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Effect of Propranolol on Angiogenic Factors in Human Hematopoietic Cell Lines *in vitro*

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ABSTRACT

Background: Beta-adrenergic blocking agents have been broadly used for treatment of many cardiovascular diseases such as arterial hypertension and ischemic heart failure. Anti-tumoral, anti-inflammatory and anti-angiogenesis effects of propranolol (a non-selective beta-adrenergic blocker) have been shown. Angiogenesis (replenish of the pre-existing vascular networks) plays a critical role in some pathological conditions such as tumor expansion and metastasis. In this study, we investigated the effects of propranolol on vascular endothelial growth factor (VEGF) production and matrix metalloproteinase-2 (MMP-2) activity (two important angiogenic factors) in human leukemic cell lines *in vitro*. **Methods:** Two human leukemic T (Molt-4 and Jurkat) and one monocyte (U937) cell lines were used in this study. The cells were cultured in complete RPMI medium and then incubated with different concentrations of propranolol (0.3-30 μM) in the presence or absence of phorbol myristate acetate (PMA, 25 ng/ml) for 48 hours. The level of VEGF secreted in the cell culture supernatants was measured with enzyme-linked immunosorbent assay kits (R and D systems) and MMP-2 activity in cell-conditioned media was evaluated by gelatin zymography. **Results:** Propranolol significantly decreased VEGF production and also MMP-2 activity in PMA-activated human leukemic cell lines Molt-4, Jurkat and U937 at 30 μM concentration of the drug compared to untreated control cells (P<0.05). **Conclusion:** Propranolol might be a useful anti-angiogenic agent in hematopoietic malignancies. Thus, propranolol along with its chronic long-term usage in cardiac problems may have potential implication in treatment of leukemia. *Iran. Biomed. J. 13 (4): 223-228, 2009*

Keywords: Propranolol, Angiogenesis, Vascular endothelial growth factor (VEGF), Matrix metalloproteinase-2 (MMP-2), Leukemia

INTRODUCTION

Beta-adrenergic blocking agents have been broadly used for treatment of many cardiovascular diseases such as arterial hypertension and ischemic heart failure [1-3] and also non-cardiovascular diseases [4] such as sever migraine attacks [5]. Anti-tumoral and anti-inflammatory effects of beta-blockers have also been reported [6-9]. Furthermore, the inhibitory effects of propranolol (a non-selective beta-adrenergic blocker) on norepinephrine-mediated vascular endothelial growth factor (VEGF) expression in adipose tissue [10] and norepinephrine-stimulated release of functional angiogenic factors in nasopharyngeal carcinoma tumor cells [11] have been described. Angiogenesis (replenish of the pre-existing vascular networks) plays a critical role in some physiological conditions such as wound healing and also in pathological conditions such as tumor expansion and metastasis, rheumatoid arthritis, diabetic retinopathy and peripheral arterial disease [12-14]. Modification of angiogenesis seems to have potential implication in treatment of different diseases such as cancers and ischemic heart failure [15]. Several agents known as angiogenic factors including cytokines such as fibroblast growth factor, (VEGF), hepatocyte growth factor (HGF), placental growth factor and stromal cell-derived factor-1alpha [16, 17], chemokines [18] and matrix metalloproteinases (MMP) [19] are implicated in angiogenesis. VEGF is a very important regulator of angiogenesis [20, 21] and has a crucial role in cancer growth and progression [22]. VEGF blockade has been validated as a therapeutic strategy in adult cancers [23]. Another important angiogenic factors

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are MMP, a group of enzymes that play an essential role in degradation of extracellular matrix macromolecules and remodeling of connective tissue matrices which consequently facilitates angiogenesis [19] and have an important role in tumor growth and metastasis [24].

