academicJournals

Vol. 8(28), pp. 747-757, 29 July, 2014
DOI: 10.5897/AJPP12.1220
Article Number: 9E24C4546905
ISSN 1996-0816

http://www.academicjournals.org/AJPP

African Journal of Pharmacy and Pharmacology

Full Length Research Paper

Biocompatibility research of a novel biodegradable ion exchange resin

Hongfei Liu[1], Weisan Pan[2], Changshan Sun[2]*, Xiaomian Zou[2], Min Fu[1], Shuang Shuang Shi[1] and Hui Ding[1]

[1]College of Pharmacy, Jiangsu University, Zhenjiang, 212013, China.
[2]Department of Pharmaceutics, Shen Yang Pharmaceutical University, Shen Yang 110016, China.

Received 19 September, 2012; Accepted 6 July, 2014

The *in vivo* pharmacological and toxicological investigation of biodegradable ion exchange resin was carried out to provide evidence for further clinical utilizations. Acute toxicity study, general pharmacological studies, hemolytic experiments, systemic hypersensitivity experiments and vascular stimulation experiments were conducted. The general pharmacological effects of the biodegradable ion exchange resin on the nervous system of mice, the functional coordination of mice, the hypnosis of mice treated with nembutal at subliminal dose, the autonomic activities of tested mice, and the heart rate, blood pressure, electrocardiograph (ECG) and breathing of the anesthetic cats. The LD_{50} of biodegradable ion exchange resin to mice by tail intravenous injection was 129.37 mg kg^{-1}, and the 95% credible limit was 121.65 ~ 137.58 mg kg^{-1}. The biodegradable ion exchange resin did not have significant influence on the animals in the general pharmacological studies in the experimental conditions described in this study. Besides, the biodegradable ion exchange resin did not have hemolytic and erythrocyte aggregate effect and was qualified for allergy test under the dose condition in this experiment. Neither did it have obvious irritant effect on the blood vessels of rabbit ears. The desirable pharmacological and toxicological behaviors of the biodegradable ion exchange resin exhibited indicated that this novel formulation had great biocompatibility and had great potential for clinical utilizations.

Key words: Pharmacology, toxicology, biodegradable ion exchange resin, biocompatibility.

INTRODUCTION

For years, considerable attentions have been drawn to injectable biodegradable microspheres for their application on drug delivery systems (Okada et al., 1989; Lee et al., 2009) which stemmed from the merits including ease of application, localized delivery for a site-specific action, prolonged delivery periods, and improved patient compliance and comfort (Levy et al., 1996; Sultana et al., 2009). However, some properties of injectable biodegradable microspheres become obstacles for their future application, such as burst, incomplete or

*Corresponding author. E-mail: liuhongfei2000@163.com. Tel: 8624-23986329. Fax: 8624-23986329.

uncontrollable drug release (Kumar et al., 2001). Among all these disadvantages, initial burst release is the most major concern because it has the potential to increase side effects. Although efforts have been made to reduce the burst release (Soriano et al., 1996; Nahata and Saini, 2008), few microspheres without burst release have been reported.

Ion exchange resins are water-insoluble, cross-linked, high-molecular weight poly-electrolyte containing salt-forming groups in repeating positions on the polymer chain. Recently, they have been widely used as carriers in drug delivery systems, showing a number of improved properties, such as better stability, better taste, fewer side effects, and more uniform absorption and sustained release (Guo et al., 2009; Kouchak and Atyabi, 2010; Salve, 2011). One of the most impressive advantages of the ion exchange polymers is that theoretically the drug release from the ion exchange polymers is determined by the ion concentration in the surrounding medium. When the ion exchange polymers are introduced into the body, the body's natural counter ion concentration stabilizes drug release, hence eliminating burst release profile. So if we can combine the biodegradable polymer with the ion exchange technology to make the biodegradable and ion exchangeable microspheres, it may provide a new solution for the "burst release" problem.

In previous studies, we successfully prepared biodegradable and ion exchangeable resin and developed a drug delivery system, using ambroxol hydrochloride (AH) as a model drug (Liu et al., 2011). Briefly, we combined a biodegradable polymer, carboxymethyl chitosan and ion exchange technology to prepare the AH lung targeting microspheres. The microspheres were characterized and studied for the drug release *in vitro* in different ionic concentration dissolution mediums.

In this study, to further investigate the possibility of biodegradable ion exchange resin for clinical utilizations, we carried out pharmacological and toxicological studies. Acute toxicity study, general pharmacological studies, hemolytic experiments, systemic hypersensitivity experiment and vascular stimulation experiment were conducted. Animals including mice, cats, rabbits and guinea pigs were employed, respectively to finish these experiments. This study will provide valuable messages for the potential applications of this novel biodegradable ion exchange resin.

