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Toxicology Studies of a Superparamagnetic iron oxide nanoparticle in vivo

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Abstract. *Objectives:* This report presents toxicological profile available on a superparamagnetic iron oxide (SPIO) nanoparticle in vivo.
Materials and Methods: Single- and repeat-dose toxicity studies were performed with SPIO given subcutaneously in mice.
Results: The SPIO nanoparticle exhibited a low toxicity profile, with no treatment-related deaths and few transient clinical signs. SPIO was taken up and distributed in heart, spleen, liver, lung, kidney, brain decreasingly within 24 hours after injection. After repeated injections for 10days, the accumulation of iron on organs studied is slight, indicating that iron is eliminated fast at 100mg/kg given subcutaneously in mice. At histopathology, no iron-positive pigment was observed in macrophages of multiple organs (mainly heart, liver, spleen, lung, kidney, brain).
Conclusion: The results of most of the studies demonstrated low hazard potential in mice following acute injection to the SPIO nanoparticle tested in this program. All effects observed are unlikely to occur in clinical practice because of the single low dosing in humans.

Introduction

Recently, medical applications of nanotechnology have attracted growing interest. Until now, a large number of new nanotechnology-based concepts for therapeutic and diagnostic medicines have emerged, and their feasibility has been demonstrated [1]. However, the risk associated with passive compounds in the less than 100 nanometer size range present new challenges in understanding potential health risks [2].

Superparamagnetic iron oxide nanoparticles have been used for years as negative magnetic resonance imaging (MRI) contrast agents [3]. However, in vivo risk assessments of SPIO have been rarely reported until now [4,5].

Our group has synthesized a series of SPIOs. In this report, single- and repeat-dose toxicity were performed with SPIO to preliminary evaluate the toxicity of SPIO we got.

Materials and methods

Materials SPIO that we manufactured has a mean particle diameter of 30 nm. SPIO was dissolved in sterile 10 mM physiological saline, 5%D-β-glucose (pH 6.4).The solution filtered through a sterile filter, and the respective dose administered subcutaneously within 30 min after dissolution.

Animals Male KM mice (17.1–22.4 g), supplied by Shanghai SLAC laboratory animal Co.,

Ltd, were housed in a temperature- and light-controlled room (25±2°C; 40–70% relative humidity,12 h light/dark cycle), acclimatized in our laboratory for a period of at least 5 days before experiment, with free access to water and food.

AAS analysis Analyses of all samples were carried out by flame atomic absorption spectrometry (Varian 240) for Fe. The organ samples were digested in a CEM MARS microwave system using HNO_3. Following digestion, samples were filtered and made up to 25 mL using UHP water for analysis. Blank levels were found to be below detection limits in all cases.

Single-dose toxicity study in mice In the single-dose toxicity study, the mice were randomized into SPIO group and a control group, 10 in each group. In SPIO group, 10 male KM mice were given a single subcutaneous (SC) injection of SPIO at 100mg/kg. The control mice were given equal volumes of saline. All animals were examined for mortality and clinical signs once per 2 hours. All of the animals were sacrificed 24 hours after injection and received a complete necropsy. The iron contained in heart, spleen, liver, lung, kidney, brain was evaluated with AAS after pretreatment .The pathological observation was also conducted.

Repeat-dose toxicity study in mice In the 10-day repeated dose toxicity study, the mice were randomized into SPIO group with dosage of 100 mg/kg and a control group. Each consisted of 10 animals. The mice in SPIO group received a SC injection of SPIO once a day for 10 days (a total of ten injections). The control group was treated with saline. All animals were examined for mortality and clinical signs daily. The mice were sacrificed 24 hours after last injection and received a complete necropsy. A small part of organ tissues (heart, spleen, liver, lung, kidney, brain) were prepared for pathology, the rest were prepared for AAS.

Data analysis Although we can not model life system by rigorous formula, we can reflect life system state by selecting parameters from organization. We choose heart, spleen, liver, lung, kidney and brain to be feature parameters from experiments.

