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Pro-inflammatory cytokine, matrix metalloproteinases and TIMP-1 are involved in wound healing after mastectomy in invasive breast cancer patients

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Abstract

Background: Mastectomy provides a good model to study the wound healing process after surgery. The involved factors include selectins, pro-inflammatory cytokines such as interleukin 6 (IL-6) and tumor necrosis factor-α (TNF-α), matrix metalloproteinases (MMPs), and their natural inhibitors (TIMPs). In the present study, we observed the kinetic changes of these factors in the process of wound healing after a mastectomy, and analyzed the relationship between these factors and the clinical outcomes.
Materials and methods: Twenty-nine patients, who received a modified radical mastectomy, were recruited to this study. The wound was inspected daily for the presence of flap necrosis, infection and seroma. Drain fluid was collected on days 1, 2 and 5. IL-6, P-selectin, MMP-2, 3, 9 and TIMP-1 were screened by ELISA kits for their impacts on wound healing after mastectomy.
Results: IL-6 demonstrated the highest level on Day 1 after operation and was negatively correlated with MMP-2, which in turn showed a consistently negative correlation with MMP-9 for days 1, 2 and 5. Incidences of seroma formation and skin flap necrosis were 27.6% and 20.7%, respectively. Seroma formation was associated with low MMP-2 levels on Day 5. While skin flap necrosis seemed to correlate with high MMP-2 levels and low levels of MMP-9 and TIMP-1.
Conclusion: IL-6, P-selectin, MMP-2, MMP-3, MMP-9 and TIMP-1 are important in the process of wound healing after mastectomy. A low MMP-2 level correlates with the formation of seroma, while MMP-2, MMP-9 and TIMP-1 are associated with skin flap necrosis.

Keywords: Interleukin 6; P-selectin; Matrix metalloproteinases; Flap necrosis; Seroma

1. Introduction

Wound healing after surgical operation is a complicated and continuous process [1]. The involved factors include selectins, pro-inflammatory cytokines such as interleukin 6 (IL-6) and tumor necrosis factor-α (TNF-α), matrix metalloproteinases (MMPs), and their natural inhibitors (TIMPs). Selectins are the cell surface lectins that mediate the adhesion of white blood cells to endothelial cells and platelets [2]. They promote the cytotoxicity of these cells [2,3]. P-selectin expression difference is observed between normal skin and injured skin [3].

Cytokines are released during the process of wound healing by the leukocytes extravasating out of the blood vessel [4–6]. They are crucial in initiating, controlling, and terminating the cellular events of wound healing [4–6]. These cellular events are responsible for the degradation of extracellular matrix, migration and proliferation of cells, angiogenesis, and matrix remodelling [1]. Cytokines can induce the production of proteases, elastases and MMPs [7,8]. An excess of TNF-α leads to damage of wound healing, resulting in a chronic wound [4]. Its effect can be blocked by a specific TNF antagonist (TNF binding protein) [4,8–10]. The expression levels of IL-6 are usually elevated during acute inflammation and trauma reflecting the degree or extent of inflammation [11].

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MMPs can digest collagen, gelatin and proteoglycans and are inhibited by natural tissue inhibitors, such as TIMP-1, TIMP-2 and membrane type inhibitors (MP-TIMP) [12]. The levels and duration of MMPs and TIMPs are tightly regulated in the whole process of acute wound healing [13–15]. An imbalance in the activity between MMPs and TIMPs can cause a chronic wound healing and even a systemic inflammatory response syndrome [1]. Many MMPs, such as MMP-3, MMP-8, MMP-9 and MMP-13, play active roles in wound healing [1,13,16–18].

MMPs are intimately involved in the wound healing process being primarily responsible for the degradation of various extracellular matrix components over the course of wound healing. Also known as matrixins, they are typically classified into five groups based on their substrate specificities. Though MMPs have been studied extensively and are well represented in the literature, many aspects of their exact interactions and functions over the full course of wound healing are still unknown. The current study attempts to shed some light on this by measuring and correlating levels of these chemicals amongst themselves and against various clinical parameters postmastectomy.

