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Cytogenetic and Developmental Effects of Antidepression Drug (Cipralex) on Female Mice and Embryos

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Abstract

Escitalopram (cipralex®) a new highly selective serotonin reuptake inhibitor, it is effective in the treatment of patients with major depression. To evaluate the cytogenetics and developmental effects of cipralex throughout major organgenesis, mice were administrated orally with a doses of 0.06, 0.12 and 0.24 mg/kg/day cipralex on gestation days 1-18 and examined on the 19th day of gestation for evidence of maternal and fetal toxicity. Cipralex at different doses tested produce significant toxic effects in reproductive parameters. Significant embryo fetotoxic effects were observed at tested dose levels as evidenced by total number of implantations, post. Implantation loss and embryo malformations. There were increases in the frequencies of micronuclei and chromosomal aberrations in both maternal and embryonic cells treated with cipalex, these increases were dose dependent. These results indicate that cipralex is considered to be cytogenetic and embryo toxic drug when administered during pregnancy.

Key words:- Escitalopram - chromosomal aberrations – micronuclei – embryo – mice

Introduction

Depression is a common and serious disorder, every year, depression affects 10% of adult humans over age 18, depression takes a big toll is suffering and can lead to suicidien severe cases. However, scientists do not know the exact mechanism that triggers depressive illness. In the past scientists believed that depression was the result of thoughts or emotions that were troubling for a person. More recently, experts realize that there can be several factors working together that will lead a person to become depressed. The three most important of these are biological, genetic and environmental factors (Croom and Plosker, 2004).

Biological causes are due to the changes in the chemistry of the brain, such as fluctuations in the levels of important hormones.

Genetic causes are the result of what you inherit from your parents, if one or both of parents have a depression, then it can be transmitted to sons. Environmental factors, result from stressful emotional situations, depression can also occur as a result of a combination of the three factors.

Escitalopram (Cipralex), a new highly selective serotonin reuptake inhibitor. It is effective in the treatment of patients with major depressive disorder (Croom and Plosker, 2003). Due to the risk associated with untreated depressive women (Cipralex) therapy is generally continued during pregnancy.

For Escitaloprom (Cipralex) no clinical data are available regarding exposed pregnancies. In rat reproductive toxicity studies performed with Escitalopram, embryo-toxic effects, but no increased incidence of malformations, were observed. So, in the present study we examined the cytogenetics effect of Escitaloprom on pregnant mice and embryos and the fetal developmental toxicity of cipralex given orally to mice during the pregnancy.

Materials and Methods

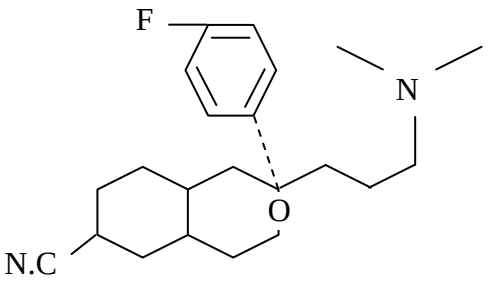
1. Test drug:

Cipralex^{R} (Escitalopram oxalate) is sparingly soluble in water slightly soluble in acetone, freely soluble in methanol, its chemical name: S(+)-1- (3-diem

ethylaminopropyl) -1-(4-fluoropheny L)-1,3-dihydros-ioben zo furan-s-carbonitrile hydrogen oxalate.

Molecular weight:

$C_{20}H_{21}FN_2O_2C_2H_2O_4$: 414-42



Animals and Treatments:

Adult fertile males and adult virgin females swiss albino mice each weighting 25 gm were used. The dose of cipralex should be administered a single oral dose of 10 mg/day, depending on individual patient response, the dose may be increased to a maximum of 20 mg daily.

