Immunohistochemical Localization of Cyclooxygenase-2 in Pregnant Rat Uterus by Sp-6 Acupuncture

Jeong-Sang Kim, Chang Su Na, Woo Jun Hwang, Byung Chul Lee, Ki Hyoung Shin and Sok Cheon Pak

*School of Oriental Medicine, Dongshin University, Naju, Korea
†School of Oriental Medicine, Wonkwang University, Iksan, Korea

Abstract: As pregnancy advances, prostaglandins (PG) increase in the uterus, leading to elevated uterine contractility. Therefore, regulating the concentration of PG in the uterus can be a key factor for controlling the duration of labor. Since the synthesis of PGs in the uterus is catalyzed by cyclooxygenase-2 (COX-2), devising a tool to regulate the expression of COX-2 could provide a method for treating complicated labor. In this study, Sp-6 acupuncture treatment was evaluated for its potential in controlling uterine motility. Immunohistochemical methods showed the COX-2 enzyme was primarily found in the endometrium and myometrium of rat uterus. COX-2 expression in these two locations were intensified by pregnancy, but reduced by acupuncture at the Sp-6 acupoint. Uterine motility monitored during Sp-6 acupuncture was reduced by 28.15% (p < 0.05) and 19.88% (p < 0.05) in pregnant rats and non-pregnant rats, respectively. The significant reduction of uterine motility in pregnant rat suggests a role for Sp-6 acupuncture in regulating the expression of COX-2 during pregnancy. These results suggest that Sp-6 acupuncture could be used as a complementary method for controlling labor in human pregnancy.

Keywords: Cyclooxygenase-2; Immunohistochemistry; Sp-6 Acupuncture; Uterus.

Introduction

During pregnancy, many morphological changes occur in the epithelial and muscle cells of the rat uterus. These two types of uterine cells are reported to be important for the implantation of the embryo, pregnancy maintenance and the starting of parturition (Fang et al., 1998). A
noticeable change associated with the morphology of these two cells in the uterus is the change in prostaglandin (PG) metabolism. The level of PG and its stage of synthesis in endometrium and myometrium vary during the estrous cycle and with the pregnancy, beginning with embryo implantation in the rat uterus (Abel and Kelly, 1979; Baskar et al., 1981; Gu et al., 1990).

PG biosynthesis starts from either free or liberated arachidonic acids in membrane phospholipids by phospholipase, and the arachidonic acids are converted to prostaglandins by cyclooxygenase (Gu and Rice, 1991). The synthesis of PG is mediated by cyclooxygenase-1 (COX-1) composed of 602 amino acids. PGs act as local hormones regulating vasomotor blood flow, flexible muscle movement, blood permeability and playing protective roles in preserving integrity of the stomach lining (Vane et al., 1998). Another isoform of COX made of 604 amino acids, cyclooxygenase-2 (COX-2) had been implicated in rat reproductive events at the time of ovulation, embryo implantation, pregnancy and parturition (Lau et al., 1973; Sananes et al., 1976; El-Banna, 1980; Vane et al., 1998). The expression of COX-2 was enhanced during pregnancy in the uterine epithelial and myometrial cells with the increase of muscle contractility, but decreased immediately after labor in rats (Dong et al., 1996), indicating that PG synthesis increases during pregnancy and a COX-2 expression is closely related to the start of labor and serves as an important modulator in parturition. Thus, the abnormally weak level of PG by low COX expression caused a difficulty in labor while the over-expression of COX caused a pre-term labor (Yusoff, 1993), and an absence of COX-2 by gene deletion caused an unsuccessful pregnancy (Lim et al., 1997). Recent studies suggest that PGs and COX-2 inhibitors could be used as potent medications for controlling abortion or pre-term labor (Pang et al., 1996; Poore et al., 1999).

In oriental medicine, complications associated with such reproductive events were explained by the disturbance in “Qi” streams that are connected by numerous acupoints. Each acupoint is connected to the specific internal organ that can be coordinated by the stimulation through the acupoint. The Sp-6 acupoint is traditionally known as one of the acupoints for human uterus, but it is uncertain yet how the acupuncture treatment has an impact on the function of uterus during pregnancy and at the time of labor. This study was performed to determine immunohistochemically how the inducible COX-2 activity in rat uterus is influenced by Sp-6 acupuncture and its relation to the uterine motility in pregnant rats.