MATERIALS AND METHODS

Biodegradable ion exchange resin was prepared in our lab. Nembutal was purchased from Shanghai Chemical Reagent Company of China Pharmaceutical Group (Shanghai, China, batch number: F20110815). Animals were kindly provided by the Experimental Animal Center of Shenyang Pharmaceutical University (Liaoning, China), including male Kunming rats weighing 250 ± 20 g, male New Zealand white rabbits weighing 2.1 kg (license: SYXK (Liaoning) 2011-0013), male and female guinea pigs weighing 300 ~ 350 g (license: SYXK (Liaoning) 2011-0013), and male and female Kunming mice weighing 18 to 22 g (license: SCXK (Liaoning) 2011-009). Cats were purchased from the market by the Experimental Animal Center of Shenyang Pharmaceutical University. Autonomic activity tester for mice was provided by Beijing pharmaceutical institute (ZIR-2, Beijing, China). RM6240CD multi-channel bio-signal acquisition and processing system was purchased from Chengdu Instrument Factory (Sichuan, China).

Acute toxicity study of Intravenous injection

Pre-experiment: 16 mice were divided into four groups randomly with 4 in each group. According to the doses of 0.40, 0.28, 0.2, 0.14 ml/10 g, 4.5 mg L^{-1} (the largest concentration) of the ion exchange resin was administered through tail intravenous injection. The mice were observed of the toxic reaction and death. 100% estimated lethal dose (Dm) and 0% estimated lethal dose (Dn) were found out.

The effect on nervous system in mice by tail intravenous injection

The tested mice were divided into four groups at random with 10 in each group, half male and half female. 3, 6 and 12 mg kg^{-1} of the biodegradable ion exchange resin was administered by intravenous injection with 0.1 ml/10 g, and the control group was administrated with 5% glucose injection with 0.1 ml/10 g. Irwin behavior experimental method was employed to investigate the influence of the biodegradable ion exchange resin on the righting reflex, passive state, muscle twitching, salivation and nystagmus of mice before administration and at 5, 10, 30, 60 and 90 min after administration (Janice and Irwin, 1993). The righting reflex and passive state of tested mice were graded according to the standard documents, and muscle twitching, salivation and nystagmus were graded according to Table 1.

The effect on functional coordination of mice (rotating rods method)

The tested mice were screened, and the mice which could climb up for 3 min on rotating rods were qualified. The qualified mice were then divided into four groups at random, half male and half female in each group. 3, 6 and 12 mg kg^{-1} of the biodegradable ion exchange resin was administered to the three experimental groups with 0.1 ml/10 g by intravenous injection. 0.1 ml/10 g of 5% glucose injection was administered to the control group. At 30 min after administration, mice were put on rotating rods (16 r min^{-1}) to calculate the percentage of mice which fell within 1 min.

The effect on hypnosis of mice treated with nembutal at subliminal dose

80 mice were divided into four groups evenly with the same condition of sex and weight, half male and half female in each group. The control group was intravenously administered with 5% glucose injection, while the rest three groups were intravenously administered with the biodegradable ion exchange resin using the dose of 3, 6 and 12 mg kg^{-1}, respectively with 0.1 ml/10 g. After 30 min, 25 mg kg^{-1} nembutal was intraperitoneally injected to each tested mouse with 0.1 ml/10 g. If the righting reflex disappeared for 1 min, the mouse was regarded as being asleep. Each group was observed for the number of mice falling asleep at 30 min after the injection of nembutal.

Table 1. The score standard of the muscle twitching, salivation and nystagmus in mice.

Item	Degree	Score
Muscle twitching	None	0
	Lightly	4
	Strongly	8
Salivation	None	0
	Few	4
	Many	8
Nystagmus	None	0
	Lightly	4
	Strongly	8

The effect of the biodegradable ion exchange resin on the autonomic activities of tested mice

The tested mice were divided into four groups at random, half male and half female in each group. Before the determination of autonomic activity, the mice were put on the autonomic activity box, ZIR-2 (Beijing drug research institute) for 3 min to be adapted. The mice were recorded for their frequency of autonomic activity within 3 min as the index before administration. Then each experimental group was given the biodegradable ion exchange resin with doses of 3, 6 and 12 mg kg^{-1}, respectively by intravenous administration, and the control group was intravenously administered with 5% glucose injection. At 15, 30, 60, and 90 min after administration, the frequency of autonomic activities of each mouse in 3 min were determined. The frequency of autonomic activities in the experimental groups was compared with that in the control group with t-test.

