MACROPHAGE BETWEEN THE FIRST AND THIRD DAY OF CLOSED FEMORAL FRACTURE

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ABSTRACT

The pathobiologic changes between first and third day after fracture is not adequately understood. Pathobiology concerns about the changes of balance to imbalance condition before symptoms and signs of disease manifest. The research’s aims were to know the difference of IL-1β and IL-10 - expressing macrophage between the first and third day after fracture. This study is a cross sectional study of 40 closed femoral fractures. We examined 20 patients which internal fixation was done on the first day and 20 patients which internal fixation was done on the third day. We examined the soft tissue biopsy (that was taken during internal fixation) for immunohistochemistry staining using IL-1β and IL-10 monoclonal antibody to see macrophage activity in expressing IL-1β and IL-10. The result showed that there was a significant lower level of IL-1β - expressing macrophage at the first day than the third day (2.37±2.98 %, vs 4.99±4.89 %, p<0.05) but no statistically difference on IL-10- expressing macrophage. From this study, can be concluded that there is lower local macrophage inflammatory reaction in the first day compare to the third day after fracture.

Keywords: IL-1β –expressing macrophage, IL-10 –expressing macrophage, pathobiology

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INTRODUCTION

Fracture or trauma is one of stress manifestation of human being. There are many coping mechanism to maintain the homeostasis. Pathobiology concerns about the changes of balance to imbalance condition before symptoms and signs of disease manifest. The changes of macrophage activity after fracture especially at the first day and the third day after fracture (when the internal fixation is done) is very important because it will influence the inflammatory response after internal fixation.

Internal fixation is one of modalities in fracture treatment. According to STEER (Succint and Timely Evaluated Evidence Review) analysis, early internal fixation is clinically more beneficial than the delayed one (Dent 2002, Pallister dan Empson 2005). Recently, that early and delayed procedure is still under debatable issues especially concerning early total care, damage control and delayed total care.

Johnson (1985) reported that an internal fixation on a major fracture with a delay more than 24 hours would cause a five times increase in occurrence of ARDS (Adult Respiratory Response Syndrome) as a complication. On an isolated femoral fracture, a 10% incidence of fat embolism syndrome will occur if the fixation was delayed after 10 hours and 0% if it was done before that (Pinney 1998). Other researcher reported that pneumonia incidence in first day on femoral fracture was 10%, however in delayed one was 38 %. This rate increased if associated with chest trauma unto 14% in first day and 48% in delayed one (Charash 1994).

These facts are due to innate immunity activation which is in synergy with the lost of tissue barrier function (Hietbrink 2006). The innate immunity in the form of systemic inflammatory response if it prolongs and occurs excessively, it will cause a Systemic Inflammatory Response Syndrome (SIRS). However, up to now the difference of local pathobiology changes between first and third day after fracture are not adequately understood.

Macrophage is a primary immune cell which produces local cytokine in tissue and in severe trauma macrophage often suffers alteration in cellular immune response (Franke 2006). With the difference in timing for internal fixation, the early or the delayed one, there are pathobiologic changes in macrophage micro environment which cause alteration on macrophage activation. Macrophage activation needs contact between ligand and receptor (Stout and Suttles 1997). Surgeon, while doing internal fixation will manipulate
tissue, therefore creating more tissue damage. This condition will induce the pre-activated macrophage to express inflammatory mediators which give influence on either local inflammatory response or systemic.

**MATERIALS AND METHODS**

This was a cross sectional study (Suryabrata 2000). Tissue biopsy was done at the time of internal fixation. We examined 40 patients with closed simple femoral fracture, 20 patients internal fixation were done at the first day and 20 patient at third day. We examined IL-1β-expressing macrophage. It is the percentage of IL-1β-expressing macrophage (number of IL-1β-expressing macrophage in 100 macrophage cells) which is counted from soft tissue biopsy surround fracture site which is taken during surgery and stained with immunohistochemistry staining using monoclonal antibody against IL-1β. We also examine IL-10-expressing macrophage. It is the percentage of IL-10-expressing macrophage (number of IL-10-expressing macrophage in 100 macrophage cells) which is counted from soft tissue biopsy surround fracture site which is taken during surgery and stained with immunohistochemistry staining using monoclonal antibody against IL-10.

