

Preclinical animal acute toxicity studies of new developed MRI contrast agent based on gadolinium

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Abstract. Acute toxicity test of new developed MRI contrast agent based on disodium salt of gadopentetic acid complex were carried out on *Mus musculus* and Sprague Dawley rats according to guidelines of preclinical studies [1]. Groups of six animals each were selected for experiment. Death and clinical symptoms of animals were recorded during 14 days. As a result the maximum tolerated dose (MTD) for female mice is 2.8 mM/kg of body weight, male mice – 1.4 mM/kg, female rats – 2.8 mM/kg, male rats – 5.6 mM/kg of body weight. No Observed Adverse Effect Dose (NOAEL) for female mice is 1.4 mM/kg, male mice – 0.7 mM/kg, male and female rats – 0.7 mM/kg. According to experimental data new developed MRI contrast agent based on Gd-DTPA complex is low-toxic.

1. Introduction

According to the rules governing medical products in Russian Federation the novel active pharmaceutical ingredient and all pharmaceuticals based on it are liable to toxicological studies. Toxicological studies consist of general toxicological action studies and specific toxicities. Toxicity studies involve wide range of tests in different species with regular monitoring for physiological or biometrical abnormalities observed in long-term administration of the drugs. General toxicological action of the drug determined in acute and subacute toxicity studies. The aim of acute toxicity studies is to estimate no observed adverse effect dose, maximum tolerated and lethal doses and get experimental animal mortality analysis. Acute toxicity describes the adverse effects of a substance that result either from a single exposure or from multiple exposures in a short space of time (usually less than 6 hours) during a 24 hours [2].

The aim of this research is acute toxicity studies of new developed MRI contrast agent based on Gd-DTPA complex using intravenous bolus injection to experimental mice and rats.

2. Materials and methods

2.1. Profile of Experimental Product

New developed MRI contrast agent is based on disodium salt of gadopentetic acid and used as solution for injection (0,5 mM/ml). It was produced in laboratory of “MedContrastSynthesis” LLC, Tomsk, Russia.

Predetermined therapeutic dose according to preclinical functional studies is 0,1 mM/kg of body weight.



2.2. Animals

To carry out the acute toxicity studies *Mus musculus* mice and Sprague Dawley rats [3] of both sexes were used according to guidelines of preclinical studies [1] as shown in table 1.

Table 1. Experimental animals

Animals	<i>Mus musculus</i> (mice)	<i>Rattus sp</i> (rats)
Stock	CD-1	SD (Sprague Dawley)
Sex	Male and female	Male and female
Age in the beginning of the research	8-10 weeks	3 – 3,5 month
Body weight in the beginning of the research (mean)	female 22,7 g male 32,0 g	female 253,0 g male 487,0 g

Groups of six animals each were selected for experiment (table 2). Administration volume and number of exposures were calculated using requirements [4].

Table 2 Grouping of the animals, the dose, administration volume and number of exposures

Group number	Dose	Animals	sex	administration volume, ml	number of exposures
Group № 1	5.6 mM/kg	mice	♀	2.2	5 (4x0.5 ml + 0.2 ml)
Group № 2	2.8 mM/kg	mice	♀	1.1	3 (2x0.5 ml + 0.1 ml)
Group № 3	1.4 mM/kg	mice	♀	0.5	1
Group № 4	0.7 mM/kg	mice	♀	0.25	1
Group № 5	0.3 mM/kg	mice	♀	0.12	1
Group № 6	5.6 mM/kg	mice	♂	3.2	4 (4x0.8 ml)
Group № 7	2,8 mM/kg	mice	♂	1.6	2 (2x0.8 ml)
Group № 8	1.4 mM/kg	mice	♂	0.8	1
Group № 9	0.7 mM/kg	mice	♂	0.4	1
Group № 10	0.3 mM/kg	mice	♂	0.2	1
Group № 11	11.2 mM/kg	rats	♀	6	3 (3x2 ml)
Group № 12	5.6 mM/kg	rats	♀	3	2 (2x1.5 ml)
Group № 13	2.8 mM/kg	rats	♀	1.5	1
Group № 14	1.4 mM/kg	rats	♀	0.75	1
Group № 15	0.7 mM/kg	rats	♀	0.4	1
Group № 16	11.2 mM/kg	rats	♂	11.5	4 (4x2.9 ml)
Group № 17	5.6 mM/kg	rats	♂	5.75	3 (3x 1.9 ml)
Group № 18	2.8 mM/kg	rats	♂	2.8	2 (2x1.4 ml)
Group № 19	1.4 mM/kg	rats	♂	1.4	1
Group № 20	0.7 mM/kg	rats	♂	0.7	1

The animals were feed laboratory chow and water ad libitum and maintained in an air-conditioned environment (20-22 °C, 45-65 % humidity) in a 12 h light/dark cycle. Animal studies conducted humanely [5].

