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RESEARCH ARTICLE

Antiproliferative Effect of H_2O_2 against Human Acute Myelogenous Leukemia KG1 Cell Line

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Abstract
It has clearly been established that oxidative stress leads to perturbation of various cellular processes resulting in either inhibition of cell proliferation or cell death. In addition, there is a growing body of evidence indicating that reactive oxygen species (ROS) are required as signal molecules that regulate different physiological processes including survival or death. Free radicals, particularly ROS, have been proposed as general mediators for apoptosis and recent studies have established that the mode of cell death depends on the severity of the oxidative damage. In this study, we determined the effect of oxidative stress on cell proliferation and characterization of cell death in human KG1 cells treated with H_2O_2. Our results indicated that oxidative stress leads to a significant decrease in cell proliferation and induction of apoptosis. Moreover, our study suggests that antiproliferative and apoptotic cell death effects of H_2O_2 took place via activation of caspase-3, affecting the expression of Bcl-2 and Bax (an antiapoptotic and a proapoptotic factor, respectively), and through deactivation of catalase enzyme, leading to accumulation of intracellular ROS and depletion of intracellular ATP level.

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1. Introduction

Oxidative damage, mediated by reactive oxygen species (ROS), is the main cause of cellular damage in a vast variety of diseases such as cancer, diabetes, neurodegenerative disorders, and cardiovascular diseases [1,2]. ROS and free radicals are continuously produced and leak from mitochondria in cells during the generation of the adenosine triphosphate (ATP) by using oxygen as a terminal electron acceptor [3]. It is well known that ROS play a key role in triggering the mitochondria-mediated apoptosis pathway in which caspases is the central enzyme. In addition, it is also well established that mitochondria is the main site for generating oxygen radicals such as superoxide anion, hydroxyl radical, singlet oxygen, and H_2O_2 [4]. Recent studies have shown that ROS and the resultant oxidative stress readily damage biological molecules; ultimately induce cell death either by apoptosis or necrosis [5].

H_2O_2, a representative ROS, can induce apoptosis in many different types of cells such as JURKAT cells, glioma cells, and human gastric carcinoma MGC803 cells [2,6]. Under normal physiological conditions, cells keep balance between the extent of intracellular ROS and endogenous cellular antioxidant enzymes including superoxide dismutase, catalase, and glutathione peroxidase. However, under oxidative stress conditions, accumulation of ROS damages cell structures such as nucleic acids, carbohydrates, proteins, and lipids [7,8].

Different reports demonstrate the dual effect of oxidative stress in the carcinogenic process [9,10]. Numerous reports illustrating the elevation of H_2O_2 levels in cancer cells, propose the key role of H_2O_2 in carcinogenesis [11]. In contrast, several studies have revealed that H_2O_2 activates hypoxia-inducible factor 1 (HIF-1), thereby leading to apoptosis resistance and immortalization [12]. Cancer cells have a lower level of antioxidant enzymes rather than normal cells. Moreover, it is proposed that the extent of ROS in cancer cells is close to the toxic threshold. Thus, cancer cells are more susceptible to oxidative stress than normal cells, thereby hyperoxygenation therapy is supposed to be a possible approach for inducing apoptosis and cancer treatment [13—15].

Leukemia is one of the most common causes of malignancy-related deaths in Iran and other countries and there are few effective therapies for a proper prevention and treatment. Leukemia is a type of cancer characterized by abnormal propagation of hematopoietic cells and lack of their differentiation into functional mature cells. Leukemia is a disease induced by the failure of cell death in hematopoietic cells to differentiate into mature cells. This cancer is often caused by neoplastic transformation of pluripotent stem cells, leading to immaturation of cell at the detectable levels of myeloblast [16]. Based on the French—American—British classification, KG-1 cells are phenotypically characterized as AML-M1 subtype, including prominent chromosomal abnormities and minimal extent of differentiation [17]. The KG-1 cell line was originally derived from bone marrow cells of a patient with erythroleukemia evolving to acute myelogenous leukemia. Current strategies of leukemia therapy mainly include chemotherapies, interferon treatment, and bone marrow transplantation as well as combination therapies that are unsatisfactory. Effective therapeutic approaches have extended patient's life for about a year [18].

