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Induction of apoptosis in leukemic cell lines treated with captopril, trandolapril and losartan: A new role in the treatment of leukaemia for these agents

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ABSTRACT

Renin–angiotensin system (RAS) in the bone marrow is related to proliferation and cellular differentiation. We investigated the effect of ACE inhibitors (ACEI) captopril (>1 mM) and trandolapril (>0.05 mM) and losartan (0.2 mM) on K562 cell line and K562 transfected with c-myc, bcl-x and bcl-2 (KmycB, Kbclx and Kbcl2 respectively). RAS components, proliferation, apoptosis and c-myc expression were analyzed. ACEI and losartan inhibited cell growth, decreased c-myc expression and increased apoptosis. These effects seem to be associated to angiotensin II-induced Smad activation. This work offers a new possible line of treatment for some acute myeloid leukemias and a new area of clinical research.

1. Introduction

The renin–angiotensin system (RAS) plays a critical role in blood pressure regulation and hemodynamic fluids. Previous data supported the existence of an endogenous RAS in some local tissues. Angiotensin produced locally exerts autocrine/paracrine influences [1]. It has been suggested that the presence of RAS in the bone marrow [2–5] might affect cellular proliferation and differentiation in physiological or pathological states [6–13]. The RAS components, namely renin, angiotensin-converting enzyme (ACE), angiotensin II, angiotensin type 1 and type 2 receptors (AT_1 and AT_2) [9,14–18], and tetrapeptide N-Acetyl-Ser-Asp-Lys-Pro (AcSDKP) which is a down regulating hematopoietic agent [17], have been isolated either in bone marrow hematopoietic cells and in bone marrow microenvironment.

Angiotensin II interacts with the AT_1 receptor to stimulate erythroid differentiation and increase hematopoietic progenitor cell proliferation; this stimulatory effect is blocked by losartan [9,14]. Stimulation of AT_1/AT_2 receptors exerts an stimulatory/inhibitory action on JAK-STAT pathway implicated in the physiologic mechanism of erythropoietin [19], thrombopoietin and other cytokines [20,21], modulating transcription factor expression like c-myc [22]. Myc is a nuclear protein of the helix-loop-helix/leucine zipper family that acts as a transcription factor involved in the control of cell proliferation, differentiation and apoptosis [23].

ACE surface antigen was shown to be over-expressed in leukemic myeloid blast cells and ACE is positively correlated with bone marrow blast count [24]. In hematopoietic bone marrow cells, ACE enhances the recruitment of primitive stem cells into the S-phase by degrading AcSDKP [25–29]. AcSDKP is a specific substrate for the N-terminal site of ACE and increases 5-fold during ACE inhibitor therapy [25,26]. Also, AcSDKP inhibits TGF-beta signal transduction through the suppression of R-Smad activation via nuclear export of Smad7 [30].

Myeloid blast cells also contain renin in their cytoplasm [15]. Bone marrow blast cells from some types of acute myeloid leukemia (AML) [16,31,32] and CD34 positive hematopoietic cells [33] do also express renin gene.

Consistent with these studies that relate the RAS to the hematopoiesis, we had previously investigated the expression of renin in bone marrow cells of patients with hematological malignancies and found significant renin expression in cells from patients with AML, but no expression in normal bone marrow from healthy donors [31,34]. Taking into consideration that leukemic myeloid cell lines are useful models to study the molecular basis

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of hematopoiesis, we studied c-myc and RAS components gene expression in K562 and KU812 (both positive for renin expression), and HL60 and U937 cell lines (both negative for renin expression) before and after being exposed to several chemical agents such as ACE inhibitors (ACEI) and AT_1 receptor antagonist. Moreover, we studied the role of RAS components expression in the development of disease as well as the response of renin positive and negative cell lines to drugs that act through this cellular pathway.

2. Materials and methods

2.1. Cell culture and assessment of growth

Leukemic myeloid cell lines K562, KU812, U937 and HL60 (obtained from American Type Culture Collection) were grown in RPMI 1640 medium (Invitrogen) supplemented with 10% fetal calf serum (Gibco BRL). K562, HL60, KU812, U937 cells (2.5×10^5 cells/ml) were treated for 3 days with 1–10 mM captopril, 0.05–2 mM trandolapril and 0.2 mM losartan. We also used K562 cells transfected with bcl-x, bcl-2 (both antiapoptotic genes) and c-myc inducible by Zinc, named by us Kbclx, Kbcl2 and KmycB respectively (yielded elegantly from Javier León-Cantabria University, Santander) [35]. Proliferation was quantified by cell counting and ^{3}H-thymidine incorporation.

2.2. Erythroid differentiation in K562

Cells were scored for erythroid differentiation by benzidine staining and by optical microscopy [36].

2.3. Apoptosis determinations

The percentage of apoptotic cells was analyzed by May–Grünwald–Giemsa staining of cytocentrifuge preparations. The apoptotic cells were identified by condensation and marginalization of chromatin, and cytoplasmic shrinkage. At least 200 cells per sample were analyzed. Binding of annexin V-FITC to cell surface was analyzed by flow cytometry using Annexin V-PE Apoptosis Detection Kit I (BD Biosciences Pharmingen) following the manufacturerĩs instructions. Cytometric analysis was carried out in a FACScan flow cytometer (Becton Dickinson) using CellQuest software.

