Evaluation of teratogenic effects of risperidone following simultaneous administration with antihypertensive and antiemetic drugs

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Abstract: Multiple drug administration is an important aspect of clinical practice particularly in specific physiological situation such as in neonates, elderly or pregnancy, since in all such situations, possibility of unwanted effects increases due to altered body physiology. In present study, the teratogenic effects of multiple drug administration risperidone, meclizine/pyridoxine and hydralazine have been compared with the teratogenic effects of individual drugs in pregnant mice. Moreover the role of folic acid and α-tocopherol if any had also been investigated in reducing the teratogenic effects of these drugs in combination.

Keywords: multiple drug administration, teratogenic effects, risperidone, meclizine/pyridoxine, hydralazine, folic acid, α- tocopherol

INTRODUCTION

Drug administration is particularly important during pregnancy (Kemp, 2002), where the choice of drugs is limited because of increased risk of teratogenic effects due to altered pharmacokinetics of drugs in pregnancy and other possible adverse effects of the drug on the baby (Chin *et al*, 1986). Hence, it is not only essential to understand the effect of drugs but also to know the point in fetal development when drugs are most toxic and when fetal organs are most susceptible (Gentile, 2010). Despite all these facts drugs have to be administered and sometimes in combination that greatly increases the probability of adverse effects. Moreover, drug combinations may precipitate a different spectrum of adverse effects that are not attributed to the individual agent and may be due to pharmacodynamic or pharmacokinetic interaction inside the body between these drugs (Menon, 2007).

It is estimated that 10% of all birth defects are caused by a prenatal exposure to drugs or teratogen. The teratogenic effects may include any malformations, growth retardation, functional deficit or death (Rogers*et al*, 1995). The time of pregnancy at which mothers are exposed to drugs is crucial to determine the type of teratogenic effect; hence the nature of these effects is determined by the gestational event during which the fetus is exposed to it. Different kinds of teratogenic effects may be seen with a similar exposure (Koren, 2001: Washington; Barnes 2010).

Women with a history of psychiatric illness had to take drugs not only for psychiatric illness but other medications for usual pregnancy complications, such as nausea, vomiting and hypertension. Maternal psychiatric disorders may have a devastating impact on the fetus and the newborn (Gentile, 2008). Thus treatment of these disorders during pregnancy is a clinical and ethical duty with the necessity to avoid or minimize fetal or neonatal drug exposure (Desai *et al*, 2009; Kohen, 2004). Hence physician taking care for a pregnant patient should have to be careful in choosing dosages and types of drugs to maximize effectiveness and minimize fetal risk (Pandey and Shukla, 2000; Newport *et al*. 2007)

Thus present study was aimed to evaluate any variations in the response of antipsychotic drug, risperidone when it is used with drugs commonly prescribed in pregnancy for usual pregnancy complications such as nausea, vomiting and hypertension to assess the safety of the combination. Moreover any possibility of reduction in teratogenic effects was also explored following simultaneous administration of folic acid and α-tocopherol.

MATERIALS AND METHODS

Experimental animals

The study was carried out on healthy, young, female mice kept in polycarbonate breeding cages with processed hardwood chips as bedding material. The animals were maintained at normal environmental conditions of temperature (18-26^{o}C) and light. They had free access to water and food *ad libitum*, equal numbers of male mice were also used for the purpose of matting.

Preparation of drugs

Reference standard and test samples of active material for the drugs used in this study were obtained after proper test analysis and certification of the samples with the courtesy of Dr. M. Tanvir Alam, Director Central Drug Laboratories, Government of Pakistan, Ministry of Health, Karachi. Risperidone, meclizine/pyridoxine and

