Abortifacient Effect of Metoclopramide in Female Albino Rats

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ABSTRACT

Background

Metoclopramide a dopamine receptor antagonist is commonly used to treat nausea and vomiting. Long term use can cause parkinsonism, galactorrhoea and gynaecomastia. As it is lipid soluble, it enters the brain, easily crosses the placental barrier and can affect the fetus. Hence, the present study is designed to assess the risk of metoclopramide in pregnant albino rats.

Objectives

To study the abortifacient effect of metoclopramide in pregnant albino rats.

Methods

Eighteen pregnant rats were divided into three groups of six rats each. The abortifacient activities of metoclopramide were studied in the doses of 1mg/kg and 3mg/kg intramuscularly. The treatments were started on the 6th day of pregnancy and continued till the 15th day. Rats were laparotomised on 19th day of pregnancy for evaluation of abortifacient action. In both the horns of the uterus, number of implantation sites, resorption sites, dead and live fetuses were observed.

Results

The mean percentage of aborted fetus was 17.22 ± 21.13 33.88 ±37.73 after 1mg/kg and 85.21±18.93 after 3mg/kg of metoclopramide. The abortifacient effect of higher dose was significantly larger compared to both control group and low dose group, but there was no significant difference between the mean percentage of abortion in control group and the low dose group of metoclopramide.

Conclusion

Metoclopramide at 3mg/kg intra muscular has abortifacient effects in female albino rats.

KEYWORDS

Abortifacient, implantation sites, metoclopramide, pregnancy, resorption sites

INTRODUCTION

Abortion is the spontaneous or induced termination of pregnancy before fetal viability. Apart from fetal chromosomal abnormalities, possible maternal causes for abortion are diabetes mellitus, hypothyroidism, infections, nutritional deficiencies and poly-cystic ovary disease. Exposure to tobacco, alcohol, caffeine, radiation, drugs and certain environmental toxins during pregnancy have been reported to be associated with the increased incidence of abortion.[1]

Metoclopramide is an antiemetic and gastroprokinetic

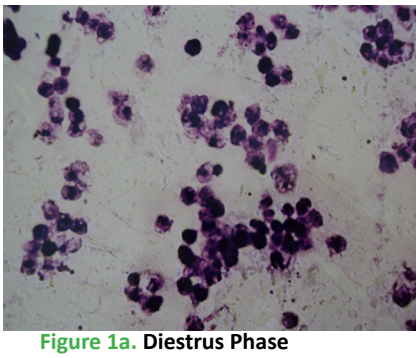

Figure 1a. Diestrus Phase

Figure 1b. Proestrus Phase

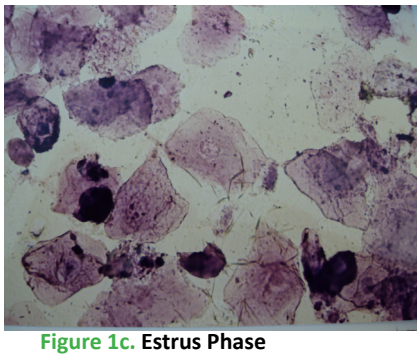

Figure 1c. Estrus Phase

Figure 1. Vaginal smear of rat showing different phases of estrus cycle.

