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Full Length Research Paper

Immuno-stabilizing effect of thymosin-alpha-1 on post-modified radical mastectomy (MRM) of breast cancer patients

Danyang Dou[1], Wanjun Tao[1], Lingling Li[1], Tingjian Jia[1], Wings TY Loo[2,3,4]*, Mary NB Cheung[3,4], Louis WC Chow[3], Yiding Dou[4] and Ziyuan Luo[5]

[1]Chengdu Family Planning Guidance Institute, Qingyang District, Chengdu, 610031, Sichuan Province, P. R. China.
[2]School of Chinese Medicine, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, P. R. China.
[3]UNIMED Medical Institute, Hng Kong.
[4]Jin Hua Dentistry, Chengdu, 610041, Sichuan Province, P. R. China.
[5]Department of Stomatology, Medical College of Jinan University, Guang Zhou, China.

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Thymosin-alpha-1 (Tα1), a synthetic thymus hormone, can increase the production of T cells. It may counteract the suppressed immunity of chemotherapy patients, thus restoring normal immune mechanism. This study aims to investigate the effect of thymosin-alpha-1 on the post-operation conditions of patients who have received modified radical mastectomy (MRM). Thirty-six consented patients with invasive breast ductal carcinoma (IDC) who were to receive MRM were randomly divided into two groups. One received hormonal therapy and the other received chemotherapy (four cycles of 5-fluorouracil, epirubicin and cyclophosphamide (FEC)) at least three weeks before surgery. Tα1 was administered in both groups, one day before surgery till day seven after surgery. Post-surgical inflammatory and metabolic responses (heart rate, wound area, body temperature, pain level, wound drainage and cytokine levels) were analyzed. Amount of drainage and cytokine levels did not show any significant difference between the groups. Temperature on Day 2 and the overall pain levels of the hormonal therapy group were significantly higher than that in the chemotherapy group. There was no significant difference in wound areas or heart rates between the two groups. Tα1 normalizes immune response parameters among chemotherapy patients to close-to-normal levels, while at the same time significantly depress level of pain, as compared to hormonal therapy-treated patients. Amount of drainage, heart rate and cytokine levels do not show any significant difference between the groups, indicating that Tα1 has normalized the metabolic and immune response of the chemotherapy patients.

Key words: Thymosin-alpha-1, invasive ductal carcinoma, modified radical mastectomy, adjuvant chemotherapy, adjuvant hormonal therapy.

INTRODUCTION

Breast cancer is the third most prevalent cancer of the world and the most common malignancy of females. In Hong Kong, it ranked first in age-standardized incidence rates for cancer (Garaci et al., 2000) and third highest mortality of all malignant neoplasms in females in 2005 (Law and Mang, 2007), and its age-specific death rate generally increased with age.

Invasive ductal carcinoma is characterized microscopically by thick strands and tubular structure. Clinically, it is stony hard on palpation, and is likely to metastasize to bone marrow, brain, lung plura and liver.

Mastectomy is usually considered in invasive breast ductal carcinoma (IDC) patients. There are a few forms to choose from: simple mastectomy involves complete removal of breast tissues without removing any lymph

*Corresponding author. E-mail: wtyloo@gmail.com. Tel: 852-9074-9468. Fax: 852-2861-1386.

nodes. Radical mastectomy is the complete removal of the breast, the overlying skin, the pectoralis muscles and all axillary and supraclavicular lymph nodes. Modified radical mastectomy (MRM) involves the removal of breast tissue, the underlying fascia of the pectoralis major muscle and some of the axillary lymph nodes, but leaves the pectoralis major intact (Borst and Ingold, 1993). The nipple-areola complex and the area around the biopsy incision are removed, but the remainder of the skin of the breast can be preserved. Total mastectomy removes the entire breast. Both pectoralis muscles and the axillary nodes are preserved.

Among the many choices, MRM is most frequently chosen for IDC patients. A study showed that immediate post-operative complications, e.g. seromas, wound infection and flap necrosis, was found to be considerably fewer following MRM. There was also a significantly lower incidence of late complications, such as limb edema and recurrent infection (Harris, 2005).

