MOUSE CALBINDIN-D_{9K} GENE EXPRESSION IN UTERUS DURING PREGNANCY AND LACTATION

Beum-Soo An , Geun-Shik Lee and Eui-Bae Jeung.

Laboratory of Veterinary Biochemistry and Molecular Biology, College of Veterinary Medicine, Chungbuk National University, Chongju, Chungbuk, Korea.

Introduction

Calbindin-D_{9k} (CaBP-9K) is a cytosolic calcium binding protein (Mr 9000) expressed predominantly in mammalian duodenum, placenta, and uterus. Intestinal expression of CaBP-9K is controlled by 1,25-dihydroxyvitamne D and is associated with calcium absorption[1,3].

However, uterine expression of that is controlled by 17-estradiol (E2) in rat and controlled by progesterone (P4) in mouse[2]. Many signals and molecular pathways have been recognized that induce or regulate the complex series of interactions required for implantation pregnancy, including cell surface adhesion molecules cytokines and growth factors[4] but many of which are still unknown. The CaBP-9K is one of the genes identified as being differently regulated. The aim of this study was to analysis the pattern of CaBP-9K gene expression and the correlation with steroid hormones (E2 and P4) in pregnant mouse uterine. In the present study, we analyzed the normal CaBP-9K and ER/PR mRNA expression through pregnant and lactation periods by Northern and Southern blot assay. To analyze the steroid hormone effect on CaBP-9K mRNA expression, the steroid hormone antagonists such as tamoxifen, ICI 182,780, and RU 486 were injected to the mouse in early pregnant period (P5) and late pregnant period (P13) when CaBP-9K was expressed highly. After treatment, the CaBP-9K and ER/PR expression levels were analyzed by Northern and RT-PCR/southern blot, respectively.

Methods and Materials

In the first experiment, six week olds female ICR mice were mated with adult males overnight and the following morning the presence of vaginal plug after mating was designated day 0 of pregnancy. Group of mice were killed on each day of pregnancy and of lactation. In the second experiment, females were mated as described above and three groups of 5 animals were injected (S.C) with 2μg/mouse of RU 486, Tamoxifen (TAM) and ICI 182,780 (ICI) dissolved in sesame oil on pregnancy day 7 and 15. The mice injected with vehicle (sesame oil) were served as a control.

After single injection of each chemical, the mice were sacrificed at 24, 48 and 72 hours. Blood was collected for hormone assay and their uteri were removed for RNA isolation. Ten microgram of total RNA were loaded on 1% agarose gel for Northern blot analysis and 18S rRNA served as an indicator of quantity of total RNA. Five microgram of total RNA were reverse transcribed using M-MLV reverse transcriptase and random primer (9 mer). For the PCR reaction, 10% of the RT products were used. The samples contained ER/PR primer for estrogen and progesterone receptor mRNA and also had primers for 1A mRNA as an internal standard. For southern blot assay, 50% of the PCR products were used.

Results and Discussion

In the results of Fig. 1., we show that the expression levels of CaBP-9K were increased up to 3-fold during P8-P10, and the levels were increased again from P15 and reached maximum at P18. The expression levels of CaBP-9K were declined drastically at birth. The expression of CaBP-9K in mouse uterus is dependent on progesterone. A shown Fig. 1., the pattern of CaBP-9K expression was similar to that of PR and ER. The expression levels of PR/ER increased 2 or 3 days before that of CaBP-9K started to express. The pattern of PR expression was coincided with peripheral plasma progesterone concentrations of pregnant mouse, while that of ER expression was not.