Females were housed in specially designed cages with adult males by ratio 3:1. After one day of mating at 09.00 am, the females which exhibiting a vaginal plug the day of the appearance of a vaginal plug was considered as the 1 day of pregnancy. The pregnant feamels were caged individually and divided into three groups each group was injected orally from the day 1 to the day 18 of gestation with a single dose of (0.06 mg/kg/day), (0.12 mg/kg/day) and 0.24 mg/kg/day) these doses equivaleut to the therapeutic dose, 2 times and 4 times the therapeutic dose l respectively. The control group injected with similar doses of water.

Pregnant females were sacrificed by cervical dislocation on the 19th day of gestation bone marrow of the females was collected and part of embryos from each group were randomly selected to study the chromosomal aberrations and micronuclei and the other part of embryos were used to study the skeletal malformations and developmental toxicity.

Fetal developmental toxicity:

Live and dead fetuses on the day 19th of gestation were measured. Part of the live fetuses from each pregnant female were preserved in 95% ethanol for subsequent skeletal malformations after staining with Alizarins.

Micronucleus tests: (In mothers)

The animals were sacrificed by cervical dislocation on the 19th day of gestation. Bone marrow smears and staining were done following the method of (Schmid 1975). Briefly, both the femora were removed and the bone marrow was flushed with 1% sodium citrate solution (20^{o}C) from a syringe. The bone marrow cells were dispersed by gentle pipetting. The cell pellet was resnspended in a small volume of 5% fetal calf serum in PBs. A drop of this suspension was smeared in a clean slide, air-dried, fixed in absolute methanol for 15 min and stained with Giemsa stain.

In Embryos:

Micronuclei were prepared according to the method by Schmid (1976). Small amount of blood from the tail of embryos was flushed with 1% sodium citrate solution then the embryonic cells were resuspended in a small volume of 5% fetal calf serum. A drop of this suspension was smeared in a clean slide, air-dried, fixed with methanol and stained with Giemsa stain. 500 cells per each female and embryo were scored.

Chromosomal preparations:

1- Bone marrow cells of pregnant females:

Chromosomes from bone marrow cells were prepared according to the method of (Ford and Hamerton 1956). Bone marrow were collected in T.C.M. 199 culture media and colchicine was added to the tube (2ml of 0.05 colchicine). Then, the cells were incubated at 37^{o}C for 90 minutes. After centrifugation, 5 ml of hypotonic solution was added and the pellet suspented and incubated at 37^{o}C for 30 minutes. After centrifugation the cells were fixed in freshly prepared 3:1 methylalchol-glacial acetic acid then, two or three drops of cell suspension were dropped on a clean slide

covered with cold ethanol and the slides were stained with 10% Giemsa stain.

2- Embryonic cells:

Chromosomal preparations from embryonic cells were prepared according to Evans *et al*., (1972). Embryos were selected from each group and placed in 5ml of T.C.M. 199 media. 2ml of 0.05 colchicine was added, cells were incubated at 37°C for 90 minutes and centrifuged, after centrifugation 5ml of hypotonic solution of 0.56% Kcl were added to the pellet. The cells were resuspended in the hypotonic solution and incubated at 37°C for 15 min, 5ml freshly prepared fixative (3 methyl alchole: 1 glacial acetic acid were added. Two or three drops of the cell suspension were dropped to the surface of cold clean slide, after dryness, they were stained with 5% Giema stain.

50 metaphase spreads were examined from each female and embryo, Numerical (polyploidy and aneuploidy) and structural (gaps, breaks, deletion rings, end to end and endometosis) aberrations were recorded.

Statistical Analysis:

The incidences of resorption, skele-tal variation, delayed ossification of fetuses between experimental and control values were calculated non-parametrically using wilcoxon's rank sum test (Siegal, 1956).

The data of chromosomal aberrations in the females and embryos were subjected to analysis of variance (ANOVA) according to snedcor and Cochran (1990). Least significant differences were used compare between means according to Waller and Duncan (1969) at probability 5%. The data of micronucleus tests were expressed as percentage.

Results

Developmental toxicity:

Treatment with cipralex during pregnancy from day 1to day 18 of gestation induces a dose-related increase in the number of resorptions and in the number of dead embryos compared with the control.

