© Wydawnictwo UR 2018
ISSN 2544-1361 (online); ISSN 2544-2406
doi: 10.15584/ejcem.2018.2.1

http://www.ejcem.ur.edu.pl/en/
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2018; 16 (2): 85–91

ORIGINAL PAPER

Anna Sęk-Mastej [1(ABCDEFGH)], Sabina Galiniak [2(DEFG)], Izabela Krawczyk-Marć [2(DFG)], Krzysztof Balawender [1(BG)], Artur Szymczak [1(CG)], Maciej Kaniewski [1(BF)], Natalia Leksa [1(CF)], Marek Biesiadecki [2(DE)], Stanisław Orkisz [1,2(F)]

Influence of Adriblastin and Bleomycin on Wistar rat mothers and fetus development

[1] Department of Human Anatomy, Chair of the Morphological Sciences, Faculty of Medicine, University of Rzeszów, Rzeszów, Poland
[2] Department of Histology and Embryology, Chair of the Morphological Sciences, Faculty of Medicine, University of Rzeszów, Rzeszów, Poland

ABSTRACT

Introduction. Gestation is a very sensitive time both to mother and child. Any substance, factor, or environmental condition disturbing homeostasis may cause congenital defects, anomalies or even death. Teratology evaluates those potential factors and their influence. Also, medicinal products used during pregnancy may be teratogenic. Adriblastin, also known as Doxorubicin, and Bleomycin are widely used cytostatic drugs in oncology.

Aim. Aim of this study was to evaluate the embryotoxic effects of Doxorubicin and Bleomycin in an animal model.

Materials and methods. Fertilised Wistar rat females were given each drug intraperitoneally between the 8th and 15th gestation day, and compared to control group receiving placebo (distilled water, 0.9% NaCl). Another group received acetyl salicylic acid, as a model, well known teratogen. Changes in mothers' weight from baseline, implantation of embryos, any discrepancies in mothers wombs and health as well as defects in fetuses were evaluated and compared. Fetus skeletons were stained by Dowson's method to visualise bone defects.

Results and conclusion. Both Adriblastin and Bleomycin were teratogenic, producing significantly more embryo absorptions, and fetal defects compared to placebo. The effects of the two cytostatics were similar to the model teratogen acetyl salicylic acid.

Keywords. pregnancy, foetus, congenital defect, teratogen

Introduction

The beginning of teratology as a science dates back to the end of the 18th and beginning of the 19th century.[1,2] Currently, it is defined as the knowledge of inherited defects in body composition developed during gestation and related to fertilisation. Its main task is to investigate causes and effects in structure and function of embryos and fetuses related to factors occurring prior to con-

Corresponding author: Anna Sęk-Mastej, e-mail: anna@mastej.pl

Participation of co-authors: A – Author of the concept and objectives of paper; B – collection of data; C – implementation of research; D – elaborate, analysis and interpretation of data; E – statistical analysis; F – preparation of a manuscript; G – working out the literature; H – obtaining funds

Received: 14.04.2018 | Accepted: 03.06.2018
Publication date: June 2018

Sęk-Mastej A, Galiniak S, Krawczyk-Marć I et al. *Influence of Adriblastin and Bleomycin on Wistar rat mothers and fetus development.* Eur J Clin Exp Med. 2018;16(2):85–91. doi: 10.15584/ejcem.2018.2.1

ception, during gestation and also after birth until early premature age.[3] A fetal defect development is defined as variation in structure and function larger in extent than those observed in standard phenotype variability specific for a given species.[3]

Teratogen, or teratogenic factor is therefore any stimulus (chemical, physical, environmental etc.), which may cause development fetal defects. Teratogenicity of chemical substances occurs when they enter the developing fetus cells or tissues and modify or damage protein synthesis at any stage of DNA translation or RNA transcription. The result of teratogenicity is either a visible malformation of the fetus/new-born, or a latent defect in physiological functioning appearing after birth, or any abnormality during pregnancy (gestation) both in mother and in offspring that leads to miscarriage.[4-6]

Specific names to defects are given based on the development phase acted upon by the teratogen(s) e.g.:

- Genopathy, when a teratogen acted on gametes (or parents) before conception, and mutations in the genes occurred, so we can also talk about chromosomes aberration,
- Blastopathy, when teratogen affected blastogenesis, in humans it is between day 1 to day 14 after conception. Usually blastopathy means total damage of structures and miscarriage.
- Embryopathy, when teratogen works during organogenesis, and congenital defects occur in organs.
- Fetopathy, when teratogen acts in late phase of pregnancy, and defects occur after birth.

