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ORIGINAL ARTICLE

Does metoclopramide exposure alter endometrial receptivity and decrease pregnancy rates?

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Abstract

Objective: The aim of this study was to investigate the effect of metoclopramide on endometrial receptivity with an immunohistochemical investigation of integrin β3 expression in pregnant rats.

Materials and methods: In the present study, the pregnant mice administrated by different doses of metoclopramide were used to explore the effect of metoclopramide on embryo implantation, especially on the endometrial receptivity.

Results: The statistical results showed that the number of implanted embryos was gradually declining along the increasing dose of metoclopramide. When the administrated dose of metoclopramide was 3 mg/kg per day, great changes were observed in the exposed uterine morphology and down-regulated integrin β3 were also found in high dose metoclopramide-exposed mice.

Conclusion: Metoclopramide exposure, especially in high doses may alter endometrial receptivity by effecting integrin expression on decidual tissue which can decrease pregnancy rates. This drug should only be recommended for use during pregnancy when benefit outweighs the risk.

Keywords

Abortion, decidua, integrin, metoclopramide, pregnancy

History

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Introduction

Endometrial receptivity, refers to the state of the endometrium when the endometrial epithelium is structurally and functionally ready to accept the embryo for implantation [1]. It is, also called the ''window'' of implantation, a spatiotemporally restricted window. In the rat, the window is only opened around midnight on day 4 of pregnancy [2].

Evaluation of the receptivity of endometrium is challenged to improve the rate of embryo implantation in reproductive medicine because endometrial receptivity is considered as the primary factor to determine the pregnancy rates. Integrin β3 has been largely accepted as the molecular biomarker of uterine receptivity in both human and mice, and its maximal expression is observed on the surface of endometrial epithelium coincident with the time of implantation [3,4]. Decreased expression of integrin β3 during embryo implantation has been observed in endometrium from unexplained infertile women and other endometrial pathologies [5].

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The decreased endometrial integrin expression may be congenital or caused by environmental factors and medications. Metoclopramide, which is a commonly used antiemetic drug to treat nausea and vomiting of pregnancy effects both centrally and peripherally [6]. Although metoclopramide is being one of the most commonly used prescription medication for nausea and vomiting in pregnancy, data on the safety of its use in pregnancy is limited [7]. In this trial, we examined whether the metoclopramide exposure decreases integrin β3 expression of decidual tissues and alters the pregnancy rates in pregnant rats.

Materials and methods

Experimental animals

A total of 30 healthy rats weighing 150–200 g, purchased from the Ankara Medical and Research Hospital Laboratory Animal Center Ankara, Turkey, after taken the ethical approval from the same center. The rats were fed routinely for 1 week before the experiment. Estrous female rats selected via the vaginal smear method were caged with male rats at a ratio of 1:1 overnight. The next morning, female rats were individually assessed, and the day of detection of the vaginal plug or sperm-positive smear was designated as the first day

of pregnancy. A total of 18 pregnant rats were selected for the study and divided into three groups of six rats each.

Acute toxicity study and study design

Acute toxicity was studied in 10 female rats. Rats were fasted overnight. They were divided into five groups of two animals each. 0.025 kg metoclopramide hydrochloride powder form was used (National Healthcare Pvt. Ltd., Birgunj, Nepal). It was diluted in 3 cc normal saline for the study purpose and applied orally after fasted overnight. Metoclopramide was administered orally to the pair of rats of each group in ascending and widely spaced doses *viz.,* 1, 3, 10, 30 and 100 mg/kg. The animals were observed continuously for 2 h and then for further 4 h. Overnight mortality was recorded finally. There were no signs of toxicity with 30 mg/kg metoclopramide. Two doses of metoclopramide (1 and 3 mg/kg) corresponding to 1/10th of the maximum tolerated dose (30 mg/kg) were chosen for the study. The drug was applied as follows:

Control: No medication (Control)