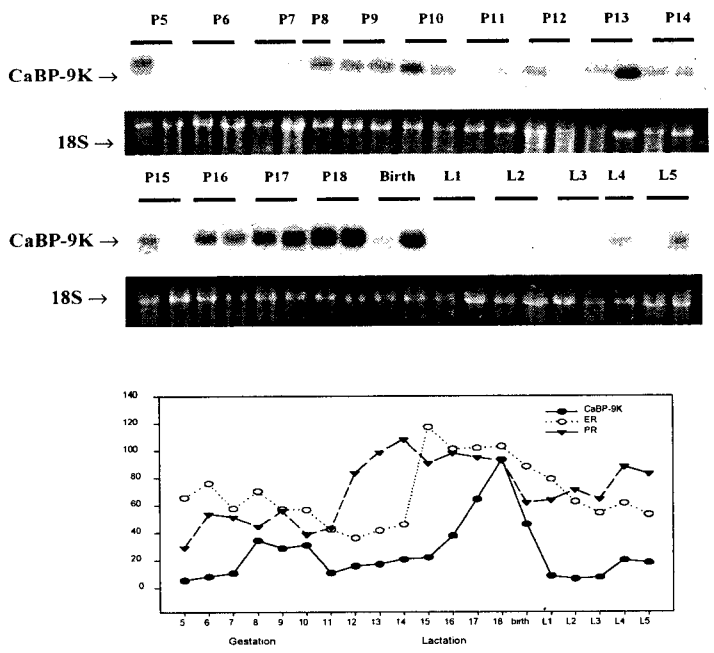

Fig. 1. Northern blot analysis for CaBP-9k mRNA expression during pregnancy and lactation period in normal pregnant mouse uterus (vaginal plug = day 0). Total RNA (10μg) was subjected to Northern blot analysis. After hybridization with the radio-labeled probes and exposure to x-ray film, the signal

was analyzed by molecular analysis program version 1.5 (Gel Doc 1000, BioRad).

In order to find a determining factor that affect on CaBP-9k expression, the steroid hormone antagonists were injected into the pregnant mice when CaBP-9K was expressed highly (Fig. 2). In early pregnant mouse, the levels of CaBP-9K were down regulated up to 3-fold when treated with RU486 at 48 hour after single injection. However the levels were up regulated significantly at TAM treated group in 24 (1.6-fold *vs* vehicle) and 48 hour (1.4-fold *vs* vehicle) after single injection. ICI induced the expression of CaBP-9K mRNA to increase at 24 hour but not 7 and 48 hour.

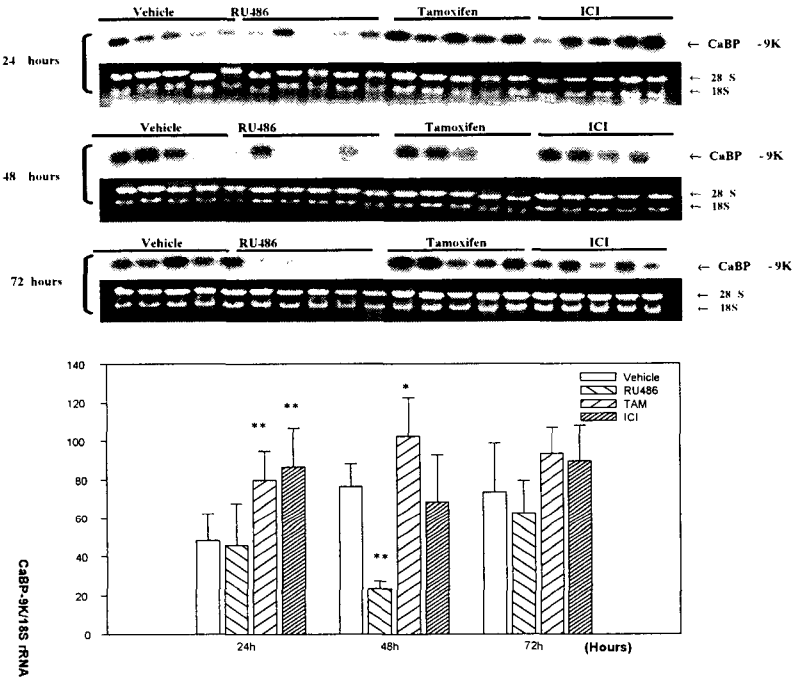

Fig. 2. Effect of TAM, RU486 and ICI on CaBP-9K mRNA in the uterus of early pregnant mouse. Pregnant mice were injected with TAM, RU486 and ICI at P7 and total RNAs were extracted in uterus at 24, 48, or 72h after injection. Total RNA (10 μg) was subjected to Northern blot analysis. After hybridization with the radio-labeled probes and exposure to x-ray film, the signal was analyzed by molecular analysis program version 1.5 (Gel Doc 1000, BioRad). Data was analyzed by one-way ANOVA, followed by Tukey's multiple comparison tests. The values represent means ± SD (n=5). *; $p < 0.05$, **; $p < 0.01$.

The antagonist effects on CaBP-9K mRNA expression were also investigated at late pregnant mice by northern analysis. Antagonist of P4, RU486 reduce the CaBP-9K gene expression at 48 (3.2-fold *vs*

vehicle) and 72 hour (3.8-fold *vs* vehicle) and the results were similar to that of early pregnant mice experiment. However other antagonists effect on CaBP-9K expression was not constant. The PR gene expression was down regulated by RU486, which is similar to CaBP-9K expression (data not shown).