Materials and Methods

Animals

Non-pregnant (n = 7, 200 ± 30 g body weight) and pregnant (n = 10, 300 ± 25 g body weight) Sprague-Dawley female rats were obtained from Daehan Laboratory (Daejun, South Korea). The animals were housed in a room kept at 25 ± 1°C and used for the study. The acupuncture was carried out in non-pregnant rats and 18-day pregnant rats. Of the non-pregnant rats, three were used for the non-acupuncture group and four for the Sp-6 acupuncture group. Of the 18-day pregnant rats, five were used for the non-acupuncture group and five for the Sp-6 acupuncture group.
Acupuncture

Acupuncture needles (Ø 0.18 mm, 15 mm H) were used for Sp-6 acupoint. It is located between calcaneal tendon and medial malleolus of hind limbs that anatomically corresponds to the human Sp-6 acupoint. Both hind limbs were treated for Sp-6 acupuncture, and the needles were inserted about 3 mm deep into the muscle layer for 30 minutes.

Uterus Motility

Each rat was weighed and anesthesia was induced by injecting Entobar™ (pentobarbital sodium, 500 mg/kg) through the cannula (PE-50) connected to the femoral vein, and then maintained by continuous injecting of less concentrated drug (10 mg/ml/h) by using a syringe pump (WPI, US) during the entire testing period. A heating pad (TR100, Fine Science Tool Inc., Canada) was used to keep the body temperature at 36°C throughout the experiment.

The uterine motility was monitored by using a small balloon tied to the tip of cannula (PE-50) that was connected to a digital pressure transducer (Harvard apparatus, US). The balloon was inserted into the rat uterus and inflated with tap water to an initial pressure of 50 mmHg. The pressure signal induced by the movement in the uterus was directly plotted onto the chart recorder (Harvard apparatus, US). The oscillographic data were also fed to computers and stored using data acquisition software (Biopac, US).

The initial movement of uterus for 30 minutes before the acupuncture was set as a normal pre-acupuncture uterine motility index. The index was calculated by summing up the peak area. The uterine motility index during the acupuncture (post-acupuncture index) was monitored for 30 minutes to compare with the indices of the pre-acupuncture stage.

Immunohistochemical Staining

Immediately after acupuncture for 30 minutes, the animals were sacrificed by cervical dislocation. The rat uterus was fixed by perfusion with 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.2 and three different locations were dissected. They were stored at 4°C for a day and immersed again in the same fixative for another day. The fixed tissue samples were paraffin-embedded for 5 µm thickness slicing, and the sliced tissue sections were mounted on 1% albumin-coated slide glasses. For the immunohistochemical reaction of COX-2, the slide glasses were deparaffinated and hydrated by immersing sequentially in xylene and descending concentration of ethanol solutions (100, 95, 90 and 80%) for 5 minutes each. The deparaffinated sections were incubated overnight with phosphate-buffered saline, pH 7.2 (PBS) solution at room temperature. On the following morning, the sections were rinsed with fresh PBS and incubated with blocking solution containing 10% horse serum for 20 minutes at room temperature and washed again with PBS for 15 minutes.

The primary COX-2 antibody was added and incubated for 2 hours at room temperature in a humid chamber and washed with PBS after the treatment. It was followed by 30 minutes incubation at room temperature with biotinylated anti-mouse IgG and washed with PBS after the incubation. Avidin-biotinylated enzyme complex (ABC) reagent (Vector Lab, USA)
was added to the section and incubated for 30 minutes at room temperature and washed with PBS solution for 15 minutes after the incubation. A few drops of DAB (3,3'-diaminobenzidine) chromogen solution were added to the section, and the excess solution was removed by dipping in tap water. After removing the moisture, the slides were counterstained in a filtered hematoxyline solution for 20 seconds and excess staining was removed by dipping in tap water. For dehydration, the slide glasses were dipped sequentially in ascending concentrations of ethanol (50, 60, 70 and 80%) for a few seconds and then rinsed for 5 minutes in the higher concentrations of ethanol (90, 95 and 100%) and in xylene solutions.

Statistical Analysis

Paired t-test was used for the significance of motility change at 5% level.

Results

Uterus Motility

All the uterine activities were expressed as the intrauterine pressure (mmHg), and the post-acupuncture values were expressed as a percentage of the pre-acupuncture values (Fig. 1). The Sp-6 acupuncture decreased uterine motility by 28.15% (p < 0.05) and 19.88% (p < 0.05) in the pregnant and non-pregnant rats, respectively (paired t-test). The statistical analysis indicated the greater effectiveness of Sp-6 acupuncture for pregnant rats than for their non-pregnant counterparts.