Also, the treatment cause a dose-related reduction in the number of live embryos but the mean fetal weight is not affected by the treatment (Table 1).

In the skeletal malformation the numbers of abnormal embryos were slightly increased in the (0.06 mg) treated group and highly increased in the (0.12 and 0.24 mg) treated groups compared with the control. The major skeletal malformations are delayed ossification and congenital defects (Table 2).

Micronucleus tests:

The frequency of micronucleus also increased as a function of the dose, up to high values with treatment with (0.24 mg) Cipralex in both mothers and embryos. The distribution of micronuclei were different between mothers and embryos, in the mothers the majority of cells containing one, two and three micronuclei but in the embryos the majority of cells were containing only one and two micronuclei (Table 3).

Chromosomal aberrations:

a- (in females)

Cytogenetic examination (Table 4) showed that the groups of females treated with cipralex (0.06, 0.12 and 0.24 mg/kg/day) during pregnancy had more frequents of chromosomal aberrations (structural and numerical) than the control group and this increase was dose-related. The most frequent structural chromosomal aberra-tions were chromatid gaps, breaks, centric rings, deletions and endometosis) and the most frequent numerical aberrations were (Aneuploidy and polyploidy).

b- In embryos:

Cytogenetic examination (Table 5) showed that the groups of embryos treated with cipralex (0.06, 0.12 and 0.24 mg/kg/day) respectively had significantly increased in the total number of structural and numerical aberrations than the control group. The most frequent structural and numerical aberrations were chromatid gaps, breaks, deletions, fragments, endometosis, aneupl-oidy and polyploidy. When comparing the female groups with the embryo groups. It can be seen that the frequencies of chromosomal aberrations in females were significantly increased than the frequencies of embryonic groups.

Table (1): Effect of (cipralex) administered on days 1 to 18 of gestation on fertility and offspring development of mice

Parameters	Daily dose (mg/kg/b.w.)			
	Control	0.06	0.12	0.24
No. of females mated	25	30	30	30
No. of females pregnant	23	25	25	25
No. of implantations	230	250	200	200
No. of resorptions	5	7	10	12
%	2.2%	2.8%	5%	6%
No. of live fetuses	220	233	178	176
%	95.6%	93.2%	89%	88%
No. of dead fetuses	5	10	12	12
%	2.2%	4%	6%	6%
Mean fetal body weight	3.92±0.3	3.80±0.3	3.57±0.2	3.57±0.2

Table (2): Skeletal observations of fetuses from female mice Receiving (cipralex) on days 1-18 of gestation.

Parameters	Daily dose (mg/kg/b.w.)			
	Control	0.06	0.12	0.24
No. Of normal fetuses/ females examined	103/23	102/25	66/25	62/25
No. Of abnormal fetuses/ females examined	7	14	23	26
Fetuses with delayed ossification	4	8	10	12
Extra ribs	1	2	3	3
Wavy ribs	0	0	2	3
No. Of fetuses with congenital defects	2	4	8	5
Bifid rib	0	0	0	3

Table (3): Results of micronucleus tests in mothers and embryos after maternal oral administration with (cipralex).

Mothers	Dose mg/kg/b.w	Number of assessed PCE	Total No. of MN	No. of cells with			
				1 MN	2MN	3MN	Frequency of Micronuclei (MN)
Control	0	500	180	85	65	30	36%
Cipralex	0.06	500	210	90	80	40	42%
	0.12	500	240	110	90	40	48%
	0.24	500	265	130	95	40	53%
Embryos	Dose mg/kg/b.w	Number of assessed PCE	Total No. of MN	1 MN	2MN	Frequency of Micronuclei (MN)	
Control	0	500	150	85	65	30%	
Cipralex	0.06	500	170	90	80	34%	
	0.12	500	200	103	97	40%	
	0.24	500	225	120	105	45%	

Table (4): Effect of (Cipralex) on maternal bone marrow cells.