2.4. RNA preparation

Total RNA was isolated from cells by the acid guanidine thiocyanate method [37]. The integrity of RNA was assessed by electrophoresis on an ethidium bromide-stained 1% agarose gel.

2.5. Northern blot analysis

Northern blots were prepared and hybridized to ^{32}P-labeled probes according to standard procedures. Probes for human c-myc were as described [35]. C-myc expression by K562 and HL60 cell lines were monitored during 3 days of treatment with the doses previously described for captopril and trandolapril.

2.6. Western blot analysis

To study of c-myc protein expression cell pellets were lysed in a solution containing 150 mM NaCl, 50 mM Tris (pH 8), 20 mM NaF, 1% NP40, 1 mM EDTA and protein inhibitor cocktail (Calbiochem), for 20 min on ice. Samples with 40 μg of protein per lane were separated in polyacrylamide gels, transferred to nitrocellulose membranes, incubated with appropriate antibodies anti-myc (N262) (was from Santa Cruz Biotechnology) and anti-α-tubulin (was a gift from Javier León-Cantabria University, Santander) and visualized with an ECL Western blotting detection system.

2.7. cDNA synthesis

First-strand cDNA was synthesized from 1 μg of total RNA using MULV reverse transcriptase (Roche Diagnostics, Mannheim, Germany) according to the manufacturerĩs instructions.

2.8. Reverse transcriptase PCR (RT-PCR)

RT-PCR was realized to determinate renin, ACE, AT_1 and AT_2 expression in K562, KU812, U937 and HL60 cell lines. For amplification of renin and the other components of RAS, primers and conditions were used as previously described [31,34,38–40]. The amplification of kidney RNA served as positive control. A negative control was included in all experiments. The effectiveness of the RT procedure was assessed by amplification of abelson mRNA.

2.9. Renin expression by real time PCR (RQ-PCR)

PCR was performed following the method described by our group [31]. Effectiveness of the RQ-PCR procedure was assessed by amplification of β-glucuronidase (β-GUS) like housekeeping gene. If the samples did not amplify the β-GUS, they were excluded from the study. Amplification of kidney cells or K562 cell line RNA was used as a positive control. A sample without cDNA was included as a negative control in all experiments. Samples which presented a melting temperature (Tm) equal to the positive control were considered positive. Moreover the products amplified were checked using electrophoresis in an agarose gel. The results obtained through RQ-PCR were compared with those obtained by RT-PCR following the Wulf et al. technique [16] and afterwards by our group [31].

2.10. Quantitative assay

The concentration of the renin gene and the GUS control gene belonging to the same sample was normalized from the β-GUS standard curve. The final results and the number of copies/μl, were shown as the ratio renin gene/β-glucuronidase gene control as we described previously [31].

3. Results

3.1. Study of proliferation cellular

3.1.1. Effects of ACEI in K562, HL60, U937 and KU812 cell lines

We found that captopril (>1 mM) and trandolapril (1 mM in K562, U937, KU812 and 0.02 mM in HL60 cell line) inhibited cell growth in these cell lines (Fig. 1a). Growth arrest was reversed when captopril was removed from the medium in all cells lines. However when trandolapril was removed growth was restored in K562 cells but not in HL60, KU812 and U937 cell lines (data not shown). The decrease in proliferation was also observed in Kbclx, Kbcl2 and KmycB with the same drug concentration. In relation to KmycB, there was no difference in proliferation between KmycB cells treated with or without Zinc. Since Zinc induces c-myc expression, we assumed that the antiproliferative effect must be c-myc independent (Fig. 1b). No morphological changes were observed in HL60, K562, Kbclx and Kbcl2 after treatment with ACEI (captopril and trandolapril).

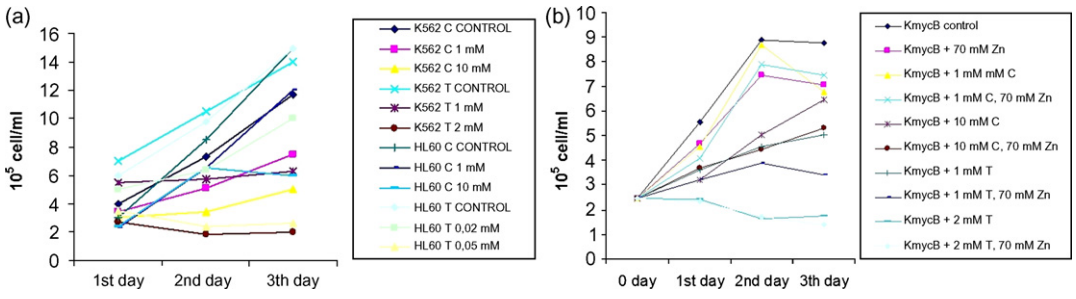


Fig. 1. Growth curves of: (a) K562 and HL60 cell lines treated with captopril (C) and trandolapril (T) and (b) KmycB cells in the absence or presence of 70 mM $ZnSO_4$ treated with captopril (C) and trandolapril (T).

