Enalapril-induced Apoptosis of Acute Promyelocytic Leukaemia Cells Involves STAT5A

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Abstract. *Background: In this study, we aimed at evaluating the cytotoxic and apoptotic effects of enalapril on human HL60 acute promyelocytic leukaemia cells and at clarifying the roles of signal transducers and activator of transcription proteins (STATs) on enalapril-induced cell death. Materials and Methods: Cell viability and cytotoxicity tests were conducted by Trypan blue dye exclusion and 2,3-Bis[2-methoxy-4-nitro-5-sulphophenyl]-2H-tetrazolium-5-carboxanilide inner salt (XTT) assays, respectively. Apoptotic analyses were performed by the AnnexinV-enhanced green fluorescent protein (EGFP) staining method and by fluorescence microscopy. Expression levels of STAT3, -5A and -5B genes were analysed by quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR). Results: The results showed that enalapril reduced viability and proliferation, and induced apoptosis in HL60 cells in a dose- and time-dependent manner as compared to untreated controls. The expression levels of STAT5A gene were significantly reduced in enalapril-treated HL60 cells as compared to untreated controls. Conclusion: Taken together, all data showed for the first time that enalapril has significant anticancer potential for the treatment of acute premyelocytic leukaemia.*

Acute promyelocytic leukaemia (APL), a distinct variant of acute myelogenous leukaemia (AML), is characterized by clonal expansion of promyelocytes in the bone marrow and in the bloodstream resulting from the arrest of the differentiation of myeloid cells at the promyelocyte phase (1).

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In the majority of APL cases, a reciprocal chromosomal translocation occurs involving the retinoic acid receptor-alpha (RARα) gene on chromosome 17 and the promyelocytic leukaemia (PML) gene on chromosome 15 (2). The resultant PML-RARα fusion protein plays a critical role in the pathogenesis of APL. Induction therapy for patients with APL used to be similar to that for AML and included a standard chemotherapy regimen of anthracycline and, cytarabine arabinoside (3, 4). Complete remission was sustained following standard chemotherapy; however, most patients experienced relapse and long-term disease-free survival was only 30-40% (5). The introduction of all-*trans* retinoic acid (ATRA) (6, 7) has changed the treatment course of APL, and its combination with anthracycline-based chemotherapy has become the standard treatment regimen for newly diagnosed APL patients. Following a combination therapy, the complete response rates were found to be more than 90% and cure rates were approximately 80% (8-10). Arsenic trioxide (ATO) in treatment of APL relapse has also given excellent results (11).

Haematopoietic cell proliferation and differentiation is regulated by cytokines that are also known as interferons, interleukins and colony-stimulating factors. Upon the binding of the cytokine with the cell surface receptor that is lacking intrinsic tyrosine kinase ability, the signalling cascade is induced and associated Janus kinases (JAKs) are activated by cross-phosphorylation. Activated JAKs first phosphorylate the receptor itself and then the signal transducers and activator of transcription proteins (STATs) (12). STATs are latent cytoplasmic transcription factors that upon activation function as signal transducers and transcription factors. Seven mammalian STAT proteins have been discovered: STAT1 to -4, and STAT5A, STAT5B, and STAT6 (13). They are known to be involved in several cellular processes, such as cell growth, cell differentiation, apoptosis and immune responses. Therefore, dysregulation of STATs, either due to constitutive activation or functional

impairment, can lead to cellular transformation (14, 15). STAT1, -3 and -5 are known to be involved in the development or suppression of malignant transformation. STAT1 mediates growth-inhibitory signals and induces apoptosis, and therefore has a tumour-suppressive role in oncogenesis. It also contributes to the host rejection of tumours (16, 17). Inhibition of the STAT3 pathway has been shown to induce apoptosis in cancerous cells (18, 19). STAT5A and -5B proteins have also been shown to be necessary for the development of malignancy (17).

The renin-angiotensin system (RAS) or the renin-angiotensin-aldosterone system (RAAS) is a hormone system that systemically regulates cardiovascular homoeostasis. However, several studies have shown that local RAS may mediate cellular processes, such as proliferation, tissue angiogenesis, apoptosis and inflammation (20). Components of the RAS were shown to be expressed in several adult tissues (21). The presence of a local bone marrow RAS, affecting physiological and pathological haematopoiesis was first hypothesized in 1996 by Haznedaroglu and co-workers. Almost all of the major components of RAS were demonstrated in haematopoietic stem cells (HSC), progenitors and bone marrow microenvironment (22-24). It is the paracrine action of locally expressed RAS, instead of its circulating counterpart, that appears vital for tumourigenesis. Expression of components of the RAS such as renin, angiotensin (Ang), angiotensin I-II receptor (AT1R, AT2R) and antigen-converting enzyme (ACE) were identified in various types of carcinoma, including brain, lung, breast, prostate, colon, and skin (20). The RAS is also a vital element in the process of development of neoplastic haematopoiesis (25). The presence of ACE surface antigen (CD143) within leukemic bone marrow (26), on the K562 erythroleukaemia cell line (27) and ACE-expressing macrophages in lymph nodes in Hodgkin's disease (28) has already been shown. In addition, CD143 has been observed to be over expressed in leukaemic blast cells, and it has been found that ACE is directly correlated to the bone marrow blast count (29). Renin activity has also been described in leukemic blasts (30, 31).

ACE inhibitors have been widely used in clinical studies for the treatment of hypertension, heart failure, coronary artery disease and diabetes mellitus. Recently, studies examining the anti-tumoural effects of ACE inhibitors have gained significance (32). Enalapril is a carboxyl-group ACE inhibitor used for the treatment of high blood pressure that works by regulating the RAAS.

Many chemotherapeutic agents have been used in the treatment of acute leukaemia yet, to date, there is no definitive treatment. In this study, we aimed to evaluate the cytotoxic and apoptotic effects of the ACE inhibitor enalapril on human HL60 acute promyelocytic leukaemia cells and to clarify the mechanisms of enalapril-induced cell death.

