Original Article

Received: 2021-12-01
Accepted: 2022-01-26

BCCR
2021, No 2, Vol 13 :119-126
www.bccrjournal.com

Response to Treatment in 4T1 Tumor Following Exposure to Paclitaxel and Doxorubicin Based on Antiangiogenic Effects

Zahra Valizadeh[1,2#], Masoomeh Beheshti[1,3#], Fatemeh Ashrafi[2], Soyar Sari[3], Raheleh Kheirbakhsh[1], Hadiseh Mohammadpour[4], Samad mohammadnejad[5], Ahad mohammadnejad[1], Saeid Amanpour[1*], Marveh Rahmati[1*]

1

1. Cancer Biology Research Center, Cancer Institute, Tehran University of Medical Sciences, Tehran, Iran
2. Department of Cellular and Molecular Biology, Faculty of Biological Sciences, North Tehran Branch, Islamic Azad University, Tehran, Iran
3. Department of Molecular and Cellular Sciences, Faculty of Advanced Science and Technology, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran
4. Dental Research Center, Dentistry Research Institute, Tehran University of Medical Sciences, Tehran, Iran
5. Cell-Based Therapies Research Center, Digestive Diseases Research Institute, Tehran University of Medical Sciences, Tehran, Iran.
#contributed equally
*Corresponding authors:
Saeid Amanpour,
Associate professor, DVSc, Cancer Biology Research Center, Cancer Institute, Tehran University of Medical Sciences, Tehran, Iran.
Telefax: +98 21 66581638,
e-mail: amanpour_s@tums.ac.ir
Marveh Rahmati,
Assistant professor, Ph.D., Cancer Biology Research Center, Cancer Institute, Tehran University of Medical Sciences, Tehran, Iran.
Tel: +98 21 66940021,
fax: +98 21 66581638,
e-mail: m_rahmati@sina.tums.ac.ir
ORCID ID: ORCID ID: 0000-0003-2676-3973

ABSTRACT

Background: 4T1 is a mice transplantable mammary carcinoma cell line with highly tumorigenic and invasive properties, making it a suitable preclinical oncology model for triple-negative breast cancer (TNBC). This pilot study aimed to create a model of clinical stages in TNBC mice and to evaluate the response to treatment with paclitaxel (PTX) and doxorubicin (DOX) based on antiangiogenic effects

Methods: Syngeneic tumors were developed in BALB/c female mice by 4T1 cell line. The mice were randomly distributed into three different groups, each containing four. A group of four was considered as healthy normal. When tumor growth reached 100-200 mm^3, two groups received the maximum tolerated dose (MTD) of PTX and DOX, respectively. Normal saline was injected into the sham control group. The tumors and tissue margins were removed by surgery one week following chemotherapy. Angiogenesis genes and microvessel density (MVD) were analyzed by real-time PCR and immunohistochemistry, respectively. Response to treatment was also assessed by standard methods of H&E staining.

Results: TNBC tumors were confirmed by pathological staining. The volume of tumors and the angiogenesis gene expressions of VEGFR1, VEGFR2, and HIF1α decreased in treated tumors compared to control ($p < 0.05$). Response to treatment to PTX was more than DOX, and the MVD decreased in both PTX and DOX chemotherapy groups.

Conclusion: Although PTX is more effective than DOX in reducing angiogenesis genes, both have the potential for treatment in the 4T1 mouse model.

Keywords: Angiogenesis, Doxorubicin, Paclitaxel, Response to treatment, Triple-Negative Breast Cancer, 4T1 Tumor

INTRODUCTION:

The cell line isolated from breast cancer in Balb/cfC3H mirrors stage IV triple-negative breast cancer (TNBC) and is identified as a single cell clone of 4T1 (ATCCCRL-2539) [1]. 4T1 tumor is known as highly invasive and metastatic. It lacks the expression of three receptors of estrogen receptor (ER), progesterone receptor (PR), and epidermal growth factor receptor 2 (HER2) [2]. In addition, it is widely used as a syngeneic tumor model to metastatic triple-negative breast cancer in preclinical research [3]. However, more inquiry is needed to establish and characterize the 4T1 model to evaluate its response to treatment. Since angiogenesis is induced by hypoxia which is common in cancers, investigating drugs that diminish or block hypoxia factors is important, especially in TNBC. Indeed under the circumstances such as central fibrosis and necrosis in TNBC, hypoxia induced by hypoxia-inducible factor 1α (HIF-1α) results in angiogenesis, proliferation, aggressive tumor phenotype, and metastasis, which eventually leads to the release of vascular endothelial growth factor (VEGF). VEGF is a signal protein involved in both vasculogenesis and angiogenesis. Part of the system restores the oxygen supply to tissues when blood circulation is inadequate such as in hypoxic conditions [4]. The members of the VEGF family induce cellular responses by binding to tyrosine kinase receptors (e.g., the VEGFR1, VEGFR2, and VEGFR3) on the cell surface. VEGFR1 function is related to VEGFR2 to modulate its signaling. VEGFR1 is also involved in vasculogenesis in the embryo [5]. The VEGFR2 receptor plays a key role in responding to the VEGF hormone and directly controlling tumor angiogenesis. In addition, VEGFR2 signaling is involved in vascular permeability, proliferation, survival, and migration of endothelial cells [6,7]. Angiogenesis is commonly evaluated by the VEGF family and microvessel density (MVD) which refers to the concentration of small blood vessels in a cancerous tumor and is measured by a vascular marker such as CD31 [8]. In various cancers, investigation for new therapeutic targets, especially drugs that inhibit angiogenesis, is under development [9]. Doxorubicin (DOX) and paclitaxel (PTX) are common drugs for chemotherapy in a wide range of cancers. They interfere in DNA repair and mitosis mechanisms, respectively [10,11]. To study the effect of drugs on tumors, in vivo models allow tumors to be examined under normal physiological conditions, interacting with the tumor's microenvironment and immune system. Among different in vivo murine models of breast cancers, syngeneic are usually fast-growing tumors in the immunocompetent host. In this model, the 4T1 mouse breast cancer cell line is used to induce tumors in mice [12,13], and the volume of tumors in mice is proportional to the stage in human tumors. For instance, 60-100 mm^3 (early stage), 100-300 mm3 (intermediated stage), 300-500 mm^3 (advanced stage) and 500-1000 mm^3 (end-stage) [14]. This pilot study aimed to model the clinical stages and evaluate the response to treatment with the PTX and DOX in tumors caused by this cell line.

Materials and Methods

Animals

Female BALB/c wild-type mice obtained from the Pasteur Institute (Iran, Tehran) were 6±8 weeks old and 20-25 grams in weight. Mice were housed under standard conditions of the animal care facility and were fed by commercial mouse diet food with a 12-hour light-dark cycle.

4T1 TNBC mouse model and treatment protocol

The 4T1 mouse cell line was purchased from the Pasteur Institute (Iran, Tehran). Cells were cultured in a complete media of RPMI, supplemented with 10% fetal bovine serum (FBS) and 100 unit/ml penicillin/streptomycin. Cells were incubated at 37°C with 5% CO2 and 95% humidity. To induce tumors in mice, 10^6 cells were injected subcutaneously in 100 µl of cell suspension in media as described before [15]. The mice's body weight and tumor volume were measured with a digital caliper every other day. To characterize the 4T1 model and determine the best protocol of treatment, different doses of PTX and DOX were applied. After gaining the appropriate amount of chemotherapy, the mice were randomly divided into three groups of four. They were monitored daily for water, food, and behavior. As the tumor volume

reached 100-300 mm^3, equivalent to the intermediate stage, one group received a single dose of 7 mg/kg DOX; the other received 20mg/kg PTX. In contrast, the third group received normal saline as a sham control group intraperitoneally (IP). One week after chemotherapy, tumor tissues and their margins were surgically removed from all mice and stored in RNA later and 10% formalin for molecular and pathological analysis, respectively.

RNA extraction and Real-time PCR

Total RNA was isolated from all tissues using an RNA X-Plus kit (Cinna Gen, Tehran, Iran) and was quantified by NanoDrop spectrophotometer (Thermo Scientific Nanodrop 2000). In the next step, cDNA was synthesized (Bio fact, South Korea), and Real-time PCR was performed with the Cyber Green method (Yekta Tajhiz Co, Cat No: YT2551) with the following program; 40 cycles of denaturation (15 seconds at 95°C), annealing (20 seconds at 60°C) and elongation process (30 seconds at 72°C). β-actin was used as the housekeeping gene and positive control. The relative expression of transcript levels of each case was calculated according to $2^{-\Delta\Delta ct}$ and analyzed by Rest 2009 software. The sequences of them are presented in Table 1.

Hematoxylin and Eosin (H&E) staining

Vital organs and tumor tissues were paraffin-embedded, and 5–10 μm cut sections were made by microtome, stained with H&E routine lab protocol, and the pathological responses were recorded under light microscopy.