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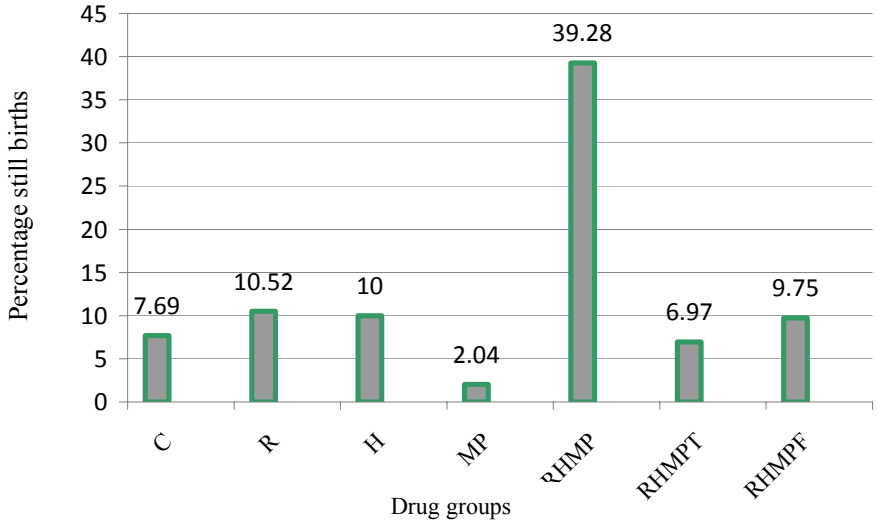

Fig. 1: Percentage of still births at low dose of risperidone and its combination with other drugs**.**
C=Control; Risperidone; H= Hydralazine; MP= Meclizine/Pyridoxine T= α-Tocopherol; F= Folic Acid

Table 1: Effect of low dose risperidone in combination with other drugs on pregnancy outcome in mice

		C	R	H	MP	RHMP	RHMPT	RHMPF
1	No. of Pregnancies	07	07	07	07	07	07	07
2	No. of litters	07	07	07	07	07	07	07
3	No. of live pups	26	34	27	48	17	40	37
4	No of Still Births	02±0.18	04±0.2*	03±0.2	01±0.14	11±0.29*	03±0.42	04±0.36
5	Malformed pups	00	00	00	00	10*	00	00
6	Normal alive pups	26	34	27	48	07*	00	00
7	Mean fetal weight	5.24±1.36	4.63±0.85	4.36±0.92	7.44±0.93	1.84±0.32*	6.76±0.53	5.55±1.12

C=Control; R= Risperidone; H= Hydralazine; MP= Meclizine/Pyridoxine;T= α-Tocopherol; F= Folic Acid; n=7

folic acid were dissolved/suspended in DMSO separately while hydralazine was dissolved in sterile water whereas α-tocopherol was administered in the form of oily liquid.

Selection of doses

All drugs except folic acid and α-tocopherol were administered orally in the therapeutic doses used in humans considering normal adult weight as 60 kg (Richards, 2008). Hydralazine was dissolved in water for injection, while all other drugs were suspended in DMSO. Risperidone was administered in two different doses 4 mg and 8 mg. Hydralazine was administered in the dose of 100 mg and meclizine/pyridoxine was administered in the doses of 50/100 mg respectively. The dose of folic acid was 0.166 mg/kg and α-tocopherol was 10 mg/kg. Animals of control group were given DMSO according to body weight.

Experimental procedure

All animals were divided into eleven groups each comprising of 7 pairs (7 males and 7 females), their body weights were recorded and each pair was housed in a separate cage to permit matting. The pairing day was counted as day one for the administration of drugs to female mice only. The drugs were administered continuously till the birth of off springs. Two animals in each group at term were randomly scarified to observe any teratogenic effect. After birth all the pups were again observed for any malformation, growth retardation, functional deficit or death and compared with the control group.

STATISTICAL ANALYSIS

Data was statistically analyzed using ANOVA and results were considered significant when *p* value was < 0.05.

RESULTS

Low dose risperidone

1. Fetal weights and still births

10.52% still births were observed when risperidone was given alone in the dose of 4 mg/60 kg as compared to still births in control animals which were 7.69% (fig. 1), however fetal weights of animals received risperidone alone were not decreased significantly i.e. 4.63 ± 0.85 gm than control which were 5.24 ± 1.36 gm (table 1, figs. 2a and b). while animals which received risperidone, hydralazine and meclizine/pyridoxine revealed a total of 28 births out of which 11 pups (39.28%)were born dead