In the 20th century, teratogens were preliminarily classified into groups.[1] In 1975 Miller and Yasuda grouped teratogens into:[1-3,5]

a. mechanical and physical, e.g. pressure, injury, irritation, radiation, hyperthermia,
b. biological, e.g. viral or bacterial infections,
c. chemical, e.g. drugs, pesticides, plant or fungal derived toxins, environmental chemical pollutants.

In 1962, drugs were considered as potential teratogens and since then broad, regular testing has begun.[4,6] Many antibiotics, alkaloids, non-steroidal anti-inflammatory, antimetabolics, but also some vitamins happened to be teratogenic.[5] Drugs cause about 5% of congenital defects in new-borns. Causes of the remaining defects have not been precisely identified yet.[5,6,20] Most of known teratogenic drugs easily pass the placenta and these drugs are the most teratogenic. Teratogenicity itself depends on protein binding properties, molecule size, and drug polarisation.[3] If a teratogen passes the placenta, polarised drugs are distributed mainly into intercellular space in fetuses. They are also quickly removed to the amnion and from there do not enter the cells easily. Contrary to this, lipophilic drugs penetrate the placenta and fetal tissues faster and their elimination is poorer. Also, protein binding enhances distribution to tissues and inside cells.[5,9,10]

Currently, many medicines are well established regarding their teratogenicity. Some are absolutely contraindicated during the entire pregnancy period; some are carefully allowed in advanced stages. Adriblastin and Bleomycin belong to the latter.

Aim of the study

The aim of this investigation was to evaluate the effects of Adriblastin and bleomycin administered to gravid Wistar rat females during organogenesis. Both mother and foetus drug effects were observed and evaluated. Comparison between drugs was performed with regards to prespecified parameters. Also, both drugs were evaluated as potential model teratogenic factors for future animal studies and comparators to other compounds, e.g. new candidates for drugs in pharmacotherapy.

Material and methods

The study was approved by the Bioethical Committee in Lublin, Poland. We used white Wistar rats females, at an age of 4-5 months, and weigh of 200 to 250 grams, derived from a certified breeding laboratory. The total number of females in the experiment was 125 of whom 595 fetuses were delivered. Animals were kept in natural day-night light exposure, at temperatures between 18-22 °C and 60% humidity. 5-6 females were placed in one standard plastic cage of 0.5 m^2, in accordance to conditions recommended in the literature.[11] Water supply and granulated feed "LSM" were available for animals ad libitum. The feed was made as per Polish Academy of Science recipe (Zakład Hodowli Zwierząt Laboratoryjnych). Regular sawdust was used as litter and stress avoidant conditions were assured. To reduce seasonal variations, the experiment was conducted in 2 three-months-long restricted cycles: March-June and September-November. Also, daily procedures were performed at regular time-schedules to avoid stress.[4] Virgin females were quarantined for 10-14 days after transferring from the breeding lab to our experiment premises to adapt to new settings.[12] Animals of doubtful health conditions were excluded during that accommodation phase. The oestrus in females was verified by vaginal smear, and matching 5 females with 2 males took place thereafter for one night. Vaginal smears followed next morning. If spermatozoa were found in the smear or sperm-slime head in the vagina the 1st gestation day was assumed since then.

Inseminated females were divided into experimental (active) and control groups, each consisting of 10-15 animals, and allocated to cages. The cages of every group were properly marked.

A. The first control group did not receive any chemical substances.
B. The second control group received 0.9% NaCl (saline) intraperitoneally, at 1 ml/kg b.w., once daily

C. The third control group received distilled water via the gastric sound, at 5 ml/kg b.w., once daily.

In the experimental (active treatment) groups the females received intraperitoneally:

A. Bleomycin at 20 mg/kg b.w. once daily (manufacturer Nippon Kayaku, Tokyo, Japan)
B. Adriblastin at 16 mg/kg b.w., once daily (mnufacturer Farmitalia Carlo Erba, Italy).

