ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

The Effect of NF-Kappa B and Angiotensin II on the Proliferation in Human Breast Adenocancer Cell Line

Ozunal ZG*[1], Bayram R[2], Yavuz MZ[2], Ucbek A[2], Aydogar A[2], Uzun Ö[2], and Gepdiremen A[2]

İstanbul University, İstanbul Faculty of Medicine, Department of Pharmacology, Turkey.
Abant İzzet Baysal University, Faculty of Medicine, Department of Pharmacology, Turkey.

ABSTRACT

There are many in-vitro studies implicating that Angiotensin (Ang)II stimulates solid organ cancer growth. Effect of AngII on cell proliferation can be related to nuclear factor NFκB. The aim of the present study is to examine the effects of pyrithione, an NFκB inhibitor and AngII on breast cancer cell proliferation. MCF-7 is treated with AII (10µM) and NFκB inhibitor, pyrithione sodium (0,1-100µM). Cells are counted and photographed. WST-1 is used to measure viability in 48h after treatment and groups are fluorescent dyed with ethidium bromide. The results of cell count showed that cell proliferation was increased in AngII treated group when compared with control group. However, this increase did not show statistically significance. Cell count was decreased in pyrithione (10 and 100µM) treated group. Morphologic changes were most apparent in 100µM pyrithione group. We concluded that pyrithione alone or in combination with AngII decreased MCF-7 cell proliferation.

Keywords: MCF-7 cell line, AngII, pyrithione, NFκB.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Renin-Angiotensin system (RAS) and AII, the main effector of Renin-Angiotensin system, regulates numerous physiological process including blood pressure, water and salt balance and is also accepted to contribute cell growth and proliferation [1,2]. However, AII may trigger an opposite effect such as growth inhibition and stimulation of apoptosis by AII type-2 receptor [3]. Moreover, studies about relationship between cancer and RAS system are contradictory: Although some of these investigations indicate that Angiotensin Converting Enzyme Inhibitors (ACEI) or Angiotensin Receptor Blockers (ARBs) may have a protective against cancer [4,5], the others suggests that ARBs may be associated with new cancer occurrence [6,7,8].

AII can be produced locally in the breast and Anjiotensin II type 1 and type 2 receptor (AT_1R or AT_2R) are present in a normal or cancerous breast tissue [9,10,11]. In addition, AII is known to induce the transcription of cytokines, chemokines, growth factors and proliferation due to activation of NFκB [12]. NFκB is a transcription factor that regulates the transcription of genes involved in inflammatory responses, cell growth control and apoptosis in normal and cancer cell [13,14]. Investigations suggest that NFκB may both inhibit and augment apoptosis in cancer cells. Apparently, the effect of NFκB in apoptosis is cell type- and signal-dependent [15].

Based on the studies described above, we aimed to examine the effects of NFκB inhibition and AngII on human breast adenocancer cell line.

MATERIAL AND METHODS

Cell Culture

MCF-7 human adenocancer cell line is obtained from Prof. Dr. Ayhan Bilir, Cell and Tissue Culture Laboratory, İstanbul Faculty of Medicine, İstanbul University. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented %10 fetal calf serum and %1 antibiotic. The cell lines were grown under standard conditions (%5 CO_2 at 37° C). Cells were seeded in 24-well tissue plate for counting and dying; 96-well tissue plate used for WST-1 analysis. Cells are counted with a hemacytometer. Cells are treated with 10µM AngII and 0,1-100µM pyrition sodium alone or in combination. Cells are visualized by a phase contrast inverted microscope at 24 and 48 hours after treatment. Cell morphology is observed dying 24 well plates with etidium bromide and examined by florescence attached inverted microscope. AngII, pyrition sodium and DMEM are obtained from Sigma-Aldrich (St. Louis, MO, USA).

Cell Viability Detection

The number of surviving cells was measured by the 4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate (WST-1) assay. Cells, grown in a 96-well tissue culture plate, were incubated with the ready-to-use WST-1(Roche, Indianapolis, IN) reagent for approximately 2 hours. After this incubation period, the formazan dye formed was

ISSN: 0975-8585

quantified with an ELISA plate reader. The measured absorbance directly correlates to the number of viable cells.

Statistics:

Each experiment was made in triplicate and all data were presented as mean ± SEM. One-way analysis of variance (ANOVA) was used when multiple comparisons were made. The significance between two groups was determined with Tukey's test.