The prognosis of breast cancer is rather good, as represented by the estimated survival rate of 73% in developed countries. This is attributed by the effective treatments of cancer, for example, surgical removal of the tumour, chemotherapy and hormonal therapy (Feigenberg et al., 1977).

In breast carcinomas, the epithelium of breast ducts is sensitive to oestrogen and progesterone. The patient is sensitive to hormonal therapy if detectable levels of intracellular protein oestrogen receptor (ER) or progesterone receptor (PgR) are present. Hormonal therapy reduces circulating oestrogen or progesterone levels to reduce proliferation of cancer cells and promote apoptosis. Adjuvant hormonal therapy would lead to tumour shrinkage for easier MRM. Previous studies have concluded that administering tamoxifen, a selective oestrogen receptor modulator, would decrease incidence of contralateral breast cancer and disease recurrence, as well as increase disease-free survival (DFS) in both premenopausal and postmenopausal female (Parkin et al., 2005). It also provides an overall survival (OS) advantage in both premenopausal and postmenopausal female (Constantino and Fisher, 2005). Side effects include hot flushes, vaginal discharge and irregular menses (Zaire, 2005).

Letrozole (Femara), a non-steroidal aromatase inhibitor, is another first-line drug used in hormonal therapy. It is documented to have better-tolerated side effects, including hot flushes, nausea and fatigue, as compared to tamoxifen. This is due to the fact that letrozole is not cytotoxic towards healthy cells.

On the molecular level of a normal human body, oestrogens are produced from converting androgens by aromatase enzyme. Letrozole competitively and reversibly inhibit the aromatase enzyme, thus the peripheral conversion of adrenally synthesized androstenedione to estradiol is blocked (Powles, 2005). Letrozole significantly decreases estrogen production in tissues, such as liver, muscle and fat (Winters et al., 2007). It was shown to result in longer disease-free survival (Chung and Clarson, 2003) and result in fewer relapses (Monnier, 2007) than tamoxifen in several Phase III clinical trials.

Letrozole has been used along with MRM in IDC patients as an adjuvant therapy, and was shown to be superior to tamoxifen (Mauriac et al., 2007). It was also used in neo-adjuvant therapy, in which letrozole was administered pre-operatively. In a study, aromatase inhibitors were shown to be able to reduce tumour volume, and were more effective than tamoxifen as a neo-adjuvant (Dixon, 2004).

On the other hand, chemotherapy is applied on most patients who have received MRM. The combined regimen of 5-fluorouracil, epirubicin and cyclophosphamide (FEC) is commonly used for MRM patients. It was shown to be able to significantly improve the five-year disease free survival in breast cancer patients (Paradiso et al., 2001). These drugs are cytotoxic, and work principally by targeting cancer cells that are fast-dividing. 5-fluorouracil is an anti-metabolite which targets the cell cycle, causing accumulation of cancer cells, specifically in the S-phase. Rate of overall DNA replication is reduced, resulting in shrinkage of the tumour (Robinson et al., 2006). Epirubicin belongs to the anthracycline group, also a strong inhibitor of DNA and RNA synthesis. It produces radicals for apoptosis and mediates DNA cleavage (Sinha and Politi, 1990). Among the many anthracyclines drugs in which most are cardiotoxic, epirubicin is less cardiotoxic and myelotoxic (Conte et al., 2000; Robert, 2007). Cyclophosphamide is a nitrogen mustard alkylating agent. In contrast to the specificity of 5-FU and epirubicin in the cycle, cyclophosphamide is cell cycle phase non-specific and generally more toxic. It also has a higher penetrating power into tumours when compared with the former two (Mayer and Burstein, 2007).

A combined regimen of FEC provides a multi-lateral targeting of cancer cells at various stages of the cell cycle and DNA replication. However, healthy cells that also undergo mitosis continuously are also targeted, for example, bone marrow cells, intestinal epithelium and hair follicular cells. Its non-specific targeting causes the notorious side effects, such as fatigue, nausea, vomiting, diarrhea, hair loss and neutropenia. Some of them are due to a deficient immune system caused by the drugs, and are believed to be lessened if the immune system is restored.

