The Decrease of Progesterone-B Receptor Roles in Abortion Ewe By Dexamethasone Administration

Peran Penurunan Receptor Progesteron Beta Dalam Arbortus Domba Bunting oleh Pemberian Dexamethason

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Abstract

In mammalian, progesterone play a pivotal role in controlling uterine function during pregnancy. It acts as a gene suppressor, down-regulate a number of genes that are essential for myometrial contraction, including its receptor. The biological actions of progesterone are mediated by two PR isoforms, PR-A and PR-B. Since during pregnancy PR-B is simply dominant regulator in order to maintain uterine muscle relaxation. On the other hand the switches of PR-B to PR-A is the momentum for uterine contraction which lead to parturition or abortion. It showed by the decrease of PR-B number in uterine muscle cells. The aim of this study was to test the hypothesis that dexamethasone (Dex) decrease PR-B roles by binding to regions ligand binding domain and inhibits PR-B transcriptional activity in pregnant ewe. The research design was Randomized Posttest Control Group Design for the expression of PR-B in uterine stromal cells at control and treatment group. Fourteen pregnant ewe (n=14) at midgestation, day 70 – 100 were recruited and distributed into control group (n=7) and treatment group of dexamethasone (n=7).

Methods: Immunohistochemical staining for the PR-B was positively decreased in the uterine stroma in treatment group.

Results: the PR-B expression in treatment compare control group were significantly differ (p<0,05).

Conclusion : in this study the decreased of PR-B expression were induced by dexamethasone treatment.

Keywords: Progesterone receptor B, dexamethasone, abortion, uterine stroma

Introduction

The concept of ewe parturition began with the activation of fetal hypothalamo-pituitary-adrenal (HPA) axis leading to an increase in cortisol by fetal adrenal gland. This cortisol is then act to alter steroidogenesis which decline in progesterone production and increase in estrogen synthesis.

Progesterone is acknowledged to play a central role in controlling uterine function during pregnancy. On the one hand, progesterone, acting as a gene suppressor, down-regulates a number of genes that are essential for myometrial contraction, including the gap junction protein, calcium channels, steroid receptor and associated proteins, and oxytocin receptor. Progesterone can also promote intrauterine PG production. High progesterone concentrations are temporally associated with the development of intrauterine PG production in pregnant sheep and woman (Wu et al., 2005).

The biological actions of progesterone are mediated by two PR isoforms, PR-A and PR-B. Progesterone receptors (PRs) have been play a key role in the control of human labor and parturition whereby the levels of the progesterone receptor B (PR-B) isoform, which is often considered to be the dominant isoform. If the levels of PR-B fall during pregnancy, by any causes such as stress, the presence of PR-B will switches to progesterone receptor A (PR-A) isoform. The PR-A act as the predominant form leading to a uterine contraction and abortion in pregnant ewe.

While PR-B tends to be a stronger activator of target genes, PR A can act as a dominant repressor of PR-B suggesting that high
PR-A expression may result in reduced progesterone responsiveness and that PR-A and PR-B may thus be, respectively, an activator and repressor of progesterone action. The repressor role of PR-A extends beyond that on PR-B, as PR-A has been shown to diminish the response of other hormone receptors such as the androgen, glucocorticoid, mineralocorticoid, and estrogen receptors to their appropriate ligands (Li X et al., 2003).

In cases such as stress that will produces cortisol, by this study the using of Dex as a synthetic cortisol in order to diminish the number and function of PR-B. So, this study hypothesis was Dex decrease PR-B roles by binding to regions ligand binding domain and inhibits PRB transcriptional activity in pregnant ewe. That a decreased in number and functions of switch in PR isoforms from the more transcriptionally dominant PR-B to the less active PR-A isoform must therefore occur in these myometrium of pregnant ewe. With the identication of PR-B, and the histological demonstrations that Dex regulate the expression of PR-B and its functions, it will become possible to more fully consider the role of PR-B decrease in uterine contraction.

