Effects of Magnetic Infrared Laser on Xylene-induced Acute Inflammation in Mice

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Abstract. [Purpose] The purpose of the present study was to investigate the effect of magnetic infrared laser (MIL) radiation on the xylene-induced acute inflammation. [Subjects] The subject animals were male ICR mice. [Methods] Mice were once irradiated (60s) by MIL at 1.33, 2.66 or 6.65 J/cm² of MIL, or treated with indomethacin or dexamethasone. Then xylene (0.03 ml) was topically applied to the anterior surface of the right ear to induced inflammation 1 h after irradiation with MIL. The changes in ear weights, histological profiles and histomorphometrical measurements of the ear were conducted upon sacrifice. All animals were sacrificed 2 h after xylene application. Results of MIL irradiation were compared to those of indomethacin and dexamethasone (15 mg/kg injected once intraperitoneally). [Results] Xylene application resulted in marked increases in xylene-insulted ear weights compared to that of the intact control. Thus, the differences between intact and insulted ears were also significantly increased. The histological characteristics of acute inflammation, such as severe vasodilation and edematous changes of skin, were detected in xylene-treated control ears with marked increase in the thickness of the ear tissues. However, these xylene-induced acute inflammatory changes were significantly and dose-dependently decreased by MIL irradiation. [Conclusion] We conclude that MIL therapy has a favorable effect in the reduction of the acute inflammatory responses induced by xylene application in mice.

Key words: Magnetic Infrared Laser, Acute inflammation, Mouse

INTRODUCTION

Inflammation is an essential protective process preserving the integrity of organisms against physical, chemical and infective insults. However, the inflammatory response to several insults frequently leads to erroneous damage to normal tissues1). Physical damage, chemical substances, micro-organisms and other agents are all possible causes of acute inflammation. The inflammatory responses to such insults consist of changes in blood flow, increased permeability of blood vessels and the subsequent escape of cells from the blood into the tissues. The changes are essentially the same regardless of the cause or its location. Acute inflammation is short-lived, typically lasting only a
few days. If the inflammation is longer lasting, however, it is referred to as chronic inflammation\(^2\).

The xylene-induced acute inflammatory mouse ear model has generally been used as one of the classic methods for detecting the efficacy of anti-inflammatory agents. In this model, the anti-inflammatory effect of a drug is based on observations of ear weight and histopathology\(^3, 4\). As control anti-inflammatory drugs, indomethacin is a cyclo-oxygenase inhibitor and dexamethasone is a well-known glucocorticoid; these drugs are the most widely used as controls in the development of the new anti-inflammatory drugs\(^5, 6\).

Besides the traditional steroidal and non-steroidal anti-inflammatory drugs, many alternative physical techniques such as electrical stimulation\(^7\), short wave\(^8\), ultrasound\(^9\) and laser\(^10\) radiation have been satisfactorily used in the treatment of inflammatory diseases. Although magnetic infrared laser (MIL) therapy has also been used in the treatment of various inflammatory diseases\(^11–13\), most therapies were clinical approaches and the effect on xylene-induced acute inflammatory responses has not been reported yet. In the present study, the effects of MIL therapy on the xylene-induced acute inflammation were investigated.

MATERIALS AND METHODS

Sixty-three male ICR mice (6 weeks old upon receipt; SLC, Japan) were used after acclimatization for 7 days. Animals were housed five or four per polycarbonate cage in a temperature (20–25°C) and humidity (40–45%) controlled room with a 12 h : 12 h light:dark cycle. Feed (Samyang, Korea) and water were supplied \textit{ad libitum}. All animals were fasted overnight before dosing and sacrifice (about 18 h with \textit{ad libitum} access to water).

A continuous MIL (Model:MOLTA-F-8-01, United Space Device Corporation, Moscow, Russia) with an output power of 1, 2 or 5 mW and a wavelength of 850 nm was used. The dosages (J/cm\(^2\)) are given with respect to spot size. The animals were distributed into seven groups with 9 mice per group: intact control, xylene-treated control, 1.33, 2.66 and 6.65 J/cm\(^2\) MIL-irradiated groups, 15 mg/kg indomethacin and dexamethasone dosing groups. MIL irradiation was applied once in contact with the ear surface for 60s, and indomethacin and dexamethasone were administered intraperitoneally once in a volume of 10 ml of saline.