The effect of the biodegradable ion exchange resin on heart rate, blood pressure, ECG and breathing of the anesthetic cats

24 cats were evenly divided into four groups with same sex and similar weight, the control group and the low, medium and high dose group, respectively. Cats were anesthetized by intraperitoneal injection of 20% urethane (1.0 g kg^{-1}) and fixed at the back. The anterior portion skin of mice was disinfected conventionally, and a central longitudinal incision with length of approximately 4 to 5 cm was made below the prominentia laryngea. The carotid artery on one side was separated, and an artery intubation was inserted and connected to RM6240 biological signal acquisition and processing system through the pressure transducer. The mean arterial pressure (MBP, mmHg) was recorded. A transverse incision was made at epigastric side with the length of approximately 4 to 5 cm and the muscle layer was incised to open the abdominal cavity. The position of duodenum was determined for administration. At the same time, ECG electrode was connected to record ECG and heart rate and processus xiphoideus was separated and connected with muscle tension transducer to synchronously record the breathing frequency and breathing depth. The administration was not conducted until each index became steady. 1, 2 and 4 $mg \cdot kg^{-1}$ of the biodegradable ion exchange resin were injected with 4 ml kg^{-1} through duodenum to jejunum. After administration we continuously observed for 90 min to respectively record changes of indexes above each cat before the administration, and 5, 15, 30, 60 and 90 min after the administration. The significance of each index between experimental group and control group was obtained by t-test of paired data.

Hemolytic experiments *in vitro*

10 ml of venous blood sample taken from rabbit ear margin was stirred for 10 min with a glass rod (top twined with absorbent cotton) to remove fiber protein, and then centrifuged (1500 rpm × 15 min). The upper clear liquid was removed and tenfold amount of 5% glucose was added to wash three times (upper clear liquid was abandoned after centrifuged) until the upper liquid showed no red color. Then 5% glucose was added to make 2% erythrocyte suspension. According to Table 2, various solutions were added into 7 test tubes. Among them, tube 6 was 5% glucose blank control and tube 7 was the positive control (distilled water). After gently shaken up, the 7 test tubes were incubated at 37°C water bath. The samples were observed for 3 h, every fifteen minutes in the first hour and every 1 h after the first hour. The result was evaluated according to the standard listed in Table 3.

The systemic hypersensitivity experiment in guinea pigs

Dose grouping

1. The low dose group of biodegradable ion exchange resin: Sensitization dose of 1 ml (0.2 mg)/time for five times (ip) and aggressive dose of 2 ml (0.4 mg)/time for one time (iv).
2. The high dose group of biodegradable ion exchange resin: Sensitization dose of 1 ml (0.4 mg)/time for five times (ip) and aggressive dose of 2 ml (0.8 mg)/time for one time (iv).
3. The positive control group of egg white injection: Sensitization dose of 1 ml/time for five times (ip) and aggressive dose of 2 ml/time for one time (iv).
4. The negative control group was 5% glucose injection with the same volume.

24 white healthy guinea pigs were randomly divided into four groups, named as the low, high dose group of biodegradable ion exchange resin, the positive control group of egg white injection and the 5% glucose negative control group, 6 in each group with half male and half female. On the next day, each guinea pig in each group was administered (ip) with the sensitization dose for continuous five times. The reaction of the guinea pigs was observed after each injection. At 10 days after the last administration, an aggressive dose was administered (iv) with 2 ml

Table 2. Application of sample in hemolytic test *in vitro.*

Application of sample (ml)	The number of the tube						
	1	2	3	4	5	6	7
2% RBC	2.5	2.5	2.5	2.5	2.5	2.5	2.5
The biodegradable ion exchange resin	0.1	0.2	0.3	0.4	0.5	0	0
5% Glu	2.4	2.3	2.2	2.1	2.0	2.5	0
Distilled water	0	0	0	0	0	0	2.5
Final concentration (mg/ml)	0.1	0.2	0.3	0.4	0.5	-	-

Table 3. The evaluation of hemolytic test result.

Degree	Sign	Phenomenon
No haemolysis	-	All of the RBC sink and the upper stratum solution is colourless and clear.
Haemolysis partly	±	The solution is red or marron, and there is a little of RBC depositing at the bottom of the tube.
Haemolysis	+	The solution is red and clear. None of the RBC deposited at the bottom of the tube.
Aggregation		The RBC is aggregating,and it didn't disperse after vibrating.

Table 4. The LD_{50} of the biodegradable ion exchange resin in mice by vein injection.

Group	Dose (mg/kg)	Number	Number of the death	Mortality (%)
1	200	10	10	100
2	180	10	9	90
3	162	10	8	80
4	146	10	8	80
5	131	10	8	80
6	118	10	5	50
7	106	10	1	10
8	95	10	0	0

of the corresponding drug or solvent. The guinea pigs were observed for 30 min to see if there were any allergic symptoms such as catching nose, standing hair, difficult breathing, spasm and shock until death. The results were comprehensively evaluated by the degree of allergic reactions, rate of appearance, dead situation and level of allergic reaction in guinea pigs.

