increased at 43°C along with differentiation of leukaemic cells, while at 45°C no significant effect on HSP70 production could be observed.

Conclusions: The encouraging results obtained here indicate that by heat treatment at 43°C, hyperthermia can be used alone or in combination with other modalities as a differentiation inducing agent without any detectable apoptotic activity. Positive correlation between HSP70 production and induction of differentiation and lack of apoptosis by hyperthermia confirm the possible role of HSP70 in the heat-induced differentiation and apoptosis in leukaemic cells.

Keywords: Hyperthermia, apoptosis, differentiation, K562 cells, heat shock protein 70

Introduction

Tumour cells, and particularly leukaemic cells, can be considered as maturation-arrested cells which have escaped some normal control and continue to proliferate. Haematopoietic cells undergo three major fates: proliferation, differentiation, and apoptosis. These processes are closely intertwined. Under normal circumstances, haematopoietic cell proliferation and cell death are carefully balanced. Considerable evidence supports the notion that leukaemias are likely to arise from the disruption of the differentiation process of haematopoietic progenitors, which fail to give birth to blood cells with restricted phenotypes, as well as from diminished ability to undergo apoptosis [1]. Recent reports support the key role of apoptotic mechanisms on the outcome of leukaemic patients [2]. Therefore, new therapeutic approaches are being developed based on the induction of apoptosis by various agents [3–6]. Preferential induction of apoptosis for primary

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human leukaemic stem cells has been achieved by some drugs [7]. Several agents can induce apoptosis as well as differentiation in leukaemic cells [8].

The maturation arrest of leukaemic cells can be reversed by differentiation inducing agents such as antitumour drugs currently used in conventional cytotoxic chemotherapy [9] or immunosuppressive agents [10], alkaloids from marine sponges [11], and physical agents such as hyperthermia [12]. In vitro differentiation of erythroleukaemia cells by chemical agents represents many aspects of the erythroid terminal differentiation programme, including haemoglobin synthesis and proliferation arrest [13], and elevated production of red cell specific determinant [14]. Despite the remarkable progress achieved in the treatment of leukaemias over the last several years, many problems still remain [15].

Hyperthermia is now a well established physical modality which is used alone or in combination with other modalities such as radiation or chemicals in the treatment of malignancies or other pathological disorders [16–20]. In almost all of these applications, hyperthermia is used as a cytotoxic agent that causes cell death or enhances the cytotoxic effects of other modalities. The cytotoxic behaviour of hyperthermia has been described and reviewed in many excellent papers [21]. It has been shown that hyperthermia could induce apoptosis in leukaemic cell lines or enhance the apoptosis induced by other chemicals [22–24].

Recently we showed that hyperthermia induced differentiation in leukaemic cell lines [25]. In that work we did not address the critical question whether apoptosis, in addition to described differentiation, was also responsible for the observed decline in colony forming ability of cancer cells induced by hyperthermia.

In the current report we have carefully analysed the differentiation versus apoptosis induction by hyperthermia in a human erythroleukaemia cell line. We have also investigated the possible role that heat shock proteins may play in the specific effect of hyperthermia on this cell line.

**Material and methods**

**Cell culture**

The K562 cells were cultured in RPMI-1640 culture medium (Gibco: UK) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 500 u/ml of penicillin (Sigma: USA) and 200 µg/ml streptomycin (Jaberebn-Hayan: Tehran). Cells were maintained at 37°C in a humidified atmosphere of 7.5% CO2 and subcultured every two to three days in order to maintain the cells in constant exponential growth phase.

**Heat treatment**

One ml of K562 cells at a density of 1 to 2 × 10⁶ cells/ml in RPMI-1640 were heated by immersing the experimental glass tubes in thermostated water bath (Haake: Germany f3 with ±0.1°C precision). Heat treatment was applied at 43°C and 45°C for different periods of time, and controls were treated similarly at 37°C. Cell viability was determined by trypan blue dye exclusion test immediately and at various times after heat treatment. Subsequently, for cell differentiation assay and apoptosis detection, heat-treated and control cells (5 × 10⁴ cells/ml) were transferred into 25 or 60 mm culture Petri dishes (Nunc: Denmark) containing RPMI-1640 supplemented with 10% FBS. Cells were incubated for an additional 120h, collected, and investigated using various assays described below. In order to measure the level of HSP70 protein, the cells were incubated for up to 8 h after heat treatment and then were used for further analysis.

