IJIRR

International Journal of Information Research and Review
Vol. 03, Issue, 02, pp.1837-1840, February, 2016

Research Article

DEVELOPMENT OF TOXICOLOGICAL EFFECTS ON THE REPRODUCTIVE SYSTEM AND EXPERIMENTAL STUDY OF THE USE OF CHITOSAN AND MODIFIED CHITOSAN IN OSTEOPOROSIS

[1,*]Marziya Abdieva and [2]Saodat Asilova

[1]Scientific Researcher, MD, Assistant of the Department of Obstetrics and Gynecology, Tashkent Medical Academy, Tashkent, Uzbekistan
[2]MD, PhD, Prof., Department of the Traumatology-Orthopedics, Military Field Surgery and Neurosurgery

ARTICLE INFO

Article History:

Received 27th November, 2015
Received in revised form
19th December, 2015
Accepted 24th January, 2016
Published online 28th February 2016

Keywords:

Chitosan,
A Modified Chitosan, Osteoporosis,
Toxicity,
Teratogenic Effects,
Embryotoxicity, Rabbits.

ABSTRACT

In this study showed the first time complex of preclinical studies of new pharmaceuticals "Modified chitosan". The drug developed at the Institute of Chemistry and Physics of Polymers of Sciences Academyof Uzbekistan. Studies conducted on various types of laboratory animals of both sexes: on 36 white mice, 122 white rats, 6 Guinea pigs and 3 rabbits. Embryotoxicity and teratogenity "Modified chitosan," not revealed absolutely.

INTRODUCTION

In highly developed countries, primary osteoporosis affects 25-40% of women during theperiod of menopause. The number of osteoporotic fractures in the world increased from 1.7 million in 1990 to 6.3 million in 2050. Osteoporotic fractures trigger disability and mortality represent a significant public health problem. Fractures of the spine, femoral neck and distal radius are the most commonly associated with osteoporosis. The risk of hip fracture for 50 years old woman, during the remained life is 16%; a fracture of the radius is 15%, a spinal fracture is 32%. This problem is especially actual among people of elderly and senile age, in which the "independent life" is closely related to mental integrity, the power of movement, providing possibility of self-care and well-being in the family. Therefore, the preservation of the functional activity of the organs of the musculoskeletal system and human movement are not only medical, but also social and political task.

Corresponidng author: Marziya Abdieva,
Scientific Researcher, MD, Assistant of the Department of Obstetrics and Gynecology, Tashkent Medical Academy, Tashkent, Uzbekistan.

Purpose: to study the effect of chitosan and Modified chitosan in the treatment of post-traumatic osteoporosis and to reveal the mechanisms of action of these therapeutic factors at micromorphological level in the experiment and conducting preclinical morphological, biochemical, angiotensinogen parametric, and toxicological studies of the Modified chitosan.

MATERIALS AND METHODS

The experiment was performed in the Central Research Laboratory on the base of the Tashkent Medical Academy (TMA). For the study were taken from 25 adult rabbits breed "Chinchilla" weighing 2500-3000g. In rabbits by operating, osteotomyof the right femur was performed under intravenous anesthesia by using barbiturates and the purpose of the study osteoporosis wasan immobilization of the operated extremities. In experimental animals through 50-55 days revealed osteoporosis. White mice were divided into 6 groups of 6 animals each, the 6th group served as a control. "Modified chitosan" was introduced to white mice once intragastric at a dose of 5000, 6500, 8000, 9000 and 10000 mg/kg. Observation of the animals was carried out within 14 days.

The experiments were conducted on white outbred female rats weighing 180 - 200 g, which were divided into 3 groups: two experimental and one control. At the stage of the cycle corresponding to late pro-estrus or early estrus female rats were placed with males under 2:1 or 3:1 at the end of the day, and the next morning vaginal smear examined under low magnification microscope. If the vaginal smear contained sperm, then a day of discovery would be considered the first day of pregnancy. Pregnant females were divided 3 groups and each group consisted of 20 animals: group 1 was control. Rats of this group were injected distilled water through intragastric. 2nd and 3rd groups of rats were experienced. In1 to 19 days of pregnancy animals of these groups were injected daily with Modified chitosanat doses of 1000 and 5000 mg/kg. The examine started to receive drug within the time from 1st to the 6th day, from 6 to 16 and from 16 to 19 days of pregnancy. Pregnant females were observed daily and their general condition, behaviour, neatness and held weekly weighing of animals were supervised. On the 20th day of pregnancy, the females were killed by decapitation. As indicators of embryotoxic action was determined pre - and postimplantation mortality, morphological (anatomical) malformations, general developmental delay of fetuses in comparison with control.