An additional control group was created to receive acetyl salicylic acid as a benchmark (model) teratogen.

Bleomycin and Adriblastin were diluted in 0.9% NaCl prior administration. Acetyl salicylic acid was diluted in water with Twin 80 prior to administration via gastric sound.

All drugs were administered between gestation day 8 to 15 which outlines organogenesis in rats.

Methods of examinations of mothers and fetuses

Daily life activities of females were noted during the experiment. Their weight was measured at day 1, 8, 15, and 21. Weight gain was calculated between the measurement days. At day 21, females were decapitated. Linear incision of the abdominal wall was cut to reach wombs with ovaries and upper part of vagina. Wombs were cut along its antimesomatrial margin. The followings were checked and counted: luteal corps, implantations, early and late resorptions, dead and alive fetuses. Alive foetuses were ranked in 3 level original scale:

1. R-1, foetus vivid, spontaneously active, pink skin
2. R-2, minimal spontaneous movements
3. R-3, alive, not active, limp, but reactive to touching.

Macroscopic evaluation of fetuses

Skin evaluation for any signs of bruises (hematomas), oedemas, excessive folds, hernias was carried out. Head size and shape, ear shapes, tongue size, limbs size and shapes, finger counts, and adhesions were noted. Any abnormalities found macroscopically resulted in placement of the foetus in formalin for microscopic evaluation.

Skeleton assessment was done according to Dowson's method.

Fetuses were first eviscerated, then dehydrated in 96% ethanol, and overexposed to 1% KOH. Staining with alizarin red dye followed and visual assessment took place. Any deficiencies, malformations, or anomalies were noted and compared to skeletons of control groups.

Numbers of intrauterine resorptions of fetuses were statistically compared between groups. Post implantation fetus mortality was counted, and rates of alive fetuses to all implantation nests in uterine were calculated. Those figures reflected eventual embryotoxic effects of studied drugs.

The teratogenic effect was estimated by comparing numbers and scale of defects in live-born fetuses in experimental groups versus control groups in which eventual defects were considered as spontaneous.

Statistical analysis

Arithmetical averages and standard deviations for obtained parameters were used to analyze and compare groups. Comparative tables were prepared for qualitative features e.g. skeleton defects. Analysis of variance (ANOVA) was used for quantitative parameters including doses and administration times of drugs. Kruskal-Wallis non-parametric test or interval estimation (Takey confidence interval) method were used in abnormal variable distribution. Chi-square statistic (Yates modification for small cardinality) was used for obtained variables and Fisher's exact test for multipartite tables. A P value < 0.05 was considered as statistically significant. Statistical analysis of the data was performed using the STATISTICA software package (version 12. StatSoft Inc. 2014, Tulsa, OK, USA, www.statsoft.com).

Results

In the Bleomycin group, mean weight gain in females from day 1 to 21 was 11.13 ± 4.1 g, which was only 10.5% of weight gains in the control group receiving distilled water. In the Adriblastin group, weight gain was 23.67 ± 5.1 g which was only 21.3% of control group receiving distilled water. In acetyl salicylic model teratogen group, weight gain was 11.00 ± 6.2 g, which was only 9.9% of control group receiving distilled water (Table 1 and 2).

Adriblastin and Bleomycin significantly decreased weight gains of pregnant females after day 15 and 21 as compared to control groups. The same effect was observed in acetyl salicylic acid model teratogen group (Table 3).

Post-implantation resorptions and fetus mortality rate was 57.9% in Bleomycin, and 37.2% in Adriblastin. Both were significantly higher than in control group. In both Adriblastin and Bleomycin receiving groups in part of fetuses the following defects were observed: spina bifida, meningocele, macroglossia, syndactyly of fingers IV and V in lower limbs, and hematomas (Table 3).