Patients use different methods to minimize side effects of chemotherapy, for example, traditional Chinese medicine against nausea and vomiting (Vogel et al., 2005), or neupogen or neulasta to raise neutrophil count (Garaci et al., 1997). More recent studies revealed that thymosin-alpha-1 was highly effective in restoring cytotoxic activities in immunosuppressed patients who were treated with chemotherapy (Tedaldi, 2005).

Thymosin-alpha-1 (Tα1) is a peptide hormone produced by the thymus. It was found to be able to augment host immune mechanisms by increasing production and activity of T cells and natural killer cells, assisting B cell development into plasma cells and thus lead to antibody production. It has been used in many fields of medicine, such as assisting in treatment of viral infections (Andreone et al., 2001), and enhancing flu vaccines in the elderly (Saki and Ajit, 2002), modulating haematopoietic functions of bone marrow cells (Malinda et al., 1998), speeding up wound healing and angiogenesis (Silecchia et al., 1999), etc. A study showed chemo-immunotherapy regimen increased the average survival time as compared to chemotherapy alone (Salvati et al., 1996). It was also shown that white blood cell count remained the same in chemo-immunotherapy treated patients, while chemotherapy-treated patients had significantly depressed CD4+, CD8+ and natural killer (NK) cell counts.

In contrast to the immunosuppressive nature of chemotherapy, only rarely do letrozole therapy report side effects of deprived immune system, e.g. leucopenia (Demark-Wahnefried et al., 1997). Compared with patients treated with FEC, hormonal therapy-treated patients would have higher white cell count and better immunity; therefore, the effect of Tα1 on these patients may be less prominent. In contrast, chemotherapy, due to its cytotoxic nature, damages many cells that require continuous regeneration, e.g. white blood cells, causing neutropenia. Using Tα1 in chemotherapy patients would hopefully increase the white cell count, thus improve immune response by its stimulatory effect on T cells.

In this study, we aim to discuss the effect of Tα1 on the immune system and post-operative effects of patients receiving hormonal therapy and chemotherapy.

MATERIALS AND METHODS

Patient selection

36 patients with invasive ductal carcinoma were recruited. The experiment protocol was first approved by the Ethics Committee of Hong Kong Hospital Authority. Written consent was obtained from all patients. The data for the status of estrogen receptor (ER), progesterone receptor (PR) and HER-2 and the lymphovascular permeation and histological grading of tumour differentiation are shown in Table 1.

Inclusion criteria

Female patients diagnosed with invasive ductal breast carcinoma were included. All required either unilateral or bilateral MRM. They should be free of distant metastasis at the time of inclusion and patients were screened to confirm normal findings.

Drug administration

Recruited patients are divided into two groups randomly. They were all hospitalized before any drugs were administered. Group 1 patients (n = 18) received hormonal therapy (2.5 mg oral femara daily) before, during and after MRM. Group 2 patients (n = 18) received four cycles of chemotherapy (500 mg/m^2 5-fluorouracil, 100 mg/m^2 epirubicin and 500 mg/m^2 cyclophosphamide) four weeks before surgery. Tα1 was administered (1.6 mg subcutaneous) to all patients daily from the day before scheduled MRM till 10 days afterwards.

Two patients from Group 1 and one patient from Group 2 had bilateral MRM. Each of these patient's data were regarded as two separate cases with different wound area, but values of the measured parameters.

Parameters measured

All patients were hospitalized and body temperature was measured and recorded before and after administering the first dose of Tα1. On the day of operation, pre-operation body temperature, pain level and heart rate were measured. After MRM but before suturing, the wound area was measured. A drainage tubing was inserted into the wound after suturing, allowing wound drainage to pass into a bottle. Post-operation body temperature and heart rate were measured. In the following weeks, temperature and heart rate was taken on days 1, 2 and 5; pain levels were ranked and wound drainage was measured from day 1 to 10. Several biochemical parameters were also measured and recorded, for example, complete blood picture (CBP), cytokine levels, etc.