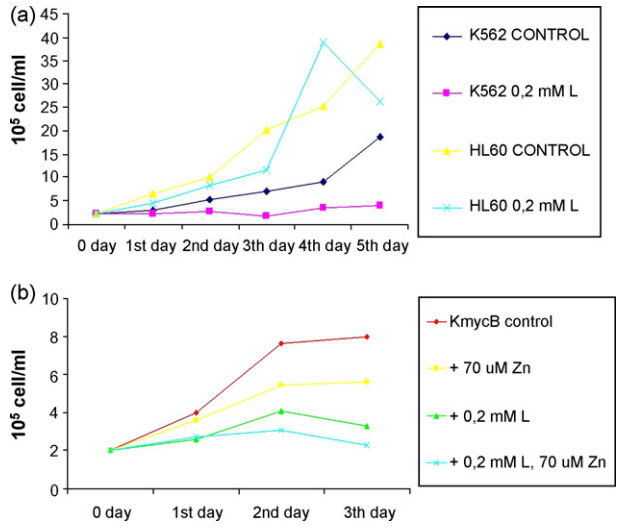


Fig. 2. Growth curves of: (a) K562 and HL60 cell lines treated with losartan (L). It is observed that the decreasing of cell proliferation in both cases and (b) KmycB cells in the absence or presence of 70 mM $ZnSO_4$ (Zn) treated with losartan (L).

3.1.2. *Effects of AT_1 receptor antagonist in K562,HL60, U937 and KU812 cell lines*

An irreversible effect in the proliferation of K562, HL60, U937 and KU812 cell lines was observed when the cells were treated with an AT_1 receptor antagonist (losartan 0.2 mM) (Fig. 2a). We observed that cellular proliferation in Kbcl2 and KmycB when treated with losartan was also hampered. As it happens with ACEI, in KmycB treated with losartan there was no proliferation difference between cells treated with or without Zinc (Fig. 2b).

3.2. *Analysis of the erythroid differentiation data*

There was no erythroid differentiation or morphological changes in K562, Kbclx and Kbcl2 cells treated with captopril and trandolapril. This was studied using benzidine staining and optical microscopy (data not shown).

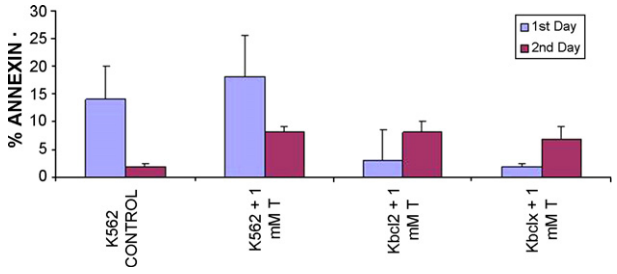


Fig. 3. Apoptosis determinated by annexin V binding in K562 and K562 transfected with bcl-2 and bcl-x (Kbcl2 and Kblx) cells treated with 1 mM trandolapril (T).

3.3. *Apoptosis determinations results*

The number of apoptotic cells, determinated by optical microscopy, was increased in K562, Kbcl2 and Kbclx cell lines treated with trandolapril (1 mM y 2 mM) during 3 days (data not shown). Annexin was analyzed in K562, Kbcl2, Kbclx cells treated with captopril (1 mM y 10 mM) and trandolapril (1 mM y 2 mM). The percentage of apoptotic cells was increased in K562 cell line treated with captopril and trandolapril in the first and second day. We observed the same apoptotic effect in Kbcl2 and Kbclx cells but unlike K562, in which apoptosis is observed from the first day, in Kbcl2 and Kbclx one more day was necessary to observe apoptosis. Since Kbcl2 y Kbclx cells over-express apoptosis inhibiting genes, this apoptotic effect could be bcl-2 and bcl-x independent (Fig. 3).

3.4. *C-myc gene and protein expression in cell lines after ACEI treatment*

The c-myc expression determinated by Northern blot was decreased in K562 and HL60 after treatment with captopril (1 mM y 10 mM) and trandolapril (1 mM y 2 mM). This results were confirmated by Western blot. We also observed a decrease in c-myc gene expression in Kbclx and Kbcl2 by Northern blot (Fig. 4). In KmycB cells after treatment with ACEI we observed the recovery of c-myc exogenous expression by Northern blot when Zinc was added to the medium (especially at third day of culture) (Fig. 5). The same results were observed by Western blot (data not shown).