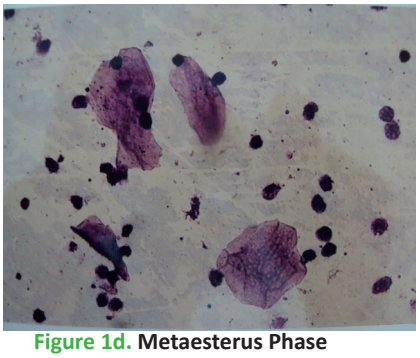

Figure 1d. Metaesterus Phase

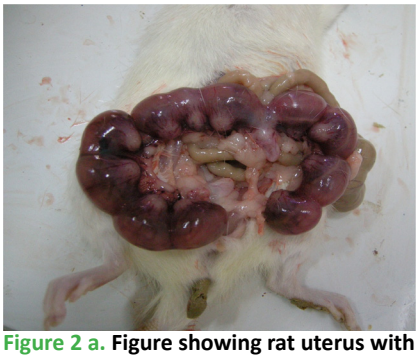

Figure 2 a. Figure showing rat uterus with fetus and implantation sites

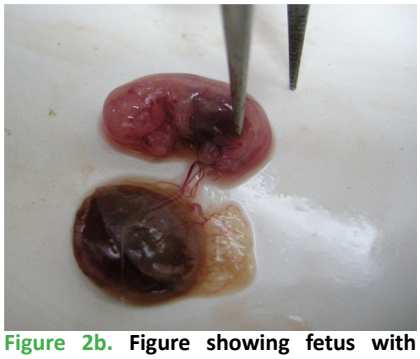

Figure 2b. Figure showing fetus with placenta

Figure 2. Uterus with the fetuses and implantation sites.

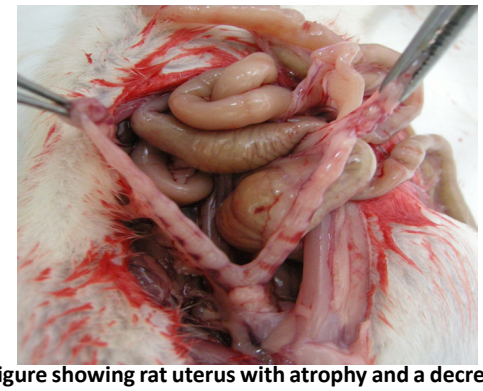

Figure 3a. Figure showing rat uterus with atrophy and a decrease in the number of fetuses.

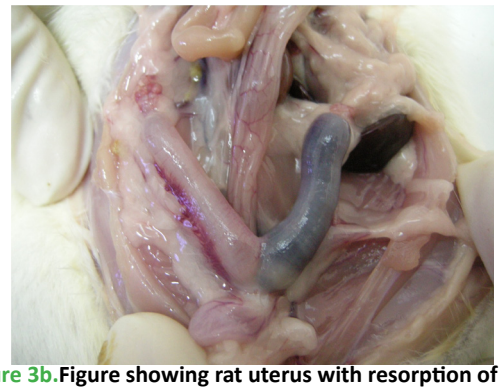

Figure 3b. Figure showing rat uterus with resorption of fetus.

agent. It is commonly used to treat nausea and vomiting. The mechanisms of action are complex and involve 5-HT_4-receptor agonism, vagal and central 5-HT_3-antagonism, and possible sensitization of muscarinic receptors on smooth muscle, in addition to dopamine receptor antagonism both centrally and peripherally.[2] Metoclopramide is rapidly absorbed orally, enters brain, easily crosses placental barrier and is secreted in milk. Long term use can cause parkinsonism, galactorrhoea and gynaecomastia.[3]

In the case of more than 90% of the drugs approved by the Food and Drug Administration in the past 20 years, there are insufficient data from human studies to determine whether the benefits of therapy exceed the risk to the fetus. In the United States and Canada, the drugs of choice for the treatment of nausea and vomiting during pregnancy are pyridoxine and doxylamine, whereas metoclopramide is used only in the most severe cases. Despite its extensive use, only a few studies have assessed the safety to the fetus of maternal exposure to metoclopramide during the first trimester and the relatively small sizes of these studies limited their power to detect adverse effects of the drug.[4,5] A survey of literature revealed that Abortifacient effects of metoclopramide in female albino rats has not been documented.

Hence, the present study is designed to assess the risk of metoclopramide in pregnant albino rats.