Evidence suggests that an increase in cellular levels of ROS may be an important event in cancer development. On the other hand, it seems that the high levels of ROS commonly observed in cancer cells are almost incompatible with cell survival and they make these cells more susceptible to ROS-induced cell death than normal cells [19,20]. So, any chemical or strategy capable of sufficiently increasing the cellular levels of ROS may therefore produce selective killing of cancer cells and be therapeutically useful. Besides, inducing cancer cell into apoptosis is one of the important therapeutic intervention approaches in cancer treatment. Based on these facts we decided to evaluate the effects of H_2O_2 on KG-1 cells apoptosis with the aim of disclosing its mechanism of action and determining the toxic threshold of H_2O_2 for this cell line.

2. Materials and methods

2.1. Materials

The cell culture medium (RPMI 1640) and penicillin/streptomycin were purchased from Gibco BRL (Life Technologies, Paisley, Renfrewshire, UK). The culture plates were obtained from Nunc (Roskilde, Denmark). H_2O_2 and DMSO were obtained from Merck (Darmstadt, Germany). Adenosine 5'-triphosphate and [3-(4,5'-dimethyltiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and propidium iodide (PI) were from Sigma Chem. Co. (Munich, Germany). 2′,7′-dichloro fluorescin diacetate (DCFH-DA) was obtained from Molecular Probe (Eugene, Oregon, USA). Ethidium bromide and acridine orange were purchased from Pharmacia LKB Biotechnology (Uppsala, Sweden). Bax, Bcl, and tubulin antibodies were obtained from Biosource (Nivelles, Belgium). Annexin-V was purchased from IQ Products (Groningen, Netherlands).

2.2. Cell culture

The human KG-1 cell line, obtained from Pasteur Institute of Iran (Tehran), was cultured in RPMI 1640 medium supplemented with 10% (v/v) fetal bovine serum and 100 units/ml penicillin/streptomycin in CO_2 humidified atmosphere at 37°C. The cell number was established using a hemocytometer and the number of viable cells was estimated by the trypan blue exclusion test.

Normal peripheral blood lymphocytes (PBL) from healthy volunteers were obtained after centrifugation on Ficoll gradient. This work was carried out in accordance with the guidelines of our Institute and with the Declaration of Helsinki (2000) of the World Medical Association. Consent was obtained from the donor after full explanation of the purpose, nature, and risk of all procedures was explained. After centrifugation, cells at the interface were removed, washed with phosphate buffered saline (PBS), and resuspended in RPMI 1640 medium containing 10% fetal bovine serum, 100 units/ml penicillin and 100 mg/ml of streptomycin.

2.3. Proliferation assay

The number of viable cells was estimated by MTT assay. Briefly, the cells (4×10^4 cells/well) were seeded in flat-bottom 96-well plates. After culturing for 24 hours, KG-1 cells were treated with various concentrations of H_2O_2 for different time intervals, and 10 μL MTT (5 mg/ ml) was added to each well 4 hours before harvesting. The medium was removed from each well and 100 μL of DMSO added. The optical density of each well was determined with a microplate reader (Elx 800 Microplate Reader; Bio-TEK, Winooski, Vermont, USA) at 570 nm [21]. Moreover, to further evaluate the involvement of caspase in toxicity induced by H_2O_2, cells were preincubated with the common caspase inhibitor, Z-VAD-FMK, at the indicated concentrations (5, 10, 20, 50, and 100 μM) for 3 h followed by exposure to H_2O_2 (300 μM) for another 12 hours. After incubation, the viabilities of KG1 cells were determined using MTT assay [21].

2.4. Catalase activity

Calalase activity was determined according to the method reported by Aebi [22]. The assay mixture consisted of 1.98 mL phosphate buffer (50 mM, pH 7.0), 1 mL H_2O_2 (10mM) and 20 μL of diluted sample. The absorbance was recorded at 240 nm every 15 seconds up to 1 minute using a UV-Vis spectrophotometer. The enzyme activity was expressed as $\times 10^{-1}$ K/mg protein in which K represents the rate constant of the first order reaction of catalase. Protein concentration was determined by the method of Lowry [23].

2.5. Measurement of intracellular ROS generation

To monitor intracellular accumulation of ROS, the fluorescent probe DCF-DA was used, which is freely permeable through the cell membrane [24]. Once it is inside the cells, the compound is hydrolyzed by the cellular esterase to DCF, which is a fluorophor and interacts with peroxides and forms fluorescent 2',7'-dichloro-fluorescein. Cells (4×10^4 cells/ ml) suspension was loaded with 10 μM DCF-DA and the mixture was incubated at 37°C for 1 hour. The cells were washed twice with PBS and examined with Varian-spectrofluorometer, model Cary Eclipse with an excitation wavelength of 485 nm and an emission wavelength of 530 nm.