Dose Mg/kg/day	Structural aberration							T.S.A	Numerical aberration		
	Chromatid gaps	Chromosomal gaps	Chromatid breaks	Centric rings	Deletions	Fragments	Endmitosis		Aneu-ploidy	Poly-ploidy	T.N.A
Control	4.667^{C}	1.000	2.000^{C}	1.33	2.667^{C}	3.000^{B}	4.667	19.33^{D}	7.000^{D}	3.333^{C}	10.33^{D}
Cipralex											
0.06 mg/kg/day	6.667^{B}	1.333	4.000^{B}	1.000	4.667^{B}	4.000^{AB}	4.667	26.33^{C}	11.33^{C}	6.000^{B}	17.33^{C}
0.12 mg/kg/day	9.667^{A}	1.667	5.333^{AB}	2.333	5.000^{AB}	5.333^{A}	3.000	32.33^{B}	12.67^{B}	7.667^{A}	20.33^{B}
0.24 mg/kg/day	8.667^{A}	2.667	6.333^{A}	2.000	6.333^{A}	4.000^{AB}	5.000	35.00^{A}	15.00^{A}	8.667^{A}	23.67^{A}

Means of different letters (A, B, C, D) in the same column are significantly different. The column without letters in not significant. 50 metaphase cells were examined from each animal.

Table (5): Effect of (Cipralex) maternal treatment on embryos at 19 days of gestations.

Dose Mg/kg/day	Structural aberration							T.S.A	Numerical aberration		
	Chromatid gaps	Chromosomal gaps	Chromatid breaks	Centric rings	Deletions	Fragments	Endmitosis		Aneu-ploidy	Poly-ploidy	T.N.A
Control	5.667^{C}	1.333	0.333^{C}	0.000^{C}	3.000^{C}	1.667	3.000	15.000^{D}	6.000^{C}	1.000^{C}	7.000^{D}
Cipralex											
0.06 mg/kg/day	5.000^{C}	2.000	3.333^{B}	0.667^{C}	3.667^{BC}	2.667	2.333	19.667^{C}	9.667^{B}	4.667^{B}	14.333^{C}
0.12 mg/kg/day	7.000^{A}	1.333	4.667^{B}	2.667^{B}	5.000^{AB}	2.333	2.667	25.667^{B}	11.67^{A}	5.333^{B}	17.000^{B}
0.24 mg/kg/day	6.000^{B}	3.333	6.333^{A}	3.667^{A}	6.000^{A}	1.667	3.000	30.000^{A}	12.00^{A}	7.000^{A}	19.000^{A}

Means of different letters (A, B, C, D) in the same column are significantly different. The column without letters in not significant. 50 metaphase cells were examined from each animal.

Discussion

The present study was carried in order to evaluate the cytogenetic and develop-mental toxicity of Escitalopram (Cipralex) a new effective drug in the treatment of depressstion disorder, on the pregnant females (mothers) and on their embryos.

A number of reviews concerning the toxicity, carcinogenicity and mutagenicity of Citalopram which is similar to Escitalopram. Citalopram did not show any carcinogeneic activity in long term oral studies using mice and rats at doses up to 40mg/kg/day. In assays of genotoxic activity, Cialopram showed no evidence of mutagenic or clastogenic activity (Croom and Plosker 2004).

The present study showed that administration of a single dose of 0.06 mg/kg/day to pregnant female, caused a slight significant increase in the chromosomal aberrations in the maternal bone marrow cells and in the embryonic cells. Also caused skeletal malformations and developmental toxicity in the embryos.

However, the administration of single dose of 0.12 mg/kg/day to pregnant mice during gestation period 1 to 18 days produced a highly significant increase in the chromosomal aberrations of the maternal bone marrow cells. Chromosomal aberrations and fetal malformations were observed in embryos on day 19 of gestation. While , in the group of pregnant females that administrated with a single dose of 0.24 mg/kg during gestation, a very highly significant increases in the chromosomal aberrations of maternal and fetal cells were observed when compared with the other two groups (0.06 and 0.12 mg/kg) respectively and controls.