METHODS

Acute Toxicity Study

Acute toxicity was studied in female rats weighing between 150-200 g. Rats were fasted overnight. They were divided into six groups of two animals each. Metoclopramide was administered intramuscularly to the pair of rats of each group in ascending and widely spaced doses viz.1, 3, 10, 30, 100, 300, 1000, 3000 mg/kg. The animals were observed continuously for two hours and then occasionally

for further four hours and finally overnight mortality was recorded.[6]

No signs of toxicity were observed only with 30 mg/kg of metoclpramide. So, two doses of metroclopramide (1mg/kg and 3mg/kg) corresponding to 1/10th of the maximum tolerated dose (30mg/kg) were chosen for the study.

Experimental Animals

The experimental protocol was approved by Institutional Review Committee (KUSMS/IRC) and animals were maintained under standard conditions in animal house approved by the Committee. Mature, healthy female albino Wistar rats weighing 150 - 200 gm were used. The rats were maintained under standard environmental condition and were provided with standard rat feed and water *ad libitum*. The animals were acclimatized for two weeks before experimentation.

Metoclopramide Hydrochloride

Metoclopramide Hydrochloride powder form was obtained from National Healthcare Pvt.Ltd, Birganj (Nepal). It was diluted in normal saline for the study purpose.

Quantity: 0.025kg

Batch number: 1005275

Manufacture Date: May 2012

Expire Date: April 2015

Estrus Cycle

Stages of estrus cycle of each rat were determined by taking vaginal smears daily between 8-9 A.M and 4-5 P.M for 15 consecutive days. Vaginal smear was prepared by introducing a drop of distilled water into the vagina with the help of a dropper and collecting back and placing it on a clean slide after adding a drop of glycerin.[7] The prepared smear was examined microscopically under low power for different type of cells [Fig 1]. There are four phases in estrus cycle of female rats. If majority of cells were mainly leucocytes, then it was labeled as in diestrus phase [Fig1a] which lasts up to 57 hours. Presence of large number of nucleated cells indicated proestrus phase [Fig 1b] which lasts for 3- 12 hours. Estrus phase was confirmed when 50% or more of the cells were cornified [Fig 1c] which lasts for 12 hours. Metaestrus phase lasts for 21 hours with many neutrophils and scattered squamous epithelial cells in the smear [Fig 1d]. Rats showing three regular estrus cycles were chosen for the study.

Fertilization of female rats

Female rats with three regular estrus cycles were caged with male rats of known fertility in the ratio of 2:1 on the evening of proestrus. Vaginal smears were examined on the following morning for the presence of sperms to confirm mating. Female rats exhibiting thick clumps of spermatozoa in the vaginal smear were chosen for the study and that day was considered as day one of pregnancy. The pregnancy rate was 100%.

Table 1. Abortifacient effect of metoclopramide in rats

Group	Treatment	Dose	Total Number of Implantation Mean ± SD	Number of live fetuses Mean ± SD	Aborted Fetuses Mean ± SD	% of Abortions Mean ± SD
Control	Vehicle	1ml	10.17 ± 1.60	10.17 ± 1.60	0	0
High Dose	Metoclopramide	3mg/kg	7.83± 2.48	1.33± 1.75	6.5±1.87	85.21 ± 18.93
Low Dose	Metoclopramide	1mg/kg	5.33 ± 3.67	4± 2.75	1.33 ±1.63	17.22 ± 21.13

Statistics

		Percent of			Total Number of
Group		Aborted Fetus	Live Fetus	Aborted Fetus	Implantation
Control	n Valid	6	6	6	6
	Missing	0	0	0	0
	Mean	.0000	10.1667	.0000	10.1667
	SD	.00000	1.60208	.00000	1.60208
	Minimum	.00	8.00	.00	8.00
	Maximum	.00	12.00	.00	12.00
High Dose	n Valid	6	6	6	6
	Missing	0	0	0	0
	Mean	85.2183	1.3333	6.5000	7.8333
	SD	18.93724	1.75119	1.87083	2.48328
	Minimum	57.14	.00	4.00	5.00
	Maximum	100.00	4.00	9.00	12.00
Low Dose N	n Valid	6	6	6	6
	Missing	0	0	0	0
	Mean	17.2222	4.0000	1.3333	5.3333
	SD	21.12573	2.75681	1.63299	3.66970
	Minimum	.00	1.00	.00	1.00
	Maximum	50.00	8.00	4.00	10.00

Sample size (n)=6 in each group
SD: standard deviation

Study Design

Eighteen pregnant rats were divided into three groups of six rats each.

