SB203580, a p38 mitogen-activated protein kinase inhibitor, suppresses the development of endometriosis by down-regulating proinflammatory cytokines and proteolytic factors in a mouse model

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BACKGROUND: p38 mitogen-activated protein kinase (p38 MAPK), a regulator of inflammation, may play a role in the pathogenesis of endometriosis (EM). We studied the effect of SB203580, a p38 MAPK inhibitor, on the development of EM in a mouse model.

METHODS: EM was induced in BALB/c mice by peritoneal injection of endometrium-rich fragments. Mice (n = 15) were injected i.p. for 24 days with SB203580 and 15 mice served as positive controls (EM group). Sham-operated mice received carrier only. Peritoneal fluid (PF) cells were collected for protein/mRNA analysis. Interleukin (IL)-1β, tumor necrosis factor (TNF)-α, matrix metalloproteinase-2 (MMP-2) and MMP-9 proteins were measured using enzyme-linked immunosorbent assay and mRNAs by RT–PCR. Phosphorylation of p38 MAPK was evaluated by western blotting.

RESULTS: SB203580 decreased the weight and size (P < 0.05 versus EM) of endometriotic lesions in BALB/c mice. IL-1β, TNF-α, MMP-2 and MMP-9 mRNA levels were decreased in peritoneal cells of the SB203580 versus EM group (P < 0.01, P < 0.05, P < 0.05 and P < 0.05, respectively). Concentrations of IL-1β, TNF-α, MMP-2 and MMP-9 proteins in PF were reduced in the SB203580 versus EM group (P < 0.05, P < 0.01, P < 0.05 and P < 0.05, respectively). Compared with the sham-operated group, phosphorylation of p38 MAPK in the EM group was increased, and this was down-regulated by SB203580 (P < 0.01).

CONCLUSIONS: SB203580 may suppress the development of EM by inhibiting expression of proinflammatory cytokines and proteolytic factors. p38 MAPK might play a key role in progression of EM.

Key words: endometriosis / mouse model / p38 mitogen-activated protein kinase / cytokines / proteolytic factors

Introduction

Endometriosis (EM), defined as the presence of endometrial tissue outside of the uterus, represents a common, benign and multifactorial gynecological condition that tends to be associated with pelvic pain and infertility (Giudice and Kao, 2004). Despite the fact that the underlying mechanisms responsible for EM remain unclear, Sampson’s theory of retrograde menstruation is the most widely accepted. Moreover, a substantial body of evidence suggests that inflammation plays an important role in the development of EM (Harada et al., 2001; Lebovic et al., 2001; Podgaec et al., 2007; Khan et al., 2010).

Some studies have demonstrated that a number of cytokines in the peritoneal fluid (PF) of EM patients are involved in the pathogenesis and progression of the disease (Bedaiwy et al., 2002). Also, the number and activity of macrophages in the PF were shown to be increased. In women with EM, the PF has high levels of
proinflammatory cytokines derived from the lesions themselves, activated macrophages and other immune cells (Giudice and Kao, 2004; Kyama et al., 2008). Expression of several proteolytic factors was also found to be abnormal in EM subjects (Wu and Ho, 2003; Pitsos and Kanakas, 2009; Cosin et al., 2010). Among others, proinflammatory cytokines, such as interleukin (IL)-1β and tumor necrosis factor (TNF-α), and proteolytic factors, such as matrix metalloproteinase-2 (MMP-2) and MMP-9, were suggested to play important roles in the development of EM (Carlberg et al., 2000; Cheong et al., 2002; Collette et al., 2006; Pitsos and Kanakas, 2009; Chen et al., 2009, 2010).

Recent studies have shown that p38 mitogen-activated protein kinase (p38 MAPK), an intracellular signal-transducing molecule, plays an important role in the regulation of a variety of inflammatory responses, including expression of proinflammatory cytokines, leukocyte adhesion and chemotaxis (New and Han, 1998; Herlaar and Brown, 1999). Increasing evidence indicates that activation of p38 MAPK might be involved in the pathogenesis of EM (Yoshino et al., 2003, 2004, 2006; Seval et al., 2006).