![Figure 1. Uterine activities expressed as percentage of sham control. During the Sp-6 acupuncture, uterine motility was decreased to 28.15% (p < 0.05), 19.88% (p < 0.05) in the pregnant and non-pregnant rats, respectively compared to each sham acupuncture.](image-url)
ACUPUNCTURE AND UTERINE MOTILITY

Immunohistochemical Staining

A basal expression of COX-2 in non-pregnant uterus was found in the epithelial cell lining facing the stromal cells (Fig. 2a) and in the myometrium (Fig. 2b). The uterine gland cells were not stained positively (Fig. 2a). Sp-6 acupunctured non-pregnant uteri showed slightly decreased level of staining on the surface of epithelial cells, stromal cells (Fig. 3a), and myometrial cell layer (Fig. 3b) as compared to non-acupunctured non-pregnant rats. The COX-2 expression for non-acupunctured pregnant rats showed a strong staining in the luminal epithelial cell lining (Fig. 4a) and myometrial cells (Fig. 4c), but not in the glandular epithelial cells (Fig. 4b). The Sp-6 acupunctured pregnant rats showed a scattered staining in the epithelial cells (Fig. 5a), stromal cells (Fig. 5b) and myometrial cells (Fig. 5c), and they were stained in lesser magnitude than in non-acupunctured pregnant rats.

Figure 2. Basal expression of COX-2 enzymes in non-acupunctured virgin rat. The staining was found on endometrial epithelial cells (a) and myometrial cell layers (b). E = Epithelial cells; UG = uterine glands; M = myometrial cells (COX-2 immunostain, × 200).

Figure 3. Sp-6 acupuncture-treated non-pregnant rat. The intensity of COX-2 staining was reduced compared with Fig. 2. E = Epithelial cells (a); UG = uterine glands (a); M = myometrial cells (b) (COX-2 immunostain, × 200).
Figure 4. COX-2 expression of a non-acupunctured pregnant rat. Strongly positive staining for COX-2 can be seen in endometrial epithelial cells (a) and the myometrial cell layer (c), but not in uterine gland (b). E = Epithelial cells; UG = uterine glands; M = myometrial cells (COX-2 immunostain, × 200).

Figure 5. COX-2 expression of an acupunctured pregnant rat. Slightly reduced staining for COX-2 can be found by the administration of Sp-6 acupuncture in endometrial epithelial cells (a) and the myometrial cell layer (c). E = Epithelial cells; UG = uterine glands (b); M = myometrial cells (COX-2 immunostain, × 200).

Discussion

The acupuncture treatment in the Sp-6 acupoint, coupled with other acupoints, was commonly prescribed and practiced for centuries in Chinese medicine for inducing labor (O’Connor et al., 1997). However, recent studies revealed two different opinions as to the effects of acupuncture on the labor. Studies by Tempfer et al. (1998) and Zeisler et al. (1998) showed a stimulating effect of acupuncture on labor, whereas other studies by Tsuei et al. (1977) and Lyrenas et al. (1987) indicated an inhibiting effect of acupuncture on labor. However, all of these studies used only statistical methods for explaining the efficacy of acupuncture and did not explain a specific role of acupuncture in regulating the uterus contractility.

In this study, an immunohistochemical analysis of COX-2 in uterine tissues was used to investigate the effectiveness of a single Sp-6 acupuncture treatment on labor. The results showed that the Sp-6 acupuncture treatment suppressed the expression of COX-2 in rat uterine tissues, which is consistent with the results of Tsuei et al. (1977). The reduced immunohistochemical expression of COX-2 by Sp-6 acupuncture was correlated to the reduced uterine motility of both pregnant and non-pregnant rats. The decreased uterine motility by Sp-6 acupuncture occurred more rapidly in pregnant rats than in non-pregnant rats. The
localization pattern of COX-2 in the endometrium and myometrium of rat uterine tissue is in agreement with Fang et al.’s study (Fang et al., 1998).

The present study demonstrated that COX-2 expression in pregnant rats could be regulated by the Sp-6 acupuncture and that the acupuncture treatment was effective for lowering the uterus motility in pregnant rats. These results are in good agreement with that of Pak et al.’s experiment that reported the effectiveness of Sp-6 acupuncture in lowering the uterine motility of pregnant rats (Pak et al., 2000). The present study suggests that the effectiveness of Sp-6 acupuncture for labor could be evaluated by an immunohistochemical assay of COX-2 in the uterine tissue, and that a single Sp-6 acupuncture treatment is useful for inhibiting the uterine motility that could be applied to regulate pre-term labor.

References