Based on the long-term and wide spread utilization of propranolol in cardiac and also non-cardiac diseases [2, 5] as well as its anti-tumor and anti-inflammatory properties [6-8] and besides its attenuating effect on angiogenic factors level in some carcinoma such as nasopharyngeal cancerous cells [11], we decided to investigate the effects of propranolol on VEGF production and MMP-2 activity (two important angiogenic factors) in three human phorbol myristat acetate (PMA)-stimulated leukemic cell lines *in vitro*. We used PMA as a potent inducer of VEGF production and MMP-2 activity [25, 26].

MATERIALS AND METHODS

Materials. RPMI-1640 medium, penicillin, streptomycin, PMA and trypan blue were purchased from Sigma (USA) and FCS from Gibco (USA). VEGF standard ELISA kit was obtained from R and D company (USA). Propranolol was a kind gift from Hakim Co. Ltd. (Tehran, Iran). Microtiter plates, flasks and tubes were from Nunc (Falcon, USA).

Cell lines. Human leukemic T cells [Molt-4 (NCBI C149) and Jurkat (NCBI C121)] and monocyte [U937 (NCBI C130)] were obtained from NCBI (National Cell Bank of Iran, Pasteur Institute of Iran, Tehran). The cells were maintained in RPMI-1640 medium supplemented with 10% FCS in 5% CO_2 at 37°C.

Preparation of propranolol. Propranolol was dissolved in RPMI-1640 medium and stored at -20°C until use. Drug was diluted in culture medium to prepare the needed concentrations before use.

Cell culture and treatment. The human leukemic cells were cultured in RPMI-1640 medium supplemented with 10% FCS, penicillin (100 IU/ml) and streptomycin (100 µg/ml) in 5% CO_2 at 37°C. The cells were seeded at a density of 2×10^6 cell/ml and then incubated with different concentrations of propranolol (0.3- 30 µM) in the presence or absence of PMA (25 ng/ml) for 48 hours. The supernatants of cell culture media were collected and used for VEGF assay and zymography. All experiments were done in triplicate.

VEGF protein assay. The amount of VEGF secreted in the cell culture supernatants by human leukemic cell lines was measured with the Quantikine human VEGF ELISA kits (R and D systems) according to the manufacturer's instructions. This assay uses the quantitative sandwich enzyme immunoassay technique. Complete RPMI medium was used as control and human recombinant $VEGF_{165}$ was employed as standard for drawing the standard curves.

Evaluation of MMP-2 activity by gelatin zymography. MMP-2 activity in cell-conditioned media was evaluated by gelatin zymography technique according to the modified Kleiner and Stetler-Stevenson method [27]. Briefly, cell culture supernatants were subjected to SDS-PAGE on 10% polyacrylamide gel copolymerized with 2 mg/ml gelatin A in the presence of 0.1% SDS under non-reducing conditions at a constant voltage of 80 V for three hours. After electrophoresis, the gels were washed in 2.5% Triton X-100 for one hour to remove SDS and then incubated in a buffer containing 0.1 M Tris-HCl, pH 7.4 and 10 mM $CaCl_2$ at 37°C overnight. Afterwards, the gels were stained with 0.5% Coomassie brilliant blue and then destained. Proteolytic activity of enzyme were detected as clear bands of gelatin lysis against a blue background. The relative intensity of lysed bands to the control band was measured by using UVI Pro gel documentation system (Vilber Lourmat, Marne-la-Vallee Cedex 1, France) and expressed as relative gelatinolytic activity.

Statistical analysis. Effect of the drug on each cell line was performed in three independent experiments and the results were expressed as mean ± SEM. Statistical comparisons among groups were made by analysis of variance (ANOVA). $P<0.05$ was considered significant. Test of multiple comparison of Tukey was applied (5%) for statistically significant differences. For statistical analysis and graph making, the software SPSS 11.5 and Excel 2003 were used, respectively.