These findings led us to hypothesize that inhibition of p38 MAPK activity might relieve EM-related inflammation and thereby suppress the development of the disease. In the present study, we established a murine model of EM and examined the effect of SB203580, a specific inhibitor of p38 MAPK (English and Cobb, 2002; Mayer and Callahan, 1999). The injection of vehicle or SB203580 equal to that of SB203580. The sham-operated mice served as the negative controls (sham-operated group, n = 15), which received no medication but did have an i.p. injection of isotonic saline solution (volume equal to that of SB203580). The injection of vehicle or SB203580 started 3 days before injection of the endometrial fragments (in SB203580 and EM groups) or the PBS (in sham-operated group) and lasted for 24 days. Then, the mice were sacrificed by cervical dislocation on the day of the last injection and surgical procedures were performed under aseptic conditions.

**Materials and Methods**

**Chemicals and reagents**

SB203580 was purchased from Calbiochem (La Jolla, CA, USA); the ELISA kits were obtained from R&D Systems (Minneapolis, MN, USA); SYBR PrimeScript RT–PCR Kit was purchased from TaKaRa Co. (Kyoto, Japan); RNA Isolation Kit was a product of GE Healthcare (Uppsala, Sweden). p38 MAP Kinase antibody (#9212L) and phospho-p38 MAP Kinase (Thr180Tyr182) antibody (#9215L) were from Cell Signaling Technology (MA, USA). Other reagents were all of analytical grade. All reagents, except the phosphate-buffered saline (PBS), were prepared the day before use and stored in a refrigerator at 4 °C. The reagents were equilibrated at room temperature for 0.5 h before use.

**Animals**

BALB/c mice were provided by the Animal Research Laboratory of Xiamen University. Forty-five female BALB/c mice (aged 8 weeks and weighing 19–21 g) were kept on a light/dark cycle of 12/12 h under conditions of a constant temperature of 21–22 °C and 60–65% humidity. The mice were caged in groups of five for a week before the start of the experiment, with free access to a commercially balanced mouse diet and tap water. The animals were obtained, maintained and used in accordance with the ‘Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals’ formulated by Xiamen University’s Animal Ethics Committee. The study was approved by the institutional review board of Xiamen University.

**Experimental design and treatment**

The EM was induced using a previously described method (Chen et al., 2009). Briefly, after the donor mice were sacrificed, the uterine horns were removed and put into a dish containing PBS. The endometrium-rich fragments, obtained by peeling off the serosa and myometrium, were finely chopped using a razor blade. Fragments suspended in 1 ml of PBS at 37 °C were injected, with an 18-gage needle and via the abdominal wall, into the peritoneal cavity of recipient mice with a ratio of 1:2 (donor:recipient). In sham-operated mice, 1 ml of PBS without fragments was injected. The operation was limited to 10 min for each mouse.

Thirty mice with experimentally induced EM were randomly divided into two groups. The first group was designated as the study group (SB203580 group, n = 15), which was injected i.p. with SB203580 at a dose of 1 μg/0.5 ml on a daily basis (this dose was established in a pre-test). The second group was the positive control group (EM group, n = 15), which received no medication except i.p. injection of isotonic saline solution (volume equal to that of SB203580). The sham-operated mice served as negative controls (sham-operated group, n = 15), which received no medication but did have an i.p. injection of isotonic saline solution (volume equal to that of SB203580). The injection of vehicle or SB203580 started 3 days before injection of the endometrial fragments (in SB203580 and EM groups) or the PBS (in sham-operated group) and lasted for 24 days. Then, the mice were sacrificed by cervical dislocation on the day of the last injection and surgical procedures were performed under aseptic conditions.

**Specimen collection**

After sacrificing the animals, 3 ml of PBS was injected into the peritoneal cavity of each mouse. After shaking the mice, the PF was collected and centrifuged at 10,000 g for 1 min. The supernatant was harvested for protein quantification and the precipitated peritoneal cells, including peritoneal macrophages, were used for RNA extraction and western blot analysis. Laparotomy was then performed and the lesions were morphologically observed. Morphologically, ectopic lesions of EM present as red, blue, brown or transparent blotches, small cysts or scars on the surface of organs or on the peritoneum of the abdominopelvic cavity; therefore, they can be identified with the naked eye. All lesions and the uteri were quickly excised and were removed from the normal surrounding tissues for identification and weighing. The lesions were put in formaldehyde solution for routine histopathological examination. The pathological diagnostic criteria for EM are the presence of any two of the four aforementioned changes, i.e. the presence of endometrial epithelial cells, glands or gland-like structures, stroma and hemorrhage (Chen et al., 2010).