Low dose: Metoclopramide (1 mg/kg) orally

High dose: Metoclopramide (3 mg/kg) orally

Drug therapies were started from the first to the 15th day of pregnancy. All rats were laparotomized under light ether anesthesia on the 19th day of pregnancy. Both horns of the uterus were observed for a number of implantation sites, resorption and dead or alive fetuses. The observations of the drug-treated groups were compared with control group.c

Immunohistochemical (IHC) evaluation of endometrial epithelium and stroma

using the Streptavidin-Biotin technique. All the 1.5 mm and 3 um cores of tissue array specimens embedded in paraffin slice on coated slides, were washed in xylene to remove the paraffin, rehydrated through serial dilutions of alcohol, followed by washings with a solution of PBS (pH 7.2). All subsequent washes were buffered via the same protocol. Treated sections were then placed in a citrate buffer (pH 6.0) and heated in a microwave for three 5 min sessions. The samples were then incubated with a rat monoclonal anti-integrin β3 antibody (EPR2417Y, ab75872, Abcam, Cambridge, UK, 1:150 dilution) for 60 min at 25 °C. The conventional biotin-streptavidin method (Thermo, Ultravision anti-Polyvalent HRP/DAB Kit TP-015-HD,) was performed for signal development, and the cells were counter-stained with hematoxylin. Positive controls were simultaneously obtained by staining tissues of the tonsil.

Statistical analysis

The data were analyzed using the SPSS version 11.5 (SPSS Inc., Chicago, IL). The categorical variables were compared using χ^2 test. The values were expressed as numbers and percentages. For permanent data, Kruskal–Wallis and one-way ANOVA analysis were used, and the results were expressed as median, minimum, maximum. $p<0.05$ was considered statistically significant.

Results

The mean number of total and living fetuses was listed in Table 1. In high dose exposed group, the number of living fetuses was lower than they were in other groups. In the drug-treated groups, especially at the higher dose there was a decrease in the number of fetuses and also atrophy (Figure 1a–c).

Table 1. Comparison of fetuses and living fetuses among groups.

	Control ($n=6$)	Low dose (1 mg/kg, $n=6$)	High dose (3 mg/kg, $n=6$)
Number of fetuses Median (Min–max)	10 (0–13)	7.3 (0–11)*	3.3 (0–8)**
Number of living fetuses Median (Min–max)	7 (0–12)	4 (0–9)*	1.3 (0–4)**

*$p<0.05$ un-treated versus high dose.

**$p<0.05$ low dose versus high dose.

Intensity and universality of immunohistochemical staining of integrin β3 for endometrial epithelium and endometrial stroma were detected different among groups. In the control group, integrin β3 staining was more intense and universal (>50%) than both high and low dose metoclopramide exposed group in endometrial stroma, while there was no statistical differences detected in endometrial epithelium among groups.(Table 2) (Figure 2).

Discussion

Implantation is a complex initial step in the establishment of a successful pregnancy and requires a receptive endometrium, a functional embryo at the blastocyst developmental stage and a synchronized dialog between maternal and embryonic tissues [8]. The cascade of signaling events that occur within the endometrial glands and stroma establishes an appropriate milieu that is critical for the development and survival of the fetus [1].

Recent studies have determined lots of genes and markers about endometrial receptivity [9]. αVβ3 integrin is one of these markers that is accepted as valuable predictors of endometrial receptivity [10]. The combined integrin αvβ3 acts as an adhesion promoter via cell–cell interactions and it has been very well characterized within the human endometrium [11]. The αvβ3 integrin is expressed in the glandular epithelium during the window of implantation and translocate into endometrial stroma, if pregnancy occurs [12].

Decreased endometrial receptivity due to decreased integrin β3 expression may be congenital or caused by environmental factors. Germeyer et al. showed that women with unexplained recurrent pregnancy loss had significantly reduced integrin expression compared to controls [13].

Reduced expression of αvβ3 has also been accepted to be related to infertility in women with endometriosis [14]. As an example of effect of environmental factors, Zhou et al. reported that sophoricoside exposure reduced the number of implanted embryos in a dose-dependent manner and failed the embryo implantation compromising the endometrial receptivity [15].

