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Notes

Effect of everolimus combined with anastrozole on serum FOXP 3 and MMP 9 in hormone receptor-positive elderly breast cancer patients

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Breast cancer currently leads among all the types of cancer in the total nmber of estimated cases worldwide, and holds fourth position in total number of deaths caused by cancer. It calls for early diagnosis and an effective treatment. Here, we explored the effect of everolimus combined with anastrozole treatment on the prognosis and serum FOXP 3 and matrix metalloproteinase 9 (MMP 9) in hormone receptor (HR) positive elderly breast cancer patients. A total of 96 elderly patients with HR positive breast cancer admitted to the hospital from April 2018 to April 2021 were selected and divided into two groups according to the random number table method. The control group (n=48) was given anastrozole, and the study group (n=48) was given everolimus and anastrozole. Recent efficacy, estrogen, adverse effects, survival rate, and test serum FOXP 3, MMP 9 levels were recorded. The results showed higher objective response rate (ORR) as (62.5%, 30/48) (41.67%, 20/48) (P <0.05), 1-year survival rate as (91.67%, 44/48) (75%, 36/48) (P <0.05), and stomatitis as (25%, 12/48) (8.33%, 4/48) (P <0.05). After treatment, estradiol (E2), luteinizing hormone (LH), FOXP 3, and MMP 9 were lower than before treatment (P <0.05), and the study group was lower than the control group (P <0.05). It has been concluded that everolimus and anastrozole therapy in HR positive elderly breast cancer can reduce estrogen and serum FOXP 3 and MMP 9 levels, with high 1-year survival rate, but with high incidence of stomatitis.

Keywords: Estradiol, Hormone receptor positive, Luteinizing hormone, Objective response rate, Stomatitis, Tumor

Breast cancer (BC) that mainly affects the epithelial cells from the mammary glands is the most diagnosed cancer worldwide with an estimated 2261419 cases. With the total number of 684996 deaths, it holds 4th position among all cancer types. These epidemiological figures are an issue of concern. As per the World Health Organization (WHO), the year 2020 witnessed over 19 million new cases of the disease and approximately 10 million related deaths globally[1,2]. The burden of breast cancer in China is relatively heavy, and the risk of breast cancer is significantly increasing due to obesity, alcohol addiction and other factors, which is mostly seen in middle-aged and elderly women, and has posed a threat to women's health[3]. Hormone receptor (hormone receptor, HR) positive geriatric breast cancer is estrogen receptor (ER) and progesterone receptor (PR) positive[4]. The breast is the target organ of endocrine hormones (e.g., estrogen)[5]. Endocrine hormones participate in the occurrence and progression of breast cancer. Therefore, breast cancer requires endocrine therapy. By affecting the synthesis and secretion of endocrine hormones is the final potential effective treatment of breast cancer[6].

Anastrozole (3rd aromatase inhibitor) effectively inhibits the growth of breast tumors by hindering the conversion process of androgen to estrogen to downregulate the expression level of estrogen[7]. However, due to individual differences, some breast cancer patients develop drug resistance after endocrine therapy (or anastrozole). Therefore, the drug regimen needs to be optimized continuously. Everolimus is an inhibitor of the mammalian protein of target of rapamycin (mammalian target of rapamycin, mTOR), and is mainly used in the clinical treatment of metastatic renal cell carcinoma and other diseases[8]. Everolimus blocks the PI3K/AKT/mTOR pathway and improves sensitivity to endocrine therapy, and this drug has been attempted for HR positive breast cancer failing anastrozole treatment[9]. In this study, we analyzed the effectiveness of everolimus combined with anastrozole in the treatment of HR positive elderly breast cancer.

Subjects and Methods

Ninety-six patients with HR-positive elderly breast cancer admitted to the hospital from April 2018 to April 2021 were selected and divided into two groups according to the random number table method. The control group (n =48) age (68.89 ± 4.38); 22 and 26

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TNM stages; 20 left breast and 28 right breast. Study group (n =48) age (69.32 ± 4.19); 25 TNM stages; 23 left breast and 25 right breast. P >0.05 for comparison between groups. The control group received anastrozole @ 1 mg once a day and the experimental group received anastrozole (same as the control group) + everolimus @ 10 mg once a day. This study was approved by the hospital ethics committee.

Inclusion and exclusion criteria

The inclusion criteria followed in this study was (i) HR positive; (ii) confirmed breast cancer; (iii) age >60 years; and (iv) normal communication and communication.

The exclusion criteria was (i) drug contraindications for everolimus and anastrozole; (ii) with other malignant tumors; (iii) vital organ dysfunction such as liver and kidney; (iv) cognitive impairment; and (v) neurological disorders.

Observation indicators

The efficacy was evaluated using the response assessment criteria for solid tumors, divided into complete response (CR), partial response (PR), disease stability (SD) and disease progression (PD). Objective response rate (ORR) =CR + PR.