Determination of tumor grade and evaluation of response to treatment

Response to treatment was evaluated by counting the cancerous cells in tissue samples and remarked as the following: R0, R1, R1a, R1b, and R1c, which showed no residual tumors, microscopic residual tumors, ≤ 30% residual tumor; 31–69% residual tumor and ≥ 70% residual tumors, respectively. The histological grade is measured during the "Bloom Richardson Scale" or "Nottingham Score", which combines three factors of nuclear grade, mitotic rate, and tubular formation. Although the histological grade predicts the aggressiveness of the tumors, it cannot affect treatment protocol. The nuclear grade reflects the rate of similarity of the cancerous nucleus to normal, in which grade 1 seems more like normal, and grade 3 looks the least like normal cells. Mitotic rate describes dividing the tumor cells and scores as 1 to 3 from the slowest to the quickest. Tubule formation shows the rate of the cancer cells to form tubules and grades as 1-3, which 1 refers to ≥ 75% of cells in a tubular shape, grade 2 is between 10 -75%, and a score of 3 shows ≤10% of cells are in tubule formation [16].

Microvessel density evaluation (MVD)

Immunohistochemistry with CD31 antibody (PECAM-1 (H-3): sc-376764) was performed according to the standard protocol, and then in 20x of light microscopy, four

Gene name	Sequence of primer	Product length
VEGFR1	Forward: 5'GCACATGACGGAAGGAAGAC 3' Reverse : 5' TTCGCAGTTCAGCAGTCCTA 3'	187bp
VEGFR2	Forward: 5'TCTGAGCATGGAACTCATGG 3' Reverse : 5'GATCTGCATTCGGACTTGGT 3'	139bp
HIF1α	Forward : 5'ATTCTCCAAGCCCTCCAAGT 3' Reverse : 5' GCCTTAGCAGTGGTCGTTTC 3'	205bp
β-actin	Forward : 5' GATCTGGCACCACACCTTCT 3' Reverse : 5' GGGTCATCTTTTCACGGTTG 3'	110bp

Table 1. Primer sequences of Hif1α, VEGFR1, VEGFR2 genes, and β-actin

hot spot areas were considered, and newly formed vessels were counted. In the end, their average was declared as MVD. Necrotic cells didn't consider in vascular counting.

Statistical analysis

All results were represented as mean ± SD from three independent experiments. The statistical analysis of genes expression VEGFR1, VEGFR2, HIF1α, and β-actin was performed using the REST 2009 and SPSS 22 software. $P < 0.05$ was considered statistically significant.

Results

TPTX reduces tumor growth and enhances response to treatment more than DOX in a 4T1 tumor.

The syngeneic mice tumors were successfully established, and the TNBC was confirmed by pathological analysis (Fig. 1a). Tumor volume measurements and pathological examinations showed that 20mg/kg of PTX and 7mg/kg of DOX were more significant than the other doses ($p < 0.05$) (Fig.1a, 1b). Results revealed a gradual increase in tumor volume until the 19th day in all groups. However, chemotherapy on the 19th day reduced tumor volume significantly until the end of the study compared to controls ($p<0.0.5$). The decrease in tumor volume was positively correlated with reduced proliferation-associated and mitotic cell count. H&E staining showed that the mitotic score in 20mg/kg of PTX was 1, which means less than or equal to 9 mitoses per 10 high power fields was observed, whereas in 7 mg/kg of DOX was 2, meaning 10-19 mitoses per 10 high power fields was calculated (Table 2). Weight changes in all animals were measured during chemotherapy, and there were no meaningful changes in treatment groups compared to the control. The presence of cancerous cells was checked in tissue samples of TNBC mice to determine whether there is a pathologic complete response (pCR) following chemotherapy. As shown in Table 2, the pCR score in 20 mg/kg of PTX was 2 (≤ 55%) in comparison to DOX, which was ≤ 40% in all groups. It should be mentioned that there was no metastasis to the lungs, kidneys, and liver of PTX-treated mice. Still, toxicity to the kidney and liver was observed, while in mice treated with DOX, metastasis to the lung with no toxicity to other organs