Skull bone evaluation in the Bleomycin group revealed lessening in the parietal bone (50% of fetuses), interparietal (28%), frontal (25%), hyoid (12.5%), and lack of 1st, 2nd, 3rd ossification spot in sternum in 25%, 18.7% and 9.4% respectively. In 6.2% of foetuses, lessening in lumbar or sacral parts of vertebral column was found.

Skull bone evaluation in the Adriblastin group revealed lessening in the parietal bone (25% of fetuses), frontal (7.5%), and a lack ossification spots in sternum in 7.5% of fetuses.

Both tested drugs became significantly teratogenic compared to control groups (Fig. 1-3).

Table 1. Weight of pregnant females in control and active treatment groups. Baseline – 1st day. *- p<0.05, ** - p<0.001 compared to Control H_2O, ^ - p<0.001 – compared to baseline.

Measurement	Administered substance at dose: mg/kg b.w.					
	Control 0	Control 0.9% NaCl	Control H_2O	Acetyl Salicylic Acid [250.0]	Bleomycin [20.0]	Adriblastin [16.0]
I day 1	233.47 ± 3.58	230.73 ± 3.69	208.3 ± 4.71	209.3 ± 6.91	186.87 ± 5.79*	215.22 ± 9.2
II day 8	257.32 ± 3.51^	252.6 ± 3.42^	244.6 ± 3.95^	223.8 ± 7.96	201.25 ± 5.29**	227.89 ± 9.1
III day 15	289.31 ± 3.57^	285.22 ± 4.36^	273.8 ± 4.0^	213.6 ± 7.7**	195.13 ± 7.34**	245.78 ± 9.33*
IV day 21	339.03 ± 3.94^	336.89 ± 3.9^	319.3 ± 6.71^	220.3 ± 10.64**	198.0 ± 13.92**	238.89 ± 11.91**

Table 2. Weight gain of females in control and active treatment groups between day 1 and 21. ANOVA was used for comparison ^ p<0.001 day 21 to baseline (day 1) * p<0.001 vs Control H_2O.

Administered substance	Dose (mg/kg b.w.)	Route of administration	Body mass (g)		Mean weight gain (g)
			1st day of gestation	21st day of gestation	
			X ± SE	X ± SE	
Control 0	-	-	233.47 ± 3.58	339.03 ± 3.94^	105.5 ± 3.14
Control H_2O	5 ml/kg	p.o	208.3 ± 4.7	319.3 ± 7.7^	111.0 ± 4.1
Control 0.9% NaCl	1 ml/kg	p.o.	230.7 ± 3.7	336.9 ± 3.9^	106 ± 3.1
Acetyl Salicylic Acid	250 mg/kg	p.o	209.3 ± 6.9	220.3 ± 10.2*	11.00 ± 6.18*
Bleomycin	20 mg/kg	i.p.	186.9 ± 5.8	198.0 ± 13.9*	11.13 ± 4.13*
Adriblastin	16 mg/kg	i.p,	215.2 ± 9.2	238.9 ± 11.9*	23.67 ± 5.14*

Table 3. Visible defects in fetus skeletons staining. Chi-square test *- p<0.01.

Finding	Administered substance											
	Control 0		Control H_2O		Control 0.9% NaCl		Acetyl Salicylic Acid		Bleomycin		Adriblastin	
	n	%	n	%	n	%	n	%	n	%	n	%
Total foetuses	121		92		115		58		48	100	64	
Stained skeletons	80	100	58	100	60	100	30	100	32		40	100
Skull												
Frontal bone loss									8	25.0*	3	7.5*
Parietal bone loss					2	3.3	10	33.33*	16	50.0*	10	25.0*
Interparietal bone loss	1	1.2					11	36.67*	9	28.13*		
Hyoid bone loss									4	12.5*		
Bone palate defect												
Sternum												
Lack of ossification points												
I												
II												
III			1	1.72					8	25.0*		
IV												
Ribs									6	18.75*	3	7.5
Shortening of ribs			1	1.72			5	16.67*	3	9.38*	2	5.0
Fusion of ribs												
Vertebral column									2	6.25*		
Vertebrae loss												
Cervical												
Thoracic							4	13.33*	1	3.12		
Lumbo-Sacral							7	23.33*	2	6.25*		

