The role of cyclooxygenase-2 and prostaglandin E2 in the pathogenesis of cutaneous lichen planus

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Summary

Background. Lichen planus (LP) is an inflammatory disease of the skin and mucous membranes. Autoimmunity has been suggested as a possible cause of this disease. The cyclooxygenase enzymes (COX-1, COX-2) are the key enzymes in the conversion of arachidonic acid into prostaglandins. Prostaglandin E2 (PGE2), a key product of COX-2, has an immunomodulatory role.

Aim. To map levels of COX-2 and PGE2 in cutaneous LP lesions and evaluate their role in the pathogenesis of the disease.

Methods. In total, 31 patients with classic cutaneous LP and 30 age- and sex-matched healthy controls were enrolled. Skin biopsies were taken from the lesional and nonlesional skin of patients, and from the normal skin of controls. COX-2 mRNA expression was detected by real-time reverse transcription quantitative PCR, and PGE2 was detected by ELISA in skin biopsies from patients and controls.

Results. Our analysis revealed a significantly higher expression of COX-2 mRNA and PGE2 in the LP skin biopsies compared with the control biopsies ($P < 0.001$ and $P < 0.001$, respectively). Lesional biopsies showed significantly higher expression of COX-2 mRNA and PGE2 compared with nonlesional biopsies. The levels of COX-2 and PGE2 were not found to be correlated with age, sex or disease duration.

Conclusions. COX-2 and its product PGE2 are strongly expressed in LP skin lesions, indicating that they have a role in the pathogenesis of LP through their immunomodulatory effects.

Introduction

Lichen planus (LP) is an inflammatory disease of the skin and mucous membranes.1 Its exact pathogenesis is still unclear, but recent studies provide evidence that autoreactive cytotoxic T lymphocytes are the effector cells that cause degeneration and destruction of keratinocytes.2

Cyclooxygenase-2 (COX-2) is an enzyme catalysing synthesis of prostaglandins (PGs) and tromboxanes from arachidonic acid. The COX-2 gene is induced by hypoxia, inflammatory cytokines, growth factors and other stress factors.3 Increased expression of COX-2 is part of the inflammatory process, and COX-2 has both promoting and inhibitory effects on this process.4,5 Increased levels of COX-2 have been reported in a variety of cancers,6 and also in autoimmune diseases7,8 and oral lichen planus (OLP) lesions.9–12

This study was performed to declare whether COX-2 and its product prostaglandin E2 (PGE2) have a role in the pathogenesis of cutaneous LP.

Methods

The study was approved by the local research ethics committee of the Faculty of Medicine, Beni Suef University. The study protocol conformed to the ethical
guidelines of the 1975 Declaration of Helsinki, as reflected in a priori approval by the human research committee. The purpose of the study was explained to each patient, and written informed consent was taken before data collection. Patient confidentiality was maintained by code numbers given to each patient, and all personal data were concealed.

Selection of the study subjects
In total, 31 patients with classic cutaneous LP referred to the dermatology clinic at Beni Suef University Hospitals (Beni Suef, Egypt) and 30 healthy controls (HCs) with no inflammatory skin disease, who were matched for age and sex, were included. Exclusion criteria included presence of malignancies or autoimmune diseases other than LP, and receipt of phototherapy.

A complete clinical examination of the skin and mucosa was carried out for exclusion of any associated diseases. Skin samples were taken by 4-mm punch biopsies from the lesional and nonlesional skin of all patients, and the normal skin of HCs. One biopsy from each participant was stained with haematoxylin and eosin for routine histopathological examination to confirm the diagnosis of LP. All other biopsies were stored at −80 °C until required.

In total, 31 patients with cutaneous LP (15 women, 16 men; mean ± SD age 37.2 ± 12.4 years), and 30 healthy controls (14 women, 16 men; mean ± SD age 32.03 ± 7.4) were enrolled.

Detection of cyclooxygenase 2 gene expression in tissue using real-time PCR
Real-time reverse transcription quantitative PCR (RT-qPCR) was used to detect COX2 gene expression. Total RNA was isolated from skin tissue homogenates using RNeasy Purification Reagent (Qiagen, Valencia, CA, USA) according to the manufacturer’s instructions. The purity (absorbance ratio: A260/A280) and concentration of RNA were obtained using spectrophotometry (GeneQuant 1300; GE Healthcare, Uppsala, Sweden). RNA quality was confirmed by gel electrophoresis.

