Original Article

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Does Valsartan Affect the Cytotoxicity of Doxorubicin When Used as a Cardioprotective Drug Against Doxorubicin-Induced Cardiotoxicity ?

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ABSTRACT

Background: Doxorubicin is widely used as a chemotherapeutic drug. It has several serious side effects, including cardiotoxicity. Valsartan is an angiotensin II receptor blocker that plays a cardioprotective role against doxorubicin-induced cardiotoxicity. **Aim**: The aim of our study is to evaluate whether the combination of valsartan and doxorubicin affects the therapeutic efficacy of doxorubicin in treating cancer using an in vitro breast cancer cell line (MCF-7). **Methods**: Different concentrations of doxorubicin, valsartan, and their combination were tested to detect their cytotoxic effects on the cell line using MTT colorimetric assay method. Three duplicates of each concentration and control were made. **Results**: Valsartan had a mild cytotoxic effect only at higher concentrations, with an estimated IC50 value of 125.8 µg/ml, while doxorubicin, had more potent cytotoxicity, with an estimated IC50 value of 87.43 µg/ml. The IC50 of the doxorubicin-valsartan combination was lower than the IC50 of both drugs when used alone, with a DRI more than 1 (3.56) and an IAI less than 1 (0.94). **Conclusions**: There is synergism between doxorubicin and valsartan on MCF-7 breast cancer cells, suggesting a potential role for the combination in cancer treatment. The combination induces cytotoxicity in lower doses than when doxorubicin used alone.

Keywords: Doxorubicin, valsartan, cardiotoxicity, synergism, angiotensin receptor blockers

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INTRODUCTION

Doxorubicin (DOX) is widely used as a chemotherapeutic drug. It plays an important role in the treatment of various malignancies, including breast cancer, sarcomas, and leukemia.[1] Doxorubicin acts on cancer cells through two mechanisms. The first involves interaction with DNA and inhibition of topoisomerase II-mediated DNA repair, while the second involves the formation of free radicals, leading to oxidative stress and apoptosis in cancer cells.[2,3] Although doxorubicin is an effective cytotoxic drug, it has several serious side effects that can limit its medical use, particularly cardiotoxicity, which poses a significant challenge in cancer treatment regimens

containing doxorubicin.[4,5] Doxorubicin induces cardiotoxicity through multiple pathways, including oxidative stress, mitochondrial damage, calcium and iron overload, inflammatory cytokines, and injury to DNA and myocyte membranes. [5-8] The challenge of combating cancer while preserving normal heart function necessitates the use of cardioprotective drugs in combination with doxorubicin. Valsartan (VAL) is an angiotensin II receptor blockerblocker.[9] It is commonly used for treating hypertension and heart failure.[10-12] Recent studies have reported that valsartan has a cardioprotective role against doxorubicin-induced cardiotoxicity through its anti-inflammatory, antioxidant, and antifibrotic effects.[13,14] The aim of our study is to evaluate whether the combination of valsartan and doxorubicin affects the therapeutic efficacy of doxorubicin in treating cancer using an in vitro breast cancer cell line (MCF-7).

MATERIALS AND METHODS

Ethical approval:

The study was approved by the ethical committee of Al-Zahraa College of Medicine (Research No. E/T 68)

Cell line:

The MCF-7 breast cancer line was obtained from the Center of Biotechnological Research at Al-Nahrain University

Chemicals and drugs:

Doxorubicin HCl (Beijing Jin Ming Biotechnology, China), valsartan (Heterolab, China), MTT assay kit (Intron Biotech, Korea), fetal bovine Serum, RPMI 1640 media kit, sodium bicarbonate (Sigma, USA), benzyl penicillin, and streptomycin (Ajanta Pharm, India). A pre-made RPMI kit with supplementary L-glutamate was utilized as the processing medium. The following chemicals were added to the medium: 0.001 g of streptomycin, 1 g of sodium bicarbonate, 10^3 IU of benzyl penicillin, and 10% fetal bovine serum. The stock solutions of 1 mg/ml of DOX and VAL were diluted to create two-fold serial dilutions in various concentrations: 12.5, 25, 50, 100, 200, and 400 µg/ml. A combination solution of DOX and VAL was prepared at a 0.5:0.5 ratio by mixing 500 µl of each stock solution and further diluting to obtain concentrations of 12.5, 25, 50, 100, 200, and 400 µg/ml. The medications were diluted with phosphate-buffered saline (PBS).