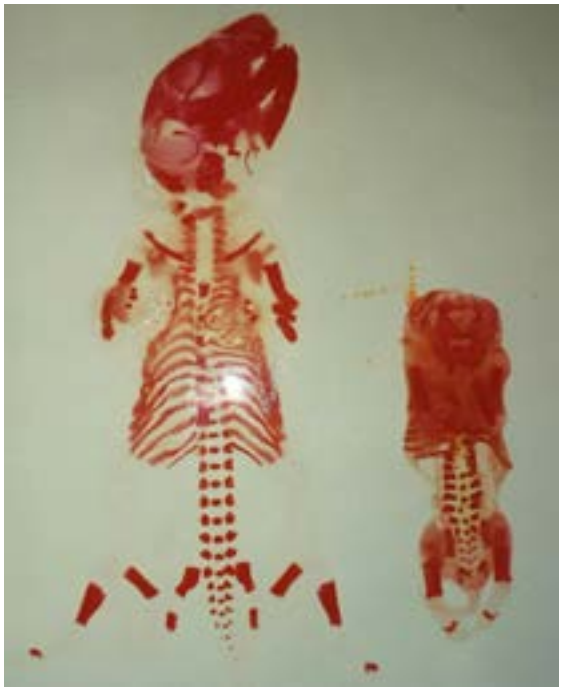

Fig. 1. *LEFT*: Normal skeleton of a foetus delivered from a mother in Control Group. *RIGHT*: Skeleton of a foetus delivered from a mother treated with Bleomycin at dose of 20 mg/kg b.w. Apparent are: ribs malformations and shortening, lack of interparietal bone, decrement of thoracic vertebral, different lengths of bodies

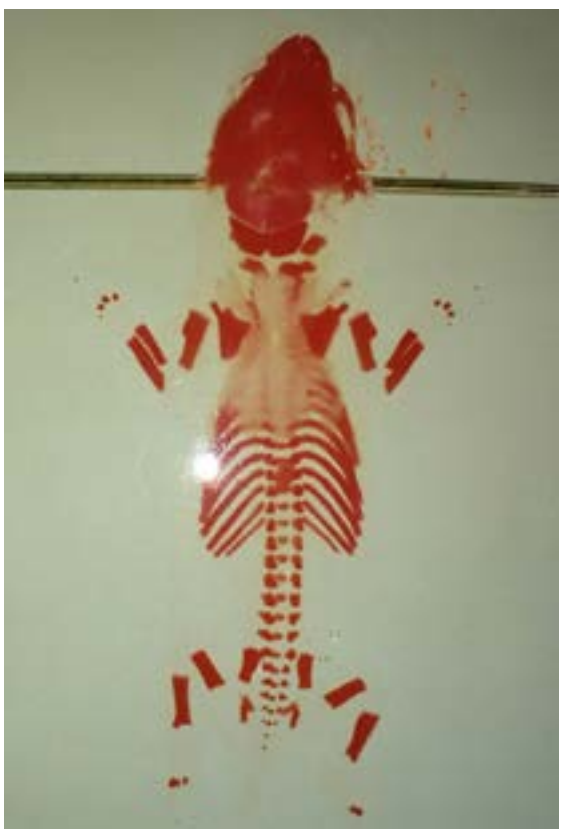

Fig. 2. Normal skeleton of a foetus delivered from a mother in the Control Group. Dowson method staining with red alizarin dye

Fig. 3. Skeleton of a foetus delivered from a mother treated with Adriblastin at dose of 16 mg/kg b.w. Apparent are: lack of 4 metacarpal bones, ribs fusion, decrement of thoracic vertebral bodies

Discussion

Teratogenic studies are part of the toxicological evaluation of all drugs in human use. Prior to any exposure in humans, especially in pregnancy, the drug or drug candidate must be evaluated in this aspect. Embryotoxic studies are recommended by WHO.[4,5] Nevertheless, it is widely agreed that we cannot exclude teratogenic effects in humans based on animal models, as no animal is identical to humans either in metabolism or placenta etc. Therefore, animal models may however support decisions in drug disapproval at early stages of drug development and help in selection of best candidates regarding safety profile. Expertise and experience in animal modelling, their reproductive processes are essential in those kinds of studies.[4,5]

