Vitamin C inhibit upregulation of plasma and joint interleukin-1β level in cold stress-exposed adjuvant arthritis

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ABSTRACT

Objective: We aimed to investigate whether cold stress increased plasma and joint IL-1β and whether vitamin C quenched ROS and reduced increased IL-1β. Secondly whether there is any correlation between plasma and joint IL-1β level.

Material and methods: Male adjuvant arthritic rats (age=10-12 weeks; n=8/group) were exposed to cold stress (5°C for 15 minutes/day for 7 days) with/without vitamin C (50 mg/day orally) and then kept for 14 days. The control group did not receive either cold exposure or vitamin C. Plasma IL-1β level was measured using indirect ELISA and joint IL-1β was measured using immunohistochemistry before treatment, day 0, 7, 14 after treatment.

Result: Cold stress significantly increased plasma IL-1β level directly after cold stress (p=0.025), 14 days after cold stress (p=0.002). Cold stress significantly increased percentage area positive of joint IL-1β day 7 (p=0.001), day 14 (p=0.001). Compared to controls vitamin C significantly reduced plasma and joint IL-1β directly after cold stress, day 7, 14 after cold stress (p<0.05). No significant correlation between plasma and joint IL-1β in all groups (p>0.05).

Conclusion: Cold stress increased plasma and joint IL-1β and vitamin C reduced increased IL-1β possibly by reducing ROS production and NF-κB activation.

1. Introduction

Rheumatoid arthritis (RA) is common autoimmune disease characterized by chronic inflammatory process of synovial joints and subsequent progressive, erosive destruction of articular tissue. RA is associated with progressive disability, systemic complications, early death and socioeconomic costs [1-3]. RA affect 1 % of human population [2]. The etiology of RA remains unknown and the exact pathogenesis still obscure [2]. Interleukin-1β IL-1β play an important role in the pathogenesis of RA. Exacerbation and advancement of RA are associated with IL-1β upregulation [4]. Interleukin-1 (IL-1) has been implicated in the dysregulation of bone and cartilage remodelling characteristic of RA [3]. IL-1 acts directly on osteoclast increasing bone resorbing capacity and adversely affect cartilage remodelling [3].

Adjuvant arthritis is animal model of rheumatoid arthritis [5]. Cold stress cause increased body heat production by thermogenesis achieved by accelerating uncoupling oxidative phosphorylation in inner membrane of mitochondria. The energy generated is shunted from ATP synthesis to heat production. Increasing oxidative phosphorylation increases reactive oxygen species production (ROS) [6]. Nuclear Factor-kappaB (NF-κB) is a key transcription factor regulating proinflammatory cytokines including IL-1β, involves in pathogenesis and progression of RA [7]. ROS triggers a cascade of events that activates NF-κB and in turn upregulates IL-1β production. Vitamin C has antioxidant effect that may quench ROS produced by oxidative phosphorylations. We aimed to investigate whether cold stress increased plasma and joint IL-1β and whether vitamin C quenched ROS and reduced increased IL-1β.
Secondly whether there is any correlation between plasma and joint IL-1β level.

2. Materials and Methods

Wistar adjuvant arthritic rats (male, 130-170 grams, age 10-12 weeks) were housed at animal house in the Laboratory of Cellular and Molecular Biology, Mathematics and Sciences Faculty, Brawijaya University, Malang, Indonesia. The study was conducted in accordance to Institution guidelines for animal research and Ethical Clearance Committe of Brawijaya University. They were exposed at room temperature with daily 12 hour light on and off cycle, fed standard chow diet and water ad libitum. Adjuvant arthritis was induced by 0.1 ml Complete Freund Adjuvant (CFA) injection at the base tail of the rats intradermally. After 14 days, the rats were injected 0.1 ml CFA intradermally at the right and left feet. After 7 days the symptoms of adjuvant arthritis can be seen as swollen and redness of the feet, ankle, limitation of movement and other symptoms.

The first group (n=8) were exposed to cold stress (5°C for 15 minutes/day for 7 days) and treated with vitamin C 50 mg/day orally and then kept for 14 days. The second (n=8) group were exposed to cold stress (5°C for 15 minutes/day for 7 days) without vitamin C 50 mg/day orally and then kept for 14 days. The control group (n=8) did not receive either cold exposure or vitamin C. At the end of experiment the rats were sacrificed by neck dislocation. Plasma IL-1β and joint IL-1β level was measured before treatment, directly after treatment, 7 and 14 days after treatment.

