Effect of Mifepristone on Transforming Growth Factor-β and its Receptor Transcription in Decidua, Villi and Serum Tumor Necrosis Factor-α Level in Early Human Gestation

Li-ping JIN¹, Da-jin LI¹, Jing-yu SHAO², Song-guo ZHENG³, Xue-zhe WU²
1. Laboratory of Reproductive Immunology, Institute of Obstetrics and Gynecology, Fudan University, Shanghai 200011, China
2. Shanghai First Maternity & Infant Health Hospital, Shanghai 200040, China
3. Laboratory of Molecular Biology, Tumor Hospital, Fudan University, Shanghai 200032, China

Objective To investigate the role of mifepristone in regulating cytokines of materno-fetal interface and serum of human early gestation

Methods Thirty-five women with early pregnancy received mifepristone 50 mg orally on study d 1 and d 2, respectively, followed by undergoing artificial abortion to get decidua and villi on study d 3. Twenty-five women with early pregnancy without mifepristone administration as control also underwent artificial abortion to get decidua and villi. The expressions of TGF-β₁ and TGF-β₁ receptor mRNA in the early decidua and villi were assessed by using RT-PCR. The concentrations of serum TNF-α were measured by radioimmunoassay.

Results The decidual expressions of TGF-β₁ mRNA and TGF-β₁ receptor mRNA in the treated group were significantly lower than those of the control (P<0.05), while the villus expressions of TGF-β₁ and TGF-β₁ receptor mRNA in the treated group were not significantly different from those of the control (P>0.05). The serum TNF-β₁ levels elevated significantly after mifepristone treatment.

Conclusion The antigestational effect of mifepristone might act through suppressing the transcription of TGF-β₁ and TGF-β₁ receptor in the decidua and increasing the serum TNF-α level, which interfered in the materno-fetal interface Th2 bias.

Keywords: Mifepristone; decidua; villi; transforming growth factor-β; tumor necrosis factor-α

Corresponding author: Li-ping JIN; Tel/fax: +86-21-63787331; E-mail: yupulis@online.sh.cn
Mifepristone is a progesterone antagonist which acts at the level of the receptor. It binds the progesterone receptor with a high affinity and antagonises progesterone biological action. It has been applied in clinical use for multiple therapeutic purposes. In obstetrics and gynecology, mifepristone has been effective in medical termination of pregnancy in the first trimester. Mifepristone followed approximately 48 h later by a prostaglandin analog has been reported to effect complete abortion in 95% to 99% of women with pregnancies as advanced as 49 days' gestation [1-3]. Thus, mifepristone plays a critical role in medical abortion.

There is clear evidence suggesting that the maternal immunity during pregnancy can enhance or inhibit the development of the feto-placental unit. Recent data support the view that many beneficial cytokines, such as TGF-β, IL-1, IL-4, IL-6 and IL-10, favor fetal survival and growth, while many deleterious cytokines, such as TNF-α, IFN-γ and IL-2, can rather compromise pregnancy. Thus, despite the complexity of the cytokine network, it appears that cytokines favoring the maintenance of fetal survival mainly belong to the Th2-type, whereas the failure of pregnancy is rather associated with the predominance of Th1-type cytokines and/or the absence of Th2-type cytokines at materno-fetal interface and in peripheral blood [4, 5]. In the present study, we try to evaluate the role of mifepristone in regulating mRNA expression of TGF-β, and TGF-β receptor (TGF-βR) on maternal-fetal interface and serum TNF-α level.

Subjects & Methods

Subjects

Between October 1997 and February 1998, 60 women were confirmed with early pregnancy by transvaginal ultrasonography in Shanghai First Maternity & Infant Health Hospital, and were recruited as subjects for this study. Moreover, all subjects met the following standards such as intrauterine pregnancy<49 d, no history of lactation and abortion within one year, no IUD, <=40 years, a history of regular cyclic menses and no history of any hormonal medications within 3 months before participation in this study. All subjects undertook artificial abortion to terminate pregnancy. The participants were randomly assigned into two groups. Thirty-five subjects received mifepristone (Shanghai Hualian Pharmaceutical Co., Ltd) 50 mg orally on study d 1 and d 2 respectively, followed by undergoing artificial abortion on study d 3. As control, 25 subjects without mifepristone administration also underwent artificial abortion. There was no significant difference between the two groups in clinical features of the subjects, including age, gravidity, parity, gestational age and weight.

Methods

The oligonucleotide primers (Shanghai Institute of Biochemistry & Cell Biology, Chinese Academy of Sciences) were designed by Oligo Primer Analysis Software. The primers were shown in Table 1.
The decidua and villi were obtained from artificial abortion. Total RNA was extracted from the decidua and villi tissues by the guanidinium isothiocyanate extraction procedure (Shanghai Huaxun Co., Ltd). The total RNA was as a template for the first strand cDNA synthesis by reverse transcription (RT reagent kit was from Shanghai Huamei Co., Ltd). Then, PCR was performed. PCR amplification (Perkin Elmer Co., USA) was carried out for 32 cycles for 50 s at 94°C, 45 s at 58°C and 45 s at 72°C. The amplification products were analyzed by electrophoresis in a 1% agarose gel, and were photographed and semi-quantified by SX-100 image system (Alpha Innotech Co., USA) with β-actin product for reference.

Serum samples were taken from 21 women before and after mifepristone treatment, and the concentration of TNF-α was measured by radioimmunoassay according to the manufacturing instructions (Tongya Immunologic Technology Academy).

Statistical analysis

Data were analyzed by SAS software and determined by Student’s t test. P value < 0.05 was considered significant.