Post-marketing surveillance of drugs is a worldwide standard as of today. Teratogenic effects are also collected from this source, and included in knowledge on

drug safety. A cautious assessment of both experimental animal studies and clinical (human use) drug safety data may improve the overall safety of medicinal products.[6,8,11,13,14] Embryotoxicity seems to be very specific to a given species, and not always translates to others in a simple manner.[6,15] Spontaneous anomalies occur quite frequently in mice at 0.4-18.6 rates, in pigs at 0.6-9.8%, in rabbits at 0.7-6.3%, but in rats only in 0.06-0.8%. Therefore, rats are much more reliable comparators than species mentioned earlier.[4,15]

In our experiment we focused on 3 most important features of teratologic techniques:

1. Phase specificity – meaning finding correlations between chemical substance exposure and severity of teratogenic effects
2. Drug specificity – differences between chemical substances themselves in teratogenicity
3. Dose specificity – differences in effects related to escalating dose of the same substance.

In our experiment females were exposed to drugs between the 8th and 15th day of gestation, which is the organogenesis stage in rats. We used drugs that are indicated in short, e.g. a few days, application in humans, not in chronic diseases.

425 fetal skeletal preparations by the Dowson method delivered substantial data on embryonic phase development, until 21st gestation day. In most skeletons exposed to active experimental drugs some defects were noted. Special attention should be paid to numerous defects in metacarpal bones and vertebral column, from 15.8% to 64.7%.

In our experiment, Bleomycin resulted in different defects as much as 47.9% of fetuses. In 57.9%, it caused premature deaths or blocked embryos implantation in wombs. Similar results were reported by Elis and DiPaolo for cytostatic antibiotics in animal models.[16]

Bleomycin is a cytostatic antibiotic, mixture of bleomycin type A2 and B2, produced naturally by Streptomyces verticillus strains or in chemical synthesis. It is a 6-aminoacid glycated peptide. It blocks DNA production in cancer cells not allowing thymidine to be linked in the DNA chain.[2,7,17,18] It is well absorbed and distributed in blood and quickly reaches all tissues. Unmetabolized drug is excreted by kidneys. It is widely used in treatment of melanoma, breast cancer, lung cancer, and lymphomas. It is well tolerated by patients, as compared to many other cytostatics. In higher doses, it causes pulmonary fibrosis.[12,19,20]

Adriblastin in our experiment caused 37.2% resorptions of embryos and defects in 59.4% of fetuses. The drug inhibits nucleic acid synthesis as well as some proteins, what is used in oncology and what also explains its teratogenic effects. Adriblastin interferes with mitochondria, lysosomes, and cell and organelle membrane transport. Therefore, Adriblastin presents a lot of serious and severe side effects, including vomiting, depression of bone marrow, and cardiotoxicity. The experiment females did not show visible symptoms or side effects, but over 50% of their alive fetuses at 21st day (decapitation) presented limited movements, were frail and limp.

Some evidence shows late and very late effects of Adriblastin. Mettler reported Adriblastin cardiotoxicity 4 to even 20 years after treatment in humans. In animal models, Adriblastin showed dose and time dependent cardiotoxicity in rabbits, mice, and rats.[4,20,21]

In necropsy of fetuses in our experiment we did not find macroscopic changes in hearts, but no microscopic data were available.

Conclusions

Bleomycin and Adriblastin proved to be embryotoxic to fetuses in our experiment. Bleomycin and Adriblastin influenced pregnant females, slowing and diminishing their weight gains. Both of tested substances may be used as a reference teratogenic substance to compare.