RESULTS

Propranolol effect on VEGF production in PMA-stimulated human leukemic cell lines. VEGF production was rather low in unstimulated Molt-4

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and Jurkat cells, but PMA (25 ng/ml) significantly increased VEGF production in both leukemic cells after 48 hour of incubation time (Fig. 1A and 1B) (P<0.05). Results depicted in Figure 1C show that U937 cells produced a large amount of VEGF without any stimulation and PMA did not show any significant effect on VEGF production by U937 cells. Propranolol significantly decreased VEGF production in the presence or absence of PMA by Molt-4 cells at 30 µM concentration of the drug after 48 hours incubation time (P<0.05) (Fig. 1A). As illustrated in Figure 1B, propranolol markedly decreased VEGF secretion in the presence or absence of PMA by Jurkat cells at 30 µM dose of the drug after 48 hours incubation time (P<0.05). As can be seen in Figure 1C, propranolol considerably

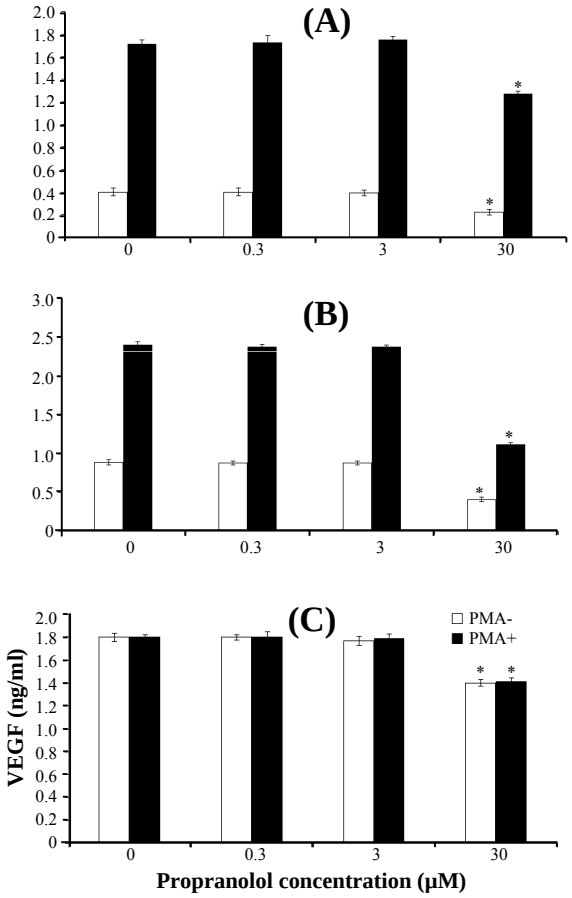

Fig. 1. Effect of propranolol on VEGF secretion by human leukemic **(A)** Molt-4 T-cell **(B)** Jurkat T-cell and **(C)** U937 monocytic cell lines. The cells (2×10^6 cells/ml) were treated with different concentrations of propranolol (0.3-30 µM) for 48 hours in the presence or absence of PMA (25 ng/ml). At the end of treatment, VEGF concentration in conditioned medium was measured by ELISA. Data are mean ± SEM of three independent experiments. *P<0.05 was considered significant.

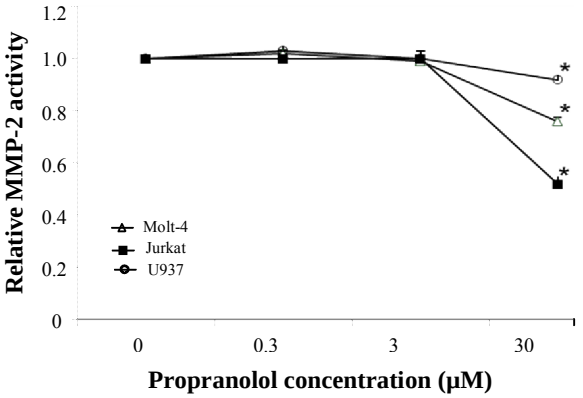

Fig. 2. Effect of propranolol on PMA-induced MMP-2 activity in human leukemic Molt-4, Jurkat and U937 cell lines. The leukemic cells (2×10^6 cells/ml) were treated with different concentrations of propranolol (0.3-30 µM) for 48 hours in the presence of PMA (25 ng/ml). At the end of treatment, MMP-2 activity in conditioned medium was measured by gelatin zymography. Data are mean ± SEM of three independent experiments. *P<0.05 was considered significant.

reduced the VEGF production by U937 cells in the presence or absence of PMA at 30 µM dose of the drug after 48 hours incubation time (P<0.05).

