ORIGINAL ARTICLE

Embryotoxicity of Mirtazapine: a study using Chick Embryotoxicity Screening Test

Eva Maňáková [1], Lucie Hubičková [1], Jana Košťálová [1], Zdena Zemanová [2]

1 CZTIS, Department of Histology and Embryology, 3rd Faculty of Medicine, Charles University in Prague, Czech Republic
2 Institute of Physiology, Academy of Sciences, Prague, Czech Republic

Correspondence to: Eva Maňáková, MD., PhD.
CZTIS, Department of Histology and Embryology,
3rd Faculty of Medicine, Charles University in Prague,
Ruská 87, 100 00 Prague, Czech Republic.
TEL: +420 267 102 310; E-MAIL: eva.manak@post.cz

Submitted: 2010-09-17 Accepted: 2010-11-16 Published online: 2010-12-28

Key words: **embryotoxicity; screening test; mirtazapine**

Neuroendocrinol Lett 2010; **31**(Suppl.2):8–10 **PMID:** 21187830 NEL31S210A02

Abstract

OBJECTIVE: Mirtazapine is a new antidepressant used in last years, however experience with it during pregnancy is unsatisfactory on the present. Its wide terapeutic range and only little proved side effects may be an advantage for treatment during pregnancy. Aim of our study was to contribute to the knowledge on possible risks.

MATERIALS AND METHODS: For embryotoxicity testing we used an alternative method – CHEST, that used chicken embryos as experimental model. Fertilized eggs of outbred Grey Leghorn stock (AVČR farm Koleč) were treated on embryonic day (ED) 4 by Mirtazapine, incubated till 9ED, when they were weighed and examined. Summing the proportions of dead and malformed embryos, the beginning of the embryotoxicity dose range was estimated.

RESULTS: Mirtazapine solved in 15% DMSO in water revealed low embryotoxicity corresponding data from preclinical studies. If 100% DMSO was used as a solvent, the dose 0.05 μg/3 μL resulted in 57% mortality (LD50). Typical malformations were microphtalmia and malformation (shortering) of limbs on left side, which is a place of contact the embryonic body with maximal Mirtazapine concentration. Approximation of doses in chick embryos to mammals is complicated by low solubility of mirtazapine.

CONCLUSIONS: If the embryotoxic dose was close to LD50, risk at therapeutical doses will be probably low. Mirtazapine according to resuls of testing and cases published in literature is relatively safe for pregnant women, only higher rate of abortions was demonstrated, however more information is needed to exclude all potentional risks.

Abbrevations:

HH	- Hamburger-Hamilton developmental stages of chick embryos
ED	- embryonic day
DMSO	- dimethysulphoxide
CHEST	- chick embryotoxicity screening test
RR	- risk ratio
NOEL	- no observable effect level
LD50	- lethal dose for 50% exposed
IUGR	- intrauterine growth retardation

INTRODUCTION

Depression is relatively frequent during pregnancy and have to be treated. Many new psychotropic drugs are introduced every year in the market, however information regarding their embryotoxicity is insufficient. Every information is valuable for the risk assessment during pregnancy. Case reports, epidemiological prospective and retrospective studies as well as experimental studies on animals. Evaluation is done according to combination of all, because the transfer of the knowledge from experiment to the practice is not easy. Drugs should not be allowed to praxis without background of experience. Mirtazapine is a new antidepressant used in last years, experience during pregnancy is unsatisfactory, moreover the animal data are based on pre-clinical testing, only. Lack of information about its embryotoxicity is a problem for righ decision during pregnancy.

MATERIAL AND METHODS

We used an alternative method for embryotoxicity testing CHick Embryotoxicity Screening Test – CHEST (Jelínek & Marhan 1994; Peterka *et al.* 1992; Veselý *et al.* 1997). Fertilized eggs of outbred Grey Leghorn stock (AVČR farm Koleč), were incubated at 37.5 ± 0.2°C and relative humidity 55–65%. Access to the embryos through an opening of eggshell was performed on 4 ED (stages HH 21 through HH 24). After treatment, the window was covered with glass slide sealed to a paraffin frame. Embryos were incubated till 9 ED, when they were withdrawn, weighed, and examined under a stereomicroscope. Summing the proportions of dead and malformed embryos, the beginning of the embryotoxicity dose range was estimated. The administrated doses were approximated to the therapeutical doses. Mirtazapine (Sigma-Aldrich, St.Louis, USA) for intra-amniotical treatment was diluted in 15% and 100% DMSO (Sigma-Aldrich, St.Louis, USA). If Mirtazapine solubility was poor, we changed protocol of CHEST. Instead of 10% DMSO we used 100%. Doses per embryo were: 0.03 μg/3 μL, 0.05 μg/3 μL, 0.1 μg/3 μL, and 0.15 μg/3 μL in 100% DMSO. Control groups were treated by distilled water, chick Ringer solution (0.7% NaCl), and 100% DMSO.