References

1. Brent RL. Utilization of animal studies to determine the effects and human risks of environmental toxicants (drug, chemicals and physical agents). *Pediatrics.* 2004;113:984-995.
2. De Santis M, Straface G, Carducci B, et al. Risc of drug--induced congenital defects. *Eur J Obst Gynec Repr Biol.* 2004;117:10-19.
3. Finell RH, Waes JGV, Eudy JD, Rosenquist TH. Molecular basis of environmentally induced birth defects. *Ann Rev Pharmacol Toxicol.* 2002;42:181-208.
4. Addis A, Sharabi S, Bonati M. Risk classification systems for drug use during pregnancy. Are they a reliable source of information? *Drug Safety.* 2000;23:245-253.
5. Felix RJ, Jones KL, Jonhnson KA, et al. Postmarceting syrveillance for drug safety in pregnancy: the organization of teratology information services project. *Birth Defects Res.* 2004;70:944-947.
6. Nemeth KA, Singh AV, Knudsen TB. Searching for biomarkers of developmnental toxicity with microarrays: Normal eye morphogenesis in rodent embryos. *Toxicol Appl Pharmacol.* 2005;206:219-228.
7. Eyre TA, Lau IJ, Mackillop L, Collins GP. Management and controversies of classical Hodgkin lymphoma in pregnancy. *Br J Haematol.* 2015;169(5):613-630.
8. Tao J, Li Q, Ma X, et al. Human placental mesenchymal stem cells of fetal origin relieves mouse pulmonary fibrosis via downregulating MyD88 and TGF-β signaling pathway. *Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi*. 2016;32(10):1347-1351.
9. Fadol AP, Lech T, Bickford C, Yusuf SW. Pregnancy in a patient with cancer and heart failure: challenges and complexities. *Adv Pract Oncol.* 2012;3(2):85-93.

10. Wang HY, Liu C, Wang Y, Zhang LL, Liu XR, Liu HL. Experimental treatment of pulmonary interstitial fibrosis with human umbilical cord blood mesenchymal stem cells. *Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi*. 2013;31(9):675-680.
11. Montemurro T, Andriolo G, Montelatici E, et al. Differentiation and migration properties of human foetal umbilical cord perivascular cells: potential for lung repair. *J Cell Mol Med.* 2011;15(4):796-808.
12. Leyder M, Laubach M, Breugelmans M, Keymolen K, De Greve J, Foulon W. Specific congenital malformations after exposure to cyclophosphamide, epirubicin, and 5-fluorouracil during the first trimester of pregnancy. *Gynecol Obstet Invest.* 2011;71(2):141-144.
13. Capeto FA, Lima FJ, Okoba W, et al. Contractile profile of esophageal and gastric fundus strips in experimental doxorubicin-induced esophageal atresia. *Braz J Med Biol Res*. 2015;48(5):458-464.
14. Murthy RK, Theriault RL, Barnett CM, et al. Outcomes of children exposed in utero to chemotherapy for breast cancer. *Breast Cancer Res.* 2014;16(6):500. doi:10.1186/s13058-014-0500-0.
15. Wilson JD. Embryotoxicity of drugs in man. Wilson JD, Frazer FC. *Hanbook of teratology*. ed. New York, USA: Plenum press 1977:309-355.
16. Elis J, DiPaolo JA. Aflatoxin B1. Induction of malformations. *Arch Pathol.* 1967;83(1):53-57.
17. Gao F, Li Q, Hou L, Li Z, Min F, Liu Z. Mesenchymal stem cell-based angiotensin-converting enzyme 2 in treatment of acute lung injury rat induced by bleomycin. *Exp Lung Res.* 2014;40(8):392-403.
18. Van Calsteren K, Verbesselt R, Beijnen J, et al. Transplacental transfer of anthracyclines, vinblastine, and 4-hydroxy-cyclophosphamide in a baboon model. *Gynecol Oncol.* 2010;119(3):594-600.
19. Faria DJ, Simões Mde J, Teixeira LC, Faria AT, Cintra ÁE, Martins JL. Effect of folic acid in a modified experimental model of anorectal malformations adriamycin-induced in rats. *Acta Cir Bras.* 2016;31(1):22-27.
20. Liu FB, Lin Q, Liu ZW. A study on the role of apoptotic human umbilical cord mesenchymal stem cells in bleomycin-induced acute lung injury in rat models. *Eur Rev Med Pharmacol Sci.* 2016;20(5):969-982.
21. Gziri MM, Pokreisz P, De Vos R, et al. Fetal rat hearts do not display acute cardiotoxicity in response to maternal Doxorubicin treatment. *J Pharmacol Exp Ther.* 2013;346(3):362-369.