2.6. Detection of apoptotic cells

Apoptotic cells were evaluated based on their morphological changes after dual staining with ethidium bromide/acridine orange for 5 minutes and visualization with Axioskop 2 plus fluorescence microscope (Ziess, Oberkochen, Germany) [25]. Viable cells have normal nuclear and green fluorescence, whereas apoptotic cells displayed condensed nuclei.

2.7. Analyses of apoptotic cells by Annexin V-FITC and PI

The cells were treated with the drug for 48 hours. After harvesting, the cells were washed twice with PBS and resuspended in 100 μL binding buffer (10mM HEPES/NaOH, pH 7.4, 140 mM NaCl and 2.5 mM $CaCl_2$). FITC-Annexin V (10 μL) was added to the cells followed by the addition of 10 μL PI (50 μg/ml of PBS) [26]. The samples were incubated for 10 minutes in the dark at 4°C and then analyzed by flow cytometry.

2.8. Caspase-3 activity assay

Activity of caspase-3 in KG1 cells in culture was detected by using a colorimetric assay kit in accordance with the protocol supplied by the manufacturer (Invitrogen, Grand Island, NY, USA). Briefly, the KG1 cells were treated with different concentrations of H_2O_2 for 12 hours and then untreated control and H_2O_2-treated cells were harvested and resuspended in 50 μL of chilled buffer and incubated on ice for 30 minutes. Lysate was centrifuged at 10,000 *g* for 1 minute at 4°C and supernatant (Cytosol extract) was used to measure the caspase-3 activity. Fifty microliters of 2x reaction buffer (containing 10mM DTT) and 10 μL of the conjugated substrate (DEVD-pNA) at a concentration of 4mM were added to each lysate. The mixture was incubated at 37°C for 2 hours in the dark, and the activity was measured at 405 nm by using a microplate reader.

2.9. Western blot

After treatment of KG1 cells with H_2O_2 for different time intervals, the cells were harvested and lysed using lysis buffer containing 1% Triton X-100, 1% SDS, 10mM Tris (pH 7.4), 100mM NaCl, 1mM EGTA, 1 mM EDTA, 20 mM sodium pyrophosphate, 2mM Na_3VO_4, 1mM NaF, 0.5% sodium deoxycholate, 10% glycerol, 1mM phenylmethylsulphonyl fluoride, 10 μg/ml leupeptin, 1 μg/ml pepstatin, and 60 μg/ ml aprotinin. Protein concentration of each sample was determined using Lowry's method. Equal quantities of protein (100 μg) were subjected to SDS polyacrylamide gel electrophoresis and transferred to a hybond-p polyvinylidene difluride membranes (Amersham Bioscience, Amersham, Buckinghamshire, UK). Transfer of proteins was assessed by Ponceau-red staining. The filter membranes were blocked in Tris-buffered saline pH 7.4 containing 0.1% Tween-20 and 5% bovine serum albumin overnight at 4°C. The blocked blots were incubated with primary antibodies for 1 hour at room temperature using antibody dilutions as recommended by the manufacturer in Tris-buffered saline pH 7.4, 0.1% Tween-20. Following 1 hour incubation with anti-rabbit or anti-mouse horseradish peroxidase conjugated secondary antibodies (Biosource, Belgium), the proteins were detected by an enhanced chemiluminescence detection system (ECL; Amersham-Pharmacia, Piscataway, NJ, USA) according to the manufacturer's instructions.

2.10. Determination of cellular ATP levels

Cellular ATP levels were measured by the bioluminescence assay. Briefly after treatment with different concentrations of H_2O_2 for 24 hours, 2×10^5 cells were resuspended in 3 mL of boiled distilled water containing 1mM magnesium sulfate, maintained at 100°C for 10 minutes, and stored

at −20 °C for further analysis. The diluted cell extract (0.2 mL) was added to 0.8 mL of luciferin-luciferase reaction buffer in a polystyrene cuvette, and the ATP-dependent luciferase activity was measured by use of a luminometer [27]. The light emission (300 ± 900 nm) was determined in a counter, and ATP standard curves were constructed each time.

2.11. Statistical analysis

Results are expressed in mean ± SD. All experiments were performed in triplicate except for the caspase 3 activity assay, which was performed in duplicate. For statistical analysis, the Student *t* test was applied. A p-value < 0.05 was considered statistically significant.