**Detection of erythroid differentiation in K562 cells**

**Benzidine staining assay for haemoglobin synthesis.** The percentage of haemoglobin-producing cells was detected by benzidine staining as reported previously [25]. Briefly, heat-treated and control K562 cells at 120 h post-incubation after heat treatment, were collected and resuspended in 20 µl of cell culture medium. Then 15 µl of the cell suspension was transferred into a cytospin tube and centrifuged at 500 rpm for 10 min. The slides were immersed in 30 ml total volume of benzidine stain containing 30% benzidine (1.5% benzidine in methanol) 20% H₂O₂ and 50% ethanol for 5 min, then rinsed in running water. The percentage of benzidine-positive cells (brown to orange coloured cells) was determined under light microscope.

**Measuring of glycophorin A expression.** The surface expression of glycophorin A antigen (CD235a) was detected by FITC-conjugated mouse anti-human glycophorin A monoclonal antibody (Clone NAM10-6G4, IQ Products, The Netherlands) using flow cytometry [26]. Heat-treated and control K562 cells at 120 h post-incubation after heat treatment, were washed and resuspended in cold PBS at a density of 10⁵ cells/ml. Then 5 µl of the appropriate FITC-conjugated antibody was added to 50 µl of cell suspension. After 15 min incubation on ice (4°C), the cells were washed with PBS and analysed by flow cytometry. The cell population for analysis was gated using forward scatter versus side scatter parameters. At least 1 × 10⁴ cells were
collected for each samples and the percentage of
glycophorin A-positive cells was determined using
LYSIS II software (Becton-Dickenson: USA). As
positive and negative control for glycophorin A
expression, cells were treated with sodium butyrate
(1 mM, Merck: Germany) and hemin (50 μM,
Sigma: USA) respectively.

Determination of apoptosis in K562 cells

Analysis of apoptosis by Annexin-V/PI double staining
assay. Apoptotic cells were detected by Annexin-
V-FITC and Propidium Iodide (PI) double staining
assay [27]. In brief, cells (5 \times 10^5) were washed with
PBS and labelled by annexin-V-FITC and PI in
binding buffer according to the instruction in the
Annexin-V-FLUOS Staining Kit provided by the
manufacturer (Roche, Germany). Fluorescent signals
of FITC and PI were detected, respectively, by
FL1 (FITC detector) at 518 nm and FL2 at 620 nm
on FACScan (Becton-Dickenson: USA). For each
analysis, 1 \times 10^4 events were recorded and data were
analysed using the LYSIS II program (Becton-
Dickenson: USA).

Morphological study of apoptosis and
necrosis. Apoptosis and necrosis was also deter-
mimed morphologically using staining with acridine
orange/ethidium bromide (AO/EtBr) by fluorescence
microscopy [28]. Cells were washed with PBS and
adjusted at a density of 1 \times 10^6 cells/ml in PBS.
Acridine orange/ethidium bromide solution (1:1 v/v)
was added to the cell suspension in a final
concentration of 100 μg/ml. The cellular morphology
was evaluated by Axoscope 2 plus fluorescence
Spimicroscopy from ZEISS (Germany). At least
100 cells were counted to calculate the fraction of
apoptotic and necrotic cells.

Protein isolation and analysis

Cells were harvested at 0, 4 and 8h after heat
treatment and then washed with PBS. The pellet was
lysed in 200 μl of cold lysis buffer at 4°C for 30 min
(Hsp70 Extraction Reagent provided in Hsp70
ELISA Kit (Stressgen, Canada) used as the lysis
buffer). The lysate was then centrifuged at 4°C, 12000 rpm for 20 min to pellet large cellular debris.
The supernatant was then collected and kept frozen
(−70°C) for further analysis to determine the protein
concentration and measure the level of HSP70.
Protein concentration was measured using the
Bradford method. The level of HSP70 protein was
determined using Hsp70 ELISA Kit (Stressgen,
Canada) following the kit instruction.