Procedure:

Each DOX and VAL were tested independently as control groups to detect their cytotoxic effects on the cell line using MTT colorimetric assay method.[15,16] A 96-well microtiter plate was used, with a final volume of 200 µl of complete culture media in each well, to cultivate around 10^4 to 10^6 cells per milliliter. Following a 24-hour incubation period at 37°C with 5% CO_2, the medium was removed, and wells were filled with successive dilutions of the necessary compounds, either alone or in combination (DOX:VAL at 1:1). Three duplicates of each concentration and control (cells preserved in serum-free medium) were made. Plates were stored at 37°C with 5% CO_2 for 24 hours. Pharmacological parameters:

The Chou-Talalay method was applied to calculate the half-maximum inhibitory concentration (IC50), the interaction index (IAI), and the dose reduction index (DRI) as follows:

A four-parameter logistic (4PL) nonlinear regression variable slope model was employed to determine the IC50 for each drug.[15]

$$Y = \text{Min} + \frac{\text{Max}-\text{Min}}{(1+\frac{X}{IC50})^{\text{Hill coefficient}}}$$

Where Y is the observed response, Max is the maximum response, Min is the minimum response, X is the concentration**,** Hill coefficient: slop of the curve**.**

Dose reduction index (DRI**):** This indicates the extent to which individual drug doses can be decreased when combined together.[17,18] It can be calculated by the following formula

DRI= $(IC50\ of drug alone/IC50\ of drugs in combination)$ X 2

For DRI>1, indicate favorable dose reduction, equal to 1; no dose reduction, <1 no favorable dose reduction.

The interaction index IAI:

$$IAI=\frac{d1}{D1}+\frac{d2}{D2}$$

Where d1, d2=IC50 of agents in combination. D1, D2= IC50 of each agent alone where IAI>1 indicates antagonism, IAI=1 indicates additive effect, IAI<1 indicates synergism.[17]

Statistical analysis: The two-way ANOVA test was employed to evaluate the statistical significance of the disparity in mean values among multiple groups. Upon obtaining a statistically significant outcome from the ANOVA model, a subsequent analysis was conducted to determine statistical significance between all paired combinations of study groups using Tukey's post hoc test. GraphPad Prism 10 was used for IC50 calculation. A statistically significant result was defined as having a p-value below the level of significance of 0.05.

RESULTS

Our study showed that treatment of MCF-7 cell lines with various concentrations of doxorubicin, valsartan, and their combination produced variable degrees of response. VAL alone exhibited a mild cytotoxic effect, with an estimated IC50 value of 125.8 µg/ml, as shown in Table 1 and Figure 1. The cytotoxic effects of VAL were significant only at higher concentrations (400, 200, 100 µg/ml) compared to untreated control cells, as shown in Figure 2. In contrast, DOX treatment was more potent, with an estimated IC50 value of 87.43 µg/ml, as shown in Table 1, with a statistically significant difference between these two drugs (p value < 0.05), as shown in Figure 2.

The IC50 of the DOX-VAL combination was lower than the IC50 of both drugs when used alone, with a dose reduction index (DRI) greater than 1 (3.56) and an interaction index (IAI) less than 1 (0.94), as shown in Table 1.

Table 1: Effects of DOX + VAL on IC50, IAI, and DRI on MCF-7 cell line

Parameters	DOX	VAL	DOX-VAL combination
IC50 (µg/ml)	87.43	125.8	49
DRI	3.56	-	-
IAI	-	-	0.94

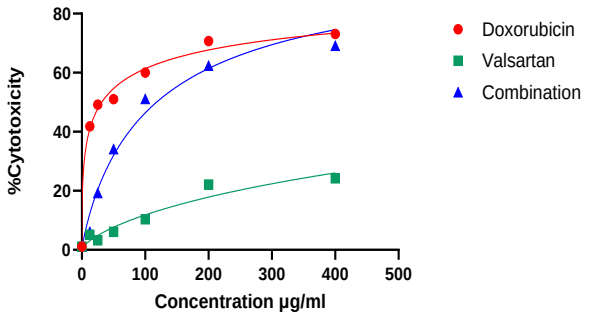

Figure 1: Dose-response effects of doxorubicin, valsartan, and their combination on MCF-7 cell line