3. Results

3.1. H_2O_2 reduced cell viability

The anti-proliferative effect of H_2O_2 was studied by measuring the total number of cells in each treated well. H_2O_2 reduced the cell viability of KG1 cells in a dose- and time-dependent manner, as shown in Fig. 1A. The cell viability decreased slightly after 24 hours, but after 48 hours of treatment it was reduced almost by 50% (IC_{50}) at 300 μM of H_2O_2. Treatment of KG1 cells with 50 μM to 400 μM of H_2O_2 for 3 days resulted in cell death by 23% to 75% relative to the control cells, whereas cytotoxic effect in terms of cell viability was less observed among healthy cells (PBL) under the same experimental conditions (Fig. 1B).

3.2. H_2O_2 induced increase in intracellular ROS

Rising of intracellular ROS has been implicated in cell death. Intracellular ROS accumulation was measured using DCF-DA, which moves freely through the cell membrane. The treatment of KG1 cells with H_2O_2 for 1 hour increased intracellular ROS generation. Compared with untreated control cells, exposure of the cells to 50, 100, 200, 300, and 400 μM of H_2O_2 caused 3, 22, 26, 32, and 64% increase in content of these species, respectively (Fig. 2).

3.3. Effect of H_2O_2 on catalase activity

The activity of catalase in treated KG1 cells is shown in Fig. 3. To study whether the effect of H_2O_2 is related to changes in intracellular catalase activity, we measure the activity of this enzyme in the cells exposed to different concentrations of H_2O_2. As shown in Fig. 3, treatment of the cells with H_2O_2 at concentrations of 50, 100, 200, 300, and 400 μM for 24 hours diminished the activity of this enzyme by 11, 30, 32, 47, and 60%, respectively compared to untreated control cells.

3.4. H_2O_2 induces apoptosis in KG-1 cells

Morphological study of the cells after ethidium bromide/acridine orange dual staining clearly documented the occurrence of apoptosis in KG1 cells after 48 hours of H_2O_2 exposure (Fig. 4A). In this figure, viable cells are homogeneously green, while apoptotic cells are orange containing bright dots in their nuclei as a consequence of chromatin condensation and nuclear fragmentation, an index for occurrence of apoptosis. To further confirm the occurrence of apoptosis, the drug-treated cells were subjected to Annexin V/PI double staining followed by flow cytometry analyses. Exposure of the cells to different concentrations of H_2O_2 induced dose-dependent apoptosis among the cells (Fig. 4B).

3.5. Activation of caspase-3 by H_2O_2

Apoptosis is often associated with the caspase family. Caspase-3 is a key factor in apoptosis. It is synthesized as an inactive proenzyme that is processed in cells undergoing apoptosis by self-proteolysis and/or cleavage by another protease. To confirm whether the activation of caspase-3 is required for the induction of apoptosis by H_2O_2, we measured the changes in caspase-3 activity in KG1 cells after 12 h. As shown in Fig. 5A, exposure of cells to single dose of H_2O_2 has caused significant activation of caspase-3

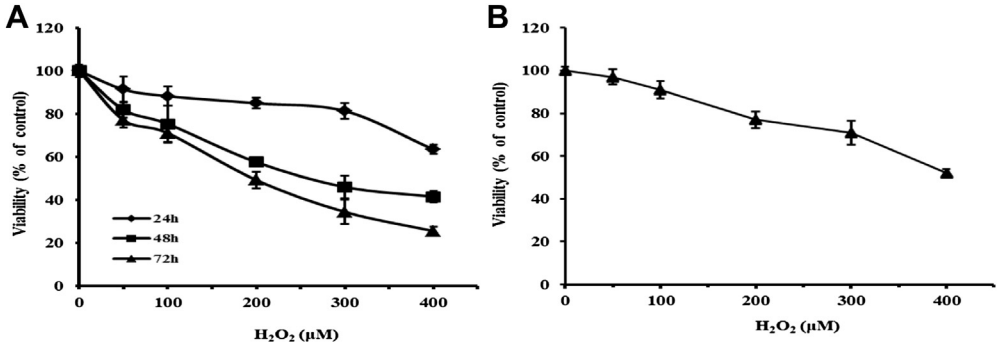

Figure 1 (A) Dose- and time-dependent effect of H_2O_2 on viability of KG1 cells. KG1 cells were incubated with different concentrations of H_2O_2 for 24, 48, and 72 hours, and MTT reduction was determined by absorption at 570 nm. Values are expressed as percentages of the values in untreated control cells and each value represents the means ± SD. (B) Effects of H_2O_2 on viability of unstimulated PBL cells after 72 hours.