Vascular stimulation experiment

1. The experimental group: The biodegradable ion exchange resin, 1 ml (5 mg)/kg/time × 1 time × 3 d.
2. The control group: 5% glucose injection, 1 ml/kg/time × 1 time × 3 d.

6 rabbits were divided into two groups randomly. The experimental group was administrated through left ear with 1 ml (5 mg)/kg/time. The control group was administrated through left ear with 5% glucose injection of the same volume as control. The two groups were administered continuously for 3 days with one time in one day. After 48 h from the last administration, the rabbits were sacrificed. The ears of the rabbits were cut at 1 and 5 cm under the needle spot. No swelling and maculopapular were observed by naked eyes. 10% formaldehyde was used for fixing, and paraffin slice was made dyed by HE. The vascular endothelium, subcutaneous tissue and thrombus were observed under an optical microscopy.

RESULTS AND DISCUSSION

Acute toxicity results of intravenous injection

The result showed that Dm was 180 mg kg^{-1} and Dn was 90 mg kg^{-1}. 8 groups were set up, 10 mice in each group with 5 male and 5 female. The dose distance was 1: 0.9. Rate of 200, 180, 162, 146, 131, 118, 106, and 95 mg kg^{-1} of the ion exchange resin was administered to the 8 groups, respectively with 0.2 ml/20 g to proceed acute toxicity experiment. 5 min after administration, dead mice began to appear in high dose group. The death time of mice mainly concentrated in 12 to 24 h. The toxic mice mainly behaved as reposing motionless, slow-moving, difficult breathing, restless moving before death, jumping and urinary incontinence. No obvious pathological changes were observed in the main organs by visual inspection. The experimental result could be seen in Table 4.

The LD_{50} of biodegradable ion exchange resin to mice by tail intravenous injection was 129.37 mg kg^{-1}, and the 95% credible limit was 121.65 ~ 137.58 mg kg^{-1}.

Table 5. Effect of the biodegradable ion exchange resin on the nervous system of mice.

Index	Before	After (min)				
	0	5	10	30	60	90
Righting reflex	0±0	0±0	0±0	0±0	0±0	0±0
Passive state	0±0	0±0	0±0	0±0	0±0	0±0
Muscle twitching	0±0	0±0	0±0	0±0	0±0	0±0
Salivation	0±0	0±0	0±0	0±0	0±0	0±0
Nystagmus	0±0	0±0	0±0	0±0	0±0	0±0

Table 6. Effect of 5% Glu on the nervous system of mice.

Index	Before	After (min)				
	0	5	10	30	60	90
Righting reflex	0±0	0±0	0±0	0±0	0±0	0±0
Passive state	0±0	0±0	0±0	0±0	0±0	0±0
Muscle twitching	0±0	0±0	0±0	0±0	0±0	0±0
Salivation	0±0	0±0	0±0	0±0	0±0	0±0
Nystagmus	0±0	0±0	0±0	0±0	0±0	0±0

Table 7. Effect of the biodegradable ion exchange resin on the functional coordination in mice (rotating rods method).

Group	Does (mg/kg)	Number	Rate of falling (%)
Control	-	10	0
The biodegradable ion exchange resin	3	10	0
	6	10	0
	12	10	0

P > 0.05, compared with control.

The effect on nervous system in mice by tail intravenous injection

Average scoring of each group was calculated, as shown in Tables 5 and 6. Though in the observation of righting reflex, passive state, muscle twitching, salivation and nystagmus of the biodegradable ion exchange resin and 5% glucose, the results indicated that by tail intravenous injection, biodegradable ion exchange resin did not have significant influence on nervous system and general behavior of sober mice with the dose used in this experiment.

The effect on functional coordination of mice (rotating rods method)

The result could be seen in Table 7. The result indicated that the biodegradable ion exchange resin with chosen range of dose by tail intravenous injection did not have significant influence on the functional coordination of mice by rotating rods method, showing good property of biodegradable ion exchange resin.

The effect on hypnosis of mice treated with nembutal at subliminal dose

The experimental groups and control group were compared by X^2 testing with the result shown in Table 8. Compared with the control group, the result of X^2 testing was $P > 0.05$, which indicated that the biodegradable ion exchange resin did not have obvious hypnotic effect on the mice treated with nembutal at subliminal dose.

The effect of the biodegradable ion exchange resin on the autonomic activities of tested mice

The result was shown in Table 9. The result showed that there was no significant difference in the frequency of autonomic activities before and after the administration of

Table 8. Effect of the biodegradable ion exchange resin on mice treated with nembutal at subliminal dose.

Group	Dose (mg/kg)	Number	Number of rats falling asleep	Rate of falling asleep (%)
Control	-	20	1	5
The biodegradable ion exchange resin	3	20	1	5
	6	20	0	0
	12	20	0	0

P > 0.05, compared with control.