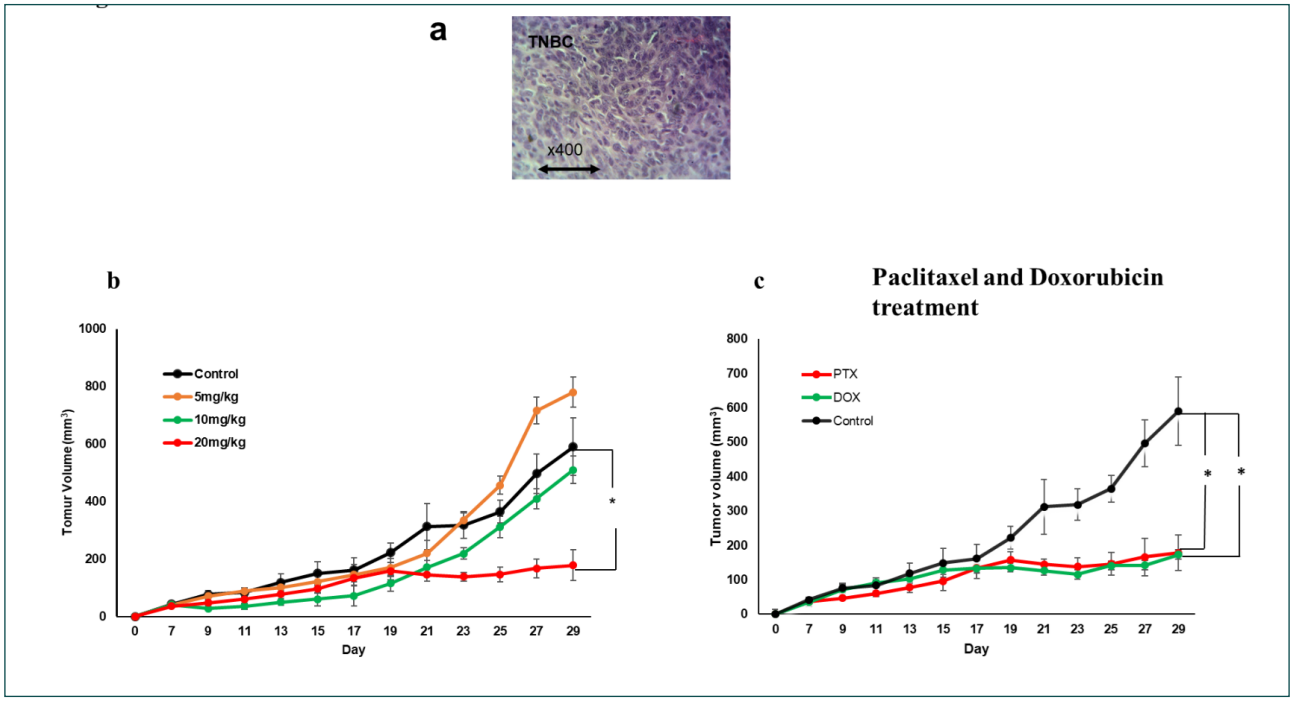

Figure.1. The effect of PTX and DOX on tumor volume in BALB/c TNBC mice
a) The TNBC tumors confirmed by pathological analysis; b) The average tumor volume in mice treated with different dosages of PTX (5, 10, and 20 mg/kg); c) Tumor volume curve in mice treated with PTX (20 mg/kg), DOX (7 mg/kg) and control groups. Statistical analysis was done with t-test, and $p < 0.05$ was considered significant.

Group	Tumor			Response to treatment	Lung		Kidney		Liver	
	T.F1	M.C2	N.PLe3	pCR4	Metas-tasis	Toxicity	Metas-tasis	Toxicity	Metas-tasis	Toxicity
Paclitaxel (5,mg/kg)	3	Score3	3	≤ 30%	Negative	+1	Negative	0	Negative	+1
Paclitaxel (10 mg/kg)	3	Score 2	3	≤ 40%	Negative	+1	Negative	0	Negative	+1
Paclitaxel (20mg/kg)	3	Score 2	2	≤ 55%	Negative	+1	Negative	+1	Negative	+2
Doxorubicin (7 mg/kg)	3	Score 2	3	<40%	positive	+1	Negative	0	Negative	0

Table 2. Tumor assessment and treatment response, toxicity, and metastasis followed by PTX and DOX treatment in BALB/c TNBC mice