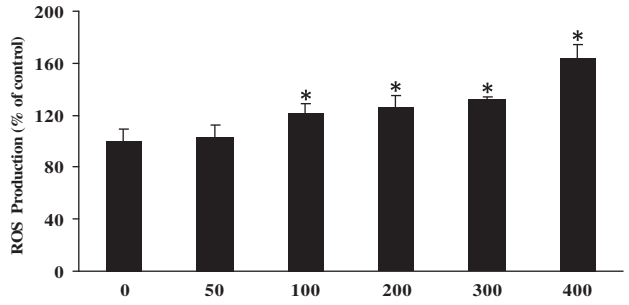

Figure 2 Dose-dependent effect of H_2O_2 on intracellular reactive oxygen species (ROS) levels in KG1 cells. Cells were exposed to different concentrations of H_2O_2 for 1 hour and ROS was detected using DCFH-DA. Values are expressed as means ± SD ($n = 3$). *Significantly different from untreated control cells ($p < 0.05$).

by 9, 21, 48, 57, and 115% at H_2O_2 concentrations of 50, 100, 200, 300, and 400 μM, respectively. This result suggests that apoptosis in H_2O_2-treated cells is mediated by the caspase-3 pathway. The data from MTT assay also revealed that Z-VAD-FMK, dose-dependently, has an inhibitory effect on H_2O_2-induced toxicity in KG1 cells (Fig. 5B). These data further confirm the apoptotic death and caspase activation induced by H_2O_2 in KG1 cells.

3.6. Effect of H_2O_2 on Bcl-2 family

To investigate the molecular mechanism of H_2O_2-induced apoptosis in KG1 cells, the expression of apoptosis-related Bcl-2 family with 300μM H_2O_2 was examined. The data presented in Fig. 5C shows that the expression of Bcl-2, an anti-apoptotic factor, decreased after 12 and 24 hours of treatment with 300μM H_2O_2 while Bax, a proapoptotic factor, was up-regulated in a time dependent manner.

3.7. Effect of H_2O_2 on intracellular ATP level

To find an association with growth inhibition, we measured cellular ATP contents in KG-1 cells after treatment with

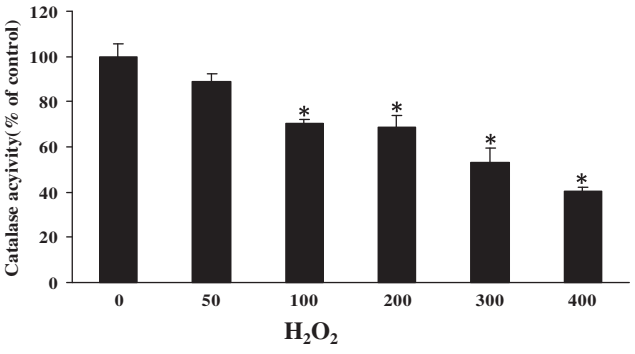

Figure 3 Inhibitory effect of H_2O_2 on the intracellular activities of catalase. The KG1 cells were incubated with various concentrations of H_2O_2 for 24 hours, and catalase activity was measured as described in Materials and methods section. Data are expressed as percent values of untreated control cells, and each value represents the means ± SD ($n = 3$). *Significantly different from control ($p < 0.05$).

300 μM H_2O_2. As shown in Fig. 6, H_2O_2 treatment has led to a decreased level of ATP to almost 44% of the control sample. Our results clearly indicates that this effect is dose-dependent, and treatment of KG1 cells with 50, 100, 200, 300, and 400μM of H_2O_2 for 24 hours imposed a concentration-dependent decrease in ATP level. In other words, at these concentrations the ATP pool has declined to 11, 12, 30, 44, and 60% of control, respectively.

4. Discussion

ROS are byproducts generated during the mitochondrial oxygen metabolism in all aerobic organisms. There is increasing evidence that ROS not only can be toxic but also are needed as critical signal molecules that regulate various physiological processes including survival or death. Studies have shown controversial effects for H_2O_2, as an oxidative stress inducer, on cancer cells depending on cell type and concentration [1,3,4]. This study was carried out to evaluate the anti-cancer properties of H_2O_2 on the human leukemia KG1 cell line and also to determine the toxic threshold of H_2O_2 for this cell line. We postulate that by understanding of toxic threshold of oxidative stress for different cancer cells, it would be possible to fight cancer cells by induction of oxidative stress with or without minimal side effects on normal cells.