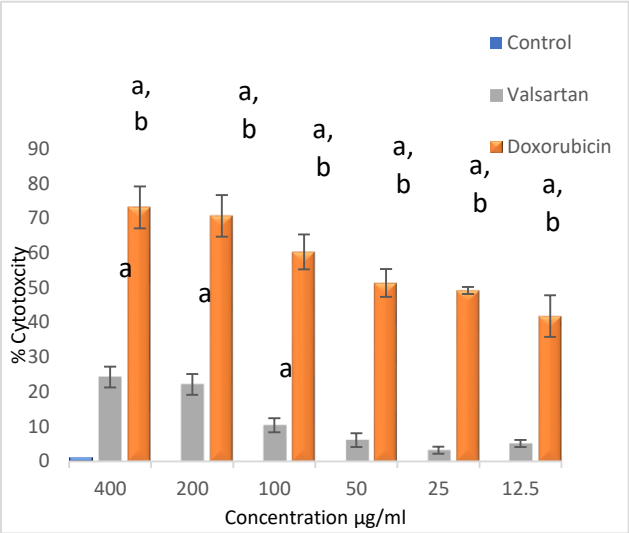

Figure 2: Comparison between cytotoxic effects of doxorubicin and valsartan on MCF-7 breast cancer cell line: a) significant from control untreated cells; b) significant from valsartan-treated cells.

DISCUSSION

We found a dose-dependent cytotoxicity of both doxorubicin and valsartan. However, the cytotoxicity of valsartan alone is mild. The analysis of the pharmacological parameters included in our study (IC50, DRI, and IAI) indicates synergism between doxorubicin and valsartan. The IC50 for the combination was lower than the IC50 of doxorubicin when used alone, suggesting that the combination induces cytotoxicity at lower doses, which may help minimize the side effects of doxorubicin. The DRI value was greater than 1 indicating a dose-sparing effect for doxorubicin when combined with valsartan, allowing for the prescription of a lower dose of the chemotherapeutic drug without impairing its antitumor activity. The IAI value was less than 1 confirming this synergistic interaction. This effect can be attributed to the inhibition of the renin-angiotensin-aldosterone system (RAAS) by valsartan. The RAAS is an important regulatory mechanism of blood pressure and fluid balance, involving the sequential activation of multiple components. It is initiated by the release of renin from juxtaglomerular cells, triggered by low sodium levels or decreased renal perfusion. Renin catalyzes the transformation of angiotensinogen into angiotensin I, which is transformed into angiotensin II, a step that is catalyzed by the enzyme angiotensin-converting enzyme (ACE). Angiotensin II is a potent vasoconstrictor and also triggers aldosterone secretion from the zona glomerulosa of the adrenal cortex, enhancing sodium reabsorption and potassium excretion in the renal tubules. Angiotensin II exerts its effects