Materials and Methods

Animals: this study was performed in pregnant ewes at midgestation with accordance and was approved by the Animal Care and Use Committee of University of Airlangga, Veterinary Faculty for ethical clearance. Using singleton pregnancies of local ewes, randomly allocated were done into two groups: the experimental groups and treatment at day 70 – 100 of gestation. Each ewe was housed individually under natural condition of their daily maintenance requirements throughout their pregnancies. Ewes in the control group (n = 7) receive normal saline (NaCl 0,9%) via intramuscular injection (i.m); while treatment group (n = 7) were allocated to receive dexamethasone injection 8 mg intramuscular (i.m).

Methods: the research design was Randomized posttest Control Group Design (Campbell, 1979) for examining the presence of PR-B in myometrial tissues. Pregnant myometrial tissues were obtained from each ewe of control group, and the treatment group which either has abortion immediately or without abortion at the day 2. The myometrial biopsies were obtained from the myometrial fundal. The myometrial tissues were immediately snap frozen and stored at minus (-) 70°C until use.

Statistics: data reported were expressed as means ± SD. Statistical significance of differences between groups was tested using Student’s t-test or one-way Anova. A level of P <0.05 was considered to be statistically significant.

Histological preparation: The myometrial tissues were fixed with formalin buffer for histology and immunohistochemistry examination in pathology laboratory of Veterinary Faculty of Airlangga University.

Immunohistochemical staining of PR-B: All staining was carried out according to the manufacturer’s instructions for paraffin section. For progesterone receptor B: was used mouse monoclonal antibody for progesterone receptor B antigen, species reactivity for sheep, immunogen from human endometrial carcinoma (EnCa 101) grown in athymic mice, made by Lab Vision Co, Warms Springs Blvd CA 94539 USA. These products are intended for research use only.

Evaluation: Every stained were evaluated in cell with details as follows: negative stain (-), weak (+), moderate (++), dense (+++). Cells number were count by visual such as 0%, 25%, 50%, or 75%. Each observation were performed at 400 magnitude (Perrot-Aplanat M et al., 1994).

Results and Discussion

In this study, we undertook a molecular approach to understand the mechanism by which Dexam manifest as antagonist activity on PR-B. This observation suggested that ligand binding domain of PR-B has any binding affinity to Dex that we can not identified. Subsequently, it has an ability to decrease the function of progesterone via PR-B.

The molecular explanation for the activity of Dex was revealed of high affinity interactions to PRB with the nuclear receptor corepressors SMRT and NCoR (Zakar, 1995). So, the present study was designed to determined a link between PR structure and biological activity with other compounds such as Dex.

With the identification of PR-B, and the histological demonstrations that Dex regulate the expression of PR-B. It has become possible to more fully consider the role of PR-B in regulating
uterine relaxation. This work has demonstrated that the number of PR-B has decreased. On the other hand it also showed that the effect of abortion in pregnant ewe was not only by the decrease of PR-B expression itself but also by the binding activity of glucocorticoid receptor (GR) to the region ligand binding domain of PR-B.

Interestingly, a possibility will be stimulated me or other researches followed up in subsequent studies. Based on my data it strongly support my hypothesis that Dex decrease PR-B roles by binding to regions ligand binding domain and inhibit PR-B transcriptional activity.

This study revealed that Dex interact with regions of PR-B ligand binding domain and inhibit PR-B transcriptional activity. This matter indicating by inhibition process of PR-B delivered to DNA. Thus, we can classify Dex as PRB antagonists or partial agonists based on how they interact with PR-B.

Lastly, for applications of Dex as a stressor and its administration in this study has displayed in reduced of number and function as well of progesterone and PR-B. This may relate to the fact that the currently available antiprogestrone are steroidal.

In conclusion, this experiment suggest that Dexa are capable of act as a stressor. These results suggest that Dexa has decreased and changed the PR-B expression is a key to tackle the challenge that will suggest novel therapeutic strategies for managing abortion or premature parturition in humans.

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References
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