Materials and Methods

Cell line, culture conditions and chemicals. Human HL60 acute promyelocytic leukaemia cells were kindly provided by Dr Serdar Bedii Omay from Ege University. HL60 cells were maintained in RPMI-1640 medium containing 10% foetal bovine serum (FBS) and 1% penicillin–streptomycin at 37˚C in 5% CO_2. Enalapril was obtained from Sigma Chemical (St. Louis, MO, USA). Enalapril (50 mg) was dissolved in 1 ml RPMI-1640 medium and stock solutions were prepared. Trypan blue dye was obtained from Sigma Chemical. 2,3-*Bis*[2-methoxy-4-nitro-5-sulphophenyl]-2*H*-tetrazolium-5-carboxanilide inner salt (XTT) Cell Proliferation Assay was obtained from Biological Industries (Israel). AnnexinV-EGFP Apoptosis Detection Kit was obtained from Biovision (CA, USA). All the other chemicals and tissue culture supplies were obtained from Sigma Chemical unless otherwise specified.

Measurement of cell viability and cytotoxicity. In order to measure the cytotoxic effect of enalapril on HL60 cells, 2×10^5 cells were seeded into 24-well plates containing 1 ml growth medium in the absence or presence of increasing concentrations of enalapril (1 nM, 10 nM, 100 nM, 5 μM and 10 μM), and incubated at 37˚C in 5% CO_2. Cell viability was assessed at 0, 24, 48, 72 and 96 h post-incubation by Trypan blue dye exclusion assay as indicated in the manufacturer's instructions.

The concentration of enalapril that inhibited cell growth by 50% (IC_{50}) was determined by XTT assay. Briefly, 2×10^4 HL60 cells were plated into 96-well plates containing 100 μl of the growth medium in the absence or presence of increasing concentrations of enalapril (1 nM, 10 nM, 100 nM, 5 μM and 10 μM) and incubated at 37˚C in 5% CO_2. After 24, 48, 72 and 96 h incubation, cells were treated with 50 μl XTT solution for 4 hours and the absorbance was recorded at 492 nm using an ELISA reader and the IC50 dose of enalapril was calculated from the cell proliferation plots (33).

Evaluation of apoptosis. Apoptosis induced by enalapril in HL60 cells was detected by fluorescence microscopy using AnnexinV-EGFP Apoptosis Detection Kit (Biovision) as described in the manufacturer's instructions. Briefly, HL60 cells were treated with 7 μM of enalapril and apoptosis was induced. At 0, 24, 48, 72 and 96 h time points, 1-5×10^5 cells were collected and 500 μl 1× binding buffer was added to the cell suspension. Subsequently, 5 μl annexinV-EGFP and 5 μl propidium iodide (PI) were added, and the cells were incubated in the dark for 5 min at room temperature. Consequently, the cells were transferred onto a coverglass and morphologically examined by fluorescence microscopy.

Total RNA isolation and quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR). Total RNA was isolated from HL60 cells treated with 7 μM (IC_{50} value) of enalapril at 24, 48, 72 and 96 h post-incubation using Trizol reagent (including guanidium thiocyanate, phenol and sodium citrate) as described by the manufacturer. One microgramme of total RNA was reverse transcribed using FirstStrand cDNA Synthesis Kit (Life Sciences GE Healthcare, Piscataway, NJ, USA) in accordance with the instructions provided by the manufacturer. After 1 h incubation at 37˚C, the reaction was stopped at 65˚C for 10 min. The resulting total cDNAs were then used to determine the mRNA levels of *STAT3, STAT5A,* and *STAT5B*. Q-PCR was performed with gene-specific primers and probes using the Fast Start DNA Master Hybridisation Probes (Roche Applied Science,

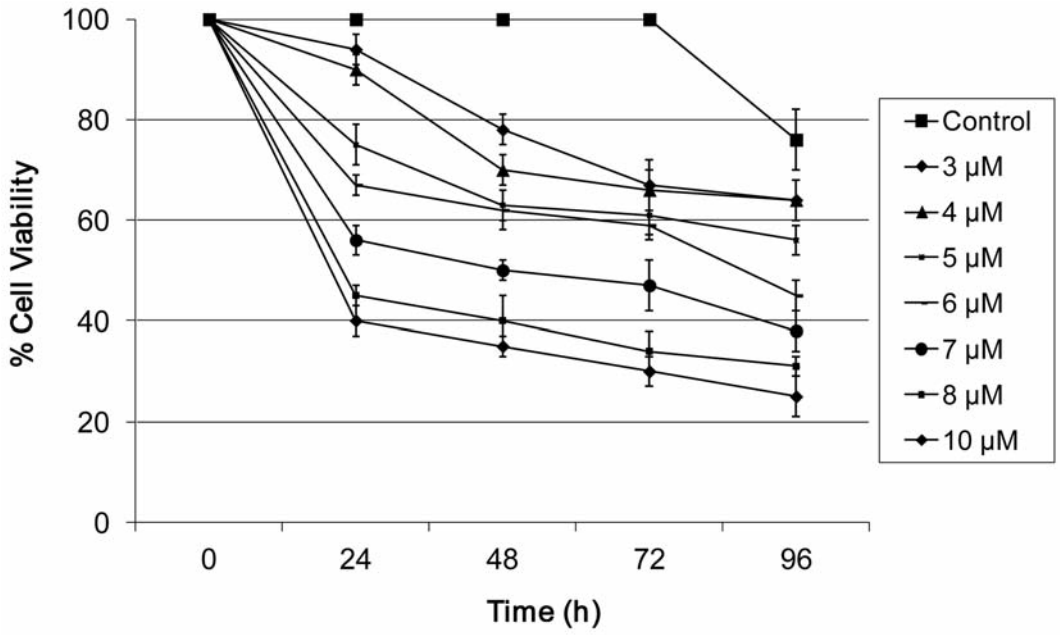


Figure 1. *Effects of enalapril on the viability of HL60 cells. Trypan blue dye exclusion assays were performed using duplicate samples in at least two independent experiments. The error bars represent the standard deviations, and when not seen, they are smaller than the thickness of the lines on the graphs. Statistical significance was determined using two-way analysis of variance, and p<0.05 was considered significant. Control: untreated control cells.*

