Endometriosis Regression in Rats Treated by Colchicine: Histopathological Evaluation and Assessment of TNF-α Levels

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Objective

To assess the efficacy of colchicine in an experimental rat endometriosis model.
Materials and methods–1

- Approval of Mustafa Kemal University Animal Laboratory Ethical Committee

- In all procedures which were applied to the animals, local ethical committee laboratory rules and rules of Guidelines for the Care and Use of Laboratory Animals of the US National Institutes of Health (Washington, DC) were obeyed.
Materials and methods – 2

- 20 Wistar–albino rats
- Endometriosis model by Vernon and Wilson method during estrous phase
- 4 weeks
- Group 1 (colchicine) (n = 8) and Group 2 (control) (n = 8), 4 were excluded
- Implant volumes were measured
- Peritoneal fluid samples were taken
- 0.1 mg/kg colchicine, po, same amount of serum physiologic
- 4 weeks
2 pieces of endometrial implants for
  - histological examination
  - Biological assessment

TNF-α levels were measured in peritoneal fluid samples and endometrial tissue samples.
The persistence of epithelial cells in endometrial autografts was evaluated semiquantitatively. The pathologic evaluation of the uterine autografts was performed according to a previously published method as follows:

- well preserved epithelial layer = score 3
- moderately preserved epithelium with leukocyte infiltrate = score 2
- poorly preserved epithelium = score 1
- no epithelium = score 0

Continuous variables → mean ± SD

The differences between numeric variables → Kruskal–Wallis test

Mann–Whitney U test for post–hoc analysis.

p < 0.05 was recognized as statistically significant.

SPSS 20.0 (SPSS Inc, Chicago, IL, USA) package program.
Rat weights

<table>
<thead>
<tr>
<th></th>
<th>before</th>
<th>after</th>
<th>(p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>colchicine</td>
<td>230±11.2 g.</td>
<td>234±9.4 g.</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>control</td>
<td>221±9.8 g.</td>
<td>231±9.23 g.</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

Implant volumes

<table>
<thead>
<tr>
<th></th>
<th>pre-treatment</th>
<th>post-treatment</th>
<th>(p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>colchicine</td>
<td>89 mm³</td>
<td>35 mm³</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>control</td>
<td>85 mm³</td>
<td>110 mm³</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>
Implant volumes before (a) and after (b) treatment
Histopathologic pictures of endometriotic implants in control (a) and colchicine (b) groups.
Histological score

Experimental group: 1.40
Control group: 2.60
Tissue TNF-α

<table>
<thead>
<tr>
<th>Group</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental group</td>
<td>45.00</td>
</tr>
<tr>
<td>Control group</td>
<td>71.00</td>
</tr>
</tbody>
</table>
Peritoneal TNF-α / Experimental group

Peritoneal TNF-α / Control group
Table-1: Comparison of implant volumes, histopathologic scores and tissue TNF-α levels between colchicine and control groups

<table>
<thead>
<tr>
<th></th>
<th>Colchicine Group (n=8)</th>
<th>Control Group (n=8)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (g)</td>
<td>230±11.2</td>
<td>221±9.8</td>
<td>NS</td>
</tr>
<tr>
<td>Initial implant volume (mm³)</td>
<td>89</td>
<td>85</td>
<td>NS</td>
</tr>
<tr>
<td>Post-treatment implant volume (mm³)</td>
<td>35</td>
<td>110</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Histopathologic score</td>
<td>1.4±0.2</td>
<td>2.6±0.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Tissue TNF-α levels (pg/ml)</td>
<td>45±8.6</td>
<td>71±11.2</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
**Table-2**: Peritoneal TNF-α levels before and after treatment in groups

<table>
<thead>
<tr>
<th></th>
<th>Colchicine Group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>Peritoneal TNF-α levels (pg/ml)</td>
<td>45±5</td>
<td>12±5</td>
</tr>
</tbody>
</table>
Activated macrophages, inflammatory cytokines, chemokines and prostaglandins are increased in peritoneal fluid of endometriosis.


Decreased cytotoxic T cells and natural killer cell activity are observed in peritoneal fluid of the patients with endometriosis.


Increased levels of IL-1, IL-6, IL-8, IL-18 and TNF-α were detected during the inflammatory process of endometriosis.


Discussion–2

- TNF-α secreted from active macrophages modulates the secretion of other cytokines and plays a crucial role in pathogenesis of endometriosis.
  

- A relation between TNF-α levels and the severity of endometriosis has been established.

Mechanism of Action of Colchicine
(Jeepakistan.blogspot.com)
According to the results of our experiment, we propose that the main effect of colchicine on endometriosis model is its inhibition of TNF–α mediated immune response.
Many immunomodulator drugs such as imiquimod, levamizole, etanercept decrease in endometrial implant volume.


Less toxic treatment modalities are needed

TNF alpha blocker drugs may cause infection and cancer.


Colchicine is currently being used safely in many diseases.
Some limitations to perform this treatment in a similar manner in humans.

Colchicine is good for endometriosis in rats

human studies are needed.
Many thanks for your attention