RESULTS AND DISCUSSION

The results of cell count after 24 hours showed that cell proliferation was increased in AngII (10 µM) treated group when compared with control group (from 15500±2,10 to 20000±2,70). However, this increase did not show statistically significance (Fig 1). Otherwise, cell count was decreased in pyrithione (10 and 100µM) treated group (from 15500±2,10 to 4,88.±0,89 and from 15500±2,10 to 6,08.±0,97). Cell count was also decreased in AngII (10 µM) and pyrithione (10 and 100µM) combination groups (from 15500±2,10 to 5,29±0,30 and from 15500±2,10 to 8,96±0,94). These results were confirmed by using phase contrast inverted microscope at 24 and 48 hours after treatment (Fig 2).

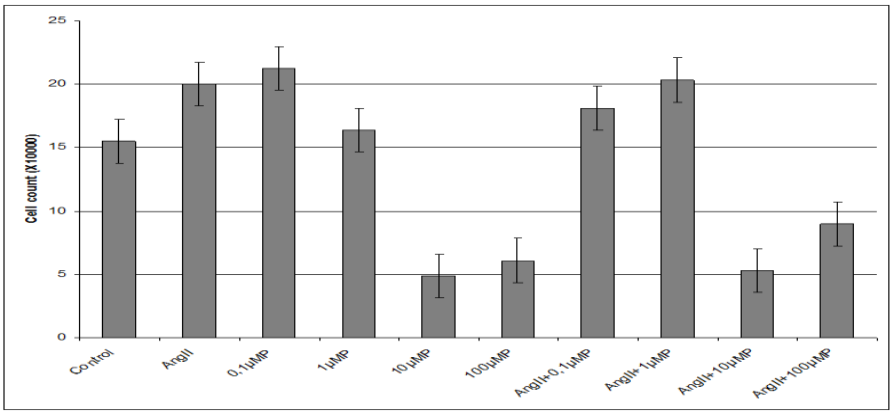
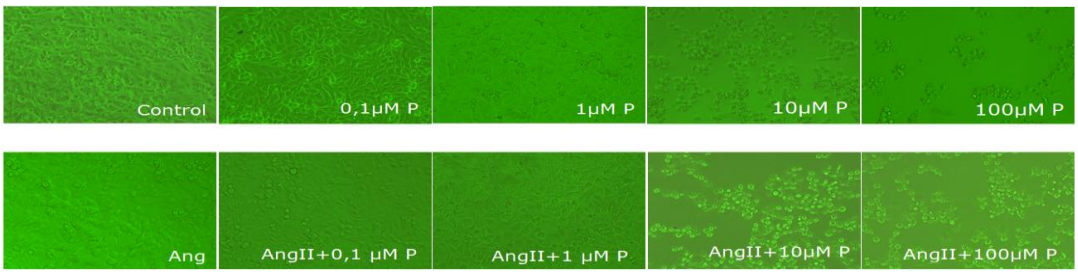


Fig 1. Cell count 24h after treatment in MCF-7 cell line. AngII(Angiotensin II), P(Pyrithione).*p<0.05 vs control, #p<0.05 vs AngII.



2a. Photographs from phase contrast inverted microscope 24h after treatment in MCF-7 cell line. AngII(Angiotensin II), P(Pyrithione).

ISSN: 0975-8585

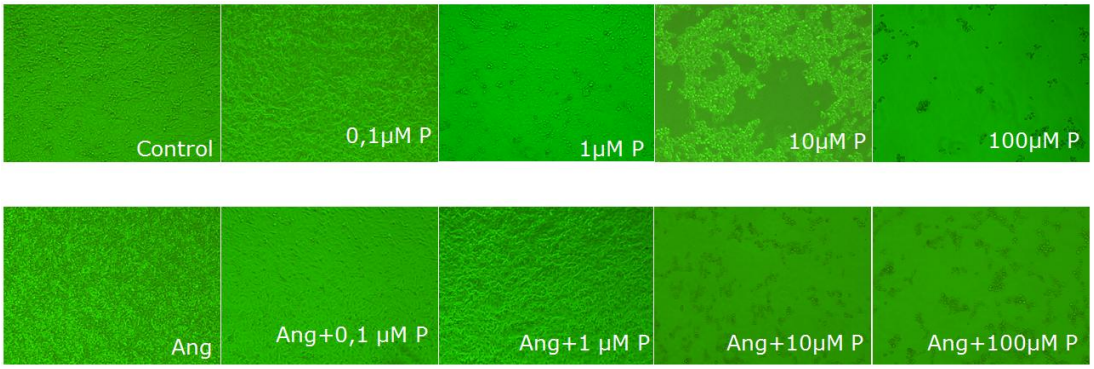


Fig 2b. Photographs from phase contrast inverted microscope 48h after treatment in MCF-7 cell line. AngII(Angiotensin II), P(Pyrithione).