2.1 Indirect ELISA for measuring plasma IL-1β level

For measuring plasma IL-1β level, 100 μl plasma and 100 μl coating buffer were poured in the microtiter plate and incubated 4°C overnight. After that added 100 μl primary antibody anti-rat IL-1β from mice and incubated 4°C overnight and washed with 300 μl TBS-Tween pH 8.00 three times. Added 100 μl secondary antibody anti-mouse IgG and incubated for 2 hours in room temperature. After that washed with 300 μl TBS-Tween pH 8.00 three times and add 100 μl Strepavidin-HRP and incubated 40 minutes at room temperature. Put 100 μl tetra methyl benzidine and incubated 10-20 minutes at room temperature and added 100 μl stop solution (NaOH 3M). The plate was read with ELISA reader at wave length 450nm and interpolated at standard curve [8,9].

2.3 Immunohistochemistry for measuring joint IL-1β level

For immunohistochemistry, rat joints were cut at 4 μm thick and was made the preparate. Preparates were put in citrate buffer pH 6 95°C for 5 minutes twice and left at room temperature for 20 minutes. Preparates were washed 3 times with destilled water for 5 minutes and incubated in 0.1% trypsin 0.1% CaCl₂ for 20 minutes, incubated in 0.05% saponin for 40 minutes. Put H₂O₂ 3% and incubated for 10 minutes and washed with PBS pH 7.4 for 5 minutes three times. Blocked the effect of non specific protein with NGS 1 % for 1 hour and absorbed with tissue blocking serum. The preparates were dropped with rat primary antibody anti-IL-1β and incubated 4°C overnight. The preparate were washed with PBS pH 7.4 for 5 minutes 3 times and dropped with secondary antibody labelled with biotin and incubated at room temperature for 60 minutes. The preparates were washed with PBS pH 7.4 for 5 minutes 3 times and dropped with SA-HRP and incubated for 40 minutes. The preparates were washed again with PBS pH 7.4 for 5 minutes 3 times and added with DAB (diaminobenzidin) for 3 minutes and washed with destilled water 3 times. After that the preparates were counterstained with Mayer’s hematoxylin and washed with tap water [modifidio]. Preparates were examined under microscope, and IL-β level were measured as percentage area positive indicated as brown colour.

3. Result

Cold stress increased plasma IL-1β level of arthritic rats directly after cold stress (39.647 ± 5.147 vs 34.766 ± 1.251), 7 days after cold stress (41.368 ± 5.714 vs 37.886 ± 4.040) and 14 days after cold stress (46.668 ± 6.108 vs 39.419 ± 2.821). Vitamin C decreased plasma IL-1β level of arthritic rats exposed to cold stress directly after cold stress (28.934 ± 4.579 vs 39.647 ± 5.147), 7 days after treatment (33.089 ± 1.675 vs 41.368 ± 5.714) and 14 days after cold stress (35.663 ± 2.866 vs 46.668 ± 6.108).
Means and standard deviations of plasma IL-1β level before treatment, directly after treatment, 7 days and 14 days after treatment were shown in Table 1.

Table 1. Means and Standard Deviations of Plasma IL-1β (pg/ml)

<table>
<thead>
<tr>
<th>Group</th>
<th>Observation 1</th>
<th>Observation 2</th>
<th>Observation 3</th>
<th>Observation 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>34.347 ± 2.066</td>
<td>28.934 ± 4.579</td>
<td>33.089 ± 1.675</td>
<td>35.663 ± 2.866</td>
</tr>
<tr>
<td>II</td>
<td>34.323 ± 1.385</td>
<td>39.647 ± 5.147</td>
<td>41.368 ± 5.714</td>
<td>46.668 ± 6.108</td>
</tr>
<tr>
<td>III</td>
<td>34.679 ± 2.888</td>
<td>34.766 ± 1.251</td>
<td>37.886 ± 4.040</td>
<td>39.419 ± 2.821</td>
</tr>
</tbody>
</table>