RESULTS

Embryos exposed to the Mirtazapine in 10% DMSO revealed the same proportion of malformation as controls. We were not able to reach the embryotoxic or letal dose. If we used 100% DMSO as a solvent, the dose 0.05 μg/3 μL expressed both embryolethality and teratogenicity. Observed malformations included eventration of body wall and defect of ventricular septum. Unilateral shortering of limb and unilateral microphatalmia were demonstrated on the lower side of the body, which was in the close contact with higher concentration of the substance. Death was caused mainly by the heart arrest that is consistent with finding in the published cases. Control groups exposed to the Ringer solution or DMSO expressed significantly higher proportion of body wall defects. Results are summarized in Tables 1 and 2.

DISCUSSION

Mirtazapine (FW 265.35) is a new antidepressant with prominent alpha 2-adrenergic auto- and heteroreceptor antagonistic properties. It has a low affinity for 5-HT1A receptors but shows 5-HT1-agonistic-like effects. The enhancement of both noradrenergic and serotonergic transmission probably underlies the therapeutic activity of mirtazapine (de Boer 1996). It is marked as a racemic mixture and both enatiomers possess pharmacological properties. Their metabolism is different resulting in the longer biological half-time of R(–)-enantiomer. It is poorly soluble in water, but it is soluble in DMSO (~8 mg/mL, Sigma-Aldrich, St.Louis, USA), chloroform, and methanol. Mirtazapine is extensively metabolized and almost completely excreated in the urine (80%) and feaces (Delbressine *et al.* 1998). Drug resorption is not changed by food intake or fat content in meal (Cohen *et al.* 1997). Mirtazapine has low acute toxicity. LD50 for rat was higher than 2000 mg/kg. There are small differences in toxicity according to sex with lower LD50 in rat females. Mirtazapine exhibits effect on cardiac vascular system and may cause hyponatremia (Wenzel *et al.* 2006). However in terms of individual adverse events, mirtazapine was significantly less likely to cause hypertension or tachycardia (RR: 0.51) and tremor (RR: 0.43) than tricyclic antidepressants (Watanabe *et al.* 2010). When safety of mirtazapine was monitored, sedation and malaise were the most frequent adverse events. Other serious suspected adverse events, however very rare, were abnormal liver function, syncope, bone marrow toxicity (Biswas *et al.* 2003). Higher incidence of tachycardia was only symptom present in cases of mirtazapine overdose (ingested dose median was 450 mg, it is 10× therapeutical dose) (Waring *et al.* 2007). Its wide therapeutic range and only little proved side effects are an advantage for treatment. Therapeutic dose is ranging from 15 mg to 45 mg/day, however the maximal dose as high as 120mg could be used in severely depressed individuals (Micromedex® Healthcare Series 2010). Mirtazapine concentration in individuals treated by therapeutic doses was in plasma 0.18 ± 0.22 mg/L in peripheral blood, 0.16 ± 0.17 mg/L in central blood, and 0.73 ± 0.68 mg/L in liver. Concentration in cases related to fatal poisoning was approximately 10× higher (Kirkton & Mc Intyre 2006). NOEL for rat embryos was 100 mg/kg/day and NOEL for rabbit was 40 mg/kg/day. Both were established during preclinical examination (Micromedex® Healthcare Series 2010). Embryotoxicity was not investigated in independent studies on animals. Only few case reports or small epidemiological stud-

Tab. 1. Embryotoxicity of Mirtazapine in 100% DMSO on 4ED. Dose 0.05 µg/3 µL corresponds to LD50. Body weight was in normal range. Table shows proportion (%) of dead and malformed embryos as well as proportion (%) of embryos with specific malformations. Body weight was in the normal range for age.

Dose	Number	Body weight	Dead	Malformed	CNS	Eye	Face	Body wall	Limbs	Rump	Heart	IUGR
0.03 µg/3µL	10	1489±119.87	20	60	0	0	0	75	0	0	0	0
0.05 µg/3µL	23	1517±280.66	57	39	0	13	25	38	0	50	25	0
0.1 µg/3µL	9	1635±190.92	78	11	0	0	0	0	50	0	0	0
0.15 µg/3µL	10	0	100	0	0	0	0	0	0	0	0	0

Tab. 2. Control groups were treated by Ringer solution (3 and 10µL) and distilled water as nonspecific noxa (inducing changes in volume and osmolarity of amnionic fluid), or by 100% DMSO, that was used as solvent, for evaluation of its embryotoxicity. Data are expressed as proportion (%) of embryos suffering from specific malformation. Critical period for CNS, eye and rumpless malformation are before treatment, therefore we considere them as spontaneous. Body wall defect, more frequent after treatment by 10 µL, may be a result of too increased amnion volume.