Statistical analysis

Data in this report are presented as Mean ± SEM
and were analysed by Student’s t-test. A P-value of
\leq 0.05 was considered statistically significant.

Results

The effects of hyperthermia on viability and
proliferation of K562 cells

K562 cells were heated at 43°C and 45°C for
different periods of time, and then were cultured
for several hours and studied for viability and
capacity of proliferation. Our results showed that
heat treatment at 43°C did not have any significant
effect on the viability of these cells, as assessed by
trypan blue dye exclusion test, since more than
90% of cells were viable after 120h of incubation at
37°C (Figure 1A). However, heat treatment at 45°C
reduced the viability of the cells in a dose- and time-
dependent manner to about 60% after 120h of
incubation at 37°C (Figure 1B). As shown in
Figure 1C and 1D, cell proliferation was affected
by hyperthermia. At 43°C, the growth of K562 cells
was inhibited in a dose- and time-dependent manner
(Figure 1C), and heat treatment at 45°C totally
inhibited the growth of these cells (Figure 1D).

Induction of differentiation by hyperthermia in K562
cells

The effect of hyperthermia on the induction of
differentiation on K562 cells was assessed by
measuring the haemoglobin synthesis and the
glycophorin A expression. Benzidine staining allows
easy and rapid distinction between differentiated
haemoglobin-containing (benzidine positive) and
undifferentiated (benzidine negative) K562 cells.
Our results showed that 90 min of heat treatment at
43°C stimulated haemoglobin synthesis by 7.1 fold
(Table I). Hyperthermia at 43°C also stimulated
the expression of the erythroid-specific membrane anti-
gen glycophorin A. Our results showed that heat
treatment at 43°C increased the fraction of glyco-
phin A positive cells by 2 folds (Table I and
Figure 2). These results supported that heat treat-
ment at 43°C induced differentiation of K562 cells
into erythroid lineage. However, we did not detect any
significant induction of differentiation in heat-treated
cells at 45°C (Table I).

Induction of apoptosis by hyperthermia in K562 cells

Apoptosis was detected by the surface exposure of
phosphatidylserine on the outer leaflet of apoptotic
cell-membranes using annexin-V-FITC. Propidium
iodide (PI) was used for the simultaneous detection
of necrotic or late apoptotic cells. Analysis of the double stained cells by flow cytometry revealed that heat treatment at 43°C did not have any significant effect on the induction of apoptosis or necrosis in K562 cells; the results of the quantitative analysis are summarized in the Table II.

Table I. The effect of hyperthermia (43°C and 45°C) on induction of differentiation (haemoglobin synthesis and glycophorin A expression) in K562 cells. Cells were heated at these temperatures for different periods of time and incubated for 120 h after hyperthermia. As positive controls, cells were treated with sodium butyrate (SB) and hemin. The fraction of benzidine positive cells and glycophorin A-expressing cells are represented as Mean±SEM from three independent experiments. B⁺ cells: Benzidine positive cells, Gly A-exp cells: Glycophorin A-expressing cells. Compared with control (37°C); *P<0.05; **P<0.01.

<table>
<thead>
<tr>
<th>Temperature (°C) &amp; Heating time (min)</th>
<th>B⁺ cells (%)</th>
<th>Gly A-exp cells (%)</th>
<th>Gly A-exp cells (%)</th>
<th>B⁺ cells (%)</th>
<th>Temperature (°C) &amp; Heating time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>37°C</td>
<td>1.36±0.19</td>
<td>14.82±0.40</td>
<td>15.78±0.14</td>
<td>0.93±0.06</td>
<td>37°C</td>
</tr>
<tr>
<td>45°C, 0</td>
<td>0.35±0.29</td>
<td>14.53±0.46</td>
<td>14.84±0.57</td>
<td>0.33±0.29</td>
<td>45°C, 0</td>
</tr>
<tr>
<td>45°C, 20</td>
<td>1.75±1.05</td>
<td>16.43±1.83</td>
<td>22.77±0.73*</td>
<td>1.95±0.41</td>
<td>43°C, 30</td>
</tr>
<tr>
<td>45°C, 40</td>
<td>1.86±0.71</td>
<td>17.02±1.40</td>
<td>26.38±0.58*</td>
<td>3.4±0.18*</td>
<td>43°C, 60</td>
</tr>
<tr>
<td>45°C, 60</td>
<td>0.76±0.47</td>
<td>16.63±0.75</td>
<td>31.15±1.83*</td>
<td>6.16±0.61*</td>
<td>43°C, 90</td>
</tr>
<tr>
<td>SB (1 mM)</td>
<td>35.91±1.02</td>
<td>14.95±0.90</td>
<td>38.83±1.77*</td>
<td>6.61±0.61*</td>
<td>Hemin (50 µM)</td>
</tr>
</tbody>
</table>