Table 9. Effect of the biodegradable ion exchange resin on autonomic activities in mice.

Group	Dose (mg/kg)	Before	After (min)			
		0	15	30	60	90
Control	-	120.5±51.4	116.5±54.8	80.7±33.8	70.4±33.4	51.3±35.6
Biodegradable ion exchange resin	3	128.3±40.9	114.1±58.8	98.6±50.7	82.6±44.1	37.5±23.7
	6	115.7±47.1	83.0±43.8	64.5±30.8	48.6±26.5	50.0±23.0
	12	120.4±58.0	94.5±36.2	70.8±25.6	49.1±18.0	61.9±21.7

Mean ± SD, n = 10, P > 0.05, compared with control.

Table 10. Effect of the biodegradable ion exchange resin on heart rate in anesthetized cats.

Time	5% Glu	Biodegradable ion exchange resin		
	4 ml/kg	1 mg/kg	2 mg/kg	4 mg/kg
0′	175±30	194±35	206±29	208±11*
5′	171±27	196±36	204±28	211±15**
15′	176±29	206±28	219±28*	210±10*
30′	190±17	203±27	219±23*	211±13*
60′	184±15	209±25	218±18**	212±11**
90′	202±17	208±30	224±25	207±14

Mean ± SD, vices/min, n=6 ; *P<0.05, **P<0.01, compared with 5% Glu.

the biodegradable ion exchange resin, compared with the control group, indicating that the biodegradable ion exchange resin did not have the effect on the automomic activities of tested mice.

The effect of the biodegradable ion exchange resin on heart rate, blood pressure, ECG and breathing of the anesthetic cats

Results were shown in Tables 10 to 18. Mice were orally administered with 3, 6 and 12 mg kg^{-1} of the biodegradable ion exchange resin and there was no significant influence on nervous system, general behavior, function coordination and hypnotic effect of mice treated with nembutal at subliminal dose. Within the 90 min after 1, 2 and 4 mg kg^{-1} of the biodegradable ion exchange resin was injected to cat duodenum, the heart rate, blood pressure, breathing and ECG of the cats did not make significant changes in each experimental group compared with the control group, showing good biocompatibility of biodegradable ion exchange.

Hemolytic experiments *in vitro*

Hemolysis or part of hemolysis was not observed in the total three hours. All the erythocytes sank in the solution with upper colorless transparent liquid and was dispersed after mixed up. The 5% glucose control group did not show hemolytic and aggregate phenomena within three hours. The distilled water control group showed hemolysis at all time points. The result was shown in Table 19. This experiment indicated that biodegradable

Table 11. Effect of the biodegradable ion exchange resin on mean arterial blood pressure (MAP) in anesthetized cats.

Time	5% Glu	Biodegradable ion exchange resin		
	4 ml/kg	1 mg/kg	2 mg/kg	4 mg/kg
0′	31±5	29±2	27±7	27±4
5′	30±5	30±1	29±3	28±3
15′	30±5	29±2	27±3	28±3
30′	31±5	28±6	27±5	28±6
60′	29±6	28±3	27±4	28±3
90′	30±7	27±3	26±8	27±3

Mean ± SD; Kpa, n = 6. *P<0.05, **P<0.01, compared with control.

Table 12. Effect of the biodegradable ion exchange resin on breathing rate in anesthetized cats mean ± SD, vices/min, n = 6), *P<0.05,**P<0.01, compared with control.

Time	5% Glu	Biodegradable ion exchange resin		
	4 ml/kg	1 mg/kg	2 mg/kg	4 mg/kg
0′	36±14	42±15	46±21	43±18
5′	40±19	44±18	40±13	45±14
15′	40±12	44±16	44±18	44±17
30′	33±6	45±20	41±10	44±18
60′	32±8	45±19	44±14	48±19
90′	32±5	49±19	42±13	46±19

Table 13. Effect of the biodegradable ion exchange resin on Respiratory depth in anesthetized cats mean ± SD, g, n=6), P>0.05, compared with control.

Time	5% Glu	Biodegradable ion exchange resin		
	4 ml/kg	1 mg/kg	2 mg/kg	4 mg/kg
0′	7.84±3.22	7.06±2.89	8.13±3.91	7.55±1.54
5′	7.38±2.73	6.69±2.82	6.93±3.56	7.16±1.68
15′	7.34±2.67	7.09±2.70	7.51±3.75	7.21±2.05
30′	7.50±2.41	7.59±2.72	7.70±2.92	7.58±2.54
60′	8.80±2.27	7.86±2.53	7.74±3.21	7.60±2.81
90′	8.98±3.02	7.83±2.99	7.43±2.60	7.99±2.91

ion exchange resin did not have hemolytic and erythrocyte aggregate effect in the experimental conditions.