Pain levels were ranked in a 0 to 10 scale, with 0 meaning no pain; 10 meaning the highest level of pain. Wound drainage is collected by a tube passing drainage from the wound to a column (Table 3).

Statistical analysis

Independent-samples t-test was used to test whether there was any statistical difference in the wound areas of groups 1 and 2. One-way ANOVA (SPSS 15.0, USA) was then used to analyze the average value of all parameters between the groups, including body temperature, pain level, amount of wound drainage and heart rate. Some of the parameters were measured over several days, therefore two sets of ANOVA analyses were done, one with each day separately analyzed, another with all data of that parameter pooled and compared with the other group.

RESULTS

There is no statistical difference between the wound areas of two groups. One-way ANOVA results show no statistical difference between the amounts of wound drainage. The same applies to heart rate, CBP and cytokine levels. Daily body temperature also showed no significant difference, except on Day 2. Table 2 shows the significance of daily body temperature.

DISCUSSION

Breast cancers are divided into invasive and non-invasive types. Non-invasive lesions are limited to the basement membrane, and can be classified as ductal carcinoma *in situ* and lobular carcinoma *in situ*. Invasive breast cancers

Table 1. Clinicopathological characteristics of 36 patients.

Mean age	**49.9 (range: 29 - 73)**
Menopausal status	
Premenopausal	20
Postmenopausal	16
Histological nodal status	
Negative	19
Positive	17
Tumor size	
≤ 2 cm (T1)	20
2 – 5 cm (T2)	10
> 5 cm (T3)	6
Histology	
Ductal	33
Lobular	3
Grading	
G1	9
G2	20
G3	7
Lymphovascular permeation	
Absence	16
Presence	20
ER status	
Negative	3
Positive	33
PR status	
Negative	8
Positive	28
Her2/neu status	
Negative	10
Positive	26

are classified by histological pattern. Epithelial tumours are most common, including intraductal papillomas, tubular carcinoma, etc. Among the many, invasive ductal carcinoma (IDC) is by far the most common type, and is responsible for around 75% of all malignant neoplasms of the breast.

Our study demonstrates that, when administered Tα1, patients under chemotherapy had similar physical and immune parameters as patients under hormonal therapy. Both groups of patients had similar heart rate, body temperature, amounts of wound drainage, CBP and cytokine levels.

The similarity in heart rates across the two groups suggests that the reduced resting metabolic rate (RMR) in patients under chemotherapy was compensated by the administration of Tα1. Normally, the cytotoxicity of chemotherapy drugs would damage the immune system by killing fast-dividing bone marrow cells, disabling adequate replenishing of healthy cells. This compromises the metabolic rate, which is a function of active cellular activities. A study showed that breast cancer patients receiving chemotherapy have lower RMR (Inoue et al., 2003), which in turn causes slower heart rate. However, when Tα1 is administered, it stimulates the patient's T cells and restores normal immune response. Thus, patients under chemotherapy would benefit from the

Table 2. Significance of daily body temperature in Groups 1 and 2 patients.

Body temperature (Daily)		Mean
Pre-administration of TA1	Group 1	36.3308
	Group 2	36.2800
Post-administration of TA2	Group 1	36.3692
	Group 2	36.1733
Post-operation	Group 1	36.5000
	Group 2	36.5462
Day 1	Group 1	36.9923
	Group 2	36.7067
Day 2	Group 1	36.7077*
	Group 2	36.2600
Day 5	Group 1	36.2769
	Group 2	36.0867

*P < 0.05.

Table 3. Significance of daily pain levels in Groups 1 and 2 patients.