Cell viability was measured by WST-1 assay. Our results demonstrated that Ang II did not increased cell proliferation significantly. But NFκB inhibition with pyrithione (10 and 100μM) was decreased cell viability (Fig 3). Cell viability was decreased in pyrithione (10 and 100μM) treated group (from 1,01±0,05 to 0,43±0,02 and from 1,01±0,05 to 0,51±0,02). Cell viability was also decreased in AngII (10 μM) and pyrithione (10 and 100μM) combination groups (from 1,01±0,05 to 0,36±0,01 and from 1,01±0,05 to 0,39±0,01).

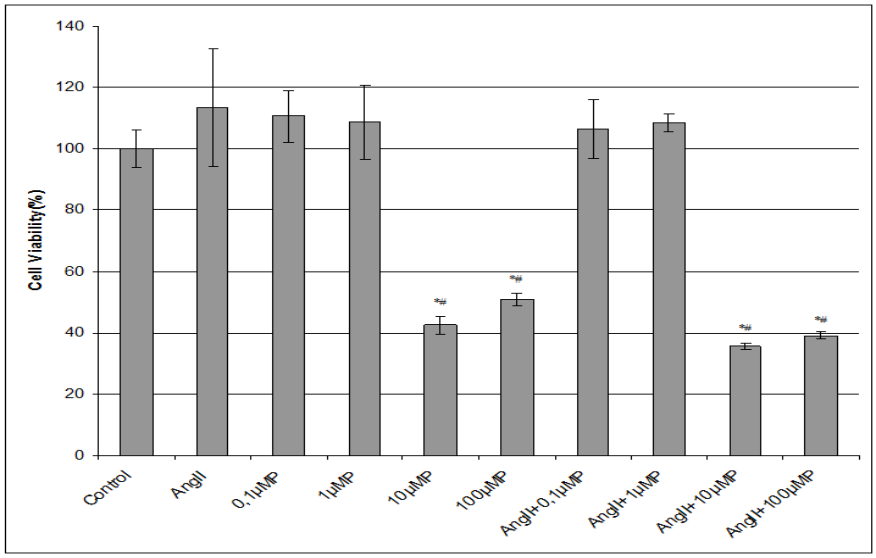


Fig 3. Cell viability 48h after treatment in MCF-7 cell line. AngII(Angiotensin II), P(Pyrithione).*p<0.05 vs control, #p<0.05 vs AngII.

Morphologic changes were determined by using ethidium bromide staining (Fig 4). According to these results 100μM pyrithione caused most apparent changes in MCF-7 cell line. Taken together, pyrithione alone or in combination with AngII decreased MCF-7 breast cancer cell proliferation.

ISSN: 0975-8585

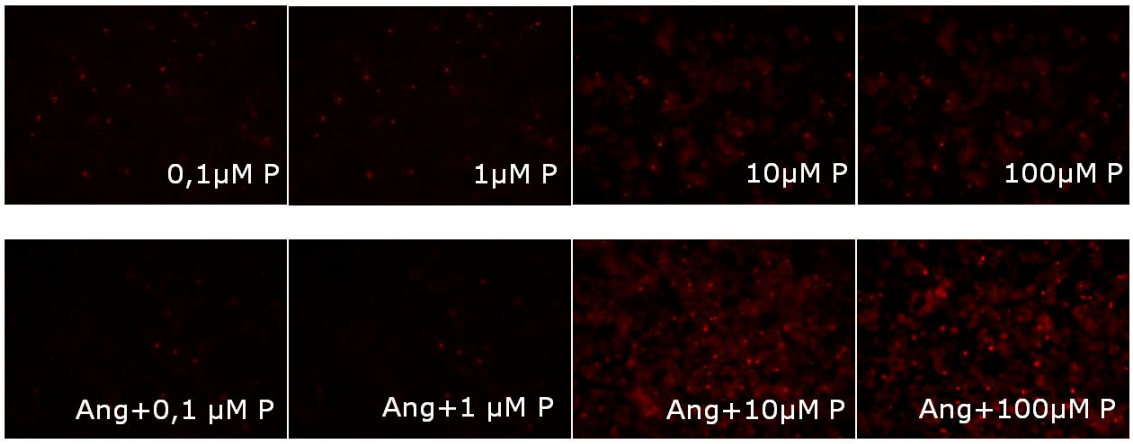


Fig 4. Photographs from florescence attached inverted microscope with ethidium bromide staining 48h after treatment in MCF-7 cell line. Ang (Angiotensin II), P(Pyrithione).

The present study aimed to test the effects of NFκB inhibition and AngII on human breast adenocancer cell line. Our results demonstrated that 1. AngII induced cell proliferation increased but this effect did not significant when compared with control group. 2. Cell count was decreased in pyrithione (10 and 100µM) treated group. 3. Cell count was decreased in AngII+pyrithione group. 4. Ethidium bromide staining show that morphologic changes were most apparent in 100µM pyrithione group.