primarily through two types of receptors: angiotensin II type 1 receptors (AT1R) and angiotensin II type 2 receptors (AT2R).[19,20] AT1R and AT2R are G protein-coupled receptors with similar affinities for angiotensin II.II.[21,22] AT1R activation is the major RAAS pathway. It plays a crucial role in variety of cellular processes, such as proliferation, migration, and inflammation. It is strongly associated with tumorigenesis, invasion, and inhibition of apoptosis.[23,24] Angiotensin exerts most of its harmful effects through the activation of AT1R, while its protective effects, such as immune modulation, neuroregeneration, anti-inflammatory, and antifibrotic actions, are exerted through the activation of AT2R.[25,26] Activation of AT1R stimulates variety of intracellular pathways mainly the phospholipase C (PLC), the phosphoinositide 3 kinase / protein kinase (PI3K/AKT), the mitogen activated protein kinase (MAPK), and Jaus kinase / signal transducer and activator of transcription (JAK-STAT) pathways. The phospholipase C (PLC) pathway converts phosphatidylinositol biphosphate (PIP2) into diacylglycerol (DAG) and inositol triphosphate (IP3). Inositol triphosphate induces the release of calcium from endoplasmic reticulum while DAG activate protein kinase C (PKC). The PI3K/AKT and pathway improves growth and survival of the cell and regulates metabolism. The MAPK pathway also stimulates cell proliferation. The (JAK-STAT) pathway modulates immunity and inflammation.[27-29] In contrast to AT1R, AT2R activation promotes nitric oxide production by the enzyme endothelial nitric oxide synthase (eNOS), activates phosphatases, and increases cyclic GMP. These pathways promote vasodilatation, cellular differentiation, and apoptosis.[30] In addition to plasma angiotensin II, various organs exhibit functional local RAAS, indicating that it has autocrine and paracrine functions. Researchers have consistently concluded that dysregulation of this local RAAS contributes to tumor progression and resistance to chemotherapy.[31,32] Increased RAAS activity has been reported in a wide variety of tumors, including gastrointestinal tumors (such as stomach, liver, colon, and pancreatic cancers) and sex hormone-related cancers (such as ovarian, breast, and prostate cancers). Additionally, tumors of the lung, skin, brain, and bone marrow also show increased RAS activity.[33,34]Among the components of RAAS, AT1R has received special attention, as it is upregulated in various malignancies, including lung, breast, and pancreatic cancers.[35] AT1R activation enhances proliferation, angiogenesis, and metastasis of tumors by activating the PI3K/AKT and MAPK pathways, which inhibit apoptosis and promote cell cycle progression, thereby facilitating tumor growth.[23,29,36,37] AT1R promote angiogenesis by increasing vascular endothelial growth factor (VEGF) and the expression of epidermal growth factor receptor (EGFR). Angiogenesis plays a crucial role in tumor survival and expansion.[38-40] Furthermore, AT1R activation enhances metastasis by decreasing antitumor immunity through the activation of STAT3.[41] and promoting the epithelial-to-mesenchymal transition (EMT), a process that enhances tumor cells motility and invasiveness.[42,43] Given the various actions of AT1R in enhancing tumor pathogenesis, angiotensin receptor blockers (ARBs) appear to have a role in cancer treatment by inhibiting the downstream pathways activated by AT1R. Accumulating evidence suggests that ARBs play role in the treatment of breast, prostate and pancreatic tumors,[44-46] and enhance overall survival of the patients.[47,48]

CONCLUSIONS

There is synergism between doxorubicin and valsartan on MCF-7 breast cancer cells, suggesting a potential role for the combination in cancer treatment. The combination induces cytotoxicity in lower doses than when doxorubicin is used alone.

REFERENCES

1. Mattioli R, Ilari A, Colotti B, Mosca L, Fazi F, Colotti G. Doxorubicin and other anthracyclines in cancers: Activity, chemoresistance, and its overcoming. *Mol Aspects Med*. 2023 Oct;93:101205. doi: 10.1016/j.mam.2023.101205.
2. Sritharan S, Sivalingam N. A comprehensive review on the time-tested anticancer drug doxorubicin. *Life Sci*. 2021 Aug 1;278:119527. doi: 10.1016/j.lfs.2021.119527.
3. Tacar O, Sriamornsak P, Dass CR. Doxorubicin: An update on anticancer molecular action, toxicity, and novel drug delivery systems. *J Pharm Pharmacol*. 2013 Feb;65(2):157-70. doi: 10.1111/j.2042-7158.2012.01567.x.
4. Renu K, V G A, P B TP, Arunachalam S. Molecular mechanism of doxorubicin-induced cardiomyopathy - An update. *Eur J Pharmacol*. 2018 Jan 5;818:241-53. doi: 10.1016/j.ejphar.2017.10.043.
5. Sheibani M, Azizi Y, Shayan M, Nezamoleslami S, Eslami F, Farjoo MH, et al. Doxorubicin-Induced Cardiotoxicity: An Overview of Pre-clinical Therapeutic Approaches. *Cardiovasc Toxicol*. 2022 Apr;22(4):292-310. doi: 10.1007/s12012-022-09721-1.
6. Hoeger CW, Turissini C, Asnani AH. Doxorubicin cardiotoxicity: Pathophysiology Updates. *Curr Treat Options Cardiovasc Med*. 2020;22:1-17. doi: 10.1007/s11936-020-00842-w.
7. Linders AN, Dias IB, López Fernández T, Tocchetti CG, Bomer N, Van der Meer P, et al. A review of the pathophysiological mechanisms of doxorubicin-induced cardiotoxicity and aging. *NPJ Aging*. 2024 Jan 23;10(1):9. doi: 10.1038/s41514-024-00135-7.
8. Vitale R, Marzocco S, Popolo A. Role of oxidative stress and inflammation in Doxorubicin-Induced Cardiotoxicity: A brief