The systemic hypersensitivity experiment in guinea pigs

When the sensitization dose was administered (ip) for 5 times, the 4 groups all showed no abnormal reaction. After each administration, the guinea pigs in the low and high dose group of biodegradable ion exchange resin exhibited normal activity, ingestion and drinking as the negative control group (5% glucose injection). On the 10th day after the last administration, each group was attacked by administering (iv) an aggressive dose. In the experimental groups of low and high dose of biodegradable ion exchange resin, the guinea pigs did not have any obvious allergic reaction so the level of allergic reaction was 0. But in the positive control group (egg white injection) appeared serious and significant allergic symptoms, containing difficult breathing, twitching, urinary incontinence and then shock to death. The death time was within one minute and the death rate was 100%. The level of allergic reaction of the positive control group was the 4th grade. No abnormal reaction was observed in the negative control group of glucose injection. The experimental result indicated that the biodegradable ion exchange resin was qualified for allergy test under the

Table 14. Effect of the biodegradable ion exchange resin on P wave of electrocardiogram in anesthetized cats.

Time	5% Glu	Biodegradable ion exchange resin		
	4 ml/kg	1 mg/kg	2 mg/kg	4 mg/kg
0′	0.17±0.07	0.16±0.04	0.19±0.05	0.22±0.09
5′	0.14±0.07	0.17±0.06	0.17±0.04	0.19±0.06
15′	0.18±0.08	0.17±0.05	0.17±0.04	0.24±0.09
30′	0.21±0.10	0.18±0.06	0.20±0.05	0.23±0.09
60′	0.21±0.07	0.17±0.04	0.18±0.02	0.25±0.08
90′	0.23±0.07	0.14±0.05*	0.18±0.03	0.21±0.05

Mean ± SD, mV, n = 6. *P<0.05, compared with control.

Table 15. Effect of the biodegradable ion exchange resin on T wave of electrocardiogram in anesthetized cats.

Time	5% Glu	Biodegradable ion exchange resin		
	4 ml/kg	1 mg/kg	2 mg/kg	4 mg/kg
0′	0.45±0.15	0.28±0.13	0.29±0.11	0.38±0.15
5′	0.45±0.14	0.28±0.12	0.29±0.09	0.39±0.13
15′	0.46±0.16	0.29±0.13	0.28±0.12	0.36±0.14
30′	0.42±0.17	0.29±0.09	0.29±0.08	0.37±0.16
60′	0.40±0.15	0.25±0.10	0.29±0.07	0.42±0.18
90′	0.37±0.14	0.27±0.09	0.25±0.08	0.32±0.14

Mean ± SD, mV, n = 6. *P<0.05, compared with control.

Table 16. Effect of the biodegradable ion exchange resin on QRS wave of electrokardiogram in anesthetized cats.

Time	5% Glu	Biodegradable ion exchange resin		
	4 ml/kg	1 mg/kg	2 ml/kg	4 mg/kg
0′	29±11	24±10	23±5	27±6
5′	29±9	27±9	25±5	29±8
15′	28±8	26±9	27±7	27±6
30′	26±8	26±7	25±9	27±5
60′	29±10	27±7	25±5	26±6
90′	25±8	23±8	28±8	26±5

Mean ± SD, ms, n=6. P>0.05, compared with control.

Table 17. Effect of the biodegradable ion exchange resin on P-Rinterval of electrokardiogram in anesthetized cats.

Time	5% Glu	Biodegradable ion exchange resin		
	4 ml/kg	1 mg/kg	2 mg/kg	4 mg/kg
0′	48±6	46±3	44±3	47±8
5′	43±5	45±7	42±3	46±7
15′	47±4	45±3	43±6	46±7
30′	45±5	47±3	46±4	45±5
60′	45±6	45±6	43±7	47±8
90′	48±4	45±8	43±4	47±5

Mean ± SD, ms, n = 6. P>0.05, compared with control.

Table 18. Effect of the biodegradable ion exchange resin on Q-T interval of electrocardiogram in anesthetized cats.

Time	5% Glu	Biodegradable ion exchange resin		
	4 ml/kg	1 mg/kg	2 mg/kg	4 mg/kg
0′	237±25	231±40	216±17	213±12
5′	241±29	232±32	216±13	212±13
15′	239±25	221±28	203±14*	213±14
30′	233±17	221±29	201±12**	211±10*
60′	229±14	221±25	204±8**	211±15
90′	219±15	214±21	205±11	217±20

Mean ± SD, ms, n = 6. *P<0.05, **P<0.01, compared with control.