Besides RAS's effect on circulation, it is known that Ang II has a role on cell proliferation. Attention focused on local RAS system and it is shown that tRAS is present in breast tissue and is distrupted in breast cancer [16]. In our experiment the main effector of RAS AngII (10 µM) increase the cell count but it is not statistically significant. Previous studies are contradictory. For example, it was showed that AngII induced cell proliferation at dose dependently at doses ranging from 0.001-1µM [16]. Conversely, Zhao et al. Obtained maximal proliferation with 100 µM doses of AngII in MCF-7 breast cancer cells. [17] Apparently Dose dependently AngII induced proliferation can vary according to laboratory or experimental conditions.

The investigations recently revealed that NFκB pathway is one of the shared dysregulated pathway for human and mice mammary cancers and there is a strong proof for high NFκB activity is critically relavant with breast cancer [16]. Some investigation showed that NFκB inhibitors don't exert growth inhibitory effect on MCF-7 laminary cell culture. But Da Hee Oh et al. demonstrated that PDTC induced apoptosis in breast cancer cell line in a dose-dependent manner at concentrations of ≥ 25000 µM [18]. Our study is the first study investigating pyrithione effect in MCF-7 cell line. Unlike the other studies made with NFκB inhibitors, pyrithion sodium (10 and 100 µM) caused growth inhibition without fetal calf serum conditions.

The results of the combination group Ang II and pyrithion sodium did not show additive antiproliferative effect when compared only pyrithion group.

ISSN: 0975-8585

CONCLUSION

Since NFκB pathway contributes to formation of cancer, NFκB inhibitors may be important in terms of new treatment approaches. Our results are promising with pyrithione in this respect.

ACKNOWLEDGEMENT

The authors are grateful to Prof. Dr. Ayhan Bilir from Istanbul Faculty of Medicine for his support to obtain MCF-7 cell line.

REFERENCES

[1] M Iwai and M Horiuchi. Hypertens Res 2009;32(7):533-6.
[2] PE Gallagher, K Cook, D Soto-Pantoja, J Menon, EA Tallant. Curr Cancer Drug Targets 2011;11(4):394-404.
[3] L Pickel, T Matsuzuka, C Doi, R Ayuzawa, DK Maurya, SX Xie, C Berkland, M Tamura. Cancer Biol Ther 2010:9(4).
[4] JL Olin, A Veverka, DS Nuzum. Am J Health Syst Pharm 2011;68(22):2139-46.
[5] YK Chae, ME Valsecchi, J Kim, AL Bianchi, D Khemasuwan, A Desai, W Tester. Cancer Invest 2011;29(9):585-93.
[6] I Sipahi, SM Debanne, DY Rowland, DI Simon, JC Fang. Lancet Oncol 2010;11(7):627-36.
[7] JP Vavalle, LK Newby. Ann Intern Med 2010;153(12):6-8.
[8] S Bangalore, S Kumar, SE Kjeldsen, H Makani, E Grossman, J Wetterslev, et al. Lancet Oncol 2011;12:65-82.
[9] M Tahmasebi, S Barker, JR Puddefoot, GP Vinson. Br J Cancer 2006;95:67-74.
[10] S Greco, MG Elia, A Muscella, C Storelli, S Marsigliante. Cell Calcium 2002;32(1):1-10.
[11] S Greco, A Muscella, MG Elia, P Salvatore, C Storelli, S Marsigliante. J Endocrinol 2002;174(2):205-14.
[12] XC Li, JL Zhuo. Curr Opin Nephrol Hypertens 2008;17(1):37-43.
[13] V Bours, G Bonizzi, M Bentires-Alj, F Bureau, J Piette, P Lekeux, M Merville. Toxicol2000; 153(1-3):27-38.
[14] S Ghosh, M Karin. Cell 2002;109:81-96.
[15] V Bours, M Bentires-Alj, AC Hellin, P Viatour, P Robe, S Delhalle, V Benoit, MP Merville. Biochem Pharmacol 2000;60(8):1085-9.
[16] A Muscella, S Greco, MG Elia, C Storelli, S Marsigliante. J Endocrinol 2002;173(2):315-23.
[17] Y Zhao, X Chen, L Cai, Y Yang, G Sui, S Fu. J Cell Physiol 2010;225(1):168-73.
[18] Da Hee Oh, Jun Soo Bang, Hyun Mi Choi, Hyung-In Yang, Myung Chul Yoo, Kyoung Soo Kim. European J Pharmacol 2010;649:135–139.