The drug treatment was as follows:

Group I: Normal saline 1ml intramuscularly (Control)

Group II: Metoclopramide (1mg/kg) intramuscularly

Group III: Metoclopramide (3mg/kg) intramuscularly

Drug therapies were started from 6th to 15th day of pregnancy which is the period of organogenesis in fetus.

Evaluation of abortifacient activity

All the above rats were laparotomised under light ether anesthesia on the 19th day of pregnancy. Both horns of the uterus were observed for number of implantation sites, resorption and dead or alive fetuses.[8] The observations of the drug treated groups were compared with control group (Fig 2a and 2b).

Percentage of abortion was calculated by the formula:

$$\% \text{ abortion} = \frac{(\text{No. implantation} - \text{No. live fetus}) \times 100}{\text{No of implantation}}$$

Statistical Analysis

Data were analyzed using SPSS V-16. Results were expressed as Mean ± SD, and the differences between experimental groups were compared using t-test.

RESULTS

Acute toxicity studies

Mortality and behavioral changes were observed in the acute toxicity study at the dose of 30mg/kg. Therefore, two doses (1mg/kg and 3mg/kg) were selected for the study.

Abortifacient effect

The mean percentage of abortion after 1mg/kg and 3mg/kg doses were 17.22 ± 21.13 33.88 ±37.73 and 85.21±18.93 respectively. At high dose of metoclopramide, abortifacient effect was highly significant compared to both control group ($p < 0.05$) and low dose group ($p < 0.05$), but there was no significant difference between the mean percentage of abortion in the control group and the low dose group of metoclopramide ($p > 0.05$). There was atrophy and a decrease in the number of fetuses in the drug- treated groups especially at the higher dose [Fig 3a and 3b].

DISCUSSION

We have taken rats for this study because they have very short estrous cycle (menstrual cycle) 4-5 days. Estrous cycle is divided into characteristic phases: proestrus, estrus, metestrus and diestrus. The stage of the estrous cycle can be determined by vaginal cytology. Ovulation occurs at the end of metestrus.[9] By seeing the rats vaginal smear we can determine fertility of the rats. Rats are very fertile. Pregnancy in rat lasts for only 21-23 days. Birth usually occurs at night with 10-12 pups.[10] The short length of the estrous cycle and duration of pregnancy of rats makes them ideal for this investigation. Many pregnant women have nausea and vomiting during the first trimester; these effects may be severe and in some women they continue beyond the first trimester.[10] The cause is likely to be multifactorial, and the disorder is a diagnosis of exclusion.[11] Since the thalidomide tragedy, there is heightened awareness of the risks of drug therapy during pregnancy in general and of antiemetic therapy in particular.[12] Metoclopramide, a D2- receptor antagonist with additional antagonism at serotonin 5-HT3 receptor and agonism at 5-HT4 receptor, has been used during pregnancy for its antiemetic and gastric prokinetic effects.[7] It is rapidly absorbed orally, crosses the blood brain barrier, so long term use of this drug can cause parkinsonism, galactorrhoea and gynaecomastia.[3] As it enters brain, easily crosses placental barrier and can affect the fetus. Therefore, this could be a possible cause for significantly increase in % of abortion seen with drug treated groups especially in high dose. There are no controlled data in human pregnancy. Metoclopramide is only recommended for use during pregnancy when benefit outweighs risk. More data are needed to evaluate safety in the pregnancy. Hence awareness needs to be developed among female populations about the health hazards of drugs during pregnancy.

CONCLUSION

Metoclopramide at high dose 3mg/kg has shown abortifacient effects in female albino rats.

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