Table 19. The hemolytic test result of the biodegradable ion exchange resin *in vitro.*

Time (h)	Number of cuvette						
	1	2	3	4	5	6	7
0.5	-	-	-	-	-	-	+
1	-	-	-	-	-	-	+
3	-	-	-	-	-	-	+
4	-	-	-	-	-	-	+

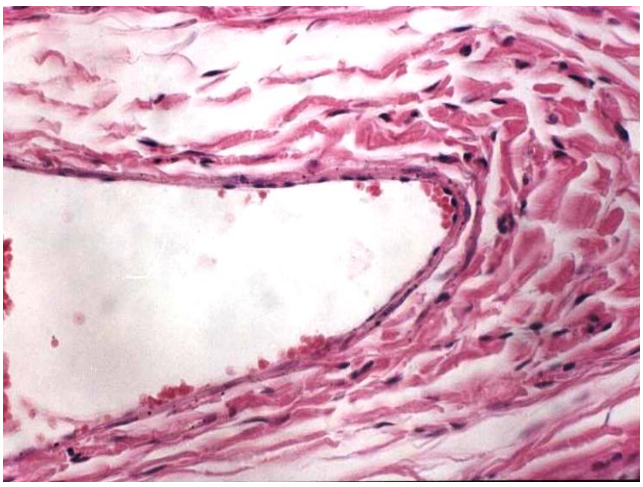

Figure 1. The pathological histology picture of the rabbit ear vein, 1 cm under the injection site of biodegradable ion exchange resin.

Vascular stimulation experiment

1. Naked eye observations: The veins in ears for injection in experimental group did not have obvious changes like congestion, exudation, edema, necrosis etc.
2. Microscopic observations: (1) The experimental group: In the 3 cases of blood vessels at 1 cm under the injection site, different amount of erythrocytes were observed. The vascular endothelium did not have swelling hyperplasia and no thrombus appeared in the vein. No infiltration and necrosis of inflammatory cells were observed in the surrounding tissues of the blood vessels. In the 3 cases at 5 cm under the injection site, the vascular endothelium did not have swelling hyperplasia and no thrombus appeared in the vein. No infiltration and necrosis of inflammatory cells were observed in the surrounding tissues of the blood vessels. (2) 5% glucose injection group: In the 3 cases at 1 cm under the injection site and the 3 cases at 5 cm under the injection site, there were no apparent pathological changes in and out of the blood vessels. Results could be seen in Figures 1 to 4. Biodegradable ion exchange resin did not have

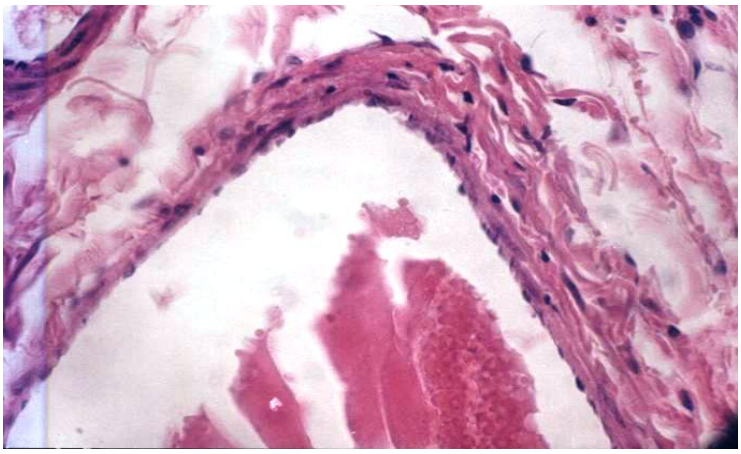

Figure 2. The pathological histology picture of the rabbit ear vein, 1 cm under the injection site of 5% glucose.

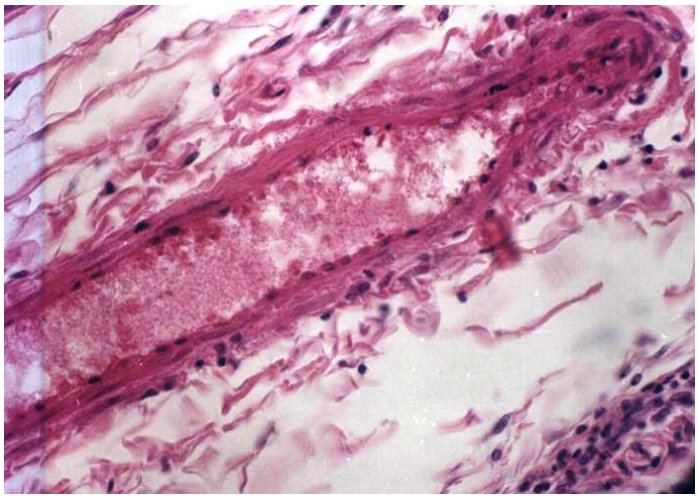

Figure 3. The pathological histology picture of the rabbit ear vein, 5 cm under the injection site of biodegradable ion exchange resin.