Figure 1. The effect of hyperthermia on the viability (A and B) and growth (C and D) of K562 cells at various times after heat treatment. Cells were heat treated at 43°C (A and C) and 45°C (B and D) for different periods of time. The number of cells and their viability was determined 24, 72 and 120 h of incubation at 37°C after heat treatment. Values are Mean ± SEM from three independent experiments.
morphologically after staining with Acridine orange and ethidium bromide by fluorescence microscopy (Figure 4). In this figure, viable cells are uniformly green, early apoptotic cells are green with bright green dots in their nuclei as a consequence of chromatin condensation and nuclear fragmentation. Ethidium bromide incorporates into the late apoptotic and necrotic cells, therefore these cells are orange, and in contrast to necrotic cells late apoptotic cells show condensed fragmented nuclei. In accordance
Figure 3. The effect of hyperthermia on Annexin-V binding/PI uptake in K562. Cells were heated at different periods of time at 45°C and incubated for 24 h (A), 72 h (B) and 120 h (C) after heat treatment. Annexin-V and PI staining were measured by flow cytometry. The dual fluorescence dot plots show the normal (viable) cells in the lower left quadrant (Annexin-V^−/PI^−), the early apoptotic cells in the lower right quadrant (Annexin-V^+/PI^-) and the late apoptotic or necrotic cells in the upper right quadrant (Annexin-V^+/PI^+). Cell debris was excluded from the analysis by conventional gating of forward scatter versus side scatter dot plots. X axis: The fluorescence intensity of Annexin-V-FITC and Y axis: The fluorescence intensity of Propidium Iodide (PI).

Figure 4. Morphological assessment of apoptosis and necrosis in K562 cells. Cells were heat treated at 45°C for different periods of time and studied 72 h after heat treatment. AO/EtBr double staining of K562 cells shows viable cells are uniformly green, early apoptotic cells are green with bright green dots in their nuclei (green arrows) and late apoptotic cells (orange arrows) are orange and in contrast to necrotic cells (orange arrows, N), they show condense fragmented nuclei. (A) Control K562 cells, (B-E) cells were heat treated at 45°C for 0, 20, 40 and 60 min, respectively. Cells were observed under fluorescence microscope at 400×.
with the data from flow cytometry, AO/EtBr double staining of K562 cells showed that heat treatment at 45°C induced apoptosis in K562 cells. In addition, these results indicate that in this temperature necrosis is mediated in cell death processes. The results of quantitative analysis are shown in Table III.

Table III. The effect of hyperthermia (45°C) on induction of apoptosis and necrosis in K562 cells. Cells were heated at 45°C for different periods of time and studied at 72 h after heat treatment. The results of quantitative analysis of AO/EtBr double staining are represented as mean ± SEM. Compared with control (37°C); *P < 0.05, **P < 0.01.

<table>
<thead>
<tr>
<th>Temperature (°C) &amp; Heating time (min)</th>
<th>Early apoptotic cells (%)</th>
<th>Late apoptotic cells (%)</th>
<th>Necrotic cells (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>37</td>
<td></td>
<td>0.66 ± 0.33</td>
<td>0</td>
</tr>
<tr>
<td>45, 0</td>
<td>2.49 ± 0.05</td>
<td>1.36 ± 0.52</td>
<td>0</td>
</tr>
<tr>
<td>45, 20</td>
<td>10.14 ± 1.16*</td>
<td>9.52 ± 0.68*</td>
<td>2.16 ± 0.23*</td>
</tr>
<tr>
<td>45, 40</td>
<td>14.59 ± 0.92*</td>
<td>2139 ± 1.00*</td>
<td>5.62 ± 0.59*</td>
</tr>
<tr>
<td>45, 60</td>
<td>15.60 ± 0.50*</td>
<td>21.65 ± 1.88*</td>
<td>6.45 ± 1.18*</td>
</tr>
</tbody>
</table>