Mastectomy does not disturb internal organ functions and, therefore, any bodily response is related to the surgical operation. Therefore, mastectomy provides a good model to study the wound healing process after surgery. In the present study, we observed the kinetic changes of these factors in the process of wound healing after a mastectomy, and analyzed the relationship between these factors and the clinical outcomes.

2. Materials and methods

2.1. Subject selection and collection of drain fluid

The 29 patients recruited were diagnosed of invasive breast cancer with their consent obtained. The mean age was 49, ranging from 29 to 68 years. All of the patients underwent a modified radical mastectomy. None of the patients received either adjuvant chemotherapy before mastectomy or immediate breast reconstruction after mastectomy. The operation involved the total removal of the breast and axillary dissection up to the level II lymph nodal station. Raising of the flaps and tissue dissection during mastectomy was performed by electro-cautery. Cautery settings were set at 20–30 Volts and only coagulation was used. Lateral skin margin of at least 2 cm from the tumor was taken. Closed suction drains were placed at the chest and axilla after the mastectomy.

Drain fluid (20 ml) was collected at 6 am on days 1, 2 and 5 by standard aseptic techniques. The wound was inspected daily and any development of flap necrosis, infection and blister formation was recorded.

2.2. Measurement of IL-6, P-selectin, MMPs and TIMP-1

The drain fluid samples were centrifuged to remove turbidity and then stored at 80 °C for further measuring IL-6, P-selectin (Roche, Germany; and Human Biotrak, USA), MMP-1, MMP-2, MMP-3, MMP-9 and TIMP-1 (Calbiochem, USA).

100 μl of sample was pipetted to designated wells. The plate was incubated and rinsed with washing buffer followed by addition of TMB substrate. The plate was ultimately read by Micro-plate Reader (Tecan, Austria) at 450 nm.

2.3. Statistical analysis

SPSS (12.0, USA) was used to compare the number of events between groups. Pearson's correlation was performed to determine correlations between the wound healing factors studied. $P < 0.05$ was considered as statistically significant.

3. Results

3.1. Clinical parameters

Four patients (13.8%) developed postoperative fever of over 38 °C. Flap necrosis was observed in six patients (20.7%). Of these, four patients (13.8%) had mild necrosis – extent of less than 1 cm from the wound edge. The remaining two patients had more extensive necrosis. One patient (3.4%) developed wound infection from *Staphylococcus aureus*. Seroma was observed in eight patients, giving an overall incidence of 27.6%. The development of seroma did not show any relationship with the age of patient, specimen weight, tumor size, or number of nodes harvested.

3.2. The level of IL-6

The mean value of IL-6 on days 1, 2 and 5 was 800.6 pg/ml, 707.3 pg/ml and 475.1 pg/ml, respectively. In comparison, the drain fluid level of IL-6 on Day 1 and Day 5 was statistically significant ($P < 0.05$) than Day 1 and Day 2 ($P < 0.05$).

3.3. Relationships between P-selectin, MMPs and TMP-1

The levels of all parameters in wound fluid samples were listed in Table 1. P-selectin level was highest in Day 2 wound fluid samples ($P < 0.001$). The levels of MMP-9 and TIMP-1 showed progressive drop from Day 1 after surgery ($P = 0.039$ and $P = 0.012$ respectively). The levels of MMP-1 and MMP-2 did not show any significant variation after operation. There was a marked rise of MMP-3 level 24 h after surgery ($P < 0.001$).

Table 1
Interval changes of the wound healing-related parameters in wound fluid samples after mastectomy (*Pearson correlation*)

	Day 1(pg/ml)	Day 2(pg/ml)	Day 5(pg/ml)	*P* value
P-selectin*	31.6 (1.0)	34.2 (1.0)	30.3 (1.0)	<0.001
MMP-1	17.2 (3.0)	10.6 (3.4)	15.0 (4.7)	0.611
MMP-2	61.0 (1.8)	63.1 (1.7)	62.5 (2.1)	0.728
MMP-3*	0.6 (0.2)	11.3 (2.0)	7.7 (1.6)	<0.001
MMP-9*	190 (8.7)	182 (8.0)	161 (7.4)	0.039
TIMP-1*	198 (2.4)	181 (4.7)	133 (5.3)	0.012

*Statistically significant ($P < 0.05$).