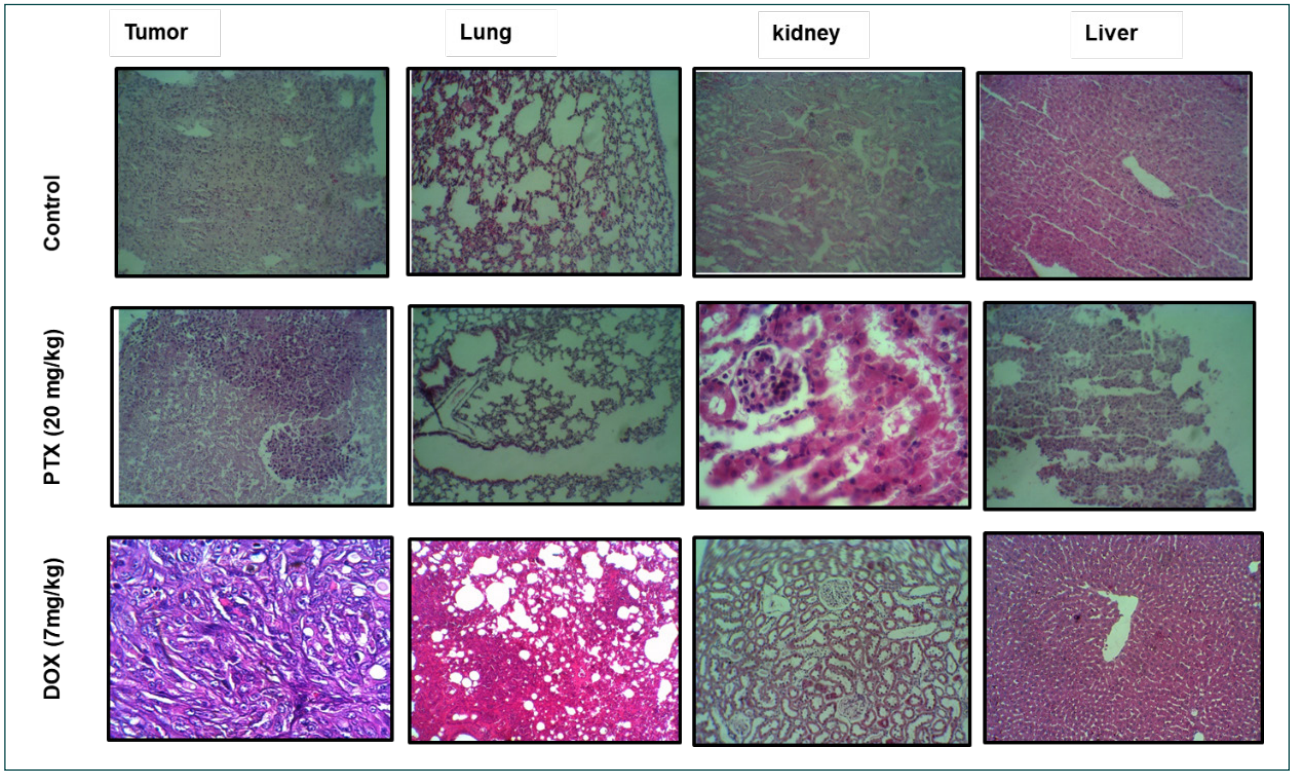

Figure.2. The effect of chemotherapy with PTX and DOX on BALB/c mice
The tumors and vital organs such as kidneys, liver, and lungs in all groups of animals were removed by surgery and monitored by H&E staining. The toxicity and metastasis associated with treatments were evaluated. Mice-treated with 5 and 10 mg/kg of PTX has no metastasis, but low toxicity to the liver was observed. However, 20mg/kg of PTX has more toxicity to the liver. In comparison, mice treated with DOX showed metastasis to the lung without toxicity to other tissues.

was observed Although, there were a little toxicity to all lungs, it showed the same toxicity in all controls (Fig.2 and Table 2). In the assessment of the tumor-treated, pathological analysis also demonstrated that the tubular differentiation of both DOX- and PTX-treated mice were 3, meaning that < 10% of tumor area formed glandular/tubular structures, the mitotic count decreased in PTX-treated in comparison with DOX-treated mice and the Nuclear Pleomorphism in PTX was 2 (cells larger than normal with open vesicular nuclei, visible nucleoli, and moderate variability in both size and shape) while it was 3 in DOX (vesicular nuclei, often with prominent nucleoli, exhibiting marked variation in size and shape, occasionally with very large and bizarre forms).

PTX reduces angiogenesis factors more than DOX in a mouse TNBC model

Gene expression was measured following chemotherapy with 7 mg/kg of DOX and 20 mg/kg of PTX to determine the critical angiogenesis factors such as VEGFR1, VEGFR2, and HIF1α. The result showed that the expression of VEGFR1, VEGFR2, and HIF1α genes decreased in treated groups compared to the sham control group. Quantitatively, in the control group, the average relative gene expression level of Hif1α, VEGFR1, and VEGFR2 were 2.22- ($P \leq 0.05$), 1.05, and 0.58-fold, respectively. In the DOX group, the average relative gene expression level of Hif1α, VEGFR1, and VEGFR2 were 0.01-($P \leq 0.05$),0.43, and 0.36-fold, respectively. In the PTX group, the average relative gene expression level of Hif1α, VEGFR1, and VEGFR2 were 0.1 and 0.06-fold, respectively, in comparison to healthy control ($p < 0.05$) (Fig. 3). The MVD analysis of PTX- and DOX-treated mice also confirmed the CD31 expression was diminished to 43.67% and 37.7%, respectively, compared to control ($p < 0.001$) (Fig. 4).