First-strand cDNA was synthesized from 4 μg of total RNA using an Oligo(dT)12–18 primer and Superscript™ II RNase Reverse Transcriptase (both part of a kit; SuperScript Choice System; Life Technologies, Breda, the Netherlands). This mixture was incubated at 42 °C for 1 h.

RT-PCR amplification was carried out using 10 μL amplification mixtures containing Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA USA), equivalent to 8 ng of reverse-transcribed RNA and 300 nmol/L primers (sequences given in Table 1). Reactions were run on an ABI PRISM 7900 HT detection system (Applied Biosystems) with cycle conditions as follows: 1 cycle at 95 °C for 10 min, followed by 40 cycles at 94 °C for 15 s and 60 °C for 1 min. Data were analysed with ABI Prism sequence detection system software (Applied Biosystems) and quantified using Sequence Detection software (v1.7; PE Biosystems, Foster City, CA, USA). Relative expression of studied genes was calculated using the comparative threshold cycle method (ΔΔCt). All values were normalized to GAPDH13 (sequences given in Table 1).

Measurement of prostaglandin E2 level
Each skin biopsy was homogenized in PBS and centrifuged at 10 000 g for 5 min, then the supernatant was examined for PGE2 level using a PGE2 EIA Kit (cat. no. 514010; Cayman Chemical, Ann Arbor, MI, USA) according to the manufacturer’s instructions. Concentrations were expressed as pg/mL.

Statistical analysis
Comparison of quantitative variables between the study groups was carried out using the Mann–Whitney U-test for independent samples. Comparison of sex distribution between the study groups was carried out using the χ² test. The correlation between various variables was assessed using the Spearman rank correlation equation for nonnormal variables. The significance level was set at P ≤ 0.05. All statistical calculations were carried out using Microsoft Excel (2007; Microsoft Corp., Redmond, WA, USA) and SPSS (v15.0 for Windows; SPSS Inc., Chicago, IL, USA).

Results

Participants
There was no difference between the groups in sex (P = 0.9) or age (P = 0.06). Disease duration ranged from 0.5 to 36 months (median 4 months).

Table 1 Primers used for PCR.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Direction</th>
<th>Sequence (5’→3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>COX-2</td>
<td>Forward</td>
<td>GCAAATCCTTGCTGTTCCAATC</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>GGGAAGGCTTCCACGTTCCTT</td>
</tr>
<tr>
<td>GADPH</td>
<td>Forward</td>
<td>AGGCCACATGCTGAGAACAC</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>GCCCAATACGACCAATCC</td>
</tr>
</tbody>
</table>

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Expression of cyclooxygenase-2 and prostaglandin E2

All patients and controls had expression of COX-2 mRNA in their skin biopsies. The mean level of COX-2 mRNA was significantly higher in the lesional biopsies from patients with LP compared with HCs (2.2 ± 0.64 vs. 0.27 ± 0.18; P < 0.001) and was also significantly higher in nonlesional biopsies than in normal control biopsies (0.89 ± 0.34 vs. 0.27 ± 0.18; P < 0.001) (Table 2, Fig. 1).

Similarly, all the patients and controls had detectable levels of PGE2 in their skin biopsies. The mean level of PGE2 was significantly higher in the lesional biopsies from patients with LP compared with normal control biopsies (499.2 ± 129.6 vs. 107.9 ± 27.4 pg/mL; P < 0.001), and was also higher in nonlesional biopsies than in normal control biopsies (Table 2, Fig. 2).

There was no significant correlation in the patient group between COX-2 mRNA expression and either age or disease duration (r = 0.01; P = 0.9 and r = 0.18; P = 0.3 respectively), or between PGE2 level and either age or disease duration (r = -0.02; P = 0.8, r = -0.12; P = 0.5 respectively).

There was no significant difference between male and female patients (both lesional and nonlesional skin) for either COX-2 mRNA expression (P = 0.5, P = 0.6 respectively) or PGE2 protein levels (P = 0.7, P = 0.6 respectively).