In the first part of our study, we assessed the effect of H_2O_2 on the viability and proliferation of the KG1 cells. Our results indicate that exposure of KG1 cells to H_2O_2 for 72 hours induces a MTT reduction around 50% relative to untreated control cells whereas cytotoxic effect in terms of cell viability, under the same experimental conditions, was much less (~15 %) observed among healthy PBLs (Fig. 1).

Hydrogen peroxide is a potent pro-oxidant that is able to induce generation and accumulation of intracellular ROS. On the other hand, several studies have demonstrated that the extent of intracellular ROS in cancer cells is very close to the lethal threshold concentration and thus cancer cells are more sensitive to further accumulation of ROS than normal cells [20,28]. Regarding this fact, we explored the anti-proliferative effect of H_2O_2 in terms of ROS accumulation. Our results, in parallel to the previous reports, indicate that treatment of KG1 cells with H_2O_2 increases the accumulation of intracellular ROS in a dose-dependent manner (Fig. 2).

To investigate the mechanism of the antiproliferative property induced by H_2O_2, we assessed the activity of main cellular H_2O_2 detoxifying enzyme, catalase, in the cells treated with different concentrations of hydrogen peroxide. Our results show that 24-hour treatment of KG1 cells with H_2O_2 diminishes the activity of catalase dose-dependently (Fig. 3). It is postulated that the accumulation of ROS induced by H_2O_2 is likely to have led to the oxidation of catalase and consequently to a significant decrease in catalase activity with certain concentrations of H_2O_2. Recent studies have established the elevation of H_2O_2 concentration in cancer cells. Moreover, it has been suggested that H_2O_2 concentration in tumor cells is managed by catalase [29]. Since the ROS level in cancer cells is close to the lethal threshold, in the present study, the inhibition of catalase may be one of the factors involved in cell death induced by H_2O_2.