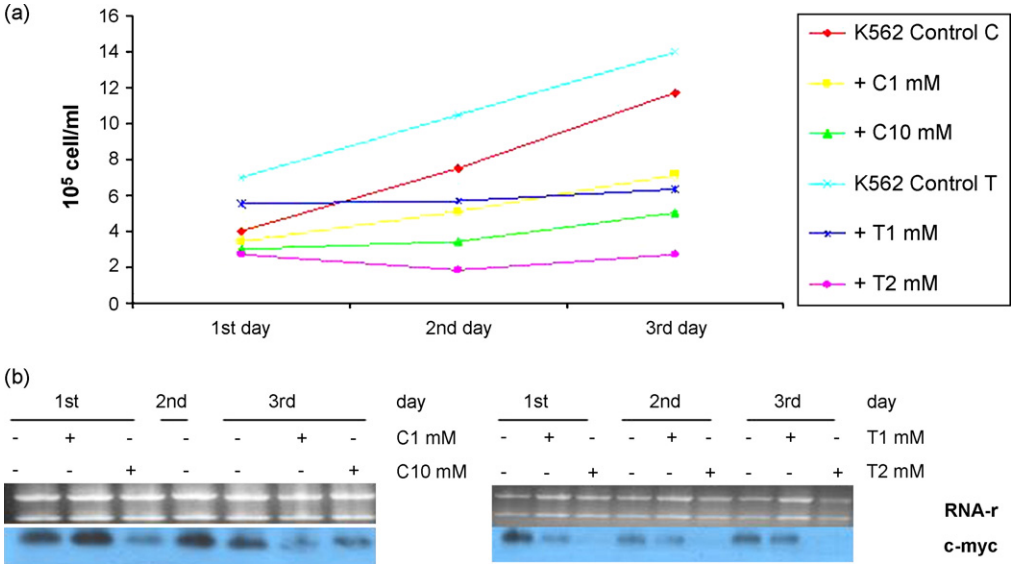


Fig. 4. (a) Proliferation in K562 after the treatment with captopril (C) and trandolapril (T) (b) C-myc gene expression in K562 after the treatment with the same drugs.

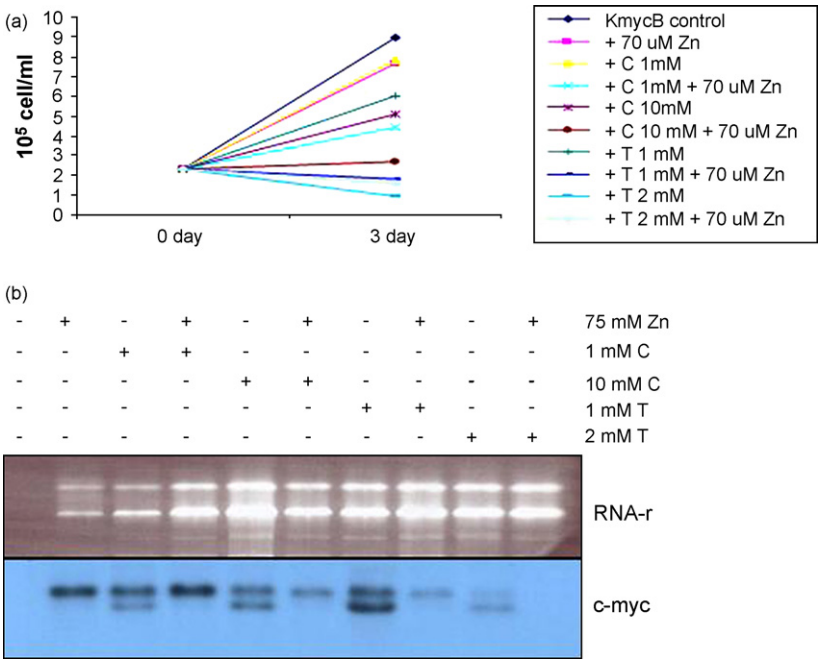


Fig. 5. (a) Proliferation and (b) C-myc gene expression in KmycB in the absence or presence of 75 mM of $ZnSO_4$ (Zn) for the indicated periods of time after the treatment with 1–10 mM captopril (C) and 1–2 mM trandolapril (T). Northern blot shows the exogen c-myc expression and not the endogen c-myc expression in cells treated with captopril and trandolapril. A picture of the rRNAs stained with ethidium bromide is shown to asses loading and integrity of the RNAs.

Table 1
RAS components gene expression in leukaemic myeloid cell lines.

Cell lines	Renin	AT_1	AT_2	ACE
K562	+	+	+	+
HL60	–	+	+	–
KU812	+	+	+	–
U937	–	+	+	–

ACE: angiotensin-converting enzyme; AT_1: angiotensin type 1 receptor; AT_2: angiotensin type 2 receptor.

3.5. Analysis of RAS components expression in leukemic cell lines without treatment by RT-PCR

Cellular lines with a more differentiated phenotype (HL60 and U937) were negative for renin expression whereas K562 and KU812 were positive. We have detected ACE transcripts in K562 but not in HL60, KU812 and U937 cells lines. AT_1 and AT_2 transcripts were positive in all cell lines (Table 1).

3.6. Quantitative assay in K562 leukemic cell lines treated with ACEI

The sensitivity of the procedure was first established taking into account that this method can detect renin in a dilution up to 10^{-4} of K562. The renin mRNA levels in K562 cell line, before and after treatment with captopril and trandolapril did not show significant changes (Fig. 6).