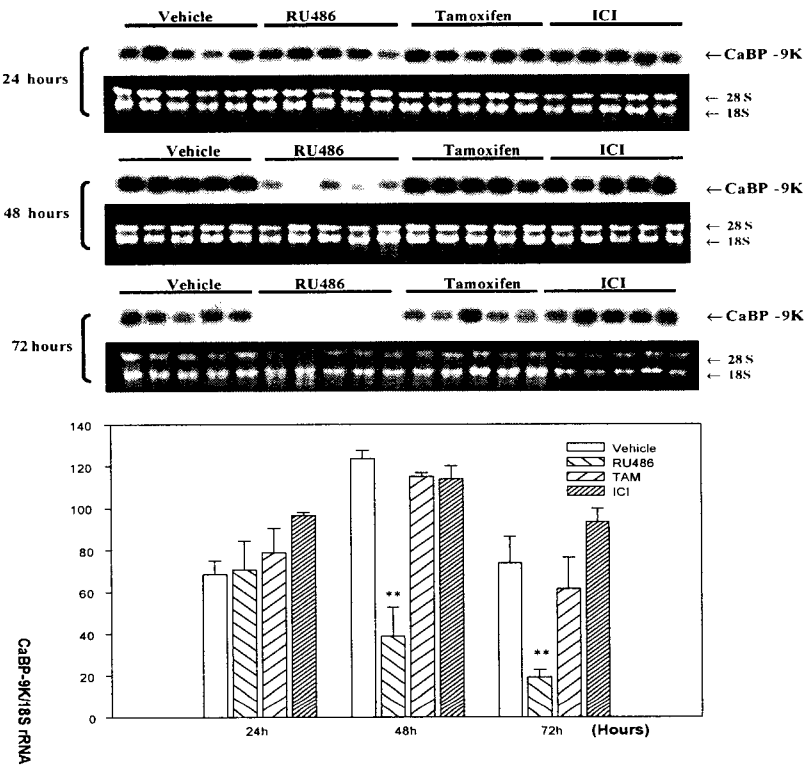

Fig. 3. . Effect of TAM, RU486 and ICI on CaBP-9K mRNA in the uterus of late pregnant mouse. Pregnant mice were injected with TAM, RU486 and ICI at P15 and total RNA were extracted in uterus at 24, 48, or 72h after injection. Total RNA (10 μg) was subjected to Northern blot analysis. After hybridization with the radio-labeled probes and exposure to x-ray film, the signal was analyzed by molecular analysis program version 1.5 (Gel Doc 1000, BioRad). Data was analyzed by one-way ANOVA, followed by Tukey's multiple comparison tests. The values represent means ± SD (n=5). *; $p < 0.05$, **; $p < 0.01$.

The CaBP-9K mRNA expression in pregnant mouse was high at post implantation period and late pregnant period. These results were also similarly presented in peripheral plasma P4 concentration and PR expression. Since the expression of CaBP-9k dependent on P4 in ovariectomized mouse uterus[2], we expected that expression of CaBP-9K in pregnant mouse is also dependent on P4 related with PR. In Figs 2 and 3, CaBP-9K mRNA was blocked by RU 486 but up regulated by ICI. The general consensus about steroid hormone receptors is that estrogen and ER induces PR in the uterus, however,

it has recently been demonstrated that estrogen has dual effects on the expression of PR, it decreases PR levels in the luminal epithelium but increases the levels of PR in the stroma and myometrium.

The CaBP-9K is mainly expressed in luminal epithelium in mouse uterus, while mainly expressed in myometrium and endometrial stroma in rat uterus, thus CaBP-9K mRNA may be regulated by not only P4 but also other sex steroid hormone complex in a spatiotemporal manner. Another possible regulating factor of CaBP-9K mRNA expression has been suggested as embryonal signal in pregnant mouse uterus and a possible role of uterine CaBP-9K has been suggested as an intracellular calcium binding protein influencing uterine activity during pregnancy. Although CaBP-9K gene play important role in not only pregnant mouse but also other mammalian uterus, the mechanism and function of that so were poorly understand. Conclusively, the CaBP-9K gene play a important role in pregnant mouse uterus through post implantation and late pregnant period and the expression was controlled by mainly PR expression in pregnant moue uterus, however factor that regulate CaBP-9K mRNA remained as further study.

Acknowledgments

This work was supported by NITR/Korea FDA Grant ED2000-4 for Endocrine Disruptors Research.

References

1. Williams J.K., Honore E.K. and Adams M.R. (1997) Circulation. 96(6),1970.

2. Tatsumi K., Higuchi T., Fujiwara H., Nakayama T., Itoh K., Mori T., Fujii S. and Fujita J. (1999) Mol Hum Reprod. 5(2),153.

3. Roche C., Bellaton C., Pansu D., Miller A. and Bronner F. (1986) Am J Physiol. 251,G314.

4. Tabibzadeh S. and Babaknia A. (1995) Hum Reprod. 10(6),1597.