One hour after dosing, 0.03 ml of xylene (Merck, Germany) was topically applied to the anterior surface of the right ear. The left ear was considered a control.

Two hours after xylene application, all animals were sacrificed and both ears were removed. Circular sections were taken using a cork borer with a 7-mm diameter and weighed as previously described\(^9\). The weight of the xylene-insulted ear was regarded as the absolute weight. To reduce errors arising from individual body weights, the relative weights of the ears were also calculated along with the differences between the intact ear and insulted ear as follows:

\[
\text{Relative weight (\%)} = \left( \frac{\text{absolute ear weight}}{\text{body weight at sacrifice}} \right) \times 100
\]

\[
\text{Differences in weights (g)} = \text{insulted ear weight} - \text{intact ear weight}
\]

Following removal, insulted ears were trimmed crosswise to include ear cartilage and fixed in 10% neutral buffered formalin, then embedded in paraffin, sectioned (3–4 μm) and stained with hematoxylin and eosin stain. The histological profiles of the ears were observed and compared to those of intact and/or xylene-treated control. The thickness of the insulted ear was calculated using automated image analysis (DMI-300; DMI, Korea) under 50X magnification (Nikon, Japan). The thicknesses from epidermis to dermis of the insulted ear skin (anterior skin thickness; μm) and anterior to posterior ear (full ear thickness, μm) were measured in histomorphometry on cross-trimmed ear specimens in regular corresponding regions in all insulted ear samples.

Multiple comparison tests of the different dose groups were carried out. The variance homogeneity was examined using the Levene test. If the Levene test indicated no significant deviations from variance homogeneity, the data obtain was analyzed using one-way ANOVA followed by a least-significant difference multi-comparison test to determine which pairs of group comparisons were significantly different. A non-parametric comparison test, the Kruskal-Wallis H test was performed in the case of significant deviations from the variance homogeneity detected by the Levene test. When a significant difference was observed in the Kruskal-Wallis H test, the Mann-Whitney U-Wilcoxon Rank Sum W test was used to determine
the specific pairs of group comparisons that were significantly different. SPSS statistical software (Release 6.1.3, SPSS Inc., USA) was used and a $p$-value $<0.05$ was considered significant.

RESULTS

A significant ($p<0.01$) increase in the absolute weight of the insulted ear was detected in the xylene-treated control as compared with the intact control and, accordingly, the differences between intact and insulted ears were also significantly ($p<0.01$) increased. However, the insulted ear weights and the differences between the intact and insulted ears of all treated groups were significantly ($p<0.01$ or $p<0.05$) decreased compared to that of the xylene-treated control. Similar changes in the relative weight of the insulted ears were also detected (Table 1).

The classic histological profile of acute inflammation, severe vasodilation and edematous changes of skin, was detected in the xylene-treated control. However, these histological indicators of acute inflammation were dramatically decreased in all dosing groups as compared with the xylene-treated control. In addition, dose-dependency was also demonstrated in the MIL irradiated groups (Fig. 1). Significant ($p<0.01$) increases in the thickness of the insulted ear (both anterior skin and full ear thicknesses) were detected in the xylene-treated control compared to that of the intact control. However, these increases in thickness of the