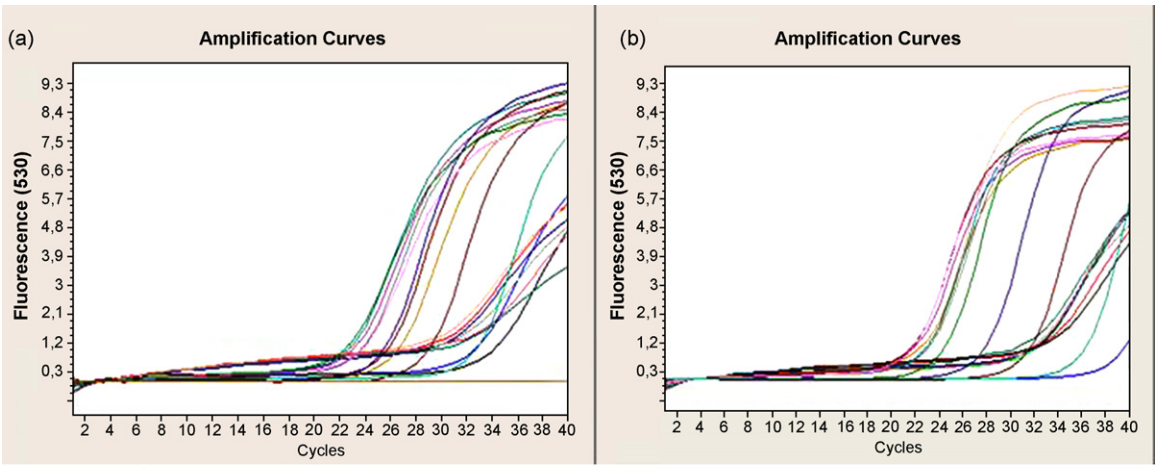
4. Discussion

The presence of all the components of the RAS (renin, angiotensinogen, angiotensin II receptor) in the bone marrow, suggests that this system is involved in normal haematopoiesis [1,5]. There are also evidences that RAS modulates primitive erythropoiesis and has a role in different stages of haematopoiesis [41,42]. Major RAS peptides can exert significant effects on primitive pluripotent hematopoietic stem cell populations [43]. Local bone marrow RAS also has been involved in leukemogenesis [15,16,7]. In this sense, we have corroborated renin expression [31] and its relevance in acute myeloid leukemia [34]. The leukemogenic role of renin and the expression of other RAS components expression, as well as the molecular pathways involved, still have not yet been fully understood. The Ebru group studied genetic polimorfisms I/D of ACE reaching the conclusion that it can affect local RAS behaviour in hematological malignancies [44].

According to that, we attempt to continue our experiments with leukemic cell lines expressing the renin gene [31] and other genes of RAS as well as to analyse the effect of ACE inhibitors on cell proliferation, cell differentiation and expression of genes involved in these processes. Our current study widens the primary results [31] and demonstrates that renin expression is related with undifferentiated phenotypic cell lines, since no expression of the renin gene was found in cellular lines that showed a highly differentiated phenotype like HL60 and U937.

In order to study the role of RAS components in haematopoiesis, we used drugs which interact with the above mentioned signalling process. These were ACE inhibitors (captopril and trandolapril) and one AT_1 receptor blocker (losartan). They all produce a stop in proliferation as well as an increase in the apoptotic rate. With in the ACEI group, induced a stop in cell proliferation in all cell lines which could be reversed after washing. However, the proliferative stop produce by trandolapril was only reversible on cell line K562. On the other cell lines treated with trandolapril, the stop in cell proliferation was irreversible which could in fact be explained due to trandolaprilĩs stronger effect and its higher ACE binding affinity, higher than captopril.

The mechanism through which AT_1 receptor antagonist exert a stop in proliferation and an increase in apoptosis seems clear, since they can prevent angiotensin II from binding its receptor. In order to explain this same effect in the use of ACEI, we have come



K562	RENIN/GUS RATIO
Control 1st d	0.00590
+ Control 1st d	0.00650
+ C 10 mM 1st d	0.00124
+ C 1 mM 2nd d	0.01800
Control 3rd d	0.00850
+ C 1 mM 3rd d	0.00540
+ C 10 mM 3rd d	0.00530

K562	RENIN/GUS RATIO
Control 1st d	0.001755
+ T 1 mM 1st d	0.000505
+ T 2 mM 1st d	0.000359
Control 2nd d	0.001877
+ T 1 mM 2nd d	0.001030
Control 3rd d	0.003000
+ T 1 mM 3rd d	0.001240

Fig. 6. Real-time PCR amplifications and fluorescence curve graphics of the renin and β-glucuronidase (GUS) cDNA target in K562 cell line and renin/GUS expression ratio in the samples of (a) K562 cell lines treated with captopril (C) and (b) K562 cell lines treated with trandolapril (T).

up with two hypothesis: namely the implication of AcSDKP and angiotensin II.

We know AcSDKP is a negative regulator of the hematopoietic precursors, that blocks the response of hemapoietic cell to a proliferative stimulus. By treating cells with ACEI, degradation of AcSDKP by ACE is avoided, experimenting an increase in concentration of this inhibitor of haematopoiesis in plasma. Nevertheless our data show that it is unlikely that the antiproliferative effect of these drugs is exerted through AcSDKP degradation since ACEI have effect both in ACE positive and negative cell lines. Our results agree with the studies reported by Hubert, reaching to the where it was concluded that the possibility that AcSDKP were responsible for the hematopoietic effect of the RAS is unlikely [42].

We have data both in favour and against ACEI producing a decrease in cell proliferation though angiotensin II inhibition. The argument in favour, of thinking that the route for which these medicaments operate is across the decrease in angiotensin II levels, is that its well known the important role angiotensin II plays on cell proliferation, since angiotensin II can indeed serve to similar functions as growth hormone over erythroid progenitors. This is done through the JAK-STAT pathway, which is linked to the transcription signal of a big number of growth factors, such as erythropoietin [17]. Moreover, we asked ourselves whether the decrease in angiotensin II levels could be responsible for the diminished expression of c-myc, which we observed in our study. We also asked ourselves whether a decrease in c-myc expression could play an important role in ACEI mechanism over leukemic cell lines, since it had been previously related to cell cycle progression, cellular growth, differentiation, apoptosis and tumorogenesis. In this way, it was described that captopril decreases c-myc expression [45]. However, we considered that the antiproliferative effect of ACEI must be c-myc independent because we observed the decrease in proliferation also in K562 cells transfected with inductible c-myc gene.