Before and after estrogen treatment, 5 mL of fasting venous blood was taken in the morning, centrifuged and serum isolated for estradiol (estradiol, E2) and luteinizing hormone (luteinizing hormone, LH) by radioimmunoassay.

Similarly, before and after treatment with the serum Forkhead Box Protein 3 (FOXP 3) and matrix metalloproteinase 9 (MMP 9) levels, the serum FOMP 3 and MMP 9 levels were tested once. Five mL of peripheral blood was drawn from sterile fasting samples, mixed with 1 mL of red blood cell lysate, and centrifuged to prepare a single cell suspension (110^7/ mL), 100 μL of cell suspension was supplemented with 10 μL FOXP3 of antibody and incubated for 30 min, and the proportion of FOXP 3 was detected by flow cytometer (FACSVerse, BD) within the specified time. Morning fasting venous blood 5 mL, centrifuged, and serum levels of MMP 9 were measured by enzyme-linked immunosorbent assay.

The occurrence of fatigue, digestive tract reactions, stomatitis, rash and other adverse reactions were noted. The survival rate of each group was calculated by outpatient review and 1-year follow-up (follow-up inpatient examination and telephone inquiry).

Statistical analysis

Data were analyzed with SPSS 23.0. If measurement data conform to normal distribution, mean standard deviation, line t test; count data, n (%), chi-square test. Differences were significant at P <0.05.

Results and Discussion

Recent comparison of efficacy

The ORR of the study group (62.50%, 30/48) was higher than that of the control group (41.67%, 20 / 48) (P <0.05) (Table 1).

Comparison of estrogen

After treatment, E2 and LH in both the groups were lower than that of before treatment values (P <0.05), and the study group was lower than the control group (P <0.05) (Table 2).

Comparison of serum FOXP 3 and MMP 9 levels

After treatment, FOXP 3 and MMP 9 in the two groups were lower than before treatment (P <0.05), and the study group was lower than the control group (P <0.05) (Table 3).

Comparison of adverse reactions

The incidence of fatigue (10.42%, 5/48), gastrointestinal reaction (10.42%, 5/48), rash (8.33%, 10/48), (P >0.48) (20.83%, 10/48), (25%, 12/48) and (8.33%, 4/48) (χ^2=4.800, P =0.028) (Table 4).

Table 1 — Comparison of recent efficacy n (%)

Group	n	CR	PR	SD	PD	ORR
Control	48	2	18	23	5	20 (41.67)
Experimental	48	7	23	16	2	30 (62.50)
χ^2						4.174
P						0.041

[CR: Complete response; PR: Partial response; SD: Disease stability; PD: Disease progression; and ORR: Objective response rate]

Table 2 — Comparison of before- and after treatment levels of oestrogen [() $\bar{x} \pm s$]

Group	n	E2 (ng/L)		LH (U/L)	
		Before	After	Before	After
Control	48	4.11±0.72	3.12±0.46*	3.96±0.38	3.32±0.26*
Experimental	48	3.94±0.67	2.85±0.38*	3.89±0.43	2.51±0.23*
t		1.198	3.135	0.845	16.166
P		0.234	0.002	0.400	0.000

[Comparison with before treatment *P < 0.05]

Table 3 — Comparison of before - and after treatment serum levels of FOXP 3 and MMP 9. [() $\bar{x} \pm s$]

Group	n	FOXP3 (%)		MMP9 (ng/mL)	
		Before	After	Before	After
Control	48	9.14±3.04	7.36±2.43*	90.23±22.08	53.28±11.14*
Experimental	48	9.92±3.28	5.11±1.67*	87.05±20.24	40.42±10.35*
t		1.208	5.287	0.736	5.859
P		0.230	0.000	0.464	0.000

[Comparison with before treatment *P < 0.05]

Table 4 — Comparison of adverse effects [n (%)]

Group		feeble	GI reaction	stomatitis	erythra
Control group (n=48)	Level 0	38	36	44	40
	I+II level	6	2	3	3
	III+IV level	4	10	1	5
	Incidence	10 (20.83)	12 (25.00)	4 (8.33)	8 (16.67)
Study group (n=48)	Level 0	43	43	36	44
	I+II level	2	2	6	2
	III+IV level	3	3	6	2
	Incidence	5 (10.42)	5 (10.42)	12 (25.00)	4 (8.33)
χ^2/P		1.975/0.160	3.503/0.061	4.800/0.028	1.524/0.217

Survival rate

The one year survival rate was higher in the study group (91.67%, 44/48) than that in the control group (75%, 36/48) (χ^2=4.800, P =0.028).