Penzberg, Upper Bavaria, Germany) and Glucose-6-phosphate dehydrogenase (G6PDH) House Keeping Gene Kit (Roche Applied Science). The *G6PDH* gene was used as an internal positive control in all PCR reactions, and its amplification product provided both a control for PCR performance and a reference for quantification of PCR products. The primer sequences were (TibMolBiol, Berlin, Germany): *STAT3* forward: 5'-ACCAACAATCCCAAGAATGT-3', reverse: 5'-CGATGCTCAGTCCTCGC-3'; *STAT5A* forward: 5'GAAGCTGAA CGTGCACATGAATC-3', reverse: 5'-GTAGGGACAGAGTCTTCA CCTGG-3'; *STAT5B* forward: 5'-AGTTTGATTCTCAGGAAAGAA TGT-3', reverse: 5'-TCCATCAACAGCTTTAG CAGT-3'. Two microlitres of the reverse transcriptase reaction was amplified using these primers for 50 cycles (95˚C, 10 s; 56˚C, 10 s; 72˚C, 5 s). The mRNA levels of *STAT3*, *-5A*, and *-5B* were determined in a Real-Time LightCycler Instrument (Roche Applied Science). The relative expression level for each gene was calculated by dividing the mRNA copy number of the target gene by the *G6PDH* mRNA copy number. qRT-PCR experiments were performed at least in three independent trials. Statistical significance was analysed by using ANOVA (analysis of variance) and $p<0.05$ was considered significant.

Results

Enalapril reduced viability and proliferation of HL60 cells in a time- and dose-dependent manner. In order to detect the potential cytotoxic effects of enalapril on human HL60 cells, we used both Trypan blue dye exclusion and XTT cell proliferation assays. The results of the Trypan blue dye exclusion assay showed that there was a dose- and time-dependent reduction in cell viability as compared to untreated controls (Figure 1). Viability of untreated HL60 cells remained constant for the initial 72 hours, before declining in number due to over-confluence in the spent medium. Viability of HL60 cells treated with increasing concentrations of enalapril (3-10 μM) was observed to drop steadily over the experiment. The IC_{50} value of enalapril was calculated from cell survival plots and was determined to be 7 μM (Figure 1).

The degree of cytotoxicity induced by enalapril was assessed by XTT assay. Enalapril reduced cell proliferation significantly in a time- and dose-dependent manner (Figure 2).

Enalapril induced early and late apoptosis in HL60 cells. Apoptotic cells were morphologically examined under fluorescence microscopy, and cellular changes were identified. Apoptotic cells were stained green, whereas necrotic cells were stained with PI and observed in red. Cells shown as both green and red were defined to be in late apoptosis (Figure 3A) and live cells were observed in blue (Figure 3B). Following the evaluation of apoptosis in HL60 cells treated with 7 μM enalapril, an increase was observed in the number of early apoptotic cells at 48 hours and the rate was found to be 20% (Figure 4). However, this value was statistically insignificant when compared to that of untreated controls, ($p>0.05$) and the difference between enalapril-treated and untreated control groups disappeared after 72 hours. At later time points, early apoptotic HL60 cells were not observed; only HL60 cells in late apoptosis were present (Figure 4).

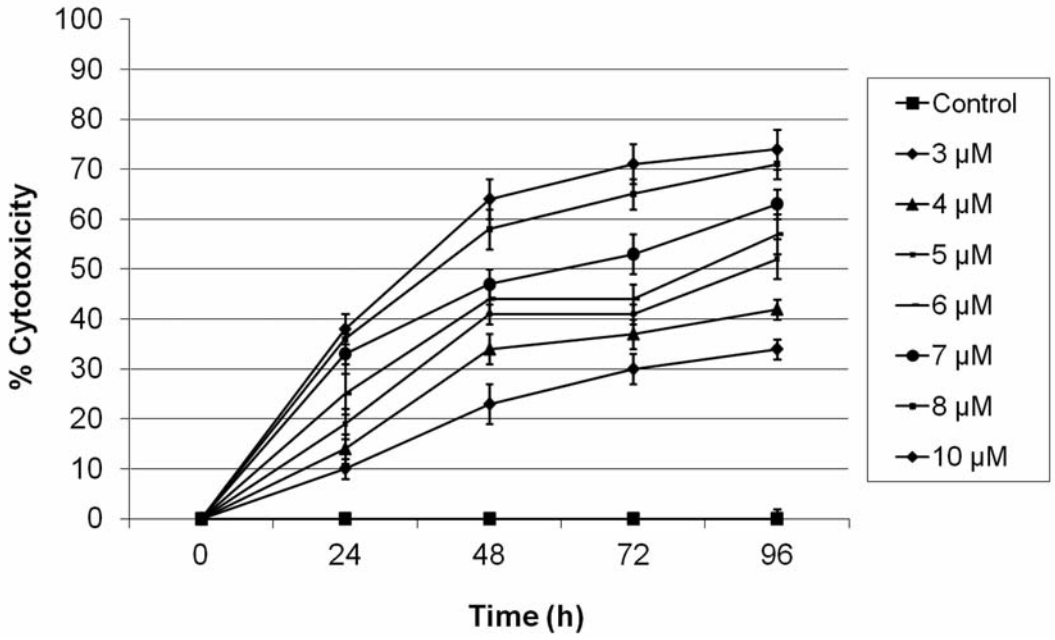


Figure 2. *Percentage changes in cytotoxicity of enalapril. The concentration of enalapril that inhibited cell growth by 50% (IC_{50}) was calculated from cytotoxicity plots. The XTT assays were performed using duplicate samples in at least two independent experiments. The error bars represent the standard deviations, and when not seen, they are smaller than the thickness of the lines on the graphs. Statistical significance was determined using two-way analysis of variance, and $p<0.05$ was considered significant. Control: untreated control cells.*