Our study shows an increase in apoptosis in cells treated with ACEI and AT1 receptor antagonist either through the annexin V method as well as optical microscopy. In this sense, Angiotensin II, though a specific receptor, is capable of activating apoptosis regulated by proteins of the Bcl-2 family [46]. However our experiments demonstrated that the antileukemic effect of ACEI seems not to be mediated by Bcl-2 or Bcl-x, since apoptosis also increases K562 cell lines transfected with these antiapoptotic genes. In addition, Rodriguez-Vita demonstrated that angiotensin II activates the Smad pathway via AT_1 receptor [47]. In this way, the decrease of c-myc expression after the treatment with captopril [45] was explained by an enhancement in expression of transforming growth factor (TGF-β1) expression [48] by Smad pathway [30]. Smad are TGF-β receptor kinase substrates that translocate into the cell nucleus to act as transcription factors. The Smad proteins are essential components of the intracellular signalling pathways used by transforming growth factor-β (TGF-β). It is also well known, that the AT_1 antagonist losartan significantly diminishes angiotensin II-induced Smad activation, whereas AT_2 antagonist had no effect. Overexpression of Smad7,which is an inhibitory Smad of transforming growth factor-β (TGF-β) signalling, induces the degradation of poly (ADP-ribose) polymerase and the activation of the caspase cascade [49].

Contrary to the theory in which the decrease in angiotensin II levels could be associated to the effects produced by ACEI and AT_1 receptor inhibitors is that, ACEI produce similar results either in ACE positive and negative cell lines, possibly meaning that this not be the only mechanism taking place. Other pathways have been also described, which may contribute to angiotensin II generation other than through ACE activity [50] and on which ACEI could also take part [51].

Recently, Krop and Danser [52] have demostrated that extrarenal sites that express the renin gene release prorenin, an "inactive" precursor of renin, instead of renin. Prorenin, as well

as the generation of angiotensin in tissues, could also act on its receptor producing angiotensin independent effects which have still not been clarified. Data from bone marrow do not clarify what it is being produced, renin or prorenin. However renin gene expresssion [16,17] as well as evidence of "renin-like" activity [15] which would then had to angiotensin production either though renin and/or prorenin have been found in some leukemia blasts. In this sense, future experiments with Aliskiren, a new orally active renin inhibitor, either alone or together with captopril, trandolapril or losartan can be carried out in order to elucidate whether renin or prorenin have angiotensin independent activity.

We thus conclude that ACE inhibitors and AT_1 receptor antagonist antiproliferative and apoptotic activity on leukemic cells can be explained through angiotensin II. This work offers a new possible treatment mechanism in some acute myeloid leukemia, since these drugs could very well have an action a genic role either associated to other drugs or by themselves, offering as well a new area of clinical research [53,54].

Conflict of Interest statement

None.

