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Up-regulation of Fas/FasL activation contribute to the apoptosis enhancement of RU486 by Gong-Qing Decoction, a traditional Chinese prescription

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ABSTRACT

Aim of the study: To elucidate the mechanisms of Gong Qing Decoction(GQD) on human trephocytes and decidual cells in vivo based upon the effective practice of alleviating uterine bleeding in RU486 medical abortion.

Materials and methods: 90 intrauterine pregnancy women within 7 weeks, presenting for elective termination of pregnancy, were divided into the GQD-RU486 group, the RU486 group and the vacuum aspiration group. Duration of uterine bleeding was recorded and volume of uterine bleeding was measured by the method of alkaline hematin photometric. Ultramicrostructure of trephocytes and decidual cells were observed with transmission electron microscope (TEM), and apoptosis rate (AR) was assessed by terminal deoxynucleotidyl transferase mediated dUTP nick end labeling (TUNEL) assay. At the same time, immunohistochemical staining was performed and integral optical density was analyzed to evaluate the protein expression of Fas, FasL, Caspase-8 and Caspase-3 in both trephocytes and decidual cells preliminarily.

Results: In comparison with the RU486 group and the vacuum aspiration group, both the duration and volume of uterine bleeding decreased significantly in the GQD-RU486 group. At the same time, both trephocytes and decidual cells in the GQD-RU486 group showed typical character of apoptotic ultramicrostructure and displayed up-regulated apoptosis rate. Synchronously, the integral optical density showed increased protein expression of Fas, FasL, Caspase-8 and Caspase-3 in both trephocytes and decidual cells in the GQD-RU486 group compared with other groups.

Conclusion: These data suggest that GQD can alleviate uterine bleeding effectively in RU486 medical abortion by way of apoptosis induction. The apoptosis enhancement of RU486 by GQD may be attributable to the activation of Fas and FasL.

1. Introduction

RU486 is a synthetic steroid which presents a high affinity for progestogen receptor without any progesterone activity; moreover, it enhances the sensitivity of gestational uterine to prostaglandin. Misoprostol (Miso) is a synthetic prostaglandin E1 analogue, which causes uterine contractions and the ripening of the cervix. Thereby, RU486 is usually used in clinical practice to terminate early pregnancy combining with Miso (Peyron et al., 1993). The method is widely used by women who wish to end unintended early pregnancy because it is less painful and more convenient than surgical abortion (Saha et al., 2007). For example, it was reported that over 80% Chinese women would like to choose medical abortion if they need to terminate an unwanted

Abbreviations: AR, apoptosis rate; FADD, Fas-associated protein with death domain; FPA, frequency of previous abortion; GA, gestational age; GQD, Gong Qing Decoction; IgG, immunoglobulin G; IOD, integral optical density; MSD, mean sac diameter; RU486, mifepristone; TCM, traditional Chinese medicine; TEM, transmission electron microscope; SD, standard deviation; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling.

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pregnancy (Cheng, 2006), and millions of women worldwide have chosen this regimen. However, there are some side effects of RU486 medical abortion and prolonged excessive uterine bleeding is perhaps the most refractory one. Previous research showed that 10% women complained of excessive bleeding in RU486 medical abortion and the hemoglobin level decreased significantly compared with surgical abortion. In addition, 1.4% and 0.25% medical abortion cases required hemostatic curettage and transfusion respectively because of the hemorrhage (Cabezas, 1998). Although abnormal uterine bleeding is a serious side effect of RU486 medical abortion, its etiopathogenesis has not been clarified clearly.

Recent studies indicated that the biological effect of RU486 is multiple. Besides affiliating with progestogen receptor competitively, it can also inhibit the proliferation of some tumor cells by means of apoptosis induction (El Etreby et al., 1998, 2000; Schneider et al., 1998). Furthermore, it was found that RU486 can induce apoptosis in trephocytes and decidual cells (Dai et al., 2000; Zhang et al., 2002), while insufficient apoptosis of trephocytes and decidual cells induced by RU486 was contributable to abnormal uterine bleeding (Wang and Zhu, 2008). Consequently, it is worth to detect the effective medicine for intensifying the apoptosis by RU486 in trephocytes and decidual cells for the sake of alleviating uterine bleeding.

In basic traditional Chinese medicine (TCM), it is considered that "blood stasis" and "Qi deficiency" are the main etiology and pathogenesis of gynecological bleeding. Therefore, drugs for invigorating blood circulation to eliminate stasis and supplementing Qi were used efficaciously in gynecological bleeding for a long period, especially in postpartum hemorrhage. RU486 medical abortion is safe and effective generally, but the intrauterine remains of trephocytes and decidual cells caused by which can lead to abnormal uterine bleeding. TCM doctors associated this etiology and pathogenesis with "blood stasis" and "Qi deficiency". Gong-Qing Decoction (GQD) consists mostly of drugs for invigorating blood circulation and supplementing Qi. It had been approved to be used in clinical research to alleviate uterine bleeding in RU486 abortion by the State Food and Drug Administration of China (Permit number: 2003L01386) and has been used in clinics as an effective preparation to alleviate uterine bleeding in RU486 medical abortion for decades. Acute toxicity testing and long-term toxicity testing had been carried out on pregnant rats. In addition, the experimental safety evaluation had been carried out in a multi-center randomized double-blind, parallel controlled clinical trial. The outcomes showed that GQD is safe for both pregnant rats and human pregnant ladies.