Differential expression patterns of STAT3, STAT5A, and STAT5B in enalapril-treated HL60 cells. Expression levels of *STAT3*, *-5A* and *-5B* genes were examined in HL60 leukaemia cells treated with 7 μM of enalapril, by qRT-PCR. The results revealed that the expression of *STAT3* decreased in enalapril-treated HL60 cells within the first 24 hours and the difference was of borderline statistical significance when compared to untreated controls ($p<0.065$). However, the change in the expression of the *STAT3* gene for the total duration of the experiment was statistically insignificant as compared to the control group ($p>0.05$) (Figure 5A). At 24, 48 and 72 hours post-incubation, the expression of the *STAT5A* gene was significantly reduced in enalapril-treated HL60 cells when compared to untreated controls ($p<0.05$) (Figure 5B). The changes in the expression levels of the *STAT5B* gene were found to be statistically insignificant in enalapril-treated HL60 cells when compared to the negative control group ($p>0.05$) (Figure 5C).

Discussion

Many chemotherapeutic agents have been used for the treatment of acute leukaemia, but no definitive treatment has yet been described. Therefore, study of novel treatment regimens, including patient-based, *in vitro* and *in vivo*, are underway. For the first time, with this study, we evaluated the potential cytotoxic effect of enalapril, an ACE inhibitor generally used for treatment of high blood pressure, on human HL60 acute PML cells.

It is well known that haematopoiesis is regulated by several factors including growth factors, cytokines, cell surface receptors and the microenvironmental signals in the bone marrow. Since haematopoietic bone marrow is an area of excessive cell growth, the presence of a local, intrinsic RAS within the bone marrow was suggested (34). As ACE and other angiotensin peptides have a role in cellular processes, such as cell proliferation and migration, but also in angiogenesis (35-37), the involvement of RAS in the development of malignant haematopoiesis has gained significance (22, 25, 38). Recent data showed the existence of ACE in human primitive lympho-haematopoietic cells, and embryonic, foetal and adult haematopoietic tissues (39, 40). ACE inhibitors are widely used as anti-hypertensive medicines but lately they have gained significance as anticancer agents (32). Their antitumoural effects were first described in a study by Lever *et al*. (41) in which breast and lung cancer incidence decreased in patients receiving ACE inhibitors, including enalapril.

In the first stage of our study, we examined the potential cytotoxic and apoptotic effects of enalapril on HL60 cells, and investigated the possible mechanisms involved in cell death. The results of Trypan blue cell viability and XTT cell

