Psoriasis is a chronic inflammatory disease affecting 1–3 % of the general population. Traditional systemic therapies for psoriasis, such as methotrexate, have a potential for long-term toxicity and may not always provide sufficient improvement of the condition.

In a recent study by van der Fits et al.,1 a mouse model of dermatitis closely resembling human psoriasis was obtained by the topical application of Imiquimod (IMQ) to mouse ear skin. IMQ, linked to the IL-23/IL-17 axis, can function as a toll-like receptor 7/8 (TLR7/8) ligand and initiate a potent innate immune system response. Mouse skin lesions showed increased epidermal proliferation, abnormal differentiation of keratinocytes, epidermal accumulation of neutrophils in micro-abcesses, neo-angiogenesis, and infiltrates consisting of CD4(+) T cells, CD11c(+) dendritic cells, and plasmacytoid dendritic cells.

In the mouse dermatitis study presented here, in vivo phagocyte-mediated myeloperoxidase (MPO) activity was monitored non-invasively by detecting bioluminescent signal resulting from MPO-specific luminol catabolism.2 MPO-luminol bioluminescence signal is known to be co-localized with histological sites of inflammation as well as infiltration of neutrophils and activated eosinophils.2
Materials and Methods

Mice (8 week-old female BALB/c; Janvier Labs) were anesthetized with an intraperitoneal injection (i.p.) of anaesthetic solution (xylazine 0.5 % (v/v), ketamine 7.5 % (v/v), 0.1 ml/10 g), and divided into 3 equal-sized groups (n = 6/group). The skin of both ears for all mice was shaved before topical administration of either vaseline (Cooper) or IMQ (Imiquimod; Aldara 5 % cream, MEDA Pharma, Sweden), from day 1 to day 4. Test Groups 2 and 3 received a total daily dose of 1.5 mg IMQ (0.75 mg IMQ/ear) per ear, while Control Group 1 received a total daily dose of 1.5 mg vaseline (0.75 mg vaseline/ear). From day 1 to day 4, Groups 1 and 2 were given the carrier (PBS; 10 ml/kg, i.p.) only, while Group 3 mice also received i.p. injection of metrotrexate (MTX) at 7.5 mg/kg (10 ml/kg) which is summarized in Table 1.

Ear Thickness

Ear thickness was measured on anesthetized mice using a micrometer (Mitutoyo) before cream application at day 1 and before imaging at day 5.

In vivo Bioluminescence Imaging

On day 5, anesthetized mice were imaged 10 min after administration of luminol. For anatomical co-registration, all images were taken with a 72 x 72 mm field of view (FOV).

Image Analysis and Presentation

Signal intensity analysis was performed using Molecular Imaging Software version 7.1 (Bruker BioSpin, Billerica, MA, USA). An elliptical region of interest (ROI) was drawn around one ear of one mouse and then 1:1 copied on the other ear. These ROIs were saved as an MRI ROI template and then applied to the other images using “Apply Template” function in the Manual ROI panel of the MRI Navigation tool. Based on the control mouse images of Group 1, a luminescence signal dynamic range threshold of 50 Psec/cm2 was selected. ROI Data were exported and saved to Microsoft Excel® files, while images were exported as JPEG and TIFF files, respectively.

Results/Discussion

At day 5, IMQ treated mice were observed to have 1.9-fold significant increase in ear thickness compared to other treatment groups (p<0.001, see Figure 1). This is consistent with results reported in the literature.1 Ear thickening was largely (but not totally) prevented by MTX treatment (see Figure 1).

After luminol administration, bioluminescence (BLI) signal intensities (photons/sec/mm2) of mouse ears were evaluated semi-quantitatively in the three treatment groups. When compared to control animals (Group 1), IMQ-treated mice (Group 2) had ear BLI signal intensities that were 70 % higher (p<0.05, see Figure 2B3). Administration of MTX to IMQ-treated mice (Group 3) resulted in a BLI signal decrease with values similar to the one observed in the control mice (again, see Figure 2 & 3).

Table 1: Experimental workflow. IMQ: Imiquimod; carrier: methylcellulose; MTX: methotrexate; BLI: Bioluminescence Imaging.

<table>
<thead>
<tr>
<th>Group 1 (control)</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Day 0</strong></td>
<td>Ear thickness validation via micrometer measurements</td>
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</tr>
<tr>
<td><strong>Day 1</strong></td>
<td>1.5 mg Vaseline &amp; carrier injection</td>
<td>1.5 mg IMQ &amp; carrier injection</td>
</tr>
<tr>
<td><strong>Day 2</strong></td>
<td>1.5 mg Vaseline &amp; carrier injection</td>
<td>1.5 mg IMQ &amp; carrier injection</td>
</tr>
<tr>
<td><strong>Day 3</strong></td>
<td>1.5 mg Vaseline &amp; carrier injection</td>
<td>1.5 mg IMQ &amp; carrier injection</td>
</tr>
<tr>
<td><strong>Day 4</strong></td>
<td>1.5 mg Vaseline &amp; carrier injection</td>
<td>1.5 mg IMQ &amp; carrier injection</td>
</tr>
<tr>
<td><strong>Day 5</strong></td>
<td>Ear thickness validation via micrometer measurements &amp; BLI Imaging</td>
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</tbody>
</table>

Mouse ear in vivo bioluminescence: a semi-quantitative analysis. Mice were imaged 1 day after the completion of 4-day treatment regimen: Ctrl: control group (Group 1); IMQ: Imiquimod treatment, Group 2; IMQ + MTX: Imiquimod and methotrexate treatment, Group 3 (see also Table 1). Effective ROI MRI software, Bruker Bioware were drawn to quantify observed bioluminescent signals.

In the psoriasis-like mouse model induced by topical application of Imiquimod (IMQ), the observed increased ear thickness was likely the result of increased keratinocyte proliferation as well as the infiltration of inflammatory cells, including neutrophils. The results presented here show that there is a clear correlation between mouse ear thickness and observed BLI signal intensity of the ear region.

Conclusion

This application note describes an experimental protocol enabling non-invasive, specific and rapid imaging of neutrophil infiltration occurring in an Imiquimod (IMQ)-induced mouse model of psoriasis-like dermatitis. Furthermore, with additional studies, it might be shown that BLI can be used along with or instead of the clinical method of ear thickness measurement, providing reliable data in a time-efficient and effective manner.
References