Previous clinical observation showed that GQD had potent curative effect to alleviate uterine bleeding in RU486 medical abortion (Wang et al., 2007). However, up to now, little information is available regarding the mechanism of GQD for alleviating uterine bleeding in RU486 medical abortion, which limits further research and development on this formula. Therefore, we performed the present study to elucidate the curative mechanism of GQD for alleviating uterine bleeding in RU486 medical abortion on the basis of clinical observation. The results indicated that GQD can shorten the duration of uterine bleeding and reduce the blood loss obviously and effectively in RU486 medical abortion by way of apoptosis enhancement. This apoptosis-intensifying effect of RU486 by GQD in trephocytes and decidual cells was contributable to the activation of caspases through Fas/FasL death receptor pathway.

2. Materials and methods

2.1. Subjects

The study protocol was approved by the Ethical Advisory Committee for Medical Sciences Human Research, Shandong University of Traditional Chinese Medicine. Healthy women, meeting the inclusion criteria and having none of the exclusion criteria following, were recruited. All participants provided written informed consent before commencing the study. *Inclusion criteria*: 18 years or older, requesting for an elective termination of early pregnancy, having an intrauterine pregnancy of no more than 49 days on the day of RU486 administration, willing and able to sign informed consent, willing to comply with the study protocol and visit schedule. *Exclusion criteria*: ultrasound evidence of an early pregnancy failure, contraindication to RU486 (chronic corticosteroid administration, adrenal disease), contraindication to Miso (glaucoma, mitral stenosis, sickle cell anemia, poorly controlled seizure disorder or known allergy to prostaglandin), known or suspected extrauterine pregnancy, known or suspected pelvic infection, hemoglobin < 10 mg/dL, known clotting defect or receiving anticoagulation therapy, cardiovascular disease (angina, valvular disease, arrhythmia or cardiac failure), current breastfeeding, intrauterine device in situ, current use of any experimental drug, suspected or confirmed uterine malformation.

From June 2007 to December 2008, 90 pregnant women who visited in affiliated hospital of Shandong university of TCM and met all the entry criteria were invited to participate in this study. Subjects were matched for age, frequency of previous abortion (FPA), gestational age (GA) and mean sac diameter (MSD). GA was determined by ultrasonography and compared with the last menstrual period. MSD was calculated according to the formula: (length of sac + width of sac + depth of sac)/3. These subjects were divided into 3 groups randomly, including GQD-RU486 group, RU486 group and vacuum aspiration group, and there were 30 subjects in each group.

2.2. Chemicals

RU486 (25 mg/table, lot number: H20000628) and Miso (200 μg/table, lot number: H10960144) were offered by Shanghai Hualian Pharmaceutical Company (Shanghai China).

2.3. Gong-Qing Decoction (GQD)

GQD consisted of 10 crude drugs including Herba Leonuri, Herba Portulacae, Radix Codonopsis, Radix Astragali, Radix Achyranthis Bidentatae, Radix Angelicae Sinensis, Herba Agrimoniae, Pollen Typhae, Rhizoma Chuanxiong and Radix Glycyrrhizae in a ratio of 10:10:6:6:6:6:5:3:3:2 in weight. All crude drugs were genuine Chinese medicine materials and were purchased from Tong-rentang Ltd. (Beijing, China, 10/9/2007).

GQD decoction was prepared by boiling the herbs in water 2 times. In every 5000 mL of water, there were 600 g Herba Leonuri, 600 g Herba Portulacae, 360 g Radix Codonopsis, 360 g Radix Astragali, 360 g Radix Achyranthis Bidentatae, 360 g Radix Angelicae Sinensis, 300 g Herba Agrimoniae, 180 g Pollen Typhae, 180 g Rhizoma Chuanxiong and 120 g Radix Glycyrrhizae. The mixture was heated at 100°C for 1 h. The mixture was filtered and the filtrate collected for later use. The filtered crude herbs were then added to another 5000 mL of water. Then, this second mixture was heated at 100°C for 1 h. As for the first, the second mixture was filtered and the filtrate collected. The 2 filtrates were mixed thoroughly and concentrated under vacuum to 2000 mL using a rotary evaporator. 8000 mL of 100% ethanol were added into the solution to a final ethanol concentration of 80%. After 12 h at room temperature, the solution was filtered through a 100 mesh sieve. The precipitate was dissolved in water and then 6000 mL of 100% ethanol were added to a final ethanol concentration of 70%. Next kept it for 12 h at room temperature to attain the extraction solution. Then the precipitate was dissolved in water and 5000 mL of 100% ethanol were added to a final ethanol concentration of 60%. The 3 extraction solutions were mixed together and concentrated to 1195.8 mL. In this