2.3. Statistics

Data obtained in the experiments were expressed in terms of mean \pm SE [6]. Statistical significance of data was assessed by analysis of variance (one-way ANOVA) followed by a comparison between different groups using Dunnet's test. Differences were considered statistically significant if the p value is not greater than 0.05 [6, 7].

3. Results

3.1. Mortality and behavioral analysis

In 14-days period the behavior pattern of experimental animals was observed 1h, 5h after the administration first and then twice a day every day, registered mortality and any symptom of toxicity.

After intravenous bolus injection mortality appeared in Group №1 (mice, female) at a dose 5.6 mM/kg: 5 of 6 animals in this group died during exposure. There was no mortality in Groups № 2, 3, 4, 5 (mice, female) at 2.8 mM/kg, 1.4 mM/kg, 0.7 mM/kg and 0.3 mM/kg correspondently.

In the Group № 6 (mice, male) at 5.6 mM/kg 5 of 6 species and in Group №7 (mice, male) at 2.8 mM/kg 3 of 6 animals died. Lethality observed in the day of exposure. There is no mortality in Groups № 8, 9 and 10 (mice, male) at 1.4 mM/kg, 0.7 mM/kg and 0.3 mM/kg correspondently.

Summary of mortality data after pharmaceutical administration are in table 3. Based on these information the value of LD₅₀ were calculated and is equal to 4.19 mM/kg for female mice and 2.8 mM/kg for male mice.

Table 3. Summary of mice mortality data after administration at different doses

Dose, mM/kg	Number of animals in group	Number of died animals	LD ₁₀	LD ₁₆	LD ₅₀ ± SE (confidence interval)	LD ₈₄
Mice, female						
5.6	6	5	2.86 mM/kg	3.12 mM/kg	4.19±0.73 (3.00 – 5.86) mM/kg	5.64 mM/kg
2.8	6	0				
1.4	6	0				
0.7	6	0				
0.3	6	0				
Mice, male						
5.6	6	5	1.33 mM/kg	1.37 mM/kg	2.80±0.88 (1.58–4.94) mM/kg	5.70 mM/kg
2.8	6	3				
1.4	6	0				
0.7	6	0				
0.3	6	0				

After intravenous bolus injection all animals in Groups №11, 12 (rats, female) at 11.2 mM/kg and 5.6 mM/kg: died during administration. In Groups № 13, 14 and 15 (rats, female) at 2.8 mM/kg, 1.4 mM/kg, 0.7 mM/kg and 0.3 mM/kg no mortality were observed.

In Group № 16 after administration at a dose 11.2 mM/kg 4 of 6 (rats, male) died in the first day. In group №17 (rats, male) at 5.6 mM/kg 1 of 6 animals died due to technical reasons and was not taken into account. In Groups № 18, 19 and 20 (rats, male) at 5.6 mM/kg, 2.8 mM/kg, 1.4 mM/kg, 0.7 mM/kg and 0.3 mM/kg correspondently no mortality were observed as shown in table 4.

Calculated LD₅₀ is equal to 2.81 mM/kg for female rats and 8.85 mM/kg for male rats.

Table 4. Summary of rats mortality data after administration at different doses

Dose, mM/kg	Number of animals in group	Number of died animals	LD ₁₀	LD ₁₆	LD ₅₀ ± SE (confidence interval)	LD ₈₄
Rats, female						
11.2	6	6	2.79 mM/kg	2.79 mM/kg	2.81±0.01 (2.80 – 2.83) mM/kg	2.84 mM/kg
5.6	6	6				
2.8	6	0				
1.4	6	0				
0.7	6	0				
Rats, male						

11.2	6	4	6.19 mM/kg	6.71 mM/kg	8.85±1.57 (6.28 – 12.47) mM/kg	11.67 mM/kg
5.6	5	0				
2.8	6	0				
1.4	6	0				
0.7	6	0				

All animals were observed daily for clinical signs. The time of onset, intensity and duration of these symptoms, if any, were recorded. There are no significant changes in behavior of female mice after admission of the drugs in doses of 1.4 mM/kg, 0.7 mM/kg и 0.3 mM/kg and male mice in doses of 0.7 mM/kg and 0.3 mM/kg. The main signs of toxicity in mice are a violation of the frequency and rhythm of respiratory movements, depression, convulsions, weakening (in high doses until the disappearance) reactions to various stimuli. Mentioned symptoms occurred within the first 15 minutes after drug administration at doses: female mice - 5.6 mM/kg and 2.8 mM/kg in male mice - 5.6 mM/kg, 2.8 mM/kg and 1.4 mM/kg.