**RNA extraction and quantitative real-time RT–PCR**

Total RNA was extracted from peritoneal cells using the RNAspin Mini Kit following the manufacturer’s instructions and was reverse transcribed in 20 μl total volume using the SYBR PrimeScript RT–PCR kit. The conditions for reverse transcription included 37 °C for 15 min (reverse transcription reaction) and one cycle at 85 °C for 5 s (denaturation of reverse transcriptase). The synthesized cDNA was stored at −80 °C.

The mRNA levels for IL-1β, TNF-α, MMP-2 and MMP-9 in each sample were determined by a real-time quantitative PCR. Primers were synthesized by TaKaRa Co. The primers used for real-time PCR were: IL-1β primers (forward 5′-TCTCAAGTGAGATGACTGAG-3′ and reverse 5′-GACACACTCAACACAGCGGT-3′); TNF-α primers (forward 5′-AAGCGCTTGGCCCAGCCT-3′ and reverse 5′-GGCCACCCTAGTTGGTTGTTACT-3′); MMP-2 primers (forward

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Suppression of endometriotic lesion growth by SB203580, a specific p38 MAPK inhibitor, in a mouse model. The total weight of all endometriotic lesions per mouse in the EM and SB203580 groups is shown. EM was successfully induced in 14 mice in both groups. Horizontal bars represent the mean weight of endometriotic lesions. $P < 0.01$ significant weight decrease in the SB203580 versus the EM group.

**Results**

Suppressing effect of SB203580 on the growth of endometriotic lesions

Twenty-one days after induction, 30 BALB/c mice with EM were sacrificed, and the endometriotic lesions were collected and examined (Fig. 1). The total weight of all endometriotic lesions per mouse in the EM group and the SB203580 group are shown in Fig. 2. The weight of endometriotic lesions in the SB203580 group (28.01 ± 6.58 mg) was significantly less than in EM group (68.89 ± 7.20 mg) ($P < 0.01$). In addition, a significant difference in endometriotic lesion diameter was observed between the two groups (Table I). EM was successfully induced in 14 mice, both in the EM and SB203580 groups. In the 15 sham-operated mice, no endometriotic lesion was observed. Furthermore, uterine weight in EM group was significantly increased compared with that of the sham-operated group ($P < 0.01$) but this increase was decreased by treatment with SB203580 ($P < 0.05$). Body weight among the three groups did not vary significantly, and there was no significant difference in lesion growth of endometriotic lesions.

**Statistical analysis**

Statistical analysis was performed by using the Statistical Package for the Social Sciences software package (version 14.0). The Lilliefors-adjusted Kolmogorov–Smirnov test was utilized to test whether the study variables were normally distributed: all the parameters studied showed a normal distribution. The unpaired Student’s $t$-test was used for comparisons. Data were expressed as the mean ± SEM. A $P$-value of <0.05 was considered to be statistically significant.

Protein quantification

Concentrations of IL-1β, TNF-α, MMP-2 and MMP-9 protein in the PF were measured using commercially available, cytokine-specific ELISA Kits. Frozen PF was thawed and then analyzed according to the manufacturer’s instructions. The intra- and interassay coefficients of variation were less than 5% in these assays.

Western blot analysis

Cells were lysed on ice with RIPA lysis buffer (Applygen Technologies Inc., China) containing 50 mM Tris–HCl (pH 7.4), 150 mM NaCl, 1% Nonidet P-40, 0.1% SDS and protease inhibitor cocktail (Roche, Switzerland). The extract was centrifuged at 12 000g for 5 min at 4°C, and the supernatant was collected. Protein concentration was determined by using a BCA assay (Applygen Technologies Inc.). Forty micrograms of total protein extract was centrifuged at 12 000g for 5 min at 4°C, and the endometriotic lesions were collected and examined for the possibility of primer–dimer formation. The reactions to check for the possibility of primer–dimer formation. The 2$−ΔΔCt$ method was used to calculate the relative mRNA level of each gene (Livak and Schmittgen, 2001).

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Suppression of endometriotic lesion growth by SB203580, a specific p38 MAPK inhibitor, in a mouse model. The total weight of all endometriotic lesions per mouse in the EM and SB203580 groups is shown. EM was successfully induced in 14 mice in both groups. Horizontal bars represent the mean weight of endometriotic lesions. $P < 0.01$ significant weight decrease in the SB203580 versus the EM group.
Table I  The number and size of endometriotic lesions, body weight and uterine weight in the sham-operated, EM and SB203580 groups of mice, 24 days after the injection of endometrial fragments.