**RESULTS**

There was a significant difference between IL-1β-expressing macrophage in the first day group and delayed one (mean 2.37% vs 4.99%, p=0.047). However there was no significant difference seen between IL-10-expressing macrophage in the first day group and delayed one (mean 3.04±3.35 vs 5.41±6.07%,p=0.135) (Table 2).

<table>
<thead>
<tr>
<th>Table 1. Subject Characteristic</th>
<th>First day group</th>
<th>Third day group</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristic</td>
<td>(n=20) (Mean±SD)</td>
<td>(n=20) (Mean±SD)</td>
<td>(n=20) (Mean±SD)</td>
</tr>
<tr>
<td>Sex:</td>
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<tr>
<td>M</td>
<td>14</td>
<td>14</td>
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<tr>
<td>F</td>
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<tr>
<td>Age (year)</td>
<td>27.8±11.47</td>
<td>25.2±10.59</td>
<td>0.461</td>
</tr>
<tr>
<td>Causes</td>
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<td>18</td>
<td>0.349</td>
</tr>
<tr>
<td>1. Traffic accident</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2. Fall from tree</td>
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<td>1</td>
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<td>3. Household accident</td>
<td>1</td>
<td>1</td>
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<td>4. etc</td>
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<tr>
<td>Diagnosis of femoral fracture:</td>
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<tr>
<td>Fracture’s line</td>
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</tr>
<tr>
<td>1. Simple tranverse/oblique</td>
<td>12</td>
<td>17</td>
<td>0.078</td>
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<tr>
<td>2. Butterfly</td>
<td>8</td>
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<tr>
<td>Hgb. Pre-internal fixation( g%)</td>
<td>12.91±1.92</td>
<td>11.8±1.28</td>
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</table>

<table>
<thead>
<tr>
<th>Table 2. IL-1β - expressing macrophage and IL-10- expressing</th>
<th>Early day group</th>
<th>Delayed day group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristic</td>
<td>(n=20) (Mean±SD)</td>
<td>(n=20) (Mean±SD)</td>
</tr>
<tr>
<td>IL-1β - expressing macrophage (%)</td>
<td>2.37±2.98</td>
<td>4.99±4.89</td>
</tr>
<tr>
<td>IL-10 - expressing macrophage (%)</td>
<td>3.04±3.35</td>
<td>5.41±6.07</td>
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Figure 1
IL-1β-Expressing Macrophage

Figure 2
IL-10-Expressing Macrophage
DISCUSSION

The Difference of IL-6 - Expressing Macrophage Between the First Day and the Third Day after Fracture.

From this study we found that the percentage of IL-1β – expressing macrophage in first day was significantly different from the delayed one (p<0.05) (Table 2).

The similar result was also shown by Einhorn (1995) who used Sprague-Dawley mouse whose legs were made broken. Then the tissue subsequence fracture site was cultured at day III and then stimulated with M-CSH. Macrophage shown a high level of activity. So, tissue macrophage at fracture site shows high activity level at day III. Ogura (1999) reported that the peak for priming index occured at day II-V.

Macrophone activation depends on tissue micro environmnet. If there is trauma or fracture, the body will respond through homeostasis proses as physiological process through neurologic, immunologic and metabolic responses (Aller 2004). Fracture acts as inflammatory focus are due to necrotic tissue, ischemic tissue surrounded by hypoxic tissue (Trentz 2000). This tissue damage is a danger signal so inflammatory process may occur. In the cellular immune response, macrophage is a very important cell and it will be activated by that danger signal. Macrophage activation classically need signal as INF-γ through INF-γ-R to express pro-inflammatory mediators (Mosser 2003). In cardiac surgery IFN-γ decreases on the first few days so there is not much macrophage activation through classic pathway and backs to normal again at day III-V (Franke 2006). Therefore active macrophage classically higher at day III-V. On trauma TLR-2 and TLR-4 will act to increase inflammatory response (Paterson 2003).