The main signs of toxicity in rats are a violation of the frequency and rhythm of respiratory movements, depression, convulsions, weakening (in high doses until the disappearance) reactions to various stimuli, increased skeletal muscle tone, pale mucous membranes, and constriction of the pupil. These symptoms were observed in males and females rats for the first 15 min after drug administration at doses of 11.2 mM/kg, 5.6 mM/kg, 2.8 mM/kg, 1.4 mM/kg and 0.7 mM/kg. Degree of manifestation of health problems, the number of animals with signs of ill health and long-term health was dose-dependent. By clinical signs of ill health it can be concluded that there are gender differences: mouse males and females rats are more sensitive to the toxic effects of the drug. Degree of manifestation of health condition, the number of animals with health deterioration symptoms and duration of expression was dose-dependent. It can be concluded that there are gender differences: mouse males and females rats are more sensitive to the toxic effects of the drug.

3.2. Body weight

The surviving mice body weight (males and females) after administration of the drug increased during research period within its physiological range.

Female rat body weight during 14 days period did not change or slightly increased within its physiological range. Male rat body weight significantly decreased in the first five days after administration, and then gradually increased, reaching the initial value at the end of the observation period.

3.3. Necropsy

If the animal died during the study time of death were determined and recorded as accurately as possible. Animals were weighed and autopsied as quick as possible. Dying animals were euthanized, weighed and autopsied. Euthanasia of dying animals were carried out after the decision of the head of the study. If it were possible, blood samples were taken for analysis of indicators of clinical pathology. Surviving experimental animals were sacrificed on the 15 day after administration except for animals which died in observed period using CO₂ inhalation technique followed by exsanguination. Necropsy of all animals was carried out and examined the body surface condition, the inner surfaces and holes, the cranial cavity, thoracic, abdominal and pelvic region with organs and tissues and neck with organs and tissues, skeleton and musculoskeletal system. All abnormalities were documented.

Examination of the dead mice (males and females administrated a dose of 5.6 mM/kg and 2.8 mM/kg of body weight) internal organs showed the hemorrhages in the diaphragm, adrenal glands, macro- and microbleeds in the lung parenchyma, subepicardial hemorrhages, hepatic congestion. The cause of death of these animals - acute respiratory and adrenal insufficiency on the background of stagnant plethora with hemorrhages in the vital organs, caused by toxic effects of the drug.

Pathological examination of mice (males and females), carried out on the 15 day after administration showed that all or most of all animals of all groups under the planned euthanasia have macro- and micro hemorrhages found in the lung parenchyma, increased blood filling in the

mesenteric and omentum. Some females also noted an increased blood supply of the fallopian tubes. Some males found hemorrhages in the diaphragm, increasing the size of the kidneys, increased blood circulation in the cortical substance.

Pathomorphological examination of the dead rats (males and females administrated a dose of 11.2 mM/kg and 5.6 mM/kg) showed hepatic congestion, hemorrhage into the diaphragm, flatulence, subepicardial hemorrhages, large hemorrhages in the lung parenchyma, petechial hemorrhages and bloody contents in the gastrointestinal tract, adrenal glands hemorrhage, bleeding in the ovaries, brain swelling, bleeding in the pia mater, microbleeds in the lungs, focal necrosis and microbleeds in the liver, congestion of mesenteric vessels, convolution smoothness and cerebellum dislocation, subdural hematoma, in one case - thymomegalia and hemorrhages in the thymus. The cause of animal death - acute respiratory and adrenal insufficiency on the background of stagnant plethora with bleeding in vital organs, caused by toxic effects of the drug.

Pathomorphological examination of rats (males and females), conducted on the 15 day after administration of the drug showed that all animals found microbleeds in the lung parenchyma, hepatic congestion, subepicardial hemorrhages; there are cases of splenomegaly, bleeding in the ovaries (female); a small number of animals with increased blood supply vessels of the mesentery and omentum, hepatomegaly, petechial hemorrhages in the intestine and ecchymosis, bleeding in the adrenal glands, extra sex glands (male), cerebral edema, and smoothness convolutions dislocation of the cerebellum, thymomegalia, congestion of the brain vessels, increased blood circulation in the kidneys, renal ischemia, renal cysts, foci of necrosis in the lungs.

Identified during necropsy examination pathologies in mice and rats are the result of minimal toxic effect of drug administered in complex with the selected method of euthanasia, the vast number of pathological findings corresponds to euthanasia using CO₂.

4. Conclusion

As a result the maximum tolerated dose (MTD) for female mice is 2.8 mM/kg of body weight, male mice – 1.4 mM/kg, female rats – 2.8 mM/kg, male rats – 5.6 mM/kg of body weight. No Observed Adverse Effect Dose (NOAEL) for female mice is 1.4 mM/kg, male mice – 0.7 mM/kg, male and female rats – 0.7 mM/kg. According to experimental data new developed MRI contrast agent based on Gd-DTPA complex is low-toxic.

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