Propranolol effect on PMA-induced MMP-2 activity in human leukemic cell lines. MMP-2 activity in unstimulated leukemic cell lines used in this study was not detectable but PMA induced MMP-2 activity in Molt-4, Jurkat and U937 cells compared with untreated control cells (Data not shown). As shown in Figure 2, propranolol significantly decreased the PMA-induced MMP-2 activity in all leukemic cells used in this study at 30 µM dose of the drug after 48 hours incubation time (P<0.05).

DISCUSSION

In this study, we showed that propranolol (at 30 µM concentration) significantly decreased the VEGF production by human leukemic T (Molt-4 and Jurkat) and monocyte (U937) cells after 48 hours incubation. These findings are consistent with Fredriksson *et al.* [10] and Yang *et al.* [11] studies reported that propranolol inhibited the norepinephrine-mediated VEGF expression in adipose tissue and norepinephrine-stimulated release of functional angiogenic factors in nasopharyngeal carcinoma tumor cells. Moreover, anti-tumoral effect of propranolol on a variety of cancers such as pulmonary adenocarcinoma [28], uterine leiomyoma

[29, 30] and a human lung adenocarcinoma cell line [31] has been reported. Concerning that VEGF has a crucial role in cancer growth and progression [22], suppressive effect of propranolol on tumor growth reported before [28-31] may be in part due to its inhibitory effect on VEGF production. Furthermore, anti-inflammatory effect of propranolol [32, 33] and other beta-blockers [7] have been reported. For example, the attenuating effect of propranolol on proinflammatory cytokines such as IL-1β mRNA expression [32] and serum TNF-α and IL-1β in severely burned children [34] have been shown. Regarding the important role of VEGF in inflammation [35, 36], the anti-inflammatory effect of propranolol [33, 34] may be partly due to its inhibitory effect on VEGF secretion.

In the present study, we also showed that propranolol significantly decreased the PMA-induced MMP-2 activity in all leukemic cells used in this study at 30 μM concentration. These results are once again consistent with Yang *et al.* [11] findings reported the inhibitory effect of propranolol on norepinephrine-mediated up-regulation of MMP-2 and MMP-9 production in nasopharyngeal carcinoma. It should be noted that we assessed the MMP-2 activity by gelatin zymography technique whereas in Yang *et al.* [11] study, MMP-2 level was detected by ELISA and MMP activity was determined by membrane invasion culture system. Nevertheless, the similar results were obtained by different techniques in this study and by Yang *et al.* [11]. Since MMP have an important role in inflammation and tumor growth [24], it seems that inhibitory effects of propranolol on MMP-2 activity may be the other cause for its anti-tumoral and anti-inflammatory effects [28-33] together with its attenuating effect on VEGF production shown in our results. Potential implication of anti-angiogenesis in treatment of ischemic heart failure has been reported [15]. So, positive effect of propranolol on ischemic heart failure [3] may be in part owing to its anti-angiogenesis effects through inhibition of VEGF production and MMP-2 activity. It should be considered that in our study, the concentration of propranolol which decreased the VEGF production and MMP-2 activity *in vitro* was higher than that of usually used in cardiovascular patients. Our previous study showed that propranolol had a significant cytotoxic effect against the same leukemic cells used in the present study at ≥ 0.2 mM (200 μM) concentration in all incubation times tested (12, 24 and 48 hours) [37].