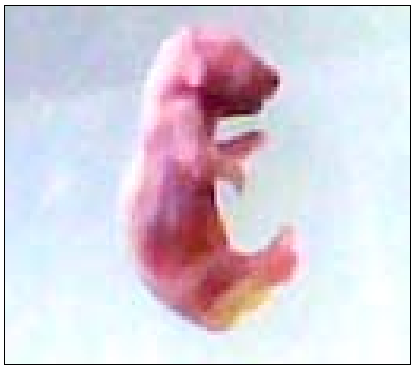

Fig. **2a**: A fetus born to control with normal birth weight

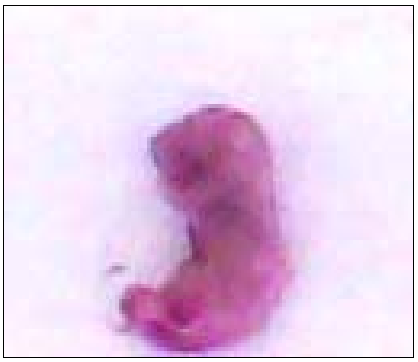

Fig. **2b**: A fetus born to low dose risperidone alone with low birth weight

Fig. **3**: Still births at low dose risperidone, hydralazine and meclizine/pyridoxine group

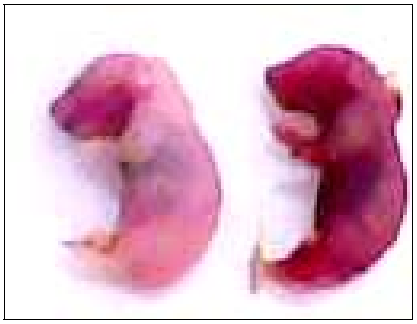

Fig. **4**: Live, normal pups born at low dose risperidone, hydralazine, meclizine/pyridoxine and α-tocopherol group

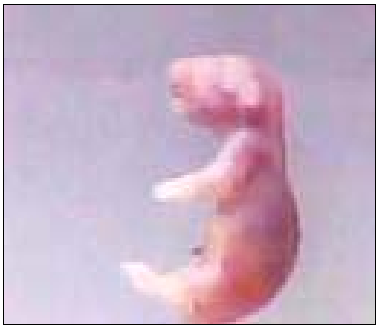

Fig. **5**: A normal pup born to low dose risperidone, hydralazine, meclizine/pyridoxine and folic acid group

(figs. 1 and 3), low birth weights were also observed in almost all the litters of this group i.e. 1.84 ± 0.32 gm (table 1), both the parameters, fetal weight and still birth differ significantly from control. Addition of α-tocopherol found to have a positive effect on still births as well as fetal weights i.e. percentage of still births reduced to 6.97% (fig. 1) birth weights were also significantly increased i.e. 6.76 ± 0.53 gm when compared with the group without α-tocopherol (table 1 and fig. 4). Similarly addition of folic acid to risperidone, hydralazine, meclizine/pyridoxine group, also reduced the percentage of still births to 9.75 (fig. 1), and a significant increase in the fetal weights to 5.55 ± 1.12 gm as compared to risperidone, hydralazine and meclizine/pyridoxine group (table 1 and fig. 5).

2. Gross malformations and functional deficit

Pups born to animals received risperidone alone appear to have normal external features on examination (fig. 6a). Animals of this group were also examined for functional deficit at maturity such as CNS activity and reproductive ability, however both were found to be normal. When risperidone was given along with hydralazine and meclizine/pyridoxine,10 pups out of 28 were found with incomplete limbs formation which died after 2 days, that was significantly different in animals given risperidone alone (fig. 6b), while remaining animals of this group that reached maturity did not show any functional deficit at the level of CNS or reproductive system. However addition of α-tocopherol to animal group received risperidone, hydralazine and meclizine/pyridoxine,

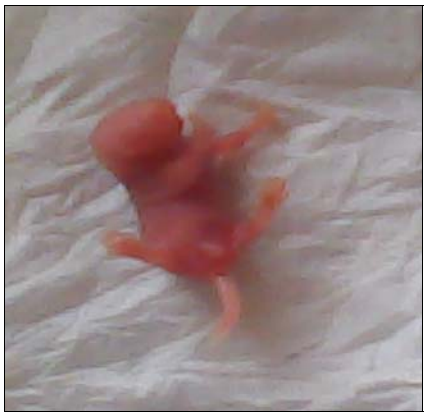

Fig. 6a: A pup of low dose risperidone without malformation

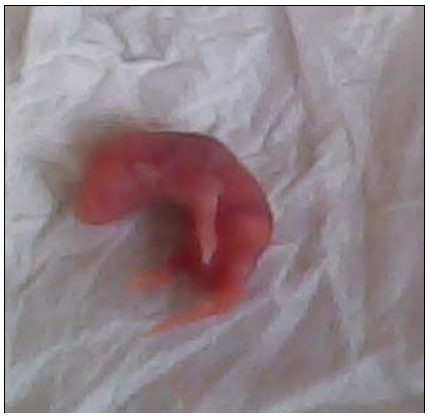

Fig. 6b: A pup of low dose risperidone hydralazine, meclizine/pyridoxine with incomplete limbs