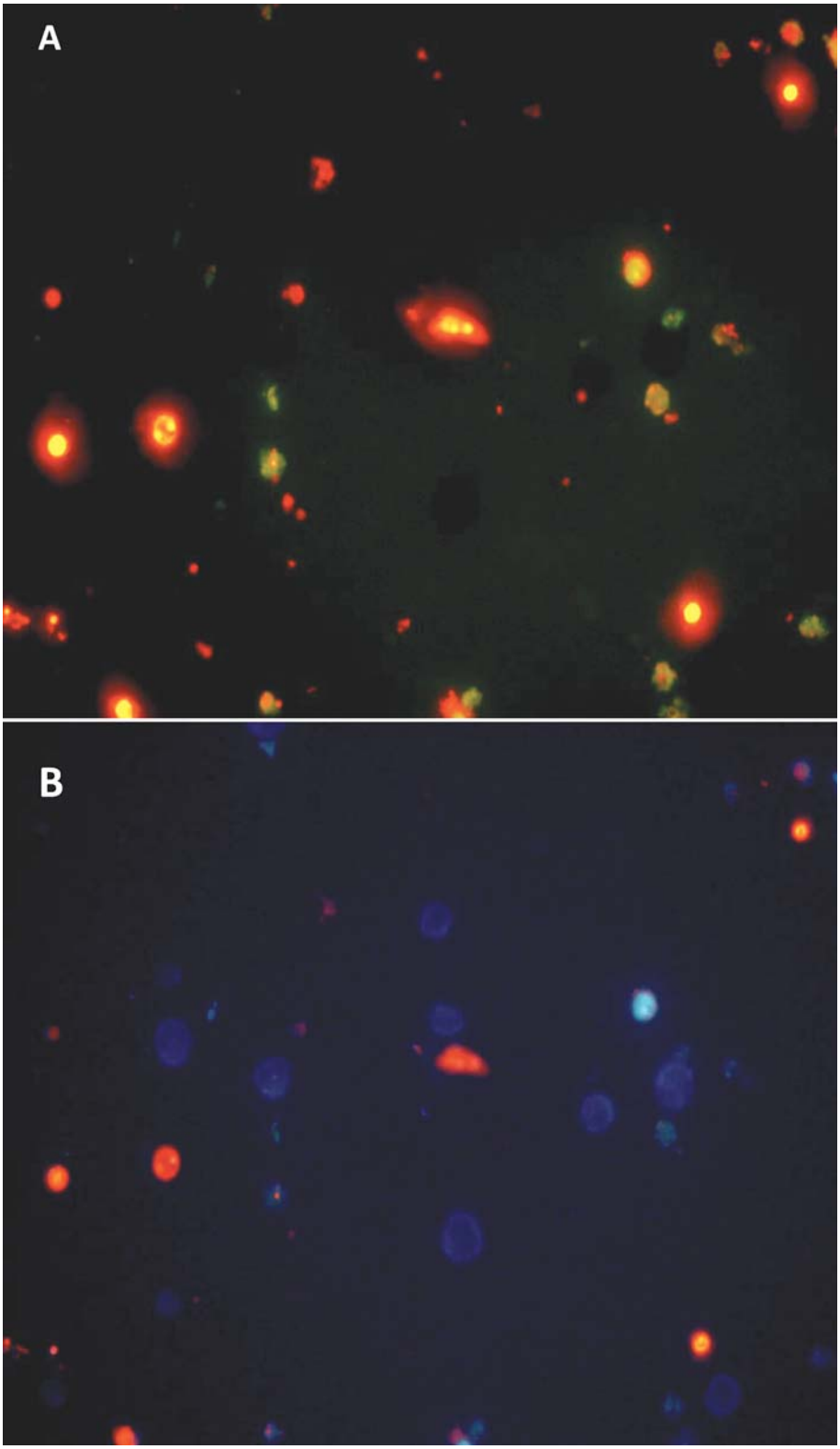


Figure 3. *Morphological examination of apoptosis in HL-60 cells treated with 7 μM of enalapril. AnnexinV-EGFP and PI staining method was used and cells were visualized by using fluorescence microscopy. The nuclei of cells that have lost membrane integrity are stained red (PI) and their plasma membranes are stained green (EGFP) (A). Cells that are stained both red and green are determined to be necrotic whereas green cells are apoptotic. Viable cells are shown in blue (B).*

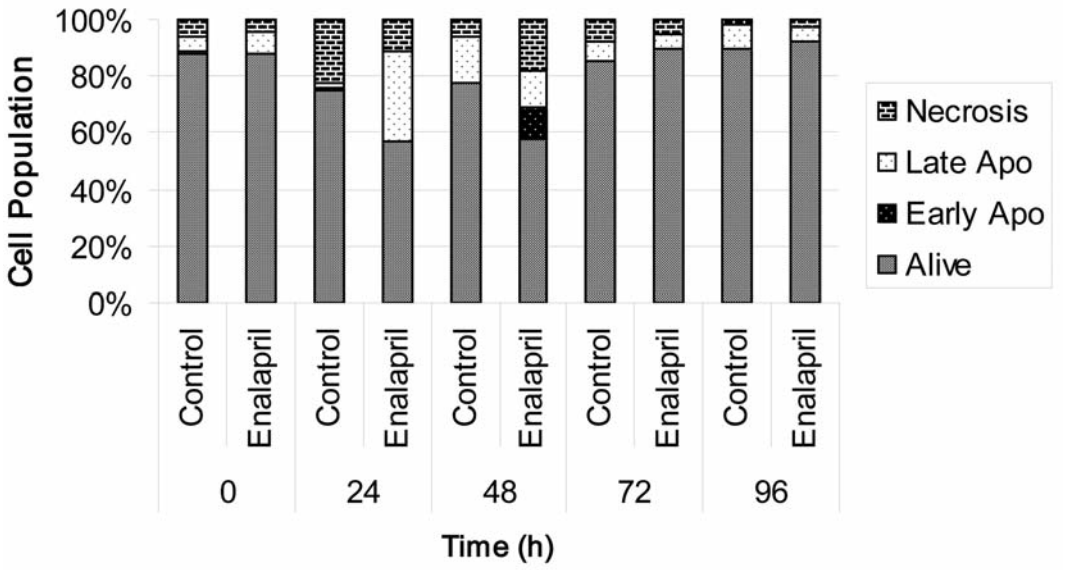
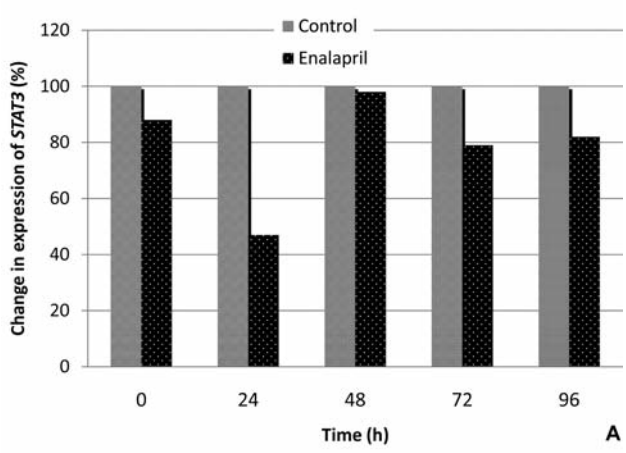


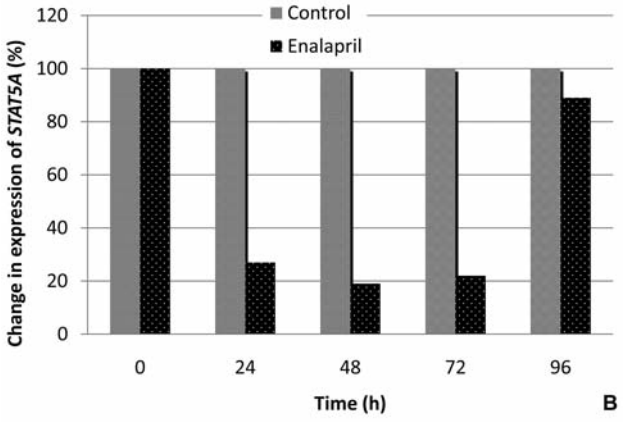
Figure 4. *Percentage changes in early and late apoptosis of HL-60 cells treated with 7 μM of enalapril, as determined by AnnexinV-EGFP method. Statistical significance was determined using the Student's t-test, and p<0.05 was considered significant. ENA: enalapril treated cells; Control: untreated control cells.*

proliferation assays revealed that enalapril reduced viability and proliferation of HL60 cells in a dose- and time-dependent manner. Previously, the potential antiproliferative effect of captopril, another ACE inhibitor, was observed on primitive haematopoietic stem and progenitor cells *in vitro* (42), however, it was shown to have no effect on proliferation of myeloid leukaemia cells (43). Similarly to our results, in a recent study, the growth and colony-forming ability of AML cells was shown to decrease *in vitro* in a dose-dependent manner following incubation with an ACE inhibitor (44). In a different study, ACE inhibitors captopril and trandolapril, and losartan, which is an angiotensin II receptor antagonist, were shown to inhibit cell proliferation and induce apoptosis in K562 chronic myeloid leukaemia cells which seemed to be related to Ang II-induced small mothers against decapentaplegic (SMAD) activation (45).