In this study, we investigated whether the metoclopramide decreases integrin β3 expression and also endometrial receptivity in pregnant rats. Metoclopramide has been extensively used to treat nausea and vomiting in pregnant women, despite a lack of data on the safety of the drug in pregnancy [7].

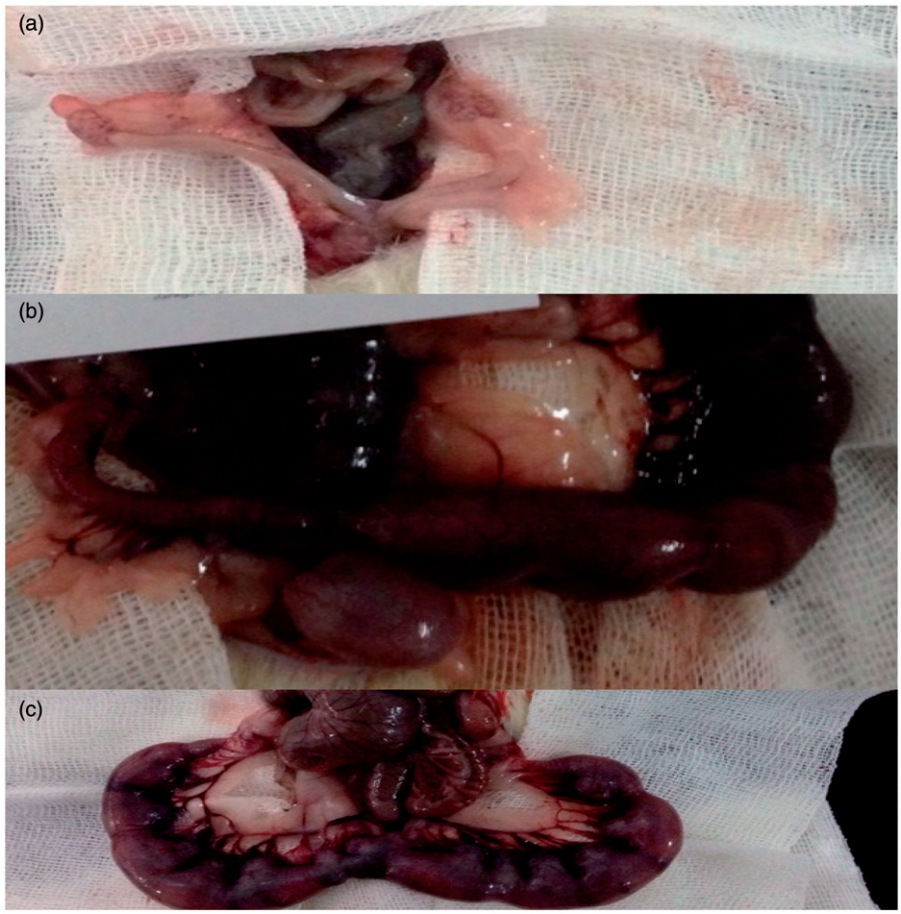

Figure 1. Picture of different numbers of implantation sites detected among the groups. (a). High dose drug applied group. (b). Low dose drug applied group. (c). Control group.

Table 2. Comparison of integrin β3 staining intensities and universalities endometrial stroma and epithelium of the groups.

	Control ($n=6$)(%)	Low dose (1 mg/kg, $n=6$)(%)	High dose (3 mg/kg, $n=6$)(%)
Endometrial epithelium			
Staining intensity			
Absent	0 (0.0)	0 (0.6)	1 (16.6)
Light	2 (33.3)	3 (50)	3 (50)
Dark	4 (66.6)	3 (50)	2 (33.3)
Staining universality			
Absent	0 (0.0)	0 (0.0)	1 (16.6)
<=50%	1 (16.6)	2 (33.3)	3 (50)
>50%	5 (83.3)	4 (66.6)	2 (33.3)
Endometrial stroma			
Staining intensity			
Absent	0 (0.0)	1 (16.6)*	4 (66.6)**
Light	2 (33.3)	3 (50)*	2 (33.3)**
Dark	4 (66.6)	2 (33.3)*	0 (12.5)**
Staining universality			
Absent	0 (0.0)	1 (16.6)*	4 (66.6)**
<=50%	2 (33.3)	2 (33.3)*	1 (16.6)**
>50%	4 (66.6)	3 (50.0)*	1 (16.6)**

*$p<0.05$ un-treated versus high dose.