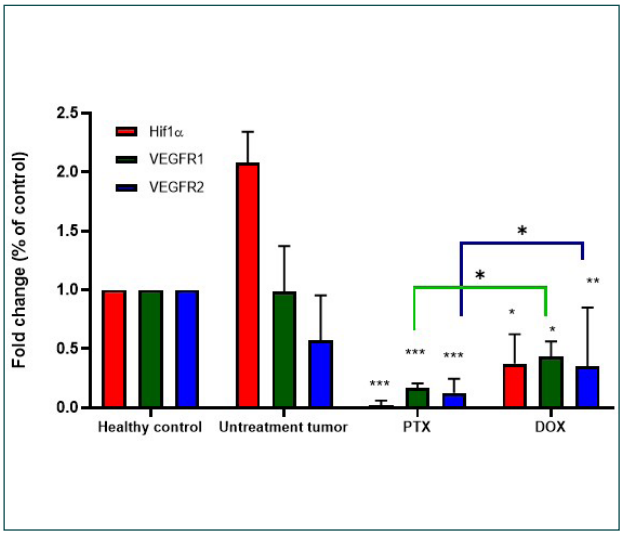

Figure.3. Angiogenesis genes following chemotherapy with PTX and DOX The mice tumor was removed by surgery, and the gene expression of Hif1α, VEGFR1, and VEGFR2 was calculated compared to healthy control. The fold change differences were statistically calculated by the T-test, and $p < 0.05$ was considered significant.

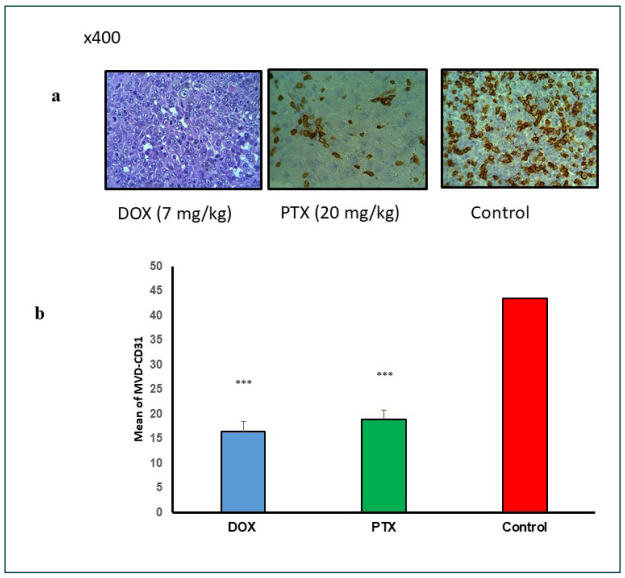

Figure.4. Microvessel density evaluation (MVD) Immunohistochemistry with CD31 antibody was done, and four hot spot areas were considered for counting the newly formed vessels. a. IHC of CD31 in PTX and DOX compared with control group b. The quantity of mean MVD evaluation.

Discussion

4T1 is a murine mammary TNBC and one of the most aggressive and metastatic cancer cell lines [17]. The development of anti-angiogenesis in breast cancer or other malignancies is a principal investigation for effective treatment [18]. As TNBC does not respond to either hormone therapy or anti-HER2 drugs, strategies targeting angiogenesis have been the central research point. This study focuses on the possible efficacy of DOX and PTX on angiogenesis activity in TNBC. The results revealed that MTD of 7 mg/kg DOX and 20 mg/kg of PTX was effective against tumors and diminished the tumor volume

compared to the control group ($p<0.05$). This agrees with the previous studies, which showed that DOX and PTX in TNBC mice should be used in MTD or in combination with other effective drugs when used in metronomic therapy [13,19,20]. During the IP injection of chemotherapy, no weight loss and no death were observed. Our remarkable achievements also agree with other experiences showing that intraperitoneal administration of injection could meaningfully reduce tumors [21].

We showed that the HIF-1α increase in syngeneic mice model of TNBC and followed by chemotherapy with DOX and PTX decreased significantly ($p < 0.05$). This is in line with the previous studies [4]. Besides, a decrease in HIF-1α expression compared to VEGFR1 and VEGFR2 expression is more meaningful than non-treatment tumors and should be considered in TNBC chemotherapy ($p < 0.05$). Although the previous experiments showed that determination of HIF-1α alone and targeted therapy couldn't be a reliable factor for TNBC, HIF-1α as a master regulator of angiogenesis should always be considered in all breast cancers [4].