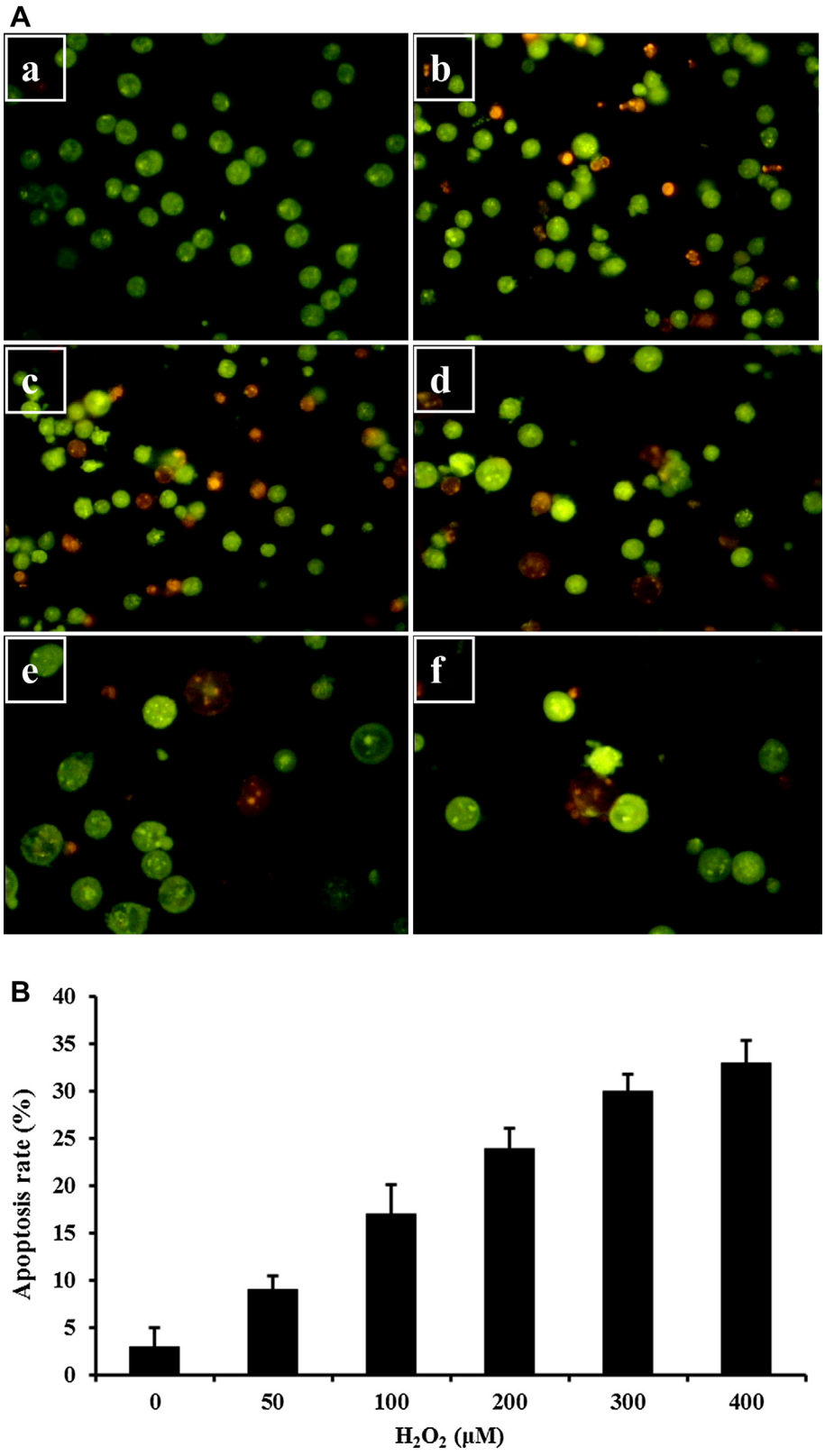

Figure 4 Induction of apoptosis by H_2O_2. (A) Fluorescence microscopic analysis of the H_2O_2-treated KG1 cells. Occurrence of apoptosis was shown with staining of the cells with ethidium bromide/acridine orange double staining and then visualized by fluorescent microscopy. Control KG1 cells (a), cells treated with 50 (b), 100 (c), 200 (d), 300 (e), and 400μM (f) of H_2O_2 after 48 hours. Chromatin condensation and ruffling of nuclear membrane are observed in apoptotic cells. Magnification 40×. (B) Apoptosis rate of H_2O_2-treated KG1 cells. KG1 cells were treated with various concentrations (0—400 μM) of H_2O_2 for 48 hours, followed by annexin V/propidium iodide flow cytometric analyses.