Measurement of plasma membrane phosphatidylserine externalization, using fluorescently labelled annexin V, is widely used for the detection of apoptotic cells and is advantageous as it offers the possibility of detecting early phases of apoptosis before the loss of cell membrane integrity (46). In order to see if enalapril induces apoptosis in HL60 acute leukaemia cells, we incubated HL60 cells with a 7 μM dose of enalapril and applied the AnnexinV-EGFP staining method at certain time points. Apoptotic cells were morphologically examined by fluorescence microscopy and the results showed that necrotic cells were in abundance when compared to apoptotic cells. The results showed that there was a slight increase in the number of early apoptotic cells in enalapril-treated HL60 cells within the early stages of experiment, however, this difference was not permanent and remained statistically insignificant when compared to untreated controls until the end of the experiment.

STAT proteins play an important role in cellular processes. Dysregulation of the STAT pathway may lead to formation of malignant cells (15). It is already known that the JAK-STAT pathway is a vital element in the interaction between the components of RAS present in the bone marrow and haematopoiesis (47). In the second stage of our study, we aimed to identify the pathway(s) that play a role in the cytotoxicity of enalapril towards HL60 cells. Therefore, we investigated the expression levels of *STAT3*, *STAT5A* and *STAT5B* genes in enalapril-treated HL60 cells by qRT-PCR. The results showed that the change in expression levels of *STAT3* and *STAT5B* were statistically insignificant ($p>0.05$) in enalapril-treated HL60 cells when compared to untreated controls, whereas the expression of *STAT5A* significantly decreased in a time-dependent manner in enalapril-treated HL60 cells when compared to the control group ($p<0.05$). These results suggest that STAT5A might have a significant role in enalapril-induced leukaemia cell death. Previously, enalapril was shown to inhibit Ang II-induced proliferation of rat cardiac fibroblasts *in vitro* by blocking the phosphorylation of STAT3 (48). In a recent study, retinal expressions of STAT3/5 were investigated in chemically induced diabetes in rats, in enalapril-treated and untreated control groups. Following the RT-PCR, *STAT3* and *STAT5* expression was found to be absent in the enalapril-treated group (49).





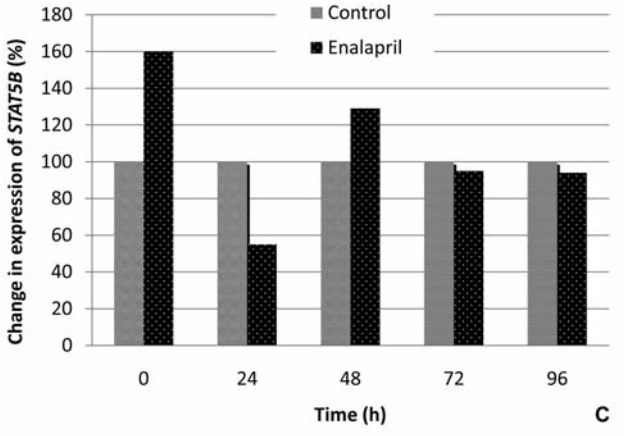


Figure 5. *Differential expression of signal transducers and activator of transcription 3 (STAT3) (A), STAT5A (B) and STAT5B (C) genes in HL-60 cells exposed to 7 μM enalapril at 24 h intervals over a 4-day period. Expression of each of the genes was normalized to 100% in untreated control cells and then data obtained in enalapril-treated HL-60 cells were plotted relative to that normalization. Statistical significance was determined using ANOVA and $p<0.05$ was considered significant. Control: untreated control cells.*

In conclusion, our study has shown for the first time that enalapril has cytotoxic and apoptotic effects on HL60 acute promyelocytic leukaemia cells and STAT5A may have significant roles in enalapril-induced cell death.

References

1 Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DA, Gralnick HR and Sultan C: Proposals for the classification of the acute leukaemias. French–American–British (FAB) co-operative group. Br J Haematol *33*: 451-458, 1976.

2 Chen Z, Tong JH, Dong S, Zhu J, Wang ZY and Chen SJ: Retinoic acid regulatory pathways, chromosomal translocations, and acute promyelocytic leukemia. Genes Chromosom Cancer *15*: 147-156, 1996.

3 Sanz MA, Jarque I, Martin G, Lorenzo I, Martínez J, Rafecas J, Pastor E, Sayas MJ, Sanz G and Gomis F: Acute promyelocytic leukemia. Therapy results and prognostic factors. Cancer *61*: 7-13, 1988.

4 Cunningham I, Gee TS, Reich LM, Kempin SJ, Naval AN and Clarkson BD: Acute promyelocytic leukemia: treatment results during a decade at Memorial Hospital. Blood *73*: 1116-1122, 1989.

5 Fenaux P, Wang ZZ and Degos L: Treatment of acute promyelocytic leukemia by retinoids. Curr Top Microbiol Immunol *313*: 101-128, 2007.

6 Huang ME, Ye YC, Chen SR, Zhao JC, Gu LJ, Cai JR, Zhao L, Xie JX, Shen ZX and Wang ZY: All-trans retinoic acid with or without low dose cytosine arabinoside in acute promyelocytic leukemia. Report of 6 cases. Chin Med J *100*: 949-953, 1987.

7 Huang ME, Ye YC, Chen SR, Chai JR, Lu JX, Zhoa L, Gu LJ and Wang ZY: Use of all-trans retinoic acid in the treatment of acute promyelocytic leukemia. Blood *72*: 567-572, 1988.