The incidence and disease burden of breast cancer in China are increasing, and the number of new cases every year is huge which seriously affects the health of female patients. Estrogen can objectively reflect the occurrence and progression of breast cancer, combine with the estrogen receptor in the nucleus, form a complex and then directly act with the corresponding target gene transcription to promote the growth of cancer cells[10]. Therefore, endocrine therapy is often selected in the clinical treatment of breast cancer and the mechanism of inhibiting the growth of tumor cells is affecting the biological signal transduction pathway[11]. Anastrozole is effective in anti-breast cancer by hindering the conversion process of androgen to estrogen to downregulate estrogen expression levels. Literature reports[12,13], Anastrozole treatment of breast cancer can inhibit oestrogen production. Clinical practice suggests that excessive activation of the PI3K/AKT/mTOR signaling pathway may instead contribute to the development of endocrine drug resistance in breast cancer patients[14]. Therefore, the direct action of the PI3K/AKT/mTOR signaling pathway was considered to improve the sensitivity to endocrine therapy. Everolimus belongs to the mTOR inhibitor, which can activate the mTOR pathway and participate in the regulation of tumor cell growth, proliferation, and differentiation.

In this study, the ORR of the study group (62.50%, 30/48) was higher than that of the control group (41.67%, 20/48) (P <0.05). It shows that the recent effect of everolimus combined with anastrozole in the treatment of HR positive elderly breast cancer is positive. Everolimus, an oral mTOR inhibitor enters the body, combines intracellular FK506BP12, forms a complex and then acts on mTOR sites to block mTOR generation, interleukin receptor transduction process, T cell generation process, inhibit tumor growth, and cooperate with endocrine therapy (or anastrozole used in this study)[15].

The growth and development of the breast requires the coordination of a variety of hormones (especially estrogen)[16]. Studies have shown that breast cancer development and progression are related to endocrine disorders[17]. For example, estrogen hypersecretion can promote mammary gland epithelial cells, fibrous tissue hyperplasia. It also suggests that it is necessary to adopt the endocrine therapy scheme for clinical treatment of breast cancer. Endocrine therapy can inhibit androgen pro-estrogen conversion through different mechanisms to downregulate estrogen expression levels[10]. Animal experiments have shown that the tumor growth and proliferation of HR-positive elderly breast cancer is affected by hormones, especially E2, which has a carcinogenic effect and can induce breast cancer[18]. LH can also stimulate the ovary, promote estrogen secretion, release and ovulation, follicle rupture, accordingly can form corpus luteum, and then secrete estrogen[19]. Table 2 shows the changes of E2 and LH before and after treatment. The results showed that after treatment, E2 and LH were lower than before treatment (P <0.05) and the study group was lower than the control group (P <0.05). It shows that everolimus combined with anastrozole in HR positive elderly breast cancer can reduce estrogen. This is because anastrozole can cause aromatase inactivation, affect the aromatization reaction, hinder estrogen secretion, combined with everolimus, can continuously reduce estrogen.

FOXP 3 is a characteristic molecular marker of HR-positive breast cancer[20]. MMP 9 is a common subtype of matrix metalloproteinase and has been shown to be associated with invasion and invasion in a variety of malignancies (including HR positive elderly breast cancer in this study)[21]. Table 3 shows that after treatment, FOXP 3 and MMP 9 were lower than before treatment (P <0.05), and the study group was lower than the control group (P <0.05). It indicated that everolimus combined with anastrozole in treating HR-positive elderly breast cancer could reduce the serum levels of FOXP 3 and MMP 9.

For adverse reactions, the incidence of stomatitis in the study group (25%, 12/48) was higher than that in the control group (8.33%, 4/48) (P <0.05). Thus, everolimus combined with anastrozole for HR positive elderly breast cancer has a high incidence of

stomatitis. Consideration was related to the dose of everolimus and anastrozole. It is recommended that the dosage should be adjusted in case of drug-related adverse reactions, or when patients have intolerance, it is recommended to stop using and replace the drug immediately to ensure the safety of medication and prevent the occurrence of adverse reactions.

In addition, the one year survival rate of the study group (91.67%, 44/48) was higher than that of the control group (75.00%, 36/48) (χ^2=4.800, P =0.028). It is suggested that everolimus combined with anastrozole improves the 1-year survival rate in elderly patients with HR positive breast cancer. This is because everolimus combined with anastrozole is slightly more effective than anastrozole alone, providing a survival benefit for HR-positive elderly breast cancer patients.

Conclusion

The recent effect of everolimus and anastrozole in HR positive elderly breast cancer can reduce estrogen and serum FOXP 3 and MMP 9 levels, with a high one-year survival rate, but a high incidence of stomatitis. This study has the limitations of small sample size (the number of HR positive elderly breast cancer cases in each group is only 48) and short follow-up time (1 year follow-up). Further, it requires comprehensive analysis of these findings verified with expanded sample size and extended follow-up.

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