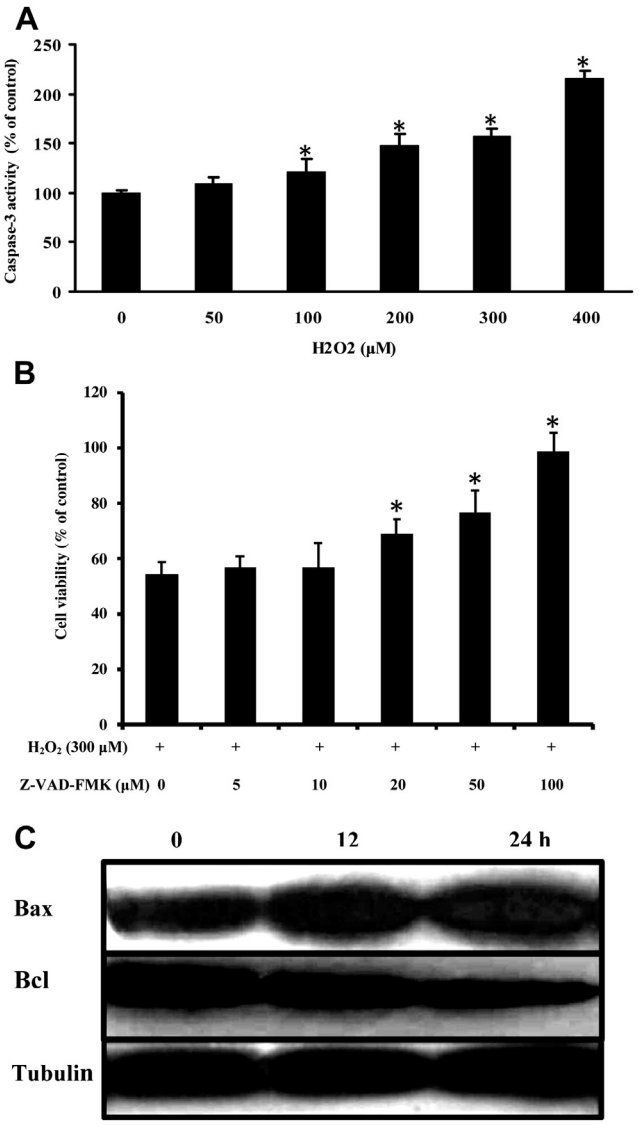

Figure 5 (A) The effect of H_2O_2 on the extent of caspase-3 activation in KG1 cells. Cells were treated with various concentrations (0—400 μM) of H_2O_2 for 12 hours. Activity of caspase-3 of KG1 cells was detected as described in Materials and methods. (B) Inhibitory effect of Z-VAD-FMK against H_2O_2-induced cytotoxicity measured by MTT assay. KG-1 cells were treated with different concentrations of Z-VAD-FMK for 3 h following by the exposure to H_2O_2 (300μM) for another 12 hours. Data are presented as means ± SD ($n = 3$). *Significantly different from control ($p < 0.05$). (C) Expression of apoptosis-related Bcl-2 family in KG1 cells after treatment with 300μM H_2O_2. Expression of Bcl-2, an antiapoptotic factor, decreased, while expression of Bax, a proapoptotic factor, increased.

Numerous studies have demonstrated that reactive oxygen species are involved in the onset of apoptosis [30]. It has been shown that H_2O_2 may induce apoptosis or necrosis depending on the exploited concentration and the cell line [31,32]. Ethidium bromide/acridine orange double staining and flow cytometry analysis using annexin V/PI revealed that 48-hour treatment of KG1 cells with H_2O_2 at different concentrations induces apoptotic cell death. The extent of H_2O_2-induced apoptotic cell death grew dose-dependently (Fig. 4).

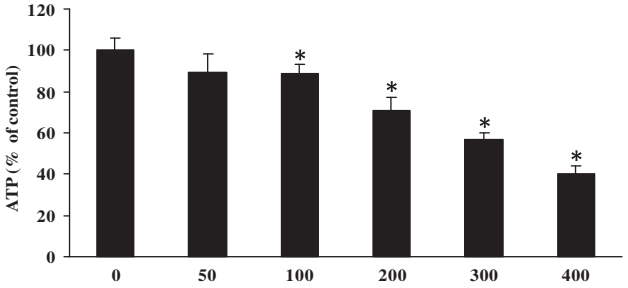

Figure 6 Time-course effect of H_2O_2 on KG1 intracellular ATP level. Intracellular ATP level of KG1 cells was measured by the bioluminescence assay after cells were incubated for 24 hours with different concentrations of H_2O_2. Values are ±SD and were presented in percentages with respect to values obtained from the untreated control cells at the same time. *Significantly different from control ($p < 0.05$).

In order to confirm the apoptotic cell death, we determined the activity of caspase-3 as a key cysteine protease involved in the execution of apoptosis [33]. Our data clearly indicate that 12-hours treatment of KG1 cells with different concentrations of H_2O_2 leads to casapase-3 activation. Moreover, a positive correlation was found between the increasing concentration of H_2O_2 and the activation of caspase-3. Treatment of the cells with 400μM H_2O_2 increased the activity of caspase-3 more than two-fold compared to the untreated control cells (Fig. 5A). In the present study, we showed that suppression of caspase-3 activity by the general caspase inhibitor, Z-VAD-FMK, also quenched the cytotoxicity induced by H_2O_2. These data indicate that H_2O_2 damages took effect by caspase-3 (Fig. 5B). In addition, this study showed that the expression of Bcl-2 as an antiapoptotic factor decreased after 12 and 24 hours of treatment with 300μM H_2O_2 while Bax, a proapoptotic factor, was up-regulated in a time dependent manner (Fig. 5C).

In the present work, we evaluated the alteration of ATP level in treated KG1 cells with different concentrations of H_2O_2. Our data clearly show that 24 hours of treatment of KG1 cells with H_2O_2 causes ATP depletion (Fig. 6). There was a negative correlation between increasing the H_2O_2 concentration and ATP depletion. Increasing evidence suggests that oxidative stress induces mitochondrial dysfunction [8]. For instance, mitochondrial membrane protein thiols are supposed to control the permeability transition pore. Oxidative damage to mitochondrial membrane protein thiols leads to permeability transition pore opening, which subsequently results in decrease in membrane potential and inhibition of ATP synthesis [34]. Numerous studies have demonstrated that ATP depletion can induce cell death [35]. Regarding these facts, in present study H_2O_2-induced ATP depletion may be an important factor for the apoptosis pathway.

In summary, our data show that H_2O_2 treatment of leukemia KG1 cells induces the apoptotic cell death. Our results suggest that observed antiproliferative effect of H_2O_2 was likely to have taken place via the deactivation of

catalase, the accumulation intracellular ROS, ATP depletion, caspase-3 activation, and the change in expression of Bcl-2 family. Our next aim is to extend and test our hypothesis for other cancer cell lines and also *in vivo* models.

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