8 Mandelli F, Diverio D, Avvisati G, Luciano A, Barbui T, Bernasconi C, Broccia G, Cerri R, Falda M, Fioritoni G, Leoni F, Liso V, Petti MC, Rodeghiero F, Saglio G,Vegna ML, Visani G, Jehn U, Willemze R, Muus P, Pelicci PG, Biondi A and Lo Coco F: Molecular remission in PML. RAR-alpha-positive acute promyelocytic leukemia by combined all-trans retinoic acid and idarubicin (AIDA) therapy. Gruppo Italiano-Malattie Ematologiche Maligne dell'Adulto and Associazione Italiana di Ematologia ed Oncologia Pediatrica Cooperative Groups. Blood *90*: 1014-1021, 1997.

9 Sanz MA, Martin G, Rayon C, Esteve J, Gonzalez M, Diaz-Mediavilla J, Bolufer P, Barragan E, Terol MJ, Gonzalez JD, Colomer D, Chillon C, Rivas C, Gomez T, Ribera JM, Bornstein R, Roman J, Calasanz MJ, Arias J, Alvarez C, Ramos F and Deben G: A modified AIDA protocol with anthracycline-based consolidation results in high antileukemic efficacy and reduced toxicity in newly diagnosed PML-RARalpha-positive acute promyelocytic leukemia. PETHEMA group. Blood *94*: 3015-3021, 1999.

10 Asou N, Kishimoto Y, Kiyoi H, Okada M, Kawai Y, Tsuzuki M, Horikawa K, Matsuda M, Shinagawa K, Kobayashi T, Ohtake S, Nishimura M, Takahashi M,Yagasaki F, Takeshita A, Kimura Y, Iwanaga M, Naoe T and Ohno R; Japan Adult Leukemia Study Group: A randomized study with or without intensified maintenance chemotherapy in patients with acute promyelocytic leukemia who have become negative for PML-RARalpha transcript after consolidation therapy: the Japan Adult Leukemia Study Group (JALSG) APL97 study. Blood *110*: 59-66, 2007.

11 Douer D and Tallman MS: Arsenic trioxide: new clinical experience with an old medication in hematologic malignancies. J Clin Oncol *23*: 2396-2410, 2005.

12 Darnell JE Jr.: STATs and gene regulation. Science *277*: 1630-1635, 1997.

13 Darnell JE Jr., Kerr IM and Stark GR: JAK-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. Science *264*: 1415-1421, 1994.

14 Garcia R and Jove R: Activation of STAT transcription factors in oncogenic tyrosine kinase signaling. J Biomed Sci *5*: 79-85, 1998.

15 Bowman T, Garcia R, Turkson J and Jove R: STATs in oncogenesis. Oncogene *19*: 2474-2488, 2000.

16 Bromberg JF, Horvath CM, Wen Z, Schreiber RD and Darnell JE Jr.: Transcriptionally active Stat1 is required for the antiproliferative effects of both interferon alpha and interferon gamma. Proc Natl Acad Sci USA *93*: 7673-7678, 1996

17 Levy DE and Gilliland DG: Divergent roles of Stat1 and Stat5 in malignancy as revealed by gene disruptions in mice. Oncogene *19*: 2505-2510, 2000.

18 Catlett-Falcone R, Landowski TH, Oshiro MM, Turkson J, Levitzki A, Savino R, Ciliberto G, Moscinski L, Fernández-Luna JL, Nunez G, Dalton WS and Jove R: Constitutive activation of Stat3 signaling confers resistance to apoptosis in human U266 myeloma cells. Immunity *10*: 105-115, 1999.

19 Puthier D, Thabard W, Rapp M, Etrillard M, Harousseau J, Bataille R and Amiot M: Interferon alpha extends the survival of human myeloma cells through an up regulation of the Mcl-1 antiapoptotic molecule. Br J Haematol *112*: 358-363, 2001.

20 Deshayes F and Nahmias C: Angiotensin receptors: A new role in cancer? Trends Endocrinol Metab *16*: 293-299, 2005.

21 Dzau V: Tissue renin–angiotensin system: physiologic and pharmacologic implications. Circulation *77*: 1-2, 1988.

22 Haznedaroglu IC and Ozturk MA: Towards the understanding of the local hematopoietic bone marrow renin–angiotensin system. Int J Biochem Cell Biol *35*: 867-880, 2003.

23 Strawn WB, Richmond RS, Tallant EA, Gallagher PE, Ferrario CM: Renin–angiotensin system expression in rat bone marrow haematopoietic and stromal cells. Br J Haematol *126*: 120-126, 2004.

24 Hubert C, Savary K, Gasc JM and Corvol P: The hematopoietic system: a new niche for the renin–angiotensin system. Nat Clin Pract Cardiovasc Med *3*: 80-85, 2006.

25 Ager EI, Neo J and Christophi C: The renin–angiotensin system and malignancy. Carcinogenesis *29*: 1675-1684, 2008.

26 Beyazit Y, Aksu S, Haznedaroglu IC, Kekilli M, Misirlioglu M, Tuncer S, Karakaya J, Koca E, Buyukasik Y, Sayinalp N and Goker H: Overexpression of the local bone marrow renin–angiotensin system in acute myeloid leukemia. J Natl Med Assoc *99*: 57-63, 2007.

27 Koca E, Haznedaroglu IC, Acar K, Beyazit Y, Aksu S, Misirlioglu M, Tuncer S, Sayinalp N, Ozcebe OI and Uner A: Renin–angiotensin system expression in the K562 human erythroleukemic cell line. J Renin Angiotensin Aldosterone Syst *8*: 145-147, 2007.

28 Koca E, Haznedaroglu IC, Uner A, Sayinalp N, Saglam AE, Goker H and Ozcebe OI: Angiotensin-converting enzyme expression of the lymphoma-associated macrophages in the lymph nodes of Hodgkin's disease. J Natl Med Assoc *99*: 1243-1247, 2007.