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight at sacrifice (g)</th>
<th>Weight of uterus (mg)</th>
<th>Number of lesions per mouse</th>
<th>Lesion diameter at sacrifice (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham (n = 15)</td>
<td>20.05 ± 0.98</td>
<td>27.91 ± 10.92</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>EM (n = 15)</td>
<td>20.39 ± 1.03</td>
<td>87.95 ± 27.95*</td>
<td>2.14 ± 0.03</td>
<td>4.13 ± 1.39</td>
</tr>
<tr>
<td>SB203580 (n = 15)</td>
<td>21.00 ± 1.33</td>
<td>45.67 ± 12.04b</td>
<td>1.90 ± 1.20</td>
<td>2.05 ± 0.44b</td>
</tr>
</tbody>
</table>

SB203580, specific inhibitor of p38 MAPK; results are mean ± SEM. EM was successfully induced in 14 mice in both the EM and SB203580 groups.

Table II  Peritoneal cell mRNA and PF protein levels of IL-1β and TNF-α in the sham-operated, EM and SB203580 groups of mice, 24 days after the injection of endometrial fragments.

<table>
<thead>
<tr>
<th>Group</th>
<th>IL-1β mRNA</th>
<th>IL-1β (pg/ml)</th>
<th>TNF-α mRNA</th>
<th>TNF-α (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>1.00 ± 1.08</td>
<td>84.98 ± 4.85</td>
<td>1.00 ± 0.41</td>
<td>291.50 ± 46.37</td>
</tr>
<tr>
<td>EM</td>
<td>11.56 ± 1.06a</td>
<td>106.49 ± 10.93b</td>
<td>3.76 ± 0.51a</td>
<td>462.54 ± 50.16b</td>
</tr>
<tr>
<td>SB203580</td>
<td>4.44 ± 0.65c</td>
<td>67.85 ± 7.39d</td>
<td>2.04 ± 0.33d</td>
<td>252.71 ± 44.03a</td>
</tr>
</tbody>
</table>

Data are the mean ± SEM (n = 15).

Increased expression of proinflammatory cytokines and proteolytic factors in the EM BALB/c mouse model

High expression of proinflammatory cytokines and proteolytic factors were observed in the EM group when compared with the sham-operated group (Tables II and III). In EM group, the mRNA and protein levels of IL-1β, TNF-α, MMP-2 and MMP-9 were significantly higher than those of the sham-operated group. To be specific, the relative mRNA levels of IL-1β, TNF-α, MMP-2 and MMP-9 increased from (1.00 ± 1.08, 1.00 ± 0.41, 1.00 ± 0.05 and 1.00 ± 0.35) to (11.56 ± 1.06, 3.76 ± 0.51, 1.62 ± 0.09 and 2.30 ± 0.23), respectively. The protein levels of IL-1β, TNF-α, MMP-2 and MMP-9 rose from (84.98 ± 4.85 pg/ml, 291.50 ± 46.37 pg/ml, 1.86 ± 0.07 ng/ml and 1.01 ± 0.33 ng/ml) to (106.49 ± 10.93 pg/ml, 462.54 ± 50.16 pg/ml, 2.70 ± 0.14 ng/ml and 3.17 ± 0.31 ng/ml), respectively, with mRNA levels decreasing from 11.56 ± 1.06 to 4.44 ± 0.65 and from 3.76 ± 0.51 to 2.04 ± 0.33, respectively (Table II). The levels of MMP-2 and MMP-9 mRNA showed the same trend as IL-1β and TNF-α. SB203580 decreased the relative mRNA levels of MMP-2 from 1.62 ± 0.09 to 1.15 ± 0.11 and MMP-9 from 2.30 ± 0.23 to 1.37 ± 0.14 (P < 0.05) (Table III).

Effects of SB203580 on IL-1β, TNF-α, MMP-2 and MMP-9 protein levels in mouse PF

Compared with the EM group, the protein concentrations of IL-1β and TNF-α in the PF were significantly lower in the SB203580 group (P < 0.05 and P < 0.01, respectively). SB203580 decreased protein concentrations of IL-1β from 106.49 ± 10.93 to 67.85 ± 7.39 pg/ml and TNF-α from 462.54 ± 50.16 to 252.71 ± 44.03 pg/ml (Table II). Similarly, the protein levels of MMP-2 and MMP-9 were significantly lower in the SB203580 than the EM group (P < 0.05). After treatment with SB203580, the protein levels of MMP-2 and MMP-9 decreased from 2.70 ± 0.14 to 1.74 ± 0.26 ng/ml and from 3.17 ± 0.31 to 1.98 ± 0.24 ng/ml, respectively (Table III).