Solvent	Number	Body weight	Dead	Malformed	CNS	Eye	Face	Body wall	Limbs	Rump	Heart	IUGR
Ringer 3 µL	4	1357±201.88	0	25	25	25	25	25	0	0	25	0
Ringer 10 µL	7	1508±90.28	0	71	0	0	0	71	0	0	0	0
100%DMSO	19	1479±229.36	11	37	5	0	5	37	0	16	0	5
Dest. water	7	1590±133.98	0	66	0	0	0	66	0	50	16	0

ies were published till now. Therapeutic dose in human ranges from 15 to 45 mg perorally. Ascertained LD50 dose (0.05 µg/3 µL) in our experiment was three orders higher than the plasma level achieved during treatment in humans. In doses that were comparable with therapeutic ones there was no difference from control group as well as in doses 10× higher. However, the practically achieved dose is not easily to determine due to poor solubility of substance. Plasma level assessment would be optimal for correlation. Our results are in agreement with experimental findings on rats and rabbits, in which the risk of resorption, lower puppy weight and degrease of viability at term were demonstrated at doses 20 times higher than therapeutic ones. Revealed malformations in experimental and control embryos, i.e. body wall and face defects, were spontaneous anomalies. Unfortunately, they were relatively frequent. The reason for significantly higher proportion of body wall defects in controls could be due to spontaneous malformation during spring season. Older hens are exchanged by young ones in this period. They still have not stable quality of eggs. Malformations typical for exposure (i.e. microphtalmia and limb defects on the lower side) could result from changes in ionts concentration (published hyponatremia) or vascular disruption. Due to this fact, the futher investigation is required. We conclude, that Mirtazapine according to our preliminary results as well as published cases is relatively safe for pregnant women, however more information is needed to exclude all potentional risks.

ACKNOWLEDGEMENTS

Work was supported by Grant of Ministry of Education Czech Republic INGO LA08034/2008.

REFERENCE

1 Biswas PN, Wilton LV, Shakir SAW (2003). The pharmacovigilance of mirtazapine: results of a prescription event monitoring study on 13 554 patients in England. J Psychopharmacology. **17**: 121–126.
2 Cohen M, Panagides J, Timmer CJ, Huisman JA (1997). Pharmacokinetics of mirtazapine from orally administred tablets: influence of a high-fat meal. Eur J Drug Metab Pharmacokinet. **22**: 103–10.
3 de Boer T (1996). The pharmacologic profile of mirtazapine. J Clin Psychiatry. **57** (Suppl 4): 19–25.
4 Delbressine LP, Moonen ME, Kaspersen FM, Wagenaars GN, Jacobs PL, Timmer CJ, Paanakkker JE, van Hal HJ, Voortman G (1998). Pharmacokinetics and biotransformation of mirtazapine in human volunteers. Clin Drug Investig.**15** (1): 45–55.
5 Jelínek R., Marhan O (1994). Validation of the chick Embryotoxicity Screening Test (CHEST). A comparative study. Funct Dev Morphol. **4**: 1–5.
6 Kirkton C, Mc Intyre IM (2006). Therapeutic and toxic concentration of mirtazapine. J Anal Toxicol. **30**: 687–91.
7 Micromedex® Healthcare Series (Internet database). Greenwood Village, Colo: Thomson Reuters (Healthcare) Inc. Updated periodically
8 Peterka M, Jelínek R., Pavlík A (1992). Embryotoxicity of 25 psychotropic drugs: a study using CHEST. Reprod Toxicol. **6**: 367–374.
9 Veselý D, Veselá D, Jelínek R (1997). The bioavailability of substances administered to chick embryos: the maximum effective route of administration. ATLA. **25**: 655–665.
10 Waring WS, Good AM, Bateman DN (2007). Lack of significant toxicity after mirtazapine overdose:a five-year review of cases admitted to a regional toxicology unit. Clin Toxicol. **45**: 45–50.
11 Watanabe N, Omori IM, Nakagawa A, Cipriani A, Barbui C, McGuire H, Churchil R., Furukawa TA, MANGA (Meta-Analysis of New Generation Antidepressants) Study Group (2010). Safety reporting and adverse-event profile of mirtazapine described in randomized controlled trials in comparison with other classes of antidepressants in the acute-phase treatment of adults with depression: systematic review and meta-analysis. CNS Drugs. **24** (1): 35–53.
12 Wenzel S, Aderjan R., Mattern R, Pedal I, Skopp G (2006). Tissue distribution of mirtazapine and desmethylmirtazapine in a case of mirtazapine poisoning. Forensic Sci Int. **156**: 229–36.