account. *Int J Mol Sci*. 2024 Jul 8;25(13):7477. doi: 10.3390/ijms25137477.
9. Potamitis C, Chatzigeorgiou P, Siapi E, Viras K, Mavromoustakos T, Hodzic A, et al. Interactions of the AT1 antagonist valsartan with dipalmitoyl-phosphatidylcholine bilayers. *Biochim Biophys Acta*. 2011 Jun;1808(6):1753-63. doi: 10.1016/j.bbamem.2011.02.002.
10. Accetto R, Chazova IY, Sirenko Y, Vincelj J, Widimsky J Jr, Barbič-Žagar B. The efficacy and safety of valsartan and the combination of valsartan and hydrochlorothiazide in the treatment of patients with mild to moderate arterial hypertension - the VICTORY trial. *Kardiol Pol*. 2017;75(1):55-64. doi: 10.5603/KP.a2016.0135.
11. Morrow DA, Velazquez EJ, Desai AS, DeVore AD, Lepage S, Park JG, et al. Sacubitril/valsartan in Patients Hospitalized With Decompensated Heart Failure. *J Am Coll Cardiol*. 2024 Mar 26;83(12):1123-32. doi: 10.1016/j.jacc.2024.01.027.
12. Chatur S, Beldhuis IE, Claggett BL, McCausland FR, Neuen BL, Desai AS, et al. Sacubitril/valsartan in patients with heart failure and deterioration in eGFR to <30 mL/min/1.73 m². *JACC Heart Fail*. 2024 Oct;12(10):1692-703. doi: 10.1016/j.jchf.2024.03.014.
13. Alhazzani K, Alotaibi MR, Alotaibi FN, Aljerian K, As Sobeai HM, Alhoshani AR, et al. Protective effect of valsartan against doxorubicin-induced cardiotoxicity: Histopathology and metabolomics in vivo study. *J Biochem Mol Toxicol*. 2021 Sep;35(9):e22842. doi: 10.1002/jbt.22842.
14. Cheng D, Chen L, Tu W, Wang H, Wang Q, Meng L, et al. Protective effects of valsartan administration on doxorubicin induced myocardial injury in rats and the role of oxidative stress and NOX2/NOX4 signaling. *Mol Med Rep*. 2020 Nov;22(5):4151-62. doi: 10.3892/mmr.2020.11521.
15. He Y, Zhu Q, Chen M, Huang Q, Wang W, Li Q, et al. The changing 50% inhibitory concentration (IC50) of cisplatin: A pilot study on the artifacts of the MTT assay and the precise measurement of density-dependent chemoresistance in ovarian cancer. *Oncotarget*. 2016 Oct 25;7(43):70803-21. doi: 10.18632/oncotarget.12223.
16. Freshney RI. Subculture and cell lines. *Culture of Animal Cells: A Manual of Basic Technique*. 2005 Oct 14.
17. Chang TT, Chou TC. Rational approach to the clinical protocol design for drug combinations: A review. *Acta Paediatr Taiwan*. 2000 Nov-Dec;41(6):294-302. PMID: 11198934.
18. Zhang N, Fu JN, Chou TC. Synergistic combination of microtubule-targeting anticancer fludelone with cytoprotective panaxytriol derived from Panax ginseng against MX-1 cells in vitro: Experimental design and data analysis using the combination index method. *Am J Cancer Res*. 2015 Dec 15;6(1):97-104.
19. Martyniak A, Tomasik PJ. A new perspective on the renin-angiotensin system. *Diagnostics (Basel)*. 2022 Dec 21;13(1):16. doi: 10.3390/diagnostics13010016.
20. Vargas Vargas RA, Varela Millán JM, Fajardo Bonilla E. Renin-angiotensin system: Basic and clinical aspects - A general perspective. *Endocrinol Diabetes Nutr (Engl Ed)*. 2022 Jan;69(1):52-62. doi: 10.1016/j.endien.2022.01.005.
21. Kambayashi Y, Bardhan S, Takahashi K, Tsuzuki S, Inui H, Hamakubo T, et al. Molecular cloning of a novel angiotensin II receptor isoform involved in phosphotyrosine phosphatase inhibition. *J Biol Chem*. 1993 Nov 25;268(33):24543-6.
22. Mukoyama M, Nakajima M, Horiuchi M, Sasamura H, Pratt RE, Dzau VJ. Expression cloning of type 2 angiotensin II receptor reveals a unique class of seven-transmembrane receptors. *J Biol Chem*. 1993 Nov 25;268(33):24539-42. PMID: 8227010.
23. Acconcia F. The network of Angiotensin Receptors in breast cancer. *Cells*. 2020 May 27;9(6):1336. doi: 10.3390/cells9061336.
24. Delforce SJ, Lumbers ER, Corbisier de Meaultsart C, Wang Y, Proietto A, Otton G, et al. Expression of renin-angiotensin system (RAS) components in endometrial cancer. *Endocr Connect*. 2017 Jan;6(1):9-19. doi: 10.1530/EC-16-0082.
25. Matavelli LC, Siragy HM. AT2 receptor activities and pathophysiological implications. *J Cardiovasc Pharmacol*. 2015 Mar;65(3):226-32. doi: 10.1097/FJC.0000000000000208.