Figure 5. The effect of hyperthermia on HSP70 level in K562 cells. Cells were heat treated at 43°C (A) and 45°C (B) for different periods of time and studied 0, 4 and 8 h after heat treatment. The x-axis shows the temperature and the time of heating, the y-axis shows the level of HSP70 as determined by the ELISA HSP70 kit. The results are expressed as the ratio of HSP70 protein/total protein from two independent experiments (each performed in duplicate). P < 0.02 compared to the control samples (37°C).

The effect of hyperthermia on HSP70 protein level

The relationship between HSP70 protein and differentiation or apoptosis induced by heat shock was studied by applying hyperthermia at 43°C and 45°C for different periods of time. The level of HSP70 was measured at 0, 4 and 8 h after heat treatment using the ELISA HFP70 kit as described in the method section.

As can be seen in Figure 5A, the expression of HSP70 was significantly increased following 30, 60, and 90 min of heat treatment at 43°C as compared to control samples. While, hyperthermia at 45°C did not induce the expression of HSP70 in heated cells and the level of HSP70 did not change significantly after this heat treatment as compared to control (Figure 5B).

Discussion

Leukaemia is a disease characterized by the failure of cell death, or inability of haematopoietic cells to differentiate into functional mature cells [1]. Therefore, induction of differentiation or cell death in immature haematopoietic cells has been applied for leukaemia prevention or therapy [29, 30]. The effect of hyperthermia on cultured tumour cells has occasionally been reported and it was shown that hyperthermia can induce differentiation [31, 32, 12, 25] or apoptosis [33] in tumour cells depending on the tumour cell type and stress intensity.

The results in this work indicated that heat treatment at 43°C reduced the growth of K562 cells without affecting their viability. Heat treatment at this temperature also induced erythroid differentiation in these cells as judged by two different markers, induction of haemoglobin synthesis and increased glycoporphin A expression. These two markers are the most important makers of erythroid differentiation of K562 cell line [34, 35]. Hyperthermia can induce glycoporphin A expression as well as haemoglobin synthesis. Therefore, this physical agent can induce terminal differentiation in...
K562 cells, although a significant but rather low degree of haemoglobin synthesis was induced by hyperthermia. Some differentiation inducing agents, such as hemin, although are more potent in the induction of haemoglobin synthesis [36] as compared to hyperthermia, however they lack the ability to induce glycophorin A. These agents did not induce terminal differentiation in K562 cells. It has been shown that certain reagents which are known to effect the differentiation of K562 cells, possess a different mechanism of action on these cells, at least in respect of haemoglobin synthesis and glycophorin A expression [35]. Our data also demonstrated that hyperthermia at 43°C did not have any significant effect on the induction of apoptosis in K562 cells as evidenced by flow cytometric methods.

While, heat treatment at 45°C inhibited the growth of K562 cells and reduced their viability, but no evidence of differentiation (haemoglobin synthesis or increased glycophorin A expression) could be detected in heat-treated cells. Apoptosis was detected in K562 heat-treated cells (45°C) by the detection of Annexin-V-positive cells and changes in cell morphology (chromatin condensation and nuclear fragmentation). It is possible that K562 cells switch from differentiation to cell death depending on the stress intensity, but little is known about the molecular mechanism underlying these phenomena.