At inflammatory process, if the homeostasis is achieved, the PMN will go through apoptosis process and its number will decrease on day III-V and its function is replaced by macrophage (Kumar 2005). In his study, Daley (2005) who used neutropenic mouse and made incision on it, found that PMN in the wound was 100 times lower than control, afterward he analyzed the supernatant from wound oozing fluid and found that the pro-inflammatory cytokine was higher than the control (TNF 68%, IL-6 168%, TGF 61% ), but no IL-10. From those studies we conclude that the inhibition of pro-inflammatory cytokines production by PMN products will cause inhibition on macrophage activation which might be due to PGE2. Since the PMN at fracture site on day I is more than day III-V, thus the macrophage activation on day III-V is higher than day I which is shown by the higher percentage of macrophage which expresses cytokines.

In his study, Browder (1990) administered glucane (a macrophage stimulator) on severe trauma case which would undergo surgery and he found a significant increase of IL-1β serum concentration on day III compared to control (143±19,3 pg/ml compared with 78,6±11,7 pg/ml, p<0,05) and this difference seemed to be related to macrophage activation. Browder’s study supported our result which shown by a significant difference (p<0,05) between the percentage of IL-1β – expressing macrophage in the first day (day I or intervention group) and the delayed one (day III-V or control group).

From the result of this study and statistical analysis, it was proven that IL-1β - expressing macrophage on intervention group was lower than control group.

The Difference of IL-10 - Expressing Macrophage Between the First Day and the Third Day After Fracture.

IL-10 - expressing macrophage in intervention group was found with mean= 3,04±3,35 % and in control group with mean= 5,41± 6,07%. Analyzed with t-test we found no significant difference between first day group and the delayed (p>0,05) (Table 2).

Data above actually showed the difference in IL-10 - expressing macrophage between the first day and the third day. The difference nearly 1: 2, however statistically that difference was not significant (p>0,05). This may be due to small sample size or due to macrophage activation which depends on micro environment of injured tissue. In the beginning of trauma, PMN is much more found in surround injured tissue and well known that the product of PMN such as PGE2 will suppress the production of pro-inflammatory mediators, but it does not suppress IL-10 since PGE2 will increase IL-10 and suppress IL-12, so indirectly will reduce pro-inflammatory cytokines (Harizi 2002, Daley 2005). In cardiac surgery it can be observed also a decrease in IFN-γ on the first few days because of the decreased in IL-12 so there is not much macrophage activation through classical pathway and will be back to normal again on day III-V (Franke 2006). Above conditions seem to give more influence to pro-inflammatory cytokines production.

The mean of IL-10 – expressing macrophage was higher on day III-V than day I. This condition was in one accord with IL-1β – expressing macrophage. In homeostasis state, IL-10 – expressing macrophage will
show a significant difference in both groups along with IL-1β – expressing macrophage because IL-10 is a cytokine regulator and also a compensation cytokine. This can be seen clearly from the correlation between IL-10 – expressing macrophage and IL-1β – expressing macrophage which was strong and significant (p<0.05) (Table 5.3) and it is presume that IL-10 inhibits IL-1β production from macrophage in autocrine or paracrine ways and vice versa.

This study has proved that IL-1β – expressing macrophage was higher significantly on day III compares to day I, but this did not happen for IL-10 – expressing macrophage. This finding showed that the function of active macrophage alternative type as a regulator or compensated cytokine to reach homeostasis is inhibited (Mosser 2003). This depends on micro environment which is more predominant in determining macrophage activity, therefore occasionally the balance between pro-inflammatory and anti-inflammatory is not reached, which local tissue is more pro-inflammatory so macrophage activation through classical pathway with pro-inflammatory cytokines is more dominant (Smith & Giannoudis 1998). From this we can see that pro-inflammatory and anti-inflammatory cytokines does not always work in parallel but depend verily to the need, which is why in this study IL-10 – expressing macrophage did not show a significant difference between intervention group and control one. From data and discussion, it can be seen that the study cannot prove if there was any significant difference between IL-10 – expressing macrophage in both intervention and control groups.

CONCLUSION

A cross sectional study on closed femoral fracture in the first and third day after fracture gave results IL-1β-expressing macrophage in first day was lower than delayed one and there was no difference between IL-10 - expressing macrophage in the first day and the delayed one.

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