Triamcinalone and Raloxifene Loaded PCL Microspheres for Treatment of Rheumatoid Arthritis

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ABSTRACT SUMMARY
In this study, it is aimed to develop an injectable controlled release system of PCL microspheres loaded with TA and/or Ral for local treatment of rheumatoid arthritis to avoid from systemic side effects of traditional administration and eliminate problems caused by direct local injections. Microspheres were prepared with different combinations of polymer and drugs (PCL:TA, PCL:Ral and as dual PCL:TA:Ral; from 10:1 to 10:4 for mono drugs and 10:4:2 for dual drugs) and were examined for particle size analysis, surface and structural characterizations, time related drug release properties, and for drug loading capacities. In order to evaluate biocompatibility of drug loaded PCL microspheres, they were also analyzed with cytotoxicity tests using 3T3 fibroblast cells. The potential of these systems, were also tested and evaluated on in vivo experimental model of RA.

INTRODUCTION
Rheumatoid arthritis (RA) is a systemic disease that mainly attacks on and degenerate the joints. As a consequence, RA disrupt the movement and thereby the life quality of patients [1, 2]. Traditional treatment of the disease requires long-term systemic drug administration which mostly results in side effects in other tissues and organs. One other drawback of systemic treatment is lower allocation of the drugs into the synovial fluid (joint space) than the serum concentration. The new approach of treatment, thus, involves intra-articular injection of drugs [3].

EXPERIMENTAL METHODS
Microspheres were prepared with solvent evaporation technique and optimized to achieve a suitable size for joint application, to sustain the delivery of the drug(s), to provide required amount of the agent with feasible amount of microsphere. In order to manage these, microspheres prepared with different combinations of polymer and drugs (PCL:TA, PCL:Ral and as dual PCL:TA:Ral; from 10:1 to 10:4 for mono drugs and 10:4:2 for dual drugs) were examined for particle size analysis, surface and structural characterizations, time related drug release properties, and for drug loading capacities. In order to evaluate biocompatibility of drug loaded PCL microspheres, they were also analyzed with cytotoxicity tests using 3T3 fibroblast cells. Biodegradation studies of polymer were done by using GPC analysis. The TA and Ral delivery systems were developed and studied for the first time in literature and they were optimized for RA treatment purposes. For evaluation of RA treatment potency of drug loaded microspheres, in vivo RA model (monoaarthritis) was developed in rats by using Complete Freund’s Adjuvant (CFA). After development of RA, microspheres were injected intra-articularly to the knee joints. In vivo results of treatment groups were evaluated by histological analysis and clinical examinations.

RESULTS AND DISCUSSION
When the results of three ratios are compared, as polymer:drug ratio decreased from 10:1 to 10:4 the cumulative amount of the drug released increases (Fig.1). As polymer:drug ratio decreased from 10:1 to 10:2 encapsulation
efficiency approximately doubled (from 23.34% to 51.33%) and loading increased approximately 6 times (from 3.09% to 17.95%) Table 1. As polymer:drug ratio decreased the mean particle size of the microspheres increased from 80.89 to 100.44 µm. It was observed that PCL:Ral (10:1) microspheres released only 0.67 µg cumulative amount of Ral at the end of 4 weeks which is 0.16% of all the loaded drug (Fig.2). Similarly PCL:Ral (10:2) microspheres had a mean cumulative release amount of 0.61 µg at the end of 4 weeks.

Table 1. Properties of microspheres

<table>
<thead>
<tr>
<th>MS Type</th>
<th>Mean Diameter (µm)</th>
<th>Encapsulation Efficiency (%)</th>
<th>Loading (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCL:TA 10:1</td>
<td>80.89</td>
<td>23.34</td>
<td>3.09</td>
</tr>
<tr>
<td>PCL:TA 10:2</td>
<td>86.72</td>
<td>51.33</td>
<td>17.95</td>
</tr>
<tr>
<td>PCL:TA 10:4</td>
<td>100.44</td>
<td>70.56</td>
<td>27.17</td>
</tr>
<tr>
<td>PCL:RAL 10:1</td>
<td>88.17</td>
<td>63.57</td>
<td>8.69</td>
</tr>
<tr>
<td>PCL:RAL 10:2</td>
<td>95.31</td>
<td>56.52</td>
<td>13.01</td>
</tr>
<tr>
<td>PCL:TA:RAL 10:4:2</td>
<td>68.77</td>
<td>42.04 TA 47.72 Ral 13.94 TA 7.91 Ral</td>
<td></td>
</tr>
</tbody>
</table>

In vivo histological studies of control groups showed formation of RA by CFA injection and degenerative progress of RA was observed in these groups upon time. In microsphere treatment groups there were considerable reduction in degeneration and the combined drug treatment group showed the most promising healing outcomes in histological evaluations.

**CONCLUSION**

In PCL:TA:Ral 10:4:2 microspheres, even though encapsulation and loading values were slightly decreased compared to PCL:TA 10:4 and PCL:Ral 10:2 microspheres, release results were still sufficiently sustained and suitable for in vivo applications. When examined separately, TA release decreased slightly while Ral release increased slightly with co-release. Besides, the lowest mean particle size was obtained with dual drug loaded PCL:TA:Ral 10:4:2 microspheres, which is advantageous for applications in intra articular injections.

**REFERENCES**