So feature vector $V = [Heart \quad Spleen \quad Liver \quad Lung \quad Kidney \quad Brain]$

We average the results to get feature under statistic mean.

$$\overline{V} = \frac{1}{n}\sum_i V_i$$

$\overline{V_C}$ represents control feature of mice. $\overline{V_S}$ represents single-dose feature. $\overline{V_R}$ represents repeat-dose feature. The norm of this feature vectors reflect SPIO's influence to mice.

If $\left|\overline{V_R}\right| > \left|\overline{V_S}\right|$, that means influence of SPIO is significant, it makes mice body state divergent, and the mice body state is far from its normal state.

If $\left|\overline{V_S}\right| > \left|\overline{V_R}\right|$, that means influence of SPIO is not significant, it makes mice body state convergent, and the mice body state regresses to its normal state.

Usually, we select 1-norm to comparison for its number reflecting the whole quantity of SPIO.

Results and discussion

The data (Table 1) showed that the iron contained in SPIO was incorporated into the body's iron store and distributed in heart, spleen, liver, lung, kidney and brain decreasingly. After repeated

injections for 10days, the accumulation of iron on organs studied is slight, indicating that SPIO was well tolerated in mice in our experimental conditions. We can also get the same result from the 1-Norm of each vector in the experiment.

At histopathology, no iron-positive pigment was observed in macrophages of multiple organs (mainly heart, liver, spleen, lung, kidney, brain).

Table 1 Single- and repeat-dose toxicity study of SPIO in vivo [mg/kg，n=10]

C — Control; S — Single-dose toxicity; R — Repeat-dose toxicity

	C	S	R
Heart	97.57	145.72	140.56
Spleen	69.86	132.26	124.39
Liver	59.66	104.25	99.65
Lung	58.51	86.75	86.03
Kidney	32.25	54.66	67.41
Brain	14.00	24.01	18.50
1-Norm	331.9	547.6	536.5

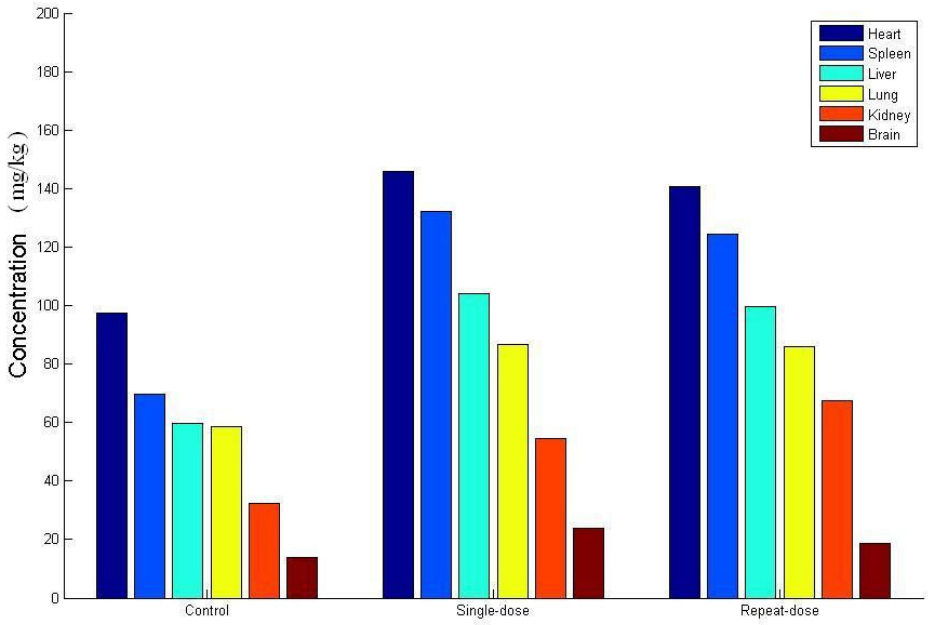

Fig. 1 Single- and repeat-dose toxicity study of SPIO in vivo

Conclusion

To summarize the findings, the results of most of the studies demonstrated low hazard potential in mice following acute injection to the SPIO nanoparticle tested in this program. All effects observed are unlikely to occur in clinical practice because of the single low dosing in humans. Future development of better animal models or in vitro approaches will depend on applying information obtained from studies on the mechanism of SPIO-human body-reaction and the identification of patient susceptibility factors.

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