Results

Transcription level of TGF-β1 and TGF-β1R in the decidua and villi after mifepristone treatment

The expression of TGF-β1 and TGF-β1 R mRNA in early human decidua of the treatment group were significantly lower than that of the control (P<0.05), while there were no significant difference in the expression of TGF-β1 and TGF-β1 R mRNA in early human villi between both groups (P>0.05, Table 2, Figure 1).

Concentration of serum TNF-α

After mifepristone administration, the concentration of serum TNF-α of 21 early pregnant women elevated significantly (0.89 ± 0.16 vs 0.56 ± 0.12) (P<0.05).

**Table 1** Sequence of the oligonuclotide primers

<table>
<thead>
<tr>
<th>Amplified product</th>
<th>Length (bp)</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGF-β1</td>
<td>48</td>
<td>5'- CAAGCAGAGTACACACAGCA-3'</td>
<td>5'-GATGCTGGCCCTCTCCAGC-3'</td>
</tr>
<tr>
<td>TGF-β1R</td>
<td>585</td>
<td>5'-CAGAGGGAAGAGTTCCCCAG-3'</td>
<td>5'-CCTTGGTCTGGTAGGAGACG-3'</td>
</tr>
<tr>
<td>β-actin</td>
<td>465</td>
<td>5'-CGGATGTCCACGTCGCACTT-3'</td>
<td>5'-GT TGCCAGTCCAGCTGCAC T-3'</td>
</tr>
</tbody>
</table>
Table 2  The transcription level of TGF-β, and TGF-β, R in decidua and villi

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Decidua</th>
<th>Villi</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>TGF-β</td>
<td>TGF-β, R</td>
</tr>
<tr>
<td>Control</td>
<td>25</td>
<td>1.68 ± 0.63</td>
<td>1.56 ± 0.52</td>
</tr>
<tr>
<td>Treatment</td>
<td>35</td>
<td>1.02 ± 0.48</td>
<td>0.91 ± 0.32</td>
</tr>
</tbody>
</table>

**Discussion**

Mifepristone has been proved to be a safe and effective medical method for termination of early pregnancy. Mifepristone blocks progesterone biological action via an effect on progesterone receptor, which leads to degeneration and necrosis of uterine decidua and villi, and inhibits the development of embryo [6]. In a series of short-term studies in women with oral mifepristone, it had effects on estrogen and progesterone receptors, plasminogen activators, gangliosides in human decidua, serum hormone levels, serum nitric oxide concentration and so forth. These observations prompted us to evaluate the mechanisms of anti-gestational action of mifepristone. These trials suggested that the anti-gestational action of mifepristone was modulated by various approaches, and multiple factors worked in coordination with each other to stop the development of pregnancy. In our study, we examined the effect of mifepristone on the transcription of TGF-β, and TGF-β, R in early human decidua, villi and serum TNF-α level for evaluating the role of mifepristone in regulating the cytokines of materno-fetal interface and serum. The present study showed that TGF-β down-regulation and TNF-α up-regulation might lead to human pregnant termination after mifepristone treatment.

As a result, recent reports shed more and more light on the effect of TGF-β on reproductive regulation. TGF-β regulates the function of hypothalamo-pituitary-ovarian axis, which
contributes to follicular growth and corpus luteum development. Moreover, TGF-β is identified as a potential modulator of endometrial and trophoblastic proliferation and differentiation. TGF-β stimulates oncofetal fibronectin synthesis by trophoblasts, which favors the establishment of the maternal environment contributing to trophoblast adhesion to decidua and allowing embryonic development. Otherwise, TGF-β is an immuno-suppressive factor that acts on a variety of immuno-competent cells. TGF-β inhibits lymphocytic proliferation, immunoglobulin secretion, TNF-α and IFN production by immuno-competent cells and IL-2 biologic activity. Accordingly, TGF-β can exert a direct protective effect on embryo from attack by activated T and NK cells. These observations provide an excellent basis for investigating the role of mifepristone in the regulation of the materno-fetal interaction. These data demonstrated that TGF-β and TGF-β receptor up-regulation in decidua and villi is beneficial to pregnancy maintenance. Interestingly, our results indicated that the expressions of TGF-β1 and TGF-β receptor in early human pregnant decidua in the treated group were significantly lower than those of the control. According to the biological effects of TGF-β, we postulate that mifepristone down-regulates the transcription of TGF-β1 and TGF-β receptor in human decidua of early gestation, leading to disorder of materno-fetal immuno-modulation and maternal rejection to embryo.

On the other hand, the low expressions of TGF-β1 and TGF-β receptor in human decidua influenced the synthesis of extracellular matrix, such as oncofetal fibronectin, which suppressed the ability of trophoblast to adhere to decidua and shed villi from decidua, and resulted in abortion.

TNF-α is produced as a result of local triggering of decidural T cells, and NK cells, as well as macrophages. Excess TNF-α proves to be harmful to trophoblasts and embryo development. TNF-α could trigger local release of prostaglandin F2α and E2 by macrophages or other cells, leading to direct or indirect negative effects on pregnancy outcome. In addition, TNF-α could act by causing local necrosis as a consequence of its action on the blood vessels that penetrate the placenta. Moreover, TNF-α could result in local NK cell activation, recruitment and/or activation of LAKs, thus leading to embryo demise. Our results showed that the concentration of serum TNF-α elevated significantly after mifepristone treatment. Perhaps overabundance of TNF-α did harm to pregnancy through the above mechanisms.

In conclusion, the antigestational effect of mifepristone might act through suppressing the transcription of TGF-β and TGF-β receptor in the decidua and increasing the serum TNF-α level, which interfered in the materno-fetal interface Th2 bias.

References