Our previous data also showed a correlation between HIF-1α and VEGF in different stages of TNBC. They revealed that the following improvement in the stages, the angiogenesis genes, and protein expression increased, and the anti-angiogenesis treatment should be administrated before advanced stages [15]. In this experiment, we showed that following chemotherapy in the intermediate phase, the expression of HIF1α decreased, and consequently, the VEGFR1 and VEGFR2 expression diminished. Regarding the effect of drugs on angiogenesis, we showed that PTX is more effective than DOX by affecting VEGFR1 and VEGFR2 ($p < 0.05$). Although studies on the effect of DOX on the 4T1 model showed that DOX alone would be able to decrease the tumor growth, combination therapy with TGFβ inhibitor enhanced the efficacy of treatment and reduced metastasis [22], the current experiment revealed that DOX alone has possibly anti-angiogenic effect on 4T1 model. Potentially, DOX and PTX could diminish tumor growth by targeting angiogenesis. Experimental studies have shown different views on the effect effect of DOX and PTX on MVD, the lymph node metastasis and/or prognosis. Some studies have shown that high MVD has a poor prognosis and high lymph node metastasis [23], while others have shown that breast cancer is independent of lymph node metastasis [24]. A systematic review and meta-analysis study found that invasive breast cancer is an angiogenesis-dependent cancer [25]. In this project, chemotherapy was initiated in the intermediate stage, where the tumor volume was 100-200 mm3. Assessment of the tumor-treated mice confirmed that the malignancy was in grade 2 and grade 3 in PTX- and DOX-treated mice, respectively. Thus, the invasion of the tumor and the decreased MVD in PTX- and DOX-treated also confirmed the effect of both drugs on the TNBC model. This agrees with the pCR effect and metastatic determination of PTX- and DOX-treated mice. However, the toxicity to vital organs is remarked in all dosages of PTX, independent of its clinical activity [26]. In conclusion, PTX and DOX are suitable in the TNBC 4T1 mouse model against angiogenesis, especially against Hif-1α.

Acknowledgment

The authors appreciate the Cancer Biology Research Center of the Cancer Institute of Iran for supporting the project.

Funding

This project has been funded and supported by Tehran University of Medical Sciences (TUMS), Tehran, Iran; Grant no: 94-04-180-31187.

Conflicts of interest:

The authors declare that they have no conflict of interest.

Ethical Issues

The project was approved by the guidelines of the Ethics Committee of Tehran University of Medical Sciences, Iran (IR.IAU.PS.REC.1397.246).

Informed consent

For this type of study, formal consent is not required.