**$p<0.05$ low dose versus high dose.

Although recent studies reported that metoclopramide treatment in the first trimester had no adverse outcomes for the fetus, including congenital malformations, perinatal death, low birth weight and low Apgar scores. Our result revealed that the number of implanted embryos was gradually fewer in high dose metoclopramide exposed group, and down-regulated integrin β3 were also found in high dose metoclopramide exposed mice [16].

This study is a preliminary study investigating the effect of metoclopramide on integrin β3 expression, and there are no controlled data in human pregnancy. Metoclopramide exposure alters the embryo implantation in a dose-dependent

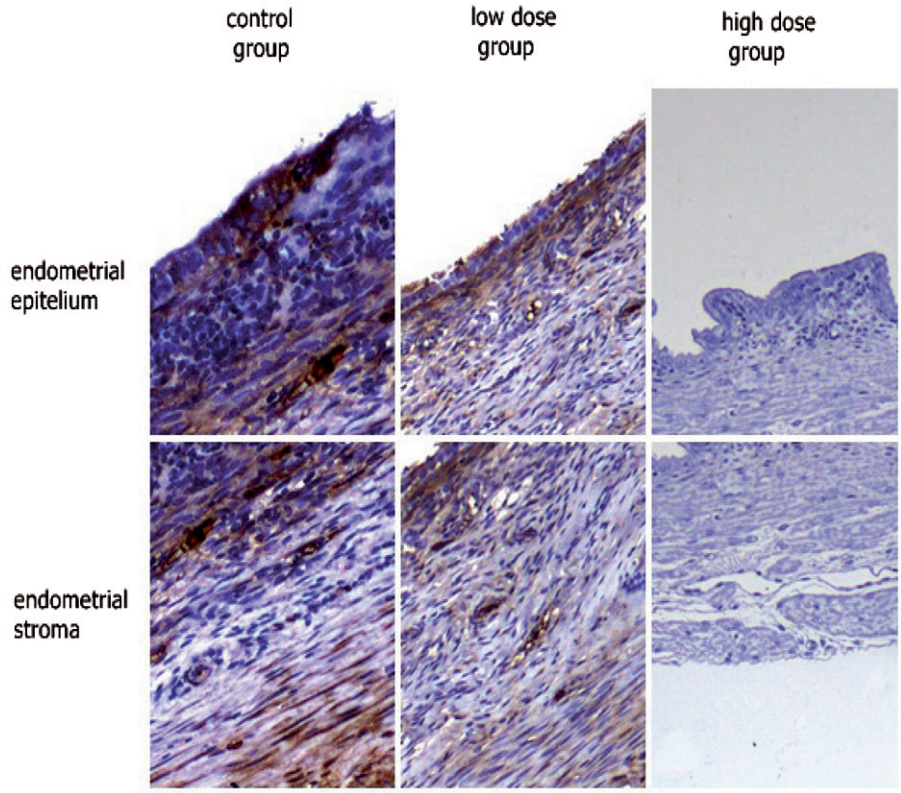

Figure 2. Immunohistochemical staining pictures of integrin β3 in different tissues. Groups were defined as control, low dose group (1 mg/kg) and high dose group (3 mg/kg) in three columns and tissues were showed as endometrial epithelium and stroma in two lines.

manner. Based on our results, we suggest that metoclopramide should only be recommended for use during pregnancy when benefit outweighs the risk. Larger studies are necessary to evaluate safety in the pregnancy.

Declaration of interest

The authors do not have any conflict of interest to disclose. No financial assistance was received in support of the study.

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