Accordingly, propranolol had not any cytotoxic effect on the cell lines examined in this study, at the mentioned concentrations (0.3-30 μM) and incubation time (48 hours). Hence, decrease of VEGF production or MMP-2 activity in the present study is not due to cytotoxic effect of propranolol.

To best of our knowledge, this is the first report about inhibitory effect of propranolol on VEGF production and MMP-2 activity in human leukemic cell lines. Taken together, our data showed that the anti-tumoral and anti-inflammatory effects of propranolol reported by other investigators [6-9] seems to be related in part to its inhibitory effect on angiogenesis through suppression of VEGF production and MMP-2 activity, as VEGF and MMP-2 are important mediators of angiogenesis [19, 21]. At the other hand, the important role of angiogenesis in leukemic patients has been reported [20, 22]. Therefore, anti-angiogenic compounds may be positive agents in treatment of leukemia. Our results suggest that propranolol with inhibitory effect on VEGF production and MMP-2 activity might be a useful anti-angiogenic mediator in hematopoietic cancers. Accordingly, propranolol may have potential implication in treatment of leukemia along with its chronic long-term usage in cardiac diseases. Further investigations about propranolol effect on angiogenic factors in peripheral blood mononuclear cells as well as hematopoietic malignancies *in vivo* are also warranted.

REFERENCES

1. Degoute, C.S. (2007) Controlled hypotension: a guide to drug choice. *Drugs 67 (7): 1053-1076.*
2. Priviero, F.B., Teixeira C.E., Claudino, M.A., De Nucci, G., Zanesco, A. and Antunes, E. (2007) Vascular effects of long-term propranolol administration after chronic nitric oxide blockade. *Eur. J. Pharmacol. 571 (2-3): 189-196*.
3. Igarashi, N., Nozawa, T., Fujii, N., Suzuki, T., Matsuki, A., Nakadate, T., Igawa, A. and Inoue, H. (2006) Influence of beta-adrenoceptor blockade on the myocardial accumulation of fatty acid tracer and its intracellular metabolism in the heart after ischemia-reperfusion injury. *Circ. J. 70 (11): 1509-1514*.
4. Frishman, W.H. (2008) Beta-adrenergic blockers: a 50-year historical perspective. *Am. J. Ther. 15 (6): 565-576*.
5. Hoffmann, J. and Reuter, U. (2007) Treatment of migraine. *Dtsch. Med. Wochenschr. 132 (41): 2153-2158.*

