Effect of Mifepristone on the Telomerase Activity in Chorion and Decidua during Early Pregnancy

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Objective To investigate telomerase activity in chorion and decidua from abortion induced by mifepristone incorporated with misoprostol at early pregnancy

Methods TRAP-SYBR Green assay was used to detect the expression of telomerase. Forty specimen were obtained from medicinal abortion (experiment group) and forty were from normal induced abortion (control group).

Results Positive expression of chorion telomerase was significantly different between the experimental group (28%, 11/40) and the control group (73%, 29/40) (P<0.05). While in decidua, the positive rate was 28% (11/40) in the experimental group and 20% (9/40) in the control group, there was no significant difference (P>0.05).

Conclusion It is suggested that miferistone may significantly decrease the telomerase activity in chorion but not in decidua.

Key Words: mifepristone; early gestation abortion; chorion; decidua; telomerase

Mifepristone incorporated with misoprostol has been widely used in abortion during early pregnancy, but the mechanism has not been clearly stated. We aimed to study the effect of mifepristone incorporated with misoprostol on telomerase expression in chorion and decidua.

Materials & Methods

Subjects
All the patients were healthy and between 21-29 years old. They were selected from patients attending our hospital who had gestation of 5-7 weeks and did not have internal

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complications as well as other diseases. They did neither take any steroid hormones in the recent half year nor take any contraceptive measure before. Result of urinary test was positive and B-ultrasound found early intrauterine pregnancy. The patients were divided into two groups randomly: 40 patients who undertook medical abortion were classified as experimental group and another 40 who undertook negative pressure aspiration were control group.

**Specimen collection**

In the experimental group, totally 150 mg mifepristone (Shanghai Hualian Pharmaceutical Company) was administrated orally. First dosage was 50 mg and 25 mg was taken after 12 h, on the day and the night of second day, and at 7 a.m. of the third day, respectively. One h after the last administration, 600 µg misoprostol (Searly, Australia) was added. All the patients were observed in the hospital, and if gestational sac was completely expelled in 2 h, the tissues were collected immediately after that; if part of the tissue remained in the uterus, curettage was done to collect chorion and decidua samples. After that, all samples were immediately preserved in liquid nitrogen. In the control group, tissues were collected and preserved in the same way after negative pressure aspiration abortion.

**Methods**

**Telomerase extraction**

Tissue of 50-100 mg were fetched from each specimen and put into the homogenizer, then 200 µL precooled splitting solution [10 mmol/L Tris-HCl (pH 7.5); 1 mmol/L MgCl₂; 1 mmol/L EGTA; 5 mmol/L 2-mercapto-ethanol; 0.5% CHAPS; 10% glycerol; 0.1 mmol/L PMSF] was added immediately. After being split on ice for 1 h and centrifuged for 20 min at 16 000 r/min and 4°C, the supernatant was extracted, protein content in extracted solution was tested by Bradford method and adjusted to 2 µg/µL. Then the supernatant was preserved at −80°C.

**Telomerase detection**

TRAP technique was used to detect the telomerase activity (test kit from Boehringer Mannheim, Germany), the reactive volume was 25 µL. The primer sequences of the primers are as follows:

- TS: 5’-AATCCGTGAGCAGAGTT-3’
- CX: 5’-CCCTTACCCTTCCCTACCCTAA-3’

Thirty three amplification cycles were processed on the PCR device according to the following parameters: 94°C for 30 s, 50°C for 30 s and 72°C for 1.5 min. The process of negative control groups included four steps: using splitting solution instead of telomerase extracted solution; inactivating telomerase extracted solution at 85°C for 10 min; adding RNase into telomerase extracted solution at 1 µg/µL and cultured at 37°C for 20 min to desuct RNA templat of telomerase; using other primer to replace TS primer in the reactive system. The positive control group was Hela 293 cells (cervix cancer cell strain).
**Electrophoresis and SYBR-Green staining**

After amplification, 5 µL production was separated by PAGE (10% polyacrylamide gel electrophoresis) at 175 V for 45 min and at 240 V for 1 h. The gelatum was dipped into SYBR-Green staining solution for 40 min which was dispensed by TBE with a proportion of 1:10 000, and then pictures were taken by brown-yellowish filter on ultraviolet (UV) transilluminators.