Acknowledgments

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References

[1] Dzau V. Tissue renin–angiotensin system: physiologic and pharmacologic implications. Circulation 1988;77:1–2.
[2] Gomez RA, Norling LL, Wilfong N, Isakson P, Lynch KR, Hock R, et al. Leukocytes synthesize angiotensinogen. Hypertension 1993;21:470–5.
[3] Haznedaroglu IC, Tuncer S, Gürsoy M. A local renin–angiotensin system in the bone marrow. Med Hypotheses 1996;46:507–10.
[4] Haznedaroglu IC, Buyukasik Y. Current evidence for the existence of a local renin–angiotensin system affecting physiological and pathological haematopoiesis in the bone marrow. Br J Haematol 1997;99:464–72.
[5] Haznedaroglu IC, Öztürk MA. Towards the understanding of the local hematopoietic bone marrow renin–angiotensin system. Int J Biochem Cell Biol 2003;35:867–80.
[6] Huckle WR, Earp HS. Regulation of cell proliferation and growth by angiotensin II. Prog Growth Factor Res 1994;5:177–94.
[7] Okamura A, Raukugi H, Ohishi M, Yanagitani Y, Takiuchi S, Moriguchi K, et al. Upregulation of renin–angiotensin system during differentiation of monocytes to macrophages. J Hypertension 1999;17:537–45.
[8] Chisi JE, Wdzieczak-Bakala J, Thierry J, Briscoe CV, Riches AC. Captopril inhibits the proliferation of hematopoietic stem and progenitor cells in murine long-term bone marrow cultures. Stem Cells 1999;17:339–44.
[9] Rodgers KE, Xiong S, Steer R, di Zerega GS. Effect of angiotensin II on hematopoietic progenitor cell proliferation. Stem Cells 2000;18:287–94.
[10] Rieger KJ, Saenz-Servent N, Papet MP, Wdzieczak-Bakala J, Morgat JL, Thierry J, et al. Involvement of human plasma angiotensin I-converting enzyme in the degradation of the hemoregulatory peptide *N*-acetyl-Seryl-Aspartyl-Lysyl-Proline. Biochem J 1993;296:373–8.
[11] Robinson S, Lenfant M, Wdzieczak-Bakala J, Melville J, Riches A. The mechanism of action of the tetrapeptide *N*-Acetyl-Ser-Asp-Lys-Pro (AcSDKP) in the control of haematopoietic stem cell. Cell Prolif 1992;25:623–32.
[12] Cole J, Ertoy D, Lin H, Sutliff RL, Ezan E, Guyene TT, et al. Lack of angiotensin II-facilitated erythropoiesis causes anemia in angiotensin-converting enzyme-deficient mice. J Clin Invest 2000;106:1391–8.
[13] Comte L, Lorgeot V, Volkov L, Allegraud A, Aldigier JC, Praloran V. Effects of the angiotensin converting enzyme inhibitor enalapril on blood hematopoietic progenitors and Acetyl-Ser-Asp-Lys-Pro concentrations. Eur J Clin Invest 1997;27:788–90.
[14] Mrug M, Stopka T, Julian BA, Prchal JF, Prchal JT. Angiotensin II stimulates proliferation of normal early erythroid progenitors. J Clin Invest 1997;100:2310–4.
[15] Wulf GG, Jahns-Streubel G, Strutz F, Basenau D, Hufner M, Buske C, et al. Paraneoplastic hypokalemia in acute myeloid leukemia: a case of renin activity in AML blast cells. Ann Hematol 1996;73:139–41.
[16] Wulf GG, Jahns-Streubel G, Nobiling R, Strutz F, Hemmerlein B, Hiddemann W, et al. Renin in acute myeloid leukaemia blast. Br J Haematol 1998;100:335–7.
[17] Abali H, Haznedaroglu IC, Goker H, Celik I, Ozatli D, Koray Z, et al. Circulating and local bone marrow renin–angiotensin system in leukemic hematopoiesis: preliminary evidences. Hematology 2002;7:75–82.
[18] Strawn WB, Richmond RS, Ann Tallant E, Gallagher PE, Ferrario CM. Renin–angiotensin system expression in rat bone marrow haematopoietic and stromal cells. Br J Haematol 2004;126:120–6.
[19] Remy I, Wilson IA, Michnick SW. Erythropietin receptor activation by ligand-induced conformation change. Science 1999;283:990–3.
[20] Horiuchi M, Hayashida W, Akisshita M, Tamura K, Daviet L, Lehtonen JY, et al. Stimulation of different subtypes of angiotensin II receptors, AT_1 y AT_2 receptors, regulates STAT activation by negative crosstalk. Circ Res 1999;84:876–82.
[21] Haznedaroglu IC, Arici M, Buyukasik Y. A unifying hypothesis for the renin–angiotensin system and hematopoiesis: sticking the pieces together with the JAK-STAT pathway. Med Hypotheses 2000;54:80–3.
[22] Xie S, Lin H, Sun T, Arlinghaus RB. Jack 2 is involved in c-myc induction by Bcr-Abl. Oncogene 2002;21:7137–46.
[23] Sheiness D, Bishop JM. DNA and RNA from uninfected vertebrate cells contain nucleotide sequences related to the putative transforming gene of avian myelocytomatosis virus. J Virol 1979;31:514–21.
[24] Aksu S, Beyazit Y, Haznedaroglu IC, Canpinar H, Kekilli M, Uner A, et al. Overexpression of angiotensin-converting enzyme (CD 143) on leukemic blasts as a clue for the activated local bone marrow RAS in AML. Leuk Lymphoma 2006;47:891–6.
[25] Azizi M, Rousseau A, Ezan E, Guyene TT, Michelet S, Grognet JM, et al. Acute angiotensin-converting enzyme inhibition increases the plasma level of the natural stem cell regulator *N*-Acetyl-Seryl-Aspartyl-Lysyl-Proline. J Clin Invest 1996;97:839–44.
[26] Michaud A, Williams TA, Chauvet MT, Corvol P. Substrate dependence of angiotensin I-converting enzyme inhibition: captopril displays a partial selectivity for inhibition of *N*-acetyl-seryl-aspartyl-lysyl-proline hydrolysis compared with that of angiotensin I. Mol Pharmacol 1997;51:1070–6.
[27] Guigon M, Bonnet D, Lemoine F, Kobari L, Parmentier C, Mary JY, et al. Inhibition of human bone marrow progenitors by the synthetic tetrapeptide AcSDKP. Exp Hematol 1990;18:1112–5.
[28] Lombard MN, Sotty D, Wdzieczak-Bakala J, Lenfant M. In vivo effect of the tetrapeptide N-acetyl-Ser-Asp-Lys-Pro on the G1-S transition of rat hepatocytes. Cell Tissue Kinet 1990;23:99–103.
[29] Volkov L, Quere P, Coudert F, Comte L, Praloran V. The terapeptide AcSDKP, a physiological inhibitor of normal cell proliferation, reduces the S phase entry of continuous cell lines. Exp Cell Res 1996;223:112–6.
[30] Kanasaki K, Koya D, Sugimoto T, Isono M, Kashiwagi A, Haneda M. N-Acetyl-seryl-aspartyl-lysyl-proline inhibits TGF-beta-mediated plasminogen activator inhibitor-1 expression via inhibition of Smad pathway in human mesangial cells. Am Soc Nephrol 2003;14:863–72.
[31] Gómez Casares MT, de la Iglesia S, Perera MM, Lemes A, Campo C, Gonzalez San Miguel JD, et al. Renin expression in hematological malignancies and its role in the regulation of hematopoiesis. Leuk Lymphoma 2002;43:2377–81.
[32] Beyazit Y, Aksu S, Haznedaroglu IC, Kekilli M, Misirlioglu M, Tuncer S, et al. Overexpression of the local bone marrow renin–angiotensin system in acute myeloid leukaemia. J Natl Med Assoc 2007;99:57–63.
[33] Gomes I, Sharma TT, Mahmud N, Kapp JD, Edassery S, Fulton N, et al. Highly abundant genes in the transcriptosome of human and baboon CD34 antigen-positive bone marrow cells. Blood 2001;98:93–9.
[34] De la Iglesia S, Gómez Casares MT, López Jorge CE, González Leiza SM, Santana G, Jiménez S, et al. Relevance of renin expression by real-time PCR in acute myeloid leukemia. Leuk Lymphoma 2006;47:409–16.
[35] Delgado MD, Lerga A, Cañelles M, Gómez-Casares MT, León J. Differential regulation of Max and role of c-myc during erythroid and myelomonocytic differentiation of K562 cells. Oncogene 1995;10:1659–65.
[36] Rowley PT, Ohlsson-Wilhelm BM, Farley BA, LaBella S. Inducers of erythroid differentiation in K562 human leukemia cells. Exp Hematol 1981;9:32–7.
[37] Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate–phenol–chloroform extraction. Anal Biochem 1987;162:156–9.
[38] Suzuki H, Shibata H, Murakami M, Sato A, Naito M, Ichihara A. Modulation of angiotensin II type 1 receptor m RNA expression in human blood cells: comparision of platelets and mononuclear leucocytes. Endocr J 1995;42:15–22.
[39] Okamura A, Raukugi H, Ohishi M, Yanagitani Y, Takiuchi S, Moriguchi K, et al. Upregulation of renin–angiotensin system during differentiation of monocytes to macrophages. J Hypertens 1999;17:537–45.
[40] Pauls K, Fink L, Franke FE. Angiotensin-converting enzyme (CD143) in neoplastic germ cells. Lab Invest 1999;79:1425–35.
[41] Savary K, Michaud A, Favier J, Larger E, Corvol P, Gasc JM. Role of the renin–angiotensin system in primitive erythropoiesis in the chick embryo. Blood 2005;105:103–10.
[42] Hubert C, Savary K, Gasc JM, Corvol P. The hematopoietic system: a new niche for the renin–angiotensin system. Nat Clin Pract Cardiovasc Med 2006;3:55–80.
[43] Goker H, Haznedaroglu IC, Beyazit Y, Aksu S, Tuncer S, Misirlioglu M, et al. Local umbilical cord blood renin–angiotensin system. Ann Hematol 2005;84:277–81.
[44] Koka E, Haznedaroglu IC, Acar K, Beyazit Y, Aksu S, Misirlioglu M, et al. Renin–angiotensin system expression in the K562 human erythroleukaemic cell line. JRAAS 2007;8:145–7.
[45] Chen S, Su J, Wu K, Hu W, Gardner DG, Chen D. Early captopril treatment prevents hypertrophy dependent gene expression in hearts of SHR. Am J Physiol Regul Integr Comp Physiol 1998;274:1511–7.