26. Namsolleck P, Recarti C, Foulquier S, Steckelings UM, Unger T. AT(2) receptor and tissue injury: Therapeutic implications. *Curr Hypertens Rep*. 2014 Feb;16(2):416. doi: 10.1007/s11906-013-0416-6.
27. Laghlam D, Jozwiak M, Nguyen LS. Renin-angiotensin-aldosterone system and immunomodulation: A State-of-the-Art Review. *Cells*. 2021 Jul 13;10(7):1767. doi: 10.3390/cells10071767.
28. Zhao Y, Wang H, Li X, Cao M, Lu H, Meng Q, et al. Ang II-AT1R increases cell migration through PI3K/AKT and NF-κB pathways in breast cancer. *J Cell Physiol*. 2014 Nov;229(11):1855-62. doi: 10.1002/jcp.24639.
29. Zhao Y, Chen X, Cai L, Yang Y, Sui G, Fu S. Angiotensin II/angiotensin II type I receptor (AT1R) signaling promotes MCF-7 breast cancer cell survival via the PI3-kinase/Akt pathway. *J Cell Physiol*. 2010 Oct;225(1):168-73. doi: 10.1002/jcp.22209.
30. Nouet S, Nahmias C. Signal transduction from the angiotensin II AT2 receptor. *Trends Endocrinol Metab*. 2000 Jan-Feb;11(1):1-6. doi: 10.1016/s1043-2760(99)00205-2.
31. Puddefoot JR, Udeozo UK, Barker S, Vinson GP. The role of angiotensin II in the regulation of breast cancer cell adhesion and invasion. *Endocr Relat Cancer*. 2006 Sep;13(3):895-903. doi: 10.1677/erc.1.01136.
32. Xie G, Cheng T, Lin J, Zhang L, Zheng J, Liu Y, et al. Local angiotensin II contributes to tumor resistance to checkpoint immunotherapy. *J Immunother Cancer*. 2018 Sep 12;6(1):88. doi: 10.1186/s40425-018-0401-3.
33. Haznedaroglu IC, Malkan UY. Local bone marrow renin-angiotensin system in the genesis of leukemia and other malignancies. *Eur Rev Med Pharmacol Sci*. 2016 Oct;20(19):4089-4111. PMID: 27775788.
34. Hanif K, Bid HK, Konwar R. Reinventing the ACE inhibitors: Some old and new implications of ACE inhibition. *Hypertens Res*. 2010 Jan;33(1):11-21. doi: 10.1038/hr.2009.184.
35. Deshayes F, Nahmias C. Angiotensin receptors: A new role in cancer? *Trends Endocrinol Metab*. 2005 Sep;16(7):293-9. doi: 10.1016/j.tem.2005.07.009.
36. Ohashi H, Takagi H, Oh H, Suzuma K, Suzuma I, Miyamoto N, et al. Phosphatidylinositol 3-kinase/Akt regulates angiotensin II-induced inhibition of apoptosis in microvascular endothelial cells by governing survivin expression and suppression of caspase-3 activity. *Circ Res*. 2004 Apr 2;94(6):785-93. doi: 10.1161/01.RES.0000121103.03275.EC.
37. Uemura H, Nakaigawa N, Ishiguro H, Kubota Y. Antiproliferative efficacy of angiotensin II receptor blockers in prostate cancer. *Curr Cancer Drug Targets*. 2005 Aug;5(5):307-23. doi: 10.2174/1568009054629663.
38. Anandanadesan R, Gong Q, Chipitsyna G, Witkiewicz A, Yeo CJ, Arafat HA, et al. Angiotensin II induces vascular endothelial growth factor in pancreatic cancer cells through an angiotensin II type 1 receptor and ERK1/2 signaling. *J Gastrointest Surg*. 2008 Jan;12(1):57-66. doi: 10.1007/s11605-007-0403-9.
39. Kosugi M, Miyajima A, Kikuchi E, Kosaka T, Horiguchi Y, Murai M. Effect of angiotensin II type 1 receptor antagonist on tumor growth and angiogenesis in a xenograft model of human bladder cancer. *Hum Cell*. 2007 Feb;20(1):1-9. doi: 10.1111/j.1749-0774.2007.00025.x.
40. Yang X, Zhu MJ, Sreejayan N, Ren J, Du M. Angiotensin II promotes smooth muscle cell proliferation and migration through the release of heparin-binding epidermal growth factor and activation of the EGF-receptor pathway. *Mol Cells*. 2005 Oct 31;20(2):263-70.
41. Kortylewski M, Kujawski M, Wang T, Wei S, Zhang S, et al. Inhibiting Stat3 signaling in the hematopoietic system elicits multicomponent antitumor immunity. *Nat Med*. 2005 Dec;11(12):1314-21. doi: 10.1038/nm1325.
42. Rasha F, Ramalingam L, Gollahon L, Rahman RL, Rahman SM, Menikdiwela K, et al. Mechanisms linking the renin-angiotensin system, obesity, and breast cancer. *Endocr Relat Cancer*. 2019 Dec 1;26(12):R653-72. doi: 10.1530/ERC-19-0314.
43. Takiguchi T, Takahashi-Yanaga F, Ishikane S, Tetsuo F, Hosoda H, Arioka M, et al. Angiotensin II promotes primary tumor growth and metastasis formation of murine TNBC 4T1 cells through the