Inhibitory effect of SB203580 on the phosphorylation of p38 MAPK in mouse peritoneal cells

Total and phosphorylated p38 MAPK was detected in peritoneal cavity cells from the sham-operated group, EM group and SB203580 group (Fig. 3).
Compared with the sham-operated group, the phosphorylation of p38 MAPK in the EM group was significantly increased. After treatment with SB203580, the phosphorylation level was significantly decreased \( (P, 0.01) \). However, the amount of total p38MAPK was unchanged in the three groups.

**Discussion**

Although EM is a common gynecological disorder, the exact pathogenesis of the condition remains elusive. Recently, many studies reported that inflammatory mediators and proteolytic factors were involved in the progression of EM. In addition, results from a number of studies also suggested that MAPKs act as pivotal intracellular signal transducers in endometrial and endometriotic cells and might play a role in the development and progression of EM (Yoshino et al., 2003, 2004). However, studies are scanty on the effect of p38 MAPK on the proinflammatory cytokines and proteolytic factors in EM.

The present study showed that treatment with SB203580, a specific p38 MAPK inhibitor, effectively decreased the weight of experimentally induced endometriotic lesions in a BALB/c mouse model. The mRNA levels of IL-1\( \beta \), TNF-\( \alpha \), MMP-2 and MMP-9 from peritoneal cavity cells and the concentrations of IL-1\( \beta \), TNF-\( \alpha \), MMP-2 and MMP-9 proteins in the PF were increased in mice with EM, whereas this increase was suppressed by SB203580 treatment. This study also demonstrated that SB203580 decreased the phosphorylation of p38 MAPK in mice with EM. These findings suggest that inhibition of p38 MAPK activity might relieve EM-related inflammation and thereby slow down the progression of the disease.

Moreover, we observed that the uterine weight of mice with EM was significantly increased when compared with the sham-operated mice, in which the weight was decreased by SB203580 treatment. Changes in the inflammatory environment in the abdominal cavity might explain this effect. The EM-related inflammation led to the edema of uterus. Inhibition of p38 MAPK activity by SB203580 might relieve the inflammation and thus relieved the edema of uterus. This is an interesting finding and warrants further study.

The p38 MAPK pathway is involved in the regulation of many cellular processes including inflammation, cell differentiation, cell growth and death. Activation of p38 MAPK by extracellular stimuli, such as bacterial pathogens and cytokines, mediates signal transduction into the nucleus to turn on the responsive genes (Ono and Han, 2000). As an intracellular signal-transducing molecule, p38 MAPK plays an important role in regulating the expression of many proinflammatory factors and proteolytic factors, such as IL-1\( \beta \), TNF-\( \alpha \), MMP-2 and MMP-9. IL-1\( \beta \) and TNF-\( \alpha \), the most frequently studied cytokines in EM, are both pleiotropic proinflammatory cytokines which might contribute to establishment of the disease by facilitating adhesion of endometrial cells to the peritoneal mesothelium (Zhang et al., 1992; Cheong et al., 2002), up-regulating the release of MMPs (Wu and Ho, 2003; Dmowski and Braun, 2004) and promoting angiogenesis (Dmowski and Braun, 2004; Groothuis et al., 2005). MMP-2 and MMP-9 have been shown to be involved in implantation and further invasion of seeded endometriotic explants (Hudelist et al., 2005;...
that SB203580 was pharmacologically active in vivo in several animal models and was a potent inhibitor of cytokine production with only minor effects on the immune system (Badger et al., 1996; Herlaar and Brown, 1999). Therefore, inhibition of the production or effects of cytokines is a rational therapeutic strategy. And the development of a low molecular weight orally active cytokine inhibitor with the pharmacological profile of SB203580 may well provide significant beneficial effects for inflammation-related diseases. In the near future, studies will need to demonstrate whether compounds currently in development have the appropriate properties for further long-term development for application to human disease.

In conclusion, our studies showed that SB203580, a specific p38 MAPK inhibitor, could suppress the progression of EM by down-regulating IL-1β, TNF-α, MMP-2 and MMP-9 levels in a mouse model, which might involve in the p38 MAPK pathway. This inhibition of proinflammatory cytokines and proteolytic factors might contribute to the suppression of endometriotic lesions by SB203580 treatment in the mouse model. However, the specific mechanisms by which the expression of the proinflammatory and proteolytic factors are regulated by p38 MAPK warrant further investigation.

Authors’ Roles


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References