Also the percentages of fetuses affected by skeletal malformations and the percentages of dead fetuses were increased significantly when compared with the other groups and the control.

The percentages of live fetuses and the embryonic weight were decreased signifycantly compared with the control group.

Moreover, the comparative analysis of the frequency of micronuclei in the mice treated with (0.06, 0.12, and 0.24 mg/kg/day) respectively showed that the frequency of micronucleated cells increased significantly in the females and embryos treated groups. These increases were dose-dependent.

These results are agreement with (Bendz, 2003) who found that oral treatment of rats with Escitalopram (Cipralex) during organogenesis at maternotoxic doses led to increased post-implantation loss and reduced fetal weight.

On the other hand, negative results had reported by (Croom, and Plosker 2003) who observed that there were no peri (Postnatal effects of Escitalopram following oral dosing of pregnant rats during gestations and no increased in the incidence of malformations observed.

Also, negative results were observed by (Croom and Plosker 2004) the effects of Esictalopram can be directly predicted from Citalopram. Citalopram did not show any mutagenic or clastogenic activity when administered during pregnancy and did not affect the female fertility.

In, conclusion it was observed that Escitalopram (Cipralex) had a slight mutagenic and developmental toxic effects on the mothers and their embryos when administered (in a therapeutic dose 0.06 mg/kg/day) during the pregnancy. In a doses of 0.12 mg and 0.24 mg/kg/day Cipralex had a very significant increases of mutagenic and developmental toxic effects on the mothers and their embryos and caused increase in the incidences of fetal malformations and in the frequency of micronucleated cells, if taken during pregnancy. Therefore, Cipralex should not be used during pregnancy unless clearly necessary and only after careful consideration of the risk/benefit.

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التأثير الوراثى والنموى لدواء السيبراليكس المضاد للاكتئاب فى إناث الفئران والأجنة

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يعتبر السبيراليكس دواء جديد وفعال فى علاج الأشخاص المصابين بالاكتئاب هذا المرض المنتشر فى جميع أنحاء العالم وبنسبة كبيرة خصوصاً فى السيدات وبما أن هذا المرض يمكن أن يسبب تداعيات خطيرة إذا ترك بدون علاج لذلك وجب على السيدات الاستمرار فى أخذ الدواء حتى أثناء الحمل ولمعرفة تأثير السبيراليكس على السيدات الحوامل والأجنة خلال فترة الحمل. تم استخدام فئران مايس صغيرة إناث وذكور وبعد حدوث عملية الإخصاب تحقن بدواء السبيراليكس بجرعات مختلفة وهى (0.24، 0.12، 0.06) مجم/كجم/ فى اليوم وهذه الجرعات تمثل الجرعة المسموح بها وضعف هذه الجرعة وأربع أضعاف الجرعة المسموح بها، وتأخذ هذه الجرعات خلال فترة الحمل من اليوم الأول للحمل حتى اليوم الـ 18 وبعد ذلك تفتح الفئران فى اليوم التاسع عشر للحمل للتعرف على مدى تأثير الدواء على الأم والأجنة ونلاحظ أن الدواء فى كل الجرعات المختلفة قد سبب نقص فى عدد الأجنة الحية وزيادة فى عدد الأجنة المشوهة والميتة وأن هذا التأثير يزداد بزيادة جرعة الدواء أما من حيث التأثير الوراثى للأجنة والأمهات فأيضاً قد سبب السبيراليكس زيادة عدد التشوهات فى كرموسومات الأجنة والأمهات من حيث التشوهات العددية والتركيبة وأيضاً سبب السبيراليكس زيادة عدد الأنوية فى خلايا الآمهات والأجنة إذا ما قورنت بمجموعات الكنترول وهذه التشوهات تعتمد أيضا على الجرعة المعطاه.

ونستخلص من ذلك أن دواء سبيراليكس له تأثير وراثى سمى على الآمهات والأجنة خلال فترة الحمل لذلك يجب أن يمنع أو يُأخذ بحذر شديد وتحت أشراف طبى وبجرعات صغيرة جداً عند الضرورة.