6. Benish, M., Bartal, I., Goldfarb, Y., Levi, B., Avraham, R., Raz, A. and Ben-Eliyahu, S. (2008) Perioperative Use of beta-blockers and COX-2 Inhibitors May Improve Immune Competence and Reduce the Risk of Tumor Metastasis. *Ann. Surg. Oncol. 15 (7): 2042-2052*.
7. Nguyen, L.P., Omoluabi, O., Parra, S., Frieske, J.M., Clement, C., Ammar-Aouchiche, Z., Ho, S.B., Ehre, C., Kesimer, M., Knoll, B.J., Tuvim, M.J., Dickey, B.F. and Bond, R.A. (2008) Chronic exposure to beta-blockers attenuates inflammation and mucin content in a murine asthma model. *Am. J. Respir. Cell Mol. Biol. 38 (3): 256-262*.
8. Kato, H., Kawaguchi, M., Inoue, S., Hirai, K. and Furuya, H. (2009) The effects of beta-adrenoceptor antagonists on proinflammatory cytokine concentrations after subarachnoid hemorrhage in rats. *Anesth. Analg. 108 (1): 288-295*.
9. Tang, F.K., Hua, N., Lu, H., Xiao, J., Tang, X.Z. and Qi, Z. (2008) Effects of bisoprolol on serum interleukin-6 and tumor necrosis factor-alpha level in patients with congestive heart failure. *Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi 24 (12): 1177-1179*.
10. Fredriksson, J.M., Lindquist, J.M., Bronnikov, G.E. and Nedergaard, J. (2000) Norepinephrine induces vascular endothelial growth factor gene expression in brown adipocytes through a beta-adrenoreceptor/ cAMP/protein kinase A pathway involving Src but independently of Erk1/2. *J. Biol. Chem. 275 (18): 13802-13811*.
11. Yang, E.V., Sood, A.K., Chen, M., Li, Y., Eubank, T.D., Marsh, C.B., Jewell, S., Flavahan, N.A., Morrison, C., Yeh, P.E., Lemeshow, S. and Glaser, R. (2006) Norepinephrine up-regulates the expression of vascular endothelial growth factor, matrix metalloproteinase (MMP)-2, and MMP-9 in nasopharyngeal carcinoma tumor cells. *Cancer Res. 66 (21): 10357-10364*.
12. Ribatti, D., Nico, B. and Crivellato, E. (2009) Morphological and molecular aspects of physiological vascular morphogenesis. *Angiogenesis. 12 (2): 101-111*.
13. Abe, R., Fujita, Y., Yamagishi, S. and Shimizu, H. (2008) Pigment epithelium-derived factor prevents melanoma growth via angiogenesis inhibition. *Curr. Pharm. Dis. 14 (36): 3802-3809*.
14. Noguer, O., Villena, J., Lorita, J., Vilaró, S. and Reina, M. (2008) Syndecan-2 down regulation impairs angiogenesis in human microvascular endothelial cells. *Exp. Cell. Res. 315 (5): 795-808*.
15. Göőz, P., Göőz, M., Baldys, A. and Hoffman, S. (2009) ADAM-17 regulates endothelial cell morphology, proliferation, and *in vitro* angiogenesis. *Biochem. Biophys. Res. Commun. 380 (1): 33-38*.
16. Heinzman, J.M., Brower, S.L. and Bush, J.E. (2008) Comparison of angiogenesis- related factor expression in primary tumor cultures under normal and hypoxic growth conditions. *Cancer Cell Int. 8: 11*.
17. Atluri, P. and Woo, Y.J. (2008) Pro-angiogenic cytokines as cardiovascular therapeutics: assessing the potential. *BioDrugs 22 (4): 209-222*.
18. Roh, M.I., Kim, H.S., Song, J.H., Lim, J.B. and Kwon, O.W. (2009) Effect of intravitreal bevacizumab injection on aqueous humor cytokine levels in clinically significant macular edema. *Ophthalmology 116 (1): 80-86*.
19. Giannopoulos, G., Pavlakis, K., Parasi, A., Kavatzas, N., Tiniakos, D., Karakosta, A., Tzanakis, N. and Peros, G. (2008) The expression of matrix metalloproteinases-2 and -9 and their tissue inhibitor 2 in pancreatic ductal and ampullary carcinoma and their relation to angiogenesis and clinic pathological parameters. *Anticancer Res. 28 (3B): 1875-1881*.
20. Kini, A.R., Peterson, L.C., Tallman, M.S. and Lingen, M.W. (2001) Angiogenesis in acute promyelocytic leukemia: induction by vascular endothelial growth factor and inhibition by all-trans retinoic acid. *Blood 97 (12): 3919-3924*.
21. Ferrara, N. (2009) Vascular Endothelial Growth Factor. *Arterioscler. Thromb. Vasc. Biol. 29 (6): 789-791*.
22. Frater, J.L., Kay, N.E., Goolsby, C.L., Crawford, S.E., Dewald, G.W. and Peterson, L.C. (2008) Dysregulated angiogenesis in B-chronic lymphocytic leukemia: Morphologic, immunohistochemical, and flow cytometric evidence. *Diagn. Pathol. 18 (3): 16-25*.
23. Zaghloul, N., Hernandez, S.L., Bae, J.O., Huang, J., Fisher, J.C., Lee, A., Kadenhe-Chiweshe, A., Kandel, J.J. and Yamashiro, D.J. (2009) Vascular endothelial growth factor blockade rapidly elicits alternative proangiogenic pathways in neuroblastoma. *Int. J. Oncol. 34 (2): 401-407*.
24. Paschos, K.A., Canovas, D. and Bird, N.C. (2009) Enzymatic function of multiple origins regulates the progression of colorectal cancer and the development of metastases. *Hippokratia. 13 (1): 23-31*.
25. Xu, H., Czerwinski, P., Hortmann, M., Sohn, H.Y., Förstermann, U. and Li, H. (2008) Protein kinase C alpha promotes angiogenic activity of human endothelial cells via induction of vascular endothelial growth factor. *Cardiovasc. Res. 78 (2): 349-355*.
26. Lee, K.J., Hwang, S.J., Choi, J.H. and Jeong, H.G. (2008) Saponins derived from the roots of Platycodon grandiflorum inhibit HT-1080 cell invasion and MMPs activities: regulation of NF-kappaB activation via ROS signal pathway. *Cancer Lett. 268 (2): 233-243*.
27. Kleiner, D.E. and Stetler-Stevenson, W.G. (1994) Quantitative zymography: detection of picogram quantities of gelatinases. *Anal. Biochem. 218 (2): 325-329*.
28. Schuller, H.M., Porter, B. and Riechert, A. (2000) Beta-adrenergic modulation of NNK-induced lung