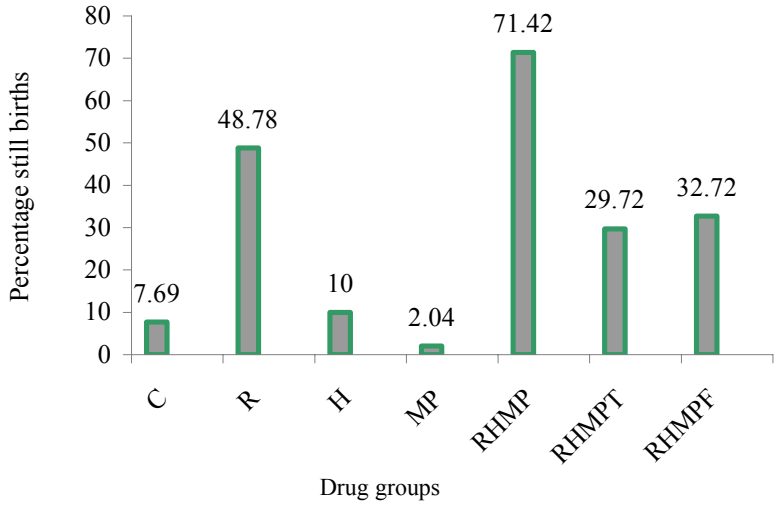

Fig. 7: Percentage of still births at high dose of risperidone and its combination with other drugs.

showed no noticeable malformation or functional deficit (fig. 4). Similarly when folic acid was added to animals received risperidone, hydralazine and meclizine/ pyridoxine again there was no malformation (fig. 5) or defects in CNS activity and reproductive behavior.

High dose risperidone

1. Fetal weights and still births

Percentage of still births was increased to 48.78% (fig. 7), when risperidone was given to animals in the dose of 8 mg/60 kg, which was significantly higher than control as well as low dose risperidone. There was significant decrease in fetal weights as compared to control i.e. 2.61±1.01 gm in comparison to 5.24±1.36 gm, however fetal weights were not significantly reduced at low dose risperidone. When risperidone was given in the dose of 8 mg/60 kg along with hydralazine and meclizine/ pyridoxine percentage of still births increased up to 71. There was also significant decrease in birth weights as compared to control i.e. 1.24 ± 0.48 gm (figs.7 and 8). While addition of α-tocopherol to risperidone, hydralazine and meclizine/pyridoxine group reduced death rate to 29% (fig. 7) and there was significant increase in the weight of fetus i.e. 5.22 ± 1.33 gm as compared to the group without α-tocopherol (table 2). Whereas addition of folic acid to the combination reduced the death rate to 32.72% (fig. 7) and birth weights significantly increased to 6.01 ± 1.45 gm (table 2).

2. Gross malformations and functional deficits

Animals received risperidone alone in high dose showed malformations in 17 pups out of 41which include incompletely formed mouth, limbs and tail (fig. 9) that is significantly higher than control. When risperidone was given along with hydralazine and meclizine/pyridoxine, 22 incompletely formed pups were born out of 35 (fig. 8) which is significantly higher than risperidone alone group. No functional deficit was found in both of these groups and the pups showed normal CNS and reproductive activity. Addition of α-tocopherol to the group prevented these malformations (fig. 10). Similarly administration of folic acid to the group produce a positive effect with no malformation of limbs or tail neither there was any functional deficit at maturity.