Group I: Rats with adjuvant arthritis exposed to cold stress and treated with vitamin C
Group II: Rats with adjuvant arthritis exposed to cold stress
Group III: Rats with adjuvant arthritis without treatment
Observation 1: Before treatment
Observation 2: Directly after treatment
Observation 3: 7 days after treatment
Observation 4: 14 days after treatment

Cold stress significantly increased plasma IL-1β level directly after cold stress (p=0.025) and 14 days after cold stress (p=0.002). Vitamin C significantly decreased plasma IL-1β level of arthritic rats exposed to cold stress directly after cold stress (p=0.001), 7 days after cold stress (p=0.001), and 14 days after cold stress (0.001).

Cold stress increased joint IL-1β level of arthritic rats directly after cold stress (2.38 ± 0.92 vs 1.75 ± 1.19), 7 days after cold stress (7.88 ± 1.81 vs 1.88 ± 0.84) and 14 days after cold stress (11.38 ± 3.42 vs 2.62 ± 1.41). Vitamin C decreased joint IL-1β level of arthritic rats exposed to cold stress directly after cold stress (1.25 ± 1.04 vs 1.75 ± 1.19), 7 days after treatment (2.25 ± 1.04 vs 7.88 ± 1.81) and 14 days after cold stress (3.38 ± 0.92 vs 11.38 ± 3.42).

Means and standard deviations of joint IL-1β level before treatment, directly after treatment, 7 days and 14 days after treatment were shown in Table 2.

Table 2. Means and Standard Deviations of Joint IL-1β (Percentage area positive)

<table>
<thead>
<tr>
<th>Group</th>
<th>Observation 1</th>
<th>Observation 2</th>
<th>Observation 3</th>
<th>Observation 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0.63 ± 0.74</td>
<td>1.25 ± 1.04</td>
<td>2.25 ± 1.04</td>
<td>3.38 ± 0.92</td>
</tr>
<tr>
<td>II</td>
<td>0.63 ± 0.74</td>
<td>2.38 ± 0.92</td>
<td>7.88 ± 1.81</td>
<td>11.38 ± 3.42</td>
</tr>
<tr>
<td>III</td>
<td>0.75 ± 0.71</td>
<td>1.75 ± 1.19</td>
<td>1.88 ± 0.84</td>
<td>2.62 ± 1.41</td>
</tr>
</tbody>
</table>

Group I: Rats with adjuvant arthritis exposed to cold stress and treated with vitamin C
Group II: Rats with adjuvant arthritis exposed to cold stress
Group III: Rats with adjuvant arthritis without treatment
Observation 1: Before treatment
Observation 2: Directly after treatment
Observation 3: 7 days after treatment
Observation 4: 14 days after treatment

4. Discussion

IL-1β functions to activate leucocytes, endothelial cells, synovial cells, osteoclasts and also induce matrix-enzyme production by chondrocytes and mediate fever [1]. This cytokine increases production of factors that stimulate cartilage matrix degradation, but also inhibits the synthesis of type II collagen and proteoglycans [3]. This study showed that cold stress increased significantly plasma and joint IL-1β level. This was possibly through increasing ROS production because of uncoupling oxidative phosphorylation in increasing heat production. IL-1β activates NF-
κB that is an inflammatory cytokines, including IL-1β and IL-6. Upregulation of IL-1β and NF-κB also has inhibitory effect on cartilage and capillary proliferation increased inflammation and inhibited apoptosis in the synovium [7,12].

This study showed that vitamin C significantly decreased plasma and joint IL-1β level of arthritic rats exposed to cold stress. Vitamin C quenched ROS produced in oxidative phosphorylation, so that in turn suppressed the activation of NF-κB and reduced plasma and joint IL-1β production. Because IL-1β mediates inflammation and joint destruction, vitamin C may improve joint condition in arthritis adjuvant exposed to cold stress.

No significant correlation between plasma and joint IL-1β in all groups. The systemic and local joint inflammation has no correlation.

5. Conclusion

Cold stress increased plasma and joint IL-1β and vitamin C reduced increased IL-1β production, possibly by reducing ROS production and NF-κB activation.

Conflict of Interest

The authors report no conflicts of interest.

References