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<tr>
<th>Table 1.</th>
<th>Changes in ear weights in intact or xylene-insulted mice</th>
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<tr>
<td><strong>Group</strong></td>
<td><strong>Absolute weight (g)</strong></td>
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<tr>
<td></td>
<td><strong>Intact ear</strong></td>
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<tr>
<td>Controls</td>
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<tr>
<td>Intact</td>
<td>0.118 ± 0.004</td>
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<tr>
<td>Xylene</td>
<td>0.115 ± 0.009</td>
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<td>References</td>
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<tr>
<td>Dexamethasone</td>
<td>0.115 ± 0.013</td>
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<tr>
<td>Indomethacin</td>
<td>0.114 ± 0.007</td>
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<tr>
<td>Magnetic Infrared Laser</td>
<td>6.65 J/cm²</td>
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<td></td>
<td>2.66 J/cm²</td>
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<td></td>
<td>1.33 J/cm²</td>
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Mean ± S.D. of nine mice.
Relative weight (%) = (absolute ear weight / body weight at sacrifice) × 100.
Differences = insulted ear weight – intact ear weight.
* $p<0.01$ and ** $p<0.05$ compared with intact control; # $p<0.01$ and ## $p<0.05$ compared with xylene control by MW test.

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<th>Table 2.</th>
<th>Histomorphometry of xylene-insulted mice ears and controls</th>
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<tr>
<td><strong>Group</strong></td>
<td><strong>Full ear thickness (µm)</strong></td>
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<td></td>
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<tr>
<td>Controls</td>
<td></td>
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<tr>
<td>Intact</td>
<td>766.765 ± 127.712</td>
</tr>
<tr>
<td>Xylene</td>
<td>1916.069 ± 147.049*</td>
</tr>
<tr>
<td>References</td>
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<tr>
<td>Dexamethasone</td>
<td>763.837 ± 103.992*</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>1059.186 ± 221.111**, #</td>
</tr>
<tr>
<td>Magnetic Infrared Laser</td>
<td>6.65 J/cm²</td>
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<td>2.66 J/cm²</td>
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<td>1.33 J/cm²</td>
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Mean ± S.D. of nine mice.
Anterior skin thickness = thicknesses from epidermis to dermis of induced ear skin; Full ear thickness = thicknesses from anterior to posterior ear of induced ear.
* $p<0.01$ compared with intact control; # $p<0.01$ compared with xylene control.
insulted ear were significantly \((p<0.01)\) decreased in all dosing groups as compared with the xylene-treated control (Table 2).

**DISCUSSION**

Xylene-induced acute inflammation of the mouse ear has generally been used as one of the classic methods for detecting the efficacy of anti-inflammatory agents\(^3, 4\). In the present study, the effects of MIL therapy on xylene-induced acute inflammation were observed, for the first time. As a result of pretreatment with MIL, changes associated with acute inflammation, such as the marked increases of insulted ear weight, vasodilation, edematous changes in the skin and increases in the thickness of ear tissues were significantly and dose-dependently decreased. These results are considered direct evidence that MIL therapy inhibits the induction of the acute inflammatory response. Previously reported antioxidative effects of MIL\(^14, 15\) are considered by us to be one of the major mechanisms of the anti-inflammatory effect of MIL detected in the present study, because NO synthase inhibitors can reverse several classic inflammatory symptoms\(^16\). In addition, immune stimulation by MIL therapy\(^17–20\) may also have been involved in the anti-inflammatory activity detected in the present study, because immunomodulatory agents can reduce inflammation\(^9\).

After topical application of xylene, marked increases in ear weight were detected as a result of the acute inflammation response; these increases in ear weight have been used as valuable markers for anti-inflammatory effects\(^21, 22\). In the present study, the increases in ear weight were dose-dependently inhibited by MIL treatment. This inhibition was considered direct evidence that the MIL therapy used in this study has favorable effects on reducing the acute inflammatory response.

Histopathologically, severe vasodilation and edematous changes of skin were detected as signs of acute inflammation after topical application of xylene\(^2, 23–25\) (De La Puerta et al., 1996; Kou et al., 2003; Rotelli et al., 2003; Kim et al., 2007). As a result of these histopathological changes, the thickness of the ear tissues was also markedly increased. However, these histopathological changes and the thickness of ear tissues were dose-dependently decreased after pretreatment with three different dosages of MIL in the present study. This inhibition was considered direct evidence that the MIL therapy used in this study has favorable effects on reducing the acute inflammatory response.

Based on the results, we conclude that the MIL therapy has a favorable effect in the reduction of the acute inflammatory response induced by xylene application in mice.
REFERENCES