Several studies demonstrated that some chemical agents could induce differentiation in leukaemic cells by blocking cell proliferation [29, 30, 37–39]. Therefore, it is possible that these chemical agents, through inhibition of proliferation, allow leukaemic cells to recover their normal programme of differentiation [1]. It has also been shown that treatment of cells with some chemical agents at subtoxic concentrations may serve to stress the cells and induce some kind of adaptive or stress response such as induction of heat shock protein expression [32]. It is possible that these events may result in changes of specific genes expression leading to cell differentiation, including changes in the expression of certain oncogenes such as myc family [40, 41], that involve in the control of cell growth and differentiation in leukaemic cells [42]. In addition, several studies have suggested a role for heat shock proteins (HSPs) during development and differentiation in different organisms [43, 44]. It has been shown that heat shock proteins (HSP70 and HSP90) can interact with multiple key component of signaling pathway that regulates growth, differentiation and cell death [45], including nuclear hormone receptors [46, 47], tyrosine-and serine/threonine kinases [48, 49] and cell death regulators [50, 51]. Several studies have also shown that HSP70 and HSP90 associate with many components and regulators of the Ras/Raf-1 pathway, which has an important role in proliferation, differentiation, growth arrest and cell death [52, 53]. In addition, the selective expression of HSP70 and HSP28 during differentiation of myeloid leukaemic cells treated with chemical agents suggested a role for these heat shock proteins in differentiation process of these cells [54, 55]. The role of heat shock proteins in differentiation of leukaemic cells has been also reviewed in many excellent papers [56, 57].

Our results also demonstrated that hyperthermia at 43°C increased HSP70 level, while, heating cells at 45°C had no significant effect on the level of HSP70 in heat-treated cells. On the other hand, the differentiation inducing effects of hyperthermia was observed at 43°C, while at 45°C hyperthermia acted as a cytotoxic and apoptosis inducing agent. Therefore, the HSP70 can act as a positive modulator in differentiation process of heat-treated leukaemic cells.

On the other hand, hyperthermia can induce both apoptosis and necrosis in a temperature-dependent manner. It is accepted that hyperthermia (at moderate temperature) triggers apoptosis, while higher temperature or more prolonged heat treatment induce necrosis in many cell types [58, 59]. The fact that hyperthermia (at 43°C) did not induce apoptosis in the K562 cells, while heat treatment at 45°C could induce apoptosis and partially necrosis in these cells could rather be explained with the well known resistance of K562 cells to drug-induced apoptosis [60]. This resistance has been attributed to the activity of P210 (bcr-abl) which transduced anti-apoptotic signals acting on different targets such as Stat1, Stat5, Bcl-xl and Akt [61, 62]. In addition, K562 cells have been shown to express high levels of the anti-apoptosis protein Bcl-xl, a member of the Bcl-2 related family of apoptosis modulators [63].

Strasser and Anderson have shown that both Bcl-2 family and heat shock proteins protected cells from heat-induced apoptosis. They suggested that these Bcl-2 and heat shock proteins act via independent mechanisms to inhibit apoptosis in heat-treated cells [64]. It is possible that heat shock induces two independent pathways leading to apoptosis; Bcl-2 may inhibit one pathway and heat shock proteins the other. However, little is known about the molecular mechanisms underlying these phenomena. Several studies have indicated that heat treatment of cells leads to many alterations, including inactivation of the normal pattern of genetics expression at various levels (transcription and translation), inhibition of cell proliferation (DNA synthesis and cell division) and alterations in cell morphology and cell adhesion [65]. Indeed, many cellular structures are affected by heat treatment [66]. The loss of structural integrity of subcellular components and consequently the loss of their cellular functions may be a consequence of
protein denaturation. Therefore, protein denaturation/aggregation may be the main process leading to thermal killing and thermotolerance may be acquired via protection against this protein damage with the aid of HSPs [67].

It has been hypothesized that HSPs are used by stressed cells either to protect against stress-induced alteration in protein structure, or to repair and restore the structure and functional integrity of damaged proteins [68, 69].

On the other hand, several studies indicated a role for HSP70 in protecting the tumour cells against the toxicity effects of different apoptosis-inducing agents. It has been also shown that over-expression of hsp70 gene protected the fibroblast cells against the lethal effect of heat treatment [69].

Considering our results, increasing the level of HSP70 in heat-treated cells (43°C) protected the cells from lethal effects of heat treatment and lead to their differentiation, while in severe treatment condition (45°C) which no increase of HSP70 was observed, the cells triggered to apoptosis. Therefore, the HSP70 may protect the cells against heat-induced apoptosis.

In conclusion, our results first suggested that hyperthermia had powerful growth inhibition, differentiation and apoptosis inducing capacity in human erythroleukaemia cell line. Although many regulating factors are involved in the pathways that regulate differentiation and apoptosis in leukaemic cells, our results shown that HSP70 may play a role in the control of these processes in heat-treated cells.

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References