RESULTS AND DISCUSSION

Endometrium is lined with a single layer of prismatic epithelium. It is well different functional and basal layers of the endothelium. There are various lengths of uterine glands, some of them extended, low cylindrical epithelium of the glands, the cytoplasm of basophile.

"Methodological guidelines for the testing of teratogenic and embryotic classical activity of new drugs" that was made in the Department of Embryology of the Institute of Experimental Medicine and guidelines for the testing of teratogenic and embryotoxic activity of new drugs". Preimplantation mortality was determined from the difference between the number of yellow bodies in ovaries and implantation sites in the uterus. Postimplantation mortality by the difference between the number of implantations and number of live fetuses.

Embryotoxic effect was evaluated in each group, was determined weight and craniocaudal fetus size. Due to the fact, deviations in the development of the fetuses may occur in a later period, some pregnant females from each group were transplanted into particular cells for natural childbirth with follow-up of the development of the offspring in the postnatal period (generation was observed for a month). The results of the study showed that daily examination of females after intragastric (i/g)injection of Modified chitosan demonstratedno significant difference in the general condition, neatness and revealed; no significant differences in gain of body masswhen it was compared with control group of pregnant female rats. The analysis of obtained results allows concluding that the fecundity of females in the experimental groups are approximately equal in comparison with the control (Table1). Statistical difference between survival and death of fetuses prior until implantation and after implantation in the studied groups of animals were not detected. Macroscopic examination of fetuses experienced rats did not reveal any differences in their appearance, body weight and craniocaudal size compared to fetuses of control group rats.

Table 1.

Indicators	Groups of animals/dose, mg/kg		
	Group 1, Control	Group 2, Modified chitosan, 1000 mg/kg	Group 3,Modified chitosan, 5000 mg/kg
The number of females in the experiment	20	20	20
Female fertility	9,05±0,54	9,5±0,76 P>0,05	9,5±0,23 P>0,05
The number of yellow bodies per 1 female	9,5±0,54	9,5±0,23 P>0,05	9,0±0,54 P>0,05
The number of dead and resorbed fetuses 1 female	0,5±0,1	0,33±0,14 P>0,05	0,6±0,14 P>0,05
Death of embrios %			
Pre-implantation	2,33±0,4	2,0±0,29 P>0,05	2,0±0,2 P>0,05
Post-implantation	3,83±0,65	3,0±0,43 P>0,05	3,16±0,58 P>0,05
Overall survival of embryos %	93,7±0,54	93,8±0,58 P>0,05	93,5±0,54 P>0,05
The mass of the embryos in g	2,55±0,07	2,62±0,07 P>0,05	2,48±0,06 P>0,05
The size of the embryos in mm	25,7±0,88	24,6±0,52 P>0,05	25,45±0,54 P>0,05

The nucleus of the extended form, occupy a large part of the cell, are painted intensively and homogeneously as possible. The mitoses are absent. Stroma is rich with cells and argyrophilic fibers. The mucous membrane passes into the submucosal layer of the muscular coat, followed by vascular and suprachoroid layers. The study of embryotoxic and teratogenic effects of Modified chitosan identified at the preclinical safety assessment of new drugs requires the solution of the question of their impact on the embryogenesis of laboratory animals. To determine the embryotoxic and teratogenic activity of Modified chitosan was guided by the

The study of fetuses' internal organs of rats'experimental groups and the skeletal system did not reveal any differences in the ossification of bones of the limbs with the introduction of the Modified chitosan in terms from 8 to 19 days of pregnancy. The influence of Modified chitosan on the course of pregnancy and intrauterine development of fetuses in rats (M±M; n =20 P). Research results of the number of edges, centers of ossification in metatarsals and metacarpal bones of the fetuses that are presented in tables 2 and 3. The results of macroscopic studies of the embryos of rats exposed to the Modified chitosan at doses of 1000 and 5000 mg/kg.

Table 2.

Indicators	Groups of animals		
	Control	Experimental group	
		5000 mg/kg	1000 мг/кг
The number of investigated embryos	72	72	72
Hemorrhage, %			
Subcutaneous	no	no	no
Facial skull	2	2	2
Into thoracic cavity	- -	1	1
In the abdominal cavity	3	3	3
Anomalies of brain development, %			
Hemorrhage	no	no	no
The expansion of the ventricles of the brain	no	no	no
Hemorrhage into the spinal cord	no	no	no

Table 3.