Discussion

LP is a relatively common, chronic, inflammatory, mucocutaneous disease that is characterized by a T cell-mediated immune response against epithelial cells, along with epithelial cell damage and subepithelial band-like infiltration of T lymphocytes.1,2

Arachidonic acid metabolites are known to have significant roles in initiating and/or terminating inflammatory processes. PGE2 is the most abundant metabolite of arachidonic acid, generated through an enzymatic cascade controlled by cyclooxygenase enzymes (COX-1, COX-2).14 COX-2 is associated with development of autoimmune diseases such as autoimmune arthritis and systemic lupus erythematosus.7,8

In human keratinocytes, the major prostaglandins produced are PGE2 and PGF2α, with small quantities of prostacyclins. PGE2 has been shown to be induced by ultraviolet rays through the upregulation of COX-2 enzyme activity.15

In the current study, we found that COX-2 expression and PGE2 level in lesional and nonlesional biopsies from patients with cutaneous LP were significantly increased compared with normal skin from HCs. These results suggested that these two major immune molecules might play a role in the pathogenesis of LP.

Although there have been many previous reports investigating the role of COX-2 and PGE2 in the

Table 2 Comparisons of prostaglandin E2 and cyclooxygenase-2 mRNA level.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients (n = 31), mean ± SD</th>
<th>Controls (n = 30)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostaglandin E2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lesional skin</td>
<td>499.2 ± 129.6</td>
<td>107.9 ± 27.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Nonlesional</td>
<td>226.1 ± 55.4</td>
<td>107.9 ± 27.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cyclooxygenase-2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lesional skin</td>
<td>2.2 ± 0.65</td>
<td>0.27 ± 0.18</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Nonlesional</td>
<td>0.89 ± 0.34</td>
<td>0.27 ± 0.18</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*All P-values were highly significant.
pathogenesis of OLP,9–12 to our knowledge this is the first study to investigate the COX-2 and PGE2 in cutaneous LP.

COX-2 and its product PGE2 have an immunomodulatory role. PGE2 may function as a double agent, initially triggering, facilitating and augmenting a stimulatory immune response, and later modulating, limiting and contributing to the turning off of this response. PGE2 promotes the migration of dendritic cells (DCs) to regional lymph nodes, and accelerates their maturation, increases their expression of costimulatory molecules and enhances their ability to stimulate T cells.16 Several studies have shown the presence and accumulation of different types of DCs in LP lesions.17

Previous findings have clearly established that PGE2 affects the differentiation and capacity of producing cytokines in T cells.18 Yao et al.19 reported that, in the presence of interleukin (IL)-12, PGE2 facilitates T helper (Th)1 differentiation. Both keratinocytes and T lymphocytes in cutaneous LP are capable of producing interferon-γ, which is a Th1 cytokine.20 Taken together, these results indicate a possible role of PGE2 in the pathogenesis of LP by driving the immune system towards a Th1 response.

PGE2 has also been shown to induce IL-23 production by DCs. IL-23 favours Th17 cell polarization and IL-17 production.21 Patients with cutaneous LP were found to have higher expression of IL1-7, IL-22 and IL-23 compared with controls.22 These findings indicate that PGE2 could share in the pathogenesis of LP by driving the immune system towards Th17 response.

In our study, the higher expression of COX-2 and PGE2 in nonlesional skin of patients compared with normal skin of HCs indicates that even clinically nonlesional skin may harbour specific cellular events that might eventually occur in the epidermis before LP lesions are clinically detectable.

Conclusion

We found higher expression of both COX-2 and PGE2 in both lesional and nonlesional skin of patients with LP compared with controls. We propose that COX-2 and PGE2 may contribute to the development of LP through facilitation of DC migration, activation and cytokine production, together with a shift of the immune response towards Th1 and Th17. Local modulation of the COX-2 and its product PGE2 could have potential as treatment regimens for LP. However, further studies are needed to evaluate the expression of various PGE2 receptors in LP lesions, and the expression of COX-2 and PGE2 in other types of cutaneous LP.

What’s already known about the topic?

- LP is a chronic inflammatory mucocutaneous disease of autoimmune aetiology.
- COX-2 levels were found to be elevated in patients with oral LP.

What does this study add?

- High levels of COX-2 and PGE2 were detected in classic cutaneous LP lesions.
- Both could contribute to the development of LP through their immunomodulatory properties.

References