[DOR: 20.1001.1.1028852.2009.13.4.2.8]
[DOI: -]

carcinogenesis in hamsters. *J. Cancer Res. Clin. Oncol. 126 (11): 624-630*.

29. Gibson, J.P., Sells, D.M., Cheng, H.C. and Yuh, L. (1987) Induction of uterine leiomyomas in mice by medroxalol and prevention by propranolol. *Toxicol. Pathol. 15 (4): 468-473*.
30. Jack, D., Poynter, D. and Spurling, N.W. (1983) Beta-adrenoceptor stimulants and mesovarian leiomyomas in the rat. *Toxicology 27 (3-4): 315-320*.
31. Schuller, H.M. and Cole, B. (1989) Regulation of cell proliferation by beta-adrenergic receptors in a human lung adenocarcinoma cell line. *Carcinogenesis 10 (9): 1753-1755*.
32. Deten, A., Volz, H.C., Holzl, A., Briest, W. and Zimmer, H.G. (2003) Effect of propranolol on cardiac cytokine expression after myocardial infarction in rats. *Mol. Cell Biochem. 251 (1-2): 127-137*.
33. Romana-Souza, B., Nascimento, A.P. and Monte-Alto-Costa, A. (2008) Low-dose propranolol improves cutaneous wound healing of burn-injured rats. *Plast. Reconstr. Surg. 122 (6): 1690-1699*.
34. Jeschke, M.G., Norbury, W.B., Finnerty, C.C., Branski, L.K. and Herndon, D.N. (2007) Propranolol does not increase inflammation, sepsis, or infectious episodes in severely burned children. *J. Trauma 62 (3): 676-681*.
35. Martin, D., Galisteo, R. and Gutkind, J.S. (2009) CXCL8/IL8 stimulates vascular endothelial growth factor (VEGF) expression and the autocrine activation of VEGFR2 in endothelial cells by activating NFκB through the CBM (Carma3/ Bcl10/Malt1) complex. *J. Biol. Chem. 284 (10): 6038-6042*.
36. Stumpf, C., Jukic, J., Yilmaz, A., Raaz, D., Schmieder, R.E., Daniel, W.G. and Garlichs, C.D. (2009) Elevated VEGF-plasma levels in young patients with mild essential hypertension. *Eur. J. Clin. Invest. 39 (1): 31-36*.
37. Hajighasemi, F. and Mirshafiey, A. (2009) *In vitro* sensitivity of leukemia cells to propranolol. *J. Clin. Med. Res. 1 (3): 144-149*.

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[DOI: -]