Indicators	Groups of animals		
	Control group	Experimental group	
		5000 мг/кг	1000 мг/кг
The numberoffetuses	50	50	50
The number of centers of ossification of the sternum	4,17±0,43	3,83±0,43 P>0,05	4,0±0,29 P>0,05
Metacarpal bones			
Right	2,72±0,06	2,72±0,06	2,62±0,06 P>0,05
On the left	2,60±0,08	2,62±0,07 P>0,05	2,77±0,04 P>0,05
The number of edges			
Right	13,0	13,0	13,0
On the left	13,0	13,0	13,0

Table 4. The study of physical development of offspring rats in early postnatal period of life

Indicators	Groups of animals		
	1-group of harried males, females	2-group males intact females	3-group control
The number of offspring	20	20	20
Otlipanie of the ear, days	2	2	2
The appearance of hair, days	5	5	5
The teething of incisors days	6	6	6
Eye opening days	12	12	12
The descent of the testes	24	24	24
The opening of the vagina day	29	29	29
	Fetus weight at 21 day, g		
21 day	29,0 ± 1,5 P>0,05	28,0 ± 1,4 P>0,05	29,0 ± 1,5

Thus, the Modified chitosan at doses of 1000 and 5000 mg/kg has no embryotoxic and teratogenic properties. Modified chitosan was injected in i/g at a dose of 1000 mg/kg body mass to males for 60 days, females for 15 days. Then the animals were paired with intact males. Control animals received saline in the same way and to the same extent. Females were placed with males being pro-estrus in the ratio 2:1 for a period of two estrous cycles. Fertilization was recorded by using vaginal smears. Replanted half the females were killed on 18^{th} day of gestation, and on the basis of the number of yellow bodies in ovaries, implantation sites in the uterus and the number of live and dead fetuses was assessed before implantation mortality of embryos. Evaluation of fertility is given by indices of fertility and mortality.

Other half of females were transplanted to natural childbirth and watched over the physical development of the offspring in early postnatal period of life. Experiments have shown that the introduction of a Modified chitosan did not affect the timing of parturition. Thus, rats was treated with drug and control, this period continued till 20-21 days. During introduction of thedrug, condition and behavior of rats did not differ from the control group. Body weight of pregnant rats,which was treated with Modified chitosan, increased on average by 46.6% and that was compared to the original. The same increase in body weight of pregnant rats was observed in the control. Reproductive function of white rats when i/pinjection of Modified chitosan at a dose of 1000 mg/kg did not differ from benchmarks.

The obtained data of the physical development of rats in early postnatal period of life were given in Table4. Medicine of Modified chitosan in the studied dose did not cause changes in reproductive functions of white rats.

Conclusion

With long-term chronic intragastric introduction of Modified chitosan has no toxic influence on the organism of laboratory animals. The drug has no toxic effect on hematological parameters, renal function and liver, as well as negative effects on the morphology of organs and tissues. In experimental models in albino rats, white mice, rabbits and Guinea pigs found that the Modified chitosan does not possess sensitizing effect, no adverse embryotoxic and teratogenic and mutagenic effect, no effect on the reproductive function of experimental animals, has no mutagenic activity.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that all experimentshave beenexamined and approved by theappropriate ethics committee and have thereforebeen performed in accordance with the ethicalstandards laid down in the 1964 Declaration ofHelsinki.

ACKNOWLEDGEMENTS

We thank junior scientific researcher BekhzodAbdullaev for excellent technical and mentalassistance. This study was supported byTashkent Medical Academy Department of Obstetrics and Gynecology for 4-5 years students; Tashkent, Republic of Uzbekistan.

COMPETING INTERESTS

Authors have declared that no competinginterests exist

REFERENCES

Asilova S. W. and Umarov G. S. 2013. Study the effect of chitosan and Modified chitosan in the process of regeneration of bone tissue in the experiment. VIII Congress of traumatologists and orthopedists of Uzbekistan, Andijan, C-121

Asilova, S. and Yugay, A. October 2013. Morphological parameters of influence of chitosan and Modified chitosan on the regeneration of bone tissue in the experiment. 34th SICOT Orthopedics World Conference, Hyderabad, India, 17-19.

Gus'kova T. A. 2008. Toxicology of medicinal substances. Moscow. - S. 27-30.

Manual pre-clinical safety testing pharmacological agents. - Tashkent – 2000

Methodical instructions on studying of embryotoxic and teratogenic effects of chemicals. Leningrad. 2002.- S. 10.

****