Pain (Daily)		Mean
Post-operation	Group 1	6.1250*
	Group 2	2.9091
Day 1	Group 1	5.3750*
	Group 2	2.9167
Day 2	Group 1	5.0000*
	Group 2	2.5833
Day 3	Group 1	4.8750*
	Group 2	2.0833
Day 4	Group 1	4.1250*
	Group 2	1.7500
Day 5	Group 1	3.5000*
	Group 2	1.5833
Day 6	Group 1	1.8750
	Group 2	1.2500
Day 7	Group 1	0.7143
	Group 2	1.0833

*P < 0.05.

addition of Tα1. On the other hand, patients who received hormonal therapy were not exposed to any chemotherapy drugs. Therefore, their immune system was relatively normal, and did not have much benefit from Tα1.

The two groups of patients did not show any significant difference in daily body temperature either, except on Day 2. Normally, if there is an infection or trauma at the surgical site, the body temperature of a post-operative patient would peak on the day of the operation, and gradually return to normal as the surgical wound heals. However, as the results of this study showed, body temperature taken on Day 2 was significantly higher in hormonal therapy-treated patients than in chemotherapy-treated patients. This is an anomaly to be explained by future research.

The amount of wound drainage was not significantly different between the two groups of patients treated with Tα1. The amount of wound drainage signifies the trauma and efficiency of the healing. Past experiments show that, without Tα1, chemotherapy patients usually have more wound drainage, thus slower wound repair. This is because cytotoxic chemotherapy drugs prevent fibroblast proliferation, collagen synthesis and deposition of collagen at the surgical area, delaying the healing process (Bland et al., 1984; Joseph and Levine, 2006). Since two groups of patients had similar amounts of wound drainage, we can deduce that the repair mechanism destroyed by cytotoxic drugs (FEC) was compensated for by Tα1. This finding is supported by a study which concluded the effect of Tα1 on endothelial cell migration, angiogenesis and wound healing (Silecchia et al., 1999).

Furthermore, we demonstrate here that, when given Tα1, chemotherapy-treated patients experience less pain at the wound as compared to hormone therapy-treated patients. Patients under chemotherapy may have their cytokine and prostaglandin levels disturbed, causing a lower sensation of pain. A recent study also reported that some chemotherapy drugs would attenuate TNF-α-induced hyperalgesia (Romani et al., 2007). In other words, patients under chemotherapy would benefit from its anti-cancer effect, as well as experience a lower level of pain. As for the chemotherapy-induced side effects, such as neutropenia, they could be resolved by administering Tα1, which stimulates T cells proliferation and minimize the effect of chemotherapy on the patient's metabolism. This allowed patients to receive a better-tolerated chemotherapy, at the same time bearing less pain.

In general, Tα1 benefits immunosuppressed patients, e.g. chemotherapy-treated patients, by working as an endogenous regulator of immune homeostasis (Briggs and Closs, 1999). Its benefits are less pronounced in immunocompetent patients, as in hormonal therapy-treated patients in this study, as their immune response is already close to standard level.

A few limitations should be noted. First, this study lacked a control group of patients who were not administered Tα1. The only control group used in this study was the group under hormonal therapy plus Tα1, who were assumed to be immunocompetent. Further studies regarding the effects of Tα1 should include a control group which could give a more reliable conclusion about the effect of Tα1.

Secondly, the sample size of this study was somewhat small, with only thirty-six patients recruited. This small sample size might not be able to detect subtle differences of certain parameters between the groups. Future studies should include more patients in order to reach a more reliable conclusion.

Thirdly, pain sensation is affected by the patient's pain tolerance, and is only a subjective, qualitative and relative measurement. In this study, a ranking scale of 1 to 10 was used. To improve reliability of the analysis of patient's pain level, the following pain scales can be adapted in future studies: faces rating scale (using facial expression to suggest pain intensities), functional pain scale (a standardized test equating patient's tolerability of pain and the impact on function, scored 0 to 5), graphic rating scale (an analogue scale by adding numbers or words between the extremes of the scale), etc (Melzack, 1975).

To summarize, Tα1 allows patients to receive chemotherapy without experiencing great pain and side effects that result from a depressed immune system. This study points us in a direction to achieve a better tolerated chemotherapy, especially if drug-induced or tumour-induced immune deficiency can be contained.

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