REFERENCES

1. Pulaski BA, Ostrand-Rosenberg S. Mouse 4T1 Breast Tumor Model. Curr Protoc Immunol. 2000;
2. Schrörs B, Boegel S, Albrecht C, Bukur T, Bukur V, Holtsträter C, et al. Multi-Omics Characterization of the 4T1 Murine Mammary Gland Tumor Model. Front Oncol. 2020;
3. Israyelyan A, Shannon EJ, Baghian A, Kearney MT, Kousoulas KG. Thalidomide suppressed the growth of 4T1 cells into solid tumors in Balb/c mice in a combination therapy with the oncolytic fusogenic HSV-1 OncdSyn. Cancer Chemother Pharmacol. 2009;
4. Yehia L, Boulos F, Jabbour M, Mahfoud Z, Fakhruddin N, El-Sabban M. Expression of HIF-1α and Markers of Angiogenesis Are Not Significantly Different in Triple Negative Breast Cancer Compared to Other Breast Cancer Molecular Subtypes: Implications for Future Therapy. Rocha S, editor. PLoS One [Internet]. 2015;10:e0129356. Available from: https://dx.plos.org/10.1371/journal.pone.0129356
5. Karkkainen MJ, Petrova T V. Vascular endothelial growth factor receptors in the regulation of angiogenesis and lymphangiogenesis. Oncogene. 2000;19:5598–605.
6. Bos R, Van Diest PJ, De Jong JS, Van Der Groep P, Van Der Valk P, Van Der Wall E. Hypoxia-inducible factor-1α is associated with angiogenesis, and expression of bFGF, PDGF-BB, and EGFR in invasive breast cancer. Histopathology. 2005;46:31–6.
7. Wong CCL, Gilkes DM, Zhang H, Chen J, Wei H, Chaturvedi P, et al. Hypoxia-inducible factor 1 is a master regulator of breast cancer metastatic niche formation. Proc Natl Acad Sci U S A. 2011;108:16369–74.
8. Mohammed RAA, Ellis IO, Mahmmod AM, Hawkes EC, Green AR, Rakha EA, et al. Lymphatic and blood vessels in basal and triple-negative breast cancers: Characteristics and prognostic significance. Mod Pathol. 2011;
9. Carey LA. Directed Therapy of Subtypes of Triple-Negative Breast Cancer. Oncologist. 2010;15:49–56.
10. De Beer EL, Bottone AE, Voest EE. Doxorubicin and mechanical performance of cardiac trabeculae after acute and chronic treatment: A review. Eur J Pharmacol. 2001;415:1–11.
11. Demidenko ZN, Kalurupalle S, Hanko C, Lim CU, Broude E, Blagosklonny M V. Mechanism of G1-like arrest by low concentrations of paclitaxel: Next cell cycle p53-dependent arrest with sub G1 DNA content mediated by prolonged mitosis. Oncogene. 2008;27:4402–10.
12. Tao K, Fang M, Alroy J, Gary GG. Imagable 4T1 model for the study of late stage breast cancer. BMC Cancer. 2008;8:1–19.
13. Gao ZG, Tian L, Hu J, Park IS, Bae YH. Prevention of metastasis in a 4T1 murine breast cancer model by doxorubicin carried by folate conjugated pH sensitive polymeric micelles. J Control Release [Internet]. Elsevier B.V.; 2011;152:84–9. Available from: http://dx.doi.org/10.1016/j.jconrel.2011.01.021
14. Malekian S, Rahmati M, Sari S, Kazemimanesh M, Kheirbakhsh R, Muhammadnejad A, et al. Expression of diverse angiogenesis factor in different stages of the 4T1 tumor as a mouse model of triple-negative breast cancer. Adv Pharm Bull. 2020;
15. Malekian S, Rahmati M, Sari S, Kazemimanesh M, Kheirbakhsh R, Muhammadnejad A, et al. Expression of Diverse Angiogenesis Factor in Different Stages of the 4T1 Tumor as a Mouse Model of Triple-Negative Breast Cancer. Adv Pharm Bull. 2020;10:323–8.
16. Ignatiadis M, Sotiriou C. Understanding the molecular basis of histologic grade. Pathobiology. 2008.
17. Ghosh A, Sarkar S, Banerjee S, Behbod F, Tawfik O, McGregor D, et al. MIND model for triple-negative breast cancer in syngeneic mice for quick and sequential progression analysis of lung metastasis. PLoS One. 2018;13:1–23.
18. Ribatti D, Nico B, Ruggieri S, Tamma R, Simone G, Mangia A. Angiogenesis and Antiangiogenesis in Triple-Negative Breast cancer. Transl Oncol [Internet]. The Authors; 2016;9:453–7. Available from: http://dx.doi.org/10.1016/j.tranon.2016.07.002
19. Reguera-Nuñez E, Xu P, Chow A, Man S, Hilberg F, Kerbel RS. Therapeutic impact of Nintedanib with paclitaxel and/or a PD-L1 antibody in preclinical models of orthotopic primary or metastatic triple negative breast cancer. J Exp Clin Cancer Res. Journal of Experimental & Clinical Cancer Research; 2019;38:1–16.
20. Shetti D, Zhang B, Fan C, Mo C, Lee BH, Wei K. Low Dose of Paclitaxel Combined with XAV939 Attenuates Metastasis, Angiogenesis and Growth in Breast Cancer by Suppressing Wnt Signaling. Cells. 2019;8:892.
21. Ami N, Sato H, Hayakawa Y. Paclitaxel-induced hypothermia and hypoperfusion increase breast cancer metastasis and angiogenesis in mice. Oncol Lett. 2018;15:2330–4.
22. Bandyopadhyay A, Wang L, Agyin J, Tang Y, Lin S, Yeh I-T, et al. Doxorubicin in Combination with a Small TGFβ Inhibitor: A Potential Novel Therapy for Metastatic Breast Cancer in Mouse Models. Anderson K, editor. PLoS One. 2010;5:e10365.
23. Choi WWL, Lewis MM, Lawson D, Yin-Goen Q, Birdsong GG, Cotsonis GA, et al. Angiogenic and lymphangiogenic microvessel density in breast carcinoma: Correlation with clinicopathologic parameters and VEGF-family gene expression. Mod Pathol. 2005;
24. Gasparini G, Toi M, Gion M, Verderio P, Dittadi R, Hanatani M, et al. Prognostic significance of vascular endothelial growth factor protein in node-negative breast carcinoma. J Natl Cancer Inst. 1997;
25. Uzzan B, Nicolas P, Cucherat M, Perret GY. Microvessel Density as a Prognostic Factor in Women with Breast Cancer: A Systematic Review of the Literature and Meta-Analysis. Cancer Res. 2004.
26. Bocci G, Di Paolo A, Danesi R. The pharmacological bases of the antiangiogenic activity of paclitaxel. Angiogenesis. 2013.