29 Aksu S, Beyazit Y, Haznedaroglu IC, Canpinar H, Kekilli M, Uner A, Sayinalp N, Büyükaşik Y, Goker H and Ozcebe OI: Overexpression of angiotensin-converting enzyme (CD 143) on leukemic blasts as a clue for the activated local bone marrow RAS in AML. Leuk Lymphoma *47*: 891-896, 2006.

30 Wulf GG, JahnsStreubel G, Strutz F, Basenau D, Hüfner M, Buske C, Wörmann B and Hiddemann W: Paraneoplastic hypokalemia in acute myeloid leukemia: A case of renin activity in AML blast cells. Ann Hematol *73*: 139-141, 1996.

31 Wulf GG, Jahns-Streubel G, Nobiling R, Strutz F, Hemmerlein B, Hiddemann W and Wörmann B: Renin in acute myeloid leukaemia blasts. Br J Haematol *100*: 335-337, 1998.

32 Lindberg H, Nielsen D, Jensen BV, Eriksen J and Skovsgaard T: Angiotensin-converting enzyme inhibitors for cancer treatment? Acta Oncol *43*: 142-152, 2004.

33 Piskin O, Ozcan MA, Ozsan GH, Ates H, Demirkan F, Alacacioglu I and Undar B: Synergistic effect of imatinib mesylate and fludarabine combination on Philadelphia chromosome-positive chronic myeloid leukemia cell lines. Turk J Haematol *24*: 23-27, 2007.

34 Haznedaroglu IC, Tuncer S and Gursoy M: A local renin–angiotensin system in the bone marrow. Med Hypotheses *46*: 507-510, 1996.

35 Gomez RA, Norling LL, Wilfong N, Isakson P, Lynch KR, Hock R and Quesenberry P: Leukocytes synthesize angiotensinogen. Hypertension *21*: 470-475, 1993.

36 Huckle WR and Earp HS: Regulation of cell proliferation and growth by angiotensin II. Prog Growth Factor Res *5*: 177-194, 1994.

37 Okamura A, Raukugi H, Ohishi M, Yanagitani Y, Takiuchi S, Moriguchi K, Fennessy PA, Higaki J and Ogihara T: Up-regulation of renin–angiotensin system during differentiation of monocytes to macrophages. J Hypertension *17*: 537-545, 1999.

38 Abali H, Haznedaroglu IC, Goker H, Celik I, Ozatli D, Koray Z and Caglar M: Circulating and local bone marrow renin–angiotensin system in leukemic hematopoiesis: preliminary evidences. Hematology *7*: 75-82, 2002.

39 Jokubaitis VJ, Sinka L, Driessen R, Whitty G, Haylock DN, Bertoncello I, Smith I, Péault B, Tavian M and Simmons PJ: Angiotensin-converting enzyme (CD143) marks hematopoietic stem cells in human embryonic, fetal, and adult hematopoietic tissues. Blood *111*: 4055-4063, 2008.

40 Zambidis ET, Soon Park T, Yu W, Tam A, Levine M, Yuan X, Pryzhkova M and Peault B: Expression of angiotensin-converting enzyme (CD143) identifies and regulates primitive hemangioblasts derived from human pluripotent stem cells. Blood *112*: 3601-3614, 2008.

41 Lever A, Hole DJ, Gillis CR, McCallum IR, McInnes GT, MacKinnon PL, Meredith PA, Murray LS, Reid JL and Robertson JW: Do inhibitors of angiotensin-I-converting enzyme protect against risk of cancer? Lancet *352*: 179-184, 1998.

42 Chisi JE, Wdzieczak-Bakala J, Thierry J, Briscoe CV and Riches AC: Captopril inhibits the proliferation of hematopoietic stem and progenitor cells in murine long term bone marrow cultures. Stem Cells *17*: 339-344, 1999.

43 Chisi JE, Briscoe CV, Ezan E, Genet R, Riches AC and Wdzieczak-Bakala J: Captopril inhibits *in vitro* and *in vivo* the proliferation of primitive haematopoietic cells induced into cell cycle by cytotoxic drug administration or irradiation but has no effect on myeloid leukaemia cell proliferation. Br J Haematol *109*: 563-570, 2000.

44 Comte L, Lorgeot V, Volkov L, Allegraud A, Aldigier JC and Praloran V: Effects of the angiotensin-converting enzyme inhibitor enalapril on blood haematopoietic progenitors and acetyl-N-Ser-Asp-Lys-Pro concentrations. Eur J Clin Invest *27*: 788-790, 1997.

45 Inigo SD, Lopez-Jorge CE, Gomez-Casares MT, Lemes Castellano A, Martin Cabrera P, Lopez Brito J, Suarez Cabrera A and Molero Labarta T: Induction of apoptosis in leukemic cell lines treated with captopril, trandolapril and losartan: A new role in the treatment of leukaemia for these agents. Leuk Res *33*: 810-816, 2009.

46 Vermes I, Haanen C, Steffens-Nakken H and Reutelingsperger C: A novel assay for apoptosis. Flow cytometric detection of phosphatidylserine expression on early apoptotic cells using fluorescein labelled annexin V. J Immunol Methods *184*: 39-51, 1995.

47 Haznedaroglu IC, Arici M and Buyukasik Y: A unifying hypothesis for the renin–angiotensin system and hematopoiesis: sticking the pieces together with the JAK-STAT pathway. Med Hypotheses *54*: 80-83, 2000.

48 van Eickels M, Grohe C, Lobbert K, Stimpel M and Vetter H: Angiotensin converting enzyme inhibitors block mitogenic signalling pathways in rat cardiac fibroblasts. Naunyn Schmiedebergs Arch Pharmacol *359*: 394-399, 1999.

49 Kim HW, Kim JL, Lee HK, Hur DY, Yun IH and Kim SD: Enalapril alters expression of key growth factors in experimental diabetic retinopathy. Curr Eye Res *34*: 976-987, 2009.

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