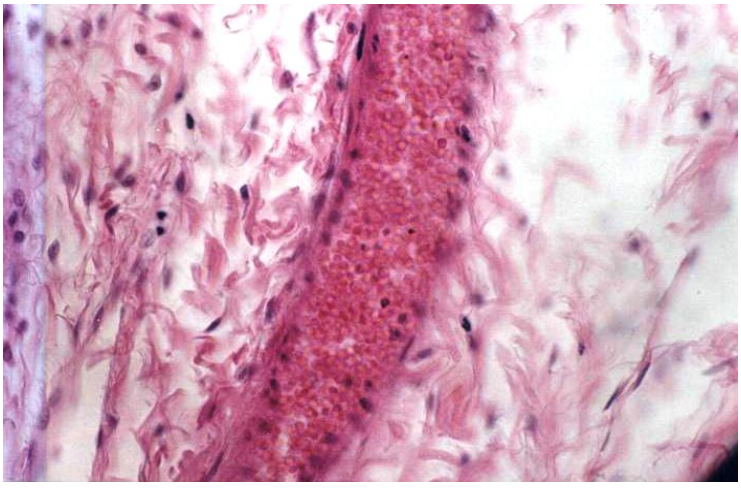

Figure 4. The pathological histology picture of the rabbit ear vein, 5 cm under the injection site of 5% glucose.

obvious irritant effect on the blood vessels of rabbit ears.

Conclusion

In the present study, *in vivo* pharmacological and toxicological research was investigated for the novel biodegradable ion exchange resin. Acute toxicity study, general pharmacological studies, hemolytic experiments, systemic hypersensitivity experiment and vascular stimulation experiment were conducted. The acute toxicity study results showed that LD_{50} of biodegradable ion exchange resin to mice by tail intravenous injection was 129.37 mg kg^{-1}. The biodegradable ion exchange resin did not have significant influence on the animals in the general pharmacological studies. Besides, the biodegradable ion exchange resin did not have hemolytic and erythrocyte aggregate effect and was qualified for allergy test under the dose condition in this experiment. Neither did it have obvious irritant effect on the blood vessels of rabbit ears. The desirable pharmacological and toxicological behaviors of the biodegradable ion exchange resin exhibited indicate that this novel formulation has great potential for clinical utilizations.

REFERENCES

Guo X, Chang RK,Hussain MA (2009). Ion-exchange resins as drug delivery carriers. J. Pharm. Sci. 98:3886-3902.

Janice ZR, Irwin LG (1993). The relationship between organizational transfer climate and positive transfer of training. Human Resourc. Dev. Qua. 4377–4390

Kouchak M, Atyabi F (2010). Ion-exchange, an approach to prepare an oral floating drug delivery system for diclofenac. Iran. J. Pharm. Res. 3:93-97.

Kumar N, Majeti NV, Ravikumar, Domb AJ (2001). Biodegradable block copolymers.Adv. Drug Deliv. Rev. 53:23-44.

Lee J, nTan CY, Lee SK, Kim YH, Lee KY (2009). Controlled delivery of heat shock protein using an injectable microsphere/hydrogel combination system for the treatment of myocardial infarction. J. Control Release 137:196-202.

Levy RJ, Labhasetwar V, Strickberger SA, Thomas U, James D (1996). Controlled release implant dosage forms for cardiac arrhythmias: Review and perspectives. Drug Deliv. 3:137-142.

Liu HF, He Y, Zhao Y,Ke P(2011). Preparation of ambroxol hydrochloride carboxymethyl chitosan micropheres without burst release. Afr. J. Pharm. Pharmacol. 5:1063-1069.

Nahata T, Saini TR (2008). D-optimal designing and optimization of long acting microsphere-based injectable formulation of aripiprazole. Drug Dev. Ind. Pharm. 34:668-675.

Okada H, Heya T, Igari Y, Ogawa Y, Toguchi H, Shimamoto T (1989). One-month release injectable microspheres of leuprolide acetate inhibit steroidogenesis and genital organ growth in rats. Int. J. Pharm. 54:231-239.

Salve P (2011). Development of sustained release beads for salbutamol sulphate using ion exchange resin. Asian J. Pharm. Tech. 1(4):104-118.

Soriano I, Delgado A, Kellaway I, Evora C (1996). Effect of surfactant agents on the in vitro release of insulin from DL-PLA microspheres. Drug Dev. Ind. Pharm. 22:1009-1012.

Sultana Y, Mall S,Maurya DP,Kumar D,Das M(2009). Preparation and in vitro characterization of diltiazem hydrochloride loaded alginate microspheres. Pharm. Dev. Technol. 14(3):321-331.