[46] Díez J. Apoptosis en las enfermedades cardiovasculares. Rev Esp Cardiol 2000;53:207–74.
[47] Rodríguez-Vita J, Sánchez-López E, Esteban V, Rupérez M, Egido J, Ruiz-Ortega M. Angiotensin II activates the Smad pathway in vascular smooth muscle cells by a transforming growth factor-beta-independent mechanism. Circulation 2005;111:2509–17.
[48] Di Paolo S, Schena A, Stallone G, Grandaliano G, Soccio M, Cerullo G, et al. Captopril enhances transforming growth factor (TGF)-beta1 expression in peripheral blood mononuclear cells: a mechanism independent from angiotensin converting enzyme inhibition? A study in cyclosporine-treated kidney-transplanted patients. Transplantation 2002;74:1710–5.
[49] Hong S, Lee C, Kim SJ. Smad7 sensitizes tumor necrosis factor induced apoptosis through the inhibition of antiapoptotic gene expression by suppressing activation of the nuclear factor-kappa B pathway. Cancer Res 2007;67:9577–783.
[50] Kumar R, Singh VP, Baker KM. The intracellular renin–angiotensin system: implications in cardiovascular remodeling. Curr Opin Nephrol Hypertens 2008;17:168–73.
[51] Zee R, Rau V, Paster, Sweet C, Lindpaintner K. Three candidate genes and angiotensin-converting enzyme inhibitor-related cough: a pharmacogenetic analysis. Hypertension 1998;31:928–1928.
[52] Krop M, Danser AH. Circulating versus tissue renin–angiotensin system: on the origin of (pro)renin. Curr Hypertens Rep 2008;10:112–8.
[53] Wuerzner G, Azizi M. Renin inhibition with aliskiren. Clin Exp Pharmacol Physiol 2008;35(April):426–30.
[54] Redondo-Müller MA, Sevanovic-Walker M, Barker S, Puddefoot JR, Vinson GP. Anti-cancer actions of a recombinant antibody (R6313/G2) against the angiotensin II AT1 receptor. Endocr Relat Cancer 2008;15:277–88.