fibroblasts surrounding cancer cells. *Eur J Pharmacol*. 2021 Oct 15;909:174415. doi: 10.1016/j.ejphar.2021.174415.

44. Chauhan VP, Martin JD, Liu H, Lacorre DA, Jain SR, Kozin SV, et al. Angiotensin inhibition enhances drug delivery and potentiates chemotherapy by decompressing tumor blood vessels. *Nat Commun*. 2013;4:2516. doi: 10.1038/ncomms3516.
45. Rhodes DR, Ateeq B, Cao Q, Tomlins SA, Mehra R, Laxman B, et al. AGTR1 overexpression defines a subset of breast cancer and confers sensitivity to losartan, an AGTR1 antagonist. *Proc Natl Acad Sci U S A*. 2009 Jun 23;106(25):10284-9. doi: 10.1073/pnas.0900351106.
46. Woo Y, Jung YJ. Angiotensin II receptor blockers induce autophagy in prostate cancer cells. *Oncol Lett*. 2017 May;13(5):3579-85. doi: 10.3892/ol.2017.5872.
47. Sun H, Li T, Zhuang R, Cai W, Zheng Y. Do renin-angiotensin system inhibitors influence the recurrence, metastasis, and survival in cancer patients? Evidence from a meta-analysis including 55 studies. *Medicine (Baltimore)*. 2017 Mar;96(13):e6394. doi: 10.1097/MD.0000000000006394.
48. Li XY, Sun JF, Hu SQ. The renin-angiotensin system blockers as adjunctive therapy for cancer: A meta-analysis of survival outcomes. *Eur Rev Med Pharmacol Sci*. 2017 Mar;21(6):1375-83. PMID: 28387887.