Table 2: Effect of high dose risperidone in combination with other drugs on pregnancy outcome in mice

		C	R	H	MP	RHMP	RHMPT	RHMPF
1	No. of pregnancies	07	07	07	07	07	07	07
2	No. of litters	07	07	07	07	07	07	07
3	No. of live fetuses	26	21	27	48	12	26	37
4	No. of still births	02±0.18	20±0.5*	03±0.2	01±0.14	23±0.42*	11±0.68	18±0.75
5	Malformed pups	00	17*	00	00	22*	00	00
6	Normal alive pups	26	04*	27	48	00*	26	37
7	Mean fetal weight	5.24±1.36	2.61±1.01*	4.36±0.92	7.44±0.93	1.24±0.48*	5.22±1.33	6.01±1.45

C=Control; Risperidone; H= Hydralazine; MP= Meclizine/Pyridoxine; T= α-Tocopherol; F= Folic Acid; n=7
Average value± SEM, * P<0.05 is considered as significant

Fig. **8**: Still birth and incomplete limb formation in fetuses at high dose risperidone, hydralazine and meclizine/ pyridoxine.

Fig. **9**: A pup born with mal-formation of mouth at high dose risperidone alone.

Fig. **10:** Healthy, normal pups born at high dose risperidone, hydrala-zine, meclizine/pyridoxine, and α-tocopherol group.

DISCUSSION

Drugs are an important source of teratogenic events and produce different teratogenic effects at various stages of development. Present study have been specifically designed to evaluate the teratogenic effects of antipsychotic drug risperidone, in combination with antihypertensive hydralazine, and antiemetic meclizine/ pyridoxine and also to explore the possibility of reducing the teratogenic effects by addition of α-tocopherol and folic acid to this combination.

Risperidone was tested at two different dose levels and all the manifestations of teratogenicity i.e. still births, growth retardation, malformation and functional deficits were observed. Animals received low dose of risperidone alone, did not reveal any malformation and growth retardation; however there were few still births and insignificant reduction in birth weights as compared to control, few previous studies also support these findings (Ernst and Goldberg, 2002; Iqbal, 2001) although very limited data is available in animals and few case reports of effect in humans (Coppola *et al*., 2007; Ratnayake and Libretto, 2002; Rodriquez, 2008; McKenna, 2005). Whereas animals received high dose of risperidone revealed not only high frequency of still births but there was significant reduction in birth weights, moreover there were also cases of malformations.

When low dose risperidone was combined with hydralazine and meclizine/pyridoxine, significant malformations, increased still births and reduced fetal weights were observed than control. The intensity of teratogenic events markedly increased in high dose risperidone combination with hydralazine and meclizine/pyridoxine than as compared with low dose risperidone combination.

Interesting results were found when α-tocopherol and folic acid were added along with these combinations in an attempt to reduce or prevent teratogenic events. Addition of α-tocopherol to low dose risperidone combination significantly prevented the teratogenicity, similarly when α-tocopherol was added to high dose risperidone combination, the ratio of still births, low birth weights and malformations again decreased significantly; however these effects were comparatively less than low dose risperidone combination.

Whereas addition of folic acid to low and high doses of risperidone combinations also prevented the teratogenic events, but comparatively low dose risperidone and α-tocopherol combination revealed more positive results than risperidone and its combination with folic acid. Similarly effects of high dose risperidone and α-tocopherol combination revealed more improvement in most of the teratogenic events except reduction in fetal birth weights. This shows that α-tocopherol and folic acid both played an important role in reducing the teratogenic effects of above combinations in mice.

Hence results of present study revealed that multiple drug

administration rather than individual drugs are more likely to be associated with increased risk of teratogenic events; however studies on large number of animals and other species are needed to reach to a definite conclusion.

Although animal studies are essential to evaluate the teratogenic effects of any drug, the possibility of variation in effects always exists in human due to specie difference. Therefore each drug should be assessed, and its risks and benefits should be evaluated (Richards, 2008). In case of psychotic illness, there should be no uncertainty in the decision to treat a pregnant woman during pregnancy, because the risks associated with remaining untreated illness is very high (Miller, 1991). Although there is consensus to use antipsychotics during pregnancy but should be used in the lowest possible dosage with regular psychiatric and obstetric monitoring and review of medication (Kohen, 2004).

Risk-benefit assessment and counseling should involve the patient and her current state of health. Counseling should actively involve the patient and the physician. The physician must consider the effects of drug exposure on the developing fetus or embryo and acknowledge specific susceptibilities at each point in fetal development, as balanced against the risks of worsening maternal illness (Czeizel, 2004).

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