**Statistics analysis**

χ² test and a conjoint table of 2 × 2 were used, P<0.05 was considered significant.

**Results**

**Detecting telomerase activity by SYBR-Green staining**

Ladder-shaped strips with spacing of 6 bp was observed in positive specimen groups, whereas there was no telomerase specificity in both negative specimen groups and negative control groups (Figure 1).

**Expression of telomerase activity in chorion and decidua during early gestation**

The telomerase positive expression in chorion was significantly different between the experimental and the control group (P<0.01), but there was no significant difference in decidua between the two groups (P>0.05)(Table 1).

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<th>Group</th>
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Discussion

Mifepristone is a kind of anti-progestogen drug capable of combining with target progestogen receptor. Previous research considered the antipregnancy mechanism of mifepristone was that high concentration of progestogen induces degeneration and necrosis of decidua and thus caused chorion trophoblast degeneration and embryo death, and finally leads to pregnancy termination[3]. Findings of resent researches further suggest that mifepristone can directly influence the chorion trophoblast cells by interacting with many immune molecules, which makes its anti-early-pregnancy mechanism even more complicated and complete. Though misoprostol is not directly related to embryo death, it can facilitate uterine smooth muscle to contract and necrosis embryo tissue to be expelled[4]. Mifepristone combined with misoprostol has been widely used in medical termination of early pregnancy.

Telomerase, a kind of ribonucleoprotein (RNP) containing RNA and protein, functions as a reverse transcriptase. As a kind of protein template, RNA in telomerase can synthesize telomere repeated sequence (for human: TTAGGG) which maintains telomere length at 3’ end of the primer in a certain reactive system. The length of telomere is directly associated with telomerase activity and reflects the status of cell proliferation[5]. Telomerase is rarely expressed in normal human tissue, however, there are high positive expressions of telomerase in limitless proliferative neoplasm tissues, cultured immortalized cells and germ cells. It was reported that expression of telomerase activity was 76% in villous tissue and 72% in embryonic tissue of normal pregnancy of 5-9 weeks[6, 7]. In our study, 78% of normal villous tissue in the control group had positive expression, which was consistent with the findings of previous reports and suggested that the villous tissue in normal pregnancy has the characteristics of proliferative stem cells. Positive expression in the experiment group (28%) was significantly lower than the control (73%), which indicated that mifepristone had influence on the villous trophoblast cells, disturbed germ cells and decreased the telomerase activity. Thus trophoblast growth and differentiation ceased as a result of embryo death. Mifepristone incorporated with misoprostol could soften cervix and facilitate uterine to contract so as to release the remaining tissue. Since misoprostol only lasts for a short period of time in medicine abortion, whether it has any effect on the chorion and decidua deserves further study.

In the control group, the positive expression was significantly lower in decidua (20%) than in chorion (72%), which indicated that decidual tissue was marked by much lower immortalization than villous tissue or it did not have proliferative ability. There was no difference between tolemorase activities in the experimental and control group, which may possibly due to that mifepristone did not have influence on proliferation and differentiation of decidual tissue; or decidua responded poorly to mifepristone since it had no proliferative and differentiative ability. It might explain why complications, such as prolonged residua of de-
cidual tissue and continuous vaginal bleeding, occur in a relatively longer period of time after medicine abortion. The different effect of mifepristone on telomerase in chorion and decidua suggests that the change of telomerase may not be dependent on anti-progestogen pathway. Telomerase may act in the process of trophoblast proliferation, differentiation and erosion via changing of adhesiveness between cells and expression of metal protein in matrix. It is considered that telomerase activity of trophoblast was modulated by TGF-β in transcription level during early pregnancy[8]. Further studies are needed to illustrate whether mifepristone modulates telomerase expression by this pathway and whether desynchronized and multipath mechanisms exist in the effect of mifepristone. At present, researches on telomerase has just begun. Trophoblasts are different from proliferated neoplasms. Though telomerase inhibitor has been reported to be applied successfully in the treatment of neoplasm, the key for its application in termination of pregnancy lies in increasing the decidual response[10].

References


(Received on October 27, 2004)