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Research Article

Preparation and Preclinical Safety Evaluation of Multivitamin Injection

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Abstract

Vitamins can maintain the enzyme activity and maintain the normal function of the humans and animals. In this study, multivitamin injection for animal use was developed (containing vitamin A, vitamin D3, vitamin E acetate, vitamin B1, riboflavin, nicotine base, vitamin B6, vitamin B12) for veterinary use, and stability and safety assessment (including hemolytic test, vascular irritation test and paw lick test) of multivitamin injection was also studied. The results showed multivitamin injection did not cause any hemolysis and blood coagulation in vitro and not cause obvious intravenous irritation. The results suggested that multivitamin injection had good safety and it can be use for veterinary use.

Introduction

As we all know, vitamins are essential for the proper physiological functions of animals . Multivitamin injection is a sterile solution for intramuscular or slow intravenous injection comprised of vitamins. It plays a very important role in maintaining the normal functions of nervous tissues and muscles .

It can supply vitamins, improve immunity, prevention of vitamin deficiencies, improve the feed conversion rate . It can enhance resistance to all kinds of stress, resistance to high intensity of stress reaction, prevent due to climate change, immunization, group, transportation stress caused by the loss of appetite, stunned, the growth and development, etc. It can regulate intestinal flora, increase appetite, gastrointestinal nerve excited is the first selection of livestock and poultry during and after illness onset rehabilitation products. It can improve livestock and poultry, cattle, sheep, pigs, chickens, weight gain, increased cow milk yield, and improve quality of breast milk. It can improve the laying rate, egg production peak, improve the quality of eggs, reduce soft shell eggs and deformity, improve the fertilization rate of livestock and poultry, reproduction rate, hatching rate and enhance the survival rate of the calves and the cub and uniformity. Although multivitamin injection plays a very important role in maintaining the normal functions of animals, it has a big challenge to develop such a formulation, the fat-soluble vitamin A, D, E in multivitamin injection is practically insoluble in water. It could not be injected directly as an aqueous solution .

Therefore, developing a new multivitamin injection preparation to overcome its insolubility is of great significance for its clinical application. In this study, veterinary multivitamin injection was prepared and the physical and chemical properties were also evaluated. Preliminary safety assessment of multivitamin injection included hemolytic test, vascular irritation test and paw lick test was also studied.

Materials and Methods

Materials

Vitamin A Palmitate,Vitamin D3,Vitamin E acetate, Thiamine Hydrochloride (Vitamin B1),Vitamin B2-5'-phosphate sodium(Calculated as riboflavin),Nicotine base,Pyridoxine Hydrochloride (Vitamin B6),Vitamin B12, Panthenol, butylated hydroxytoluene (BHA), Butyl hydroxyanisd, (BHT), Polyoxyl 40 hydrogenated castor oil (RH40), glycerin, Benzyl alcohol, EDTA-2Na, was kindly provided by HeBeiYuanZheng Pharmaceutical Co. Other reagents used in this experiment were analytical or chromatographic grade.

Preparation of multivitamin injection

Multivitamin injection was prepared as follows:

(1) The amount of the drugs and excipients were weighed first and glycerol and RH-40 were added into an appropriate vessel with constant stirring.

(2) The amounts of BHA, BHT, and benzyl alcohol were added into another container, dissolved by stirring. Then the solution was added to the solution in step (1) and mixed. The amounts of vitamin A palmitate, panthenol, vitamin E acetate were added into the above solution, dissolved by stirring. The water for injection was added to the final volume with constant stirring, after filtration through a 0.22μm filter, the prepared solution was filled into vials and sealed tightly to obtain multivitamin injection (100ml/bottle). The operation is processed under an aseptic condition with the protection from light. This product is a kind of yellow transparent or almost transparent liquid with green fluorescence.

Safety assessment

Hemolytic Test

The hemolytic test was employed as a reliable measure for determining the membrane damage caused by preparation. Rabbit blood was used to assess the hemolytic effect. Blood was obtained from the ear vein of the rabbits, and fibrin was removed by stirring with glass rod for several minutes. Then, appropriate amount of 0.9% sodium chloride solution was added to the rabbit blood. The supernatant was discarded after centrifuged at 4000 rpm for 10 minutes. Precipitated red erythrocyte cells were washed (after centrifuged) 4 to 5 times with 0.9% sodium chloride solution. Finally, a suitable amount of 0.9% sodium chloride solution was added to the red blood cells to gain 2% erythrocyte dispersion. Different amounts of multivitamin injections (0.1, 0.2, 0.3, 0.4, and 0.5 mL) were added to the test tubes together with 2.5 mL volumes of 2% e erythrocyte dispersion in the order of Table 1. Then 0.9% sodium chloride solution was added to the tubes to obtain a final volume of 5 mL. 0.9% sodium chloride solution and distilled water were used as negative and positive controls, respectively. The tube numbered 1-5 was test tube,

number 6 for the negative control and number 7 for the positive control. The tubes were placed at 37°C and observed for 4 h.

Optical microscope was used to observe appearance of solution.

Table 1: Hemolytic test design

Tube Number	1	2	3	4	5	6	7
2% red blood cell suspension (ml)	2.5	2.5	2.5	2.5	2.5	2.5	2.5
0.9% sodium chloride solution (ml)	2	2.1	2.2	2.3	2.4	2.5	
Distilled water(ml)							2.5
The test injection (ml)	0.5	0.4	0.3	0.2	0.1		
total volume (ml)	5.0	5.0	5.0	5.0	5.0	5.0	5.0

Intravenous irritation

Intravenous irritation test was assessed with rabbits. They were randomly divided to two groups: Multivitamins injection group and negative control (0.9%, normal saline) group. Before the administration, the rabbits were placed in a rabbit fixed box, and the head was fixed. Multivitamins injection was diluted with normal saline, then 0.5 mL diluted solution was injected intravenously into ear-border vein for 3 days as the experimental group, the same amount of normal saline was injected. The injection site was carefully examined. The rabbits were sacrificed 24 h after the last administration, and the ears were cut and fixed in phosphate-buffered saline containing 10% formalin for histological examination.

Licking experiment

The rats were randomly divided into two groups with 5 rats in each group. Experimental group was injected 0.2 mL of multivitamins injection to the foot pad of the right hind paw of the rat. Meantime, control group injected 0.2 mL saline solution. The onset time of paw licking, the average time of paw licking within 30min and the total time of licking were recorded.

Results and Discussion

Hemolysis test

The hemolysis test results of multivitamin injection are shown in Figure 1. It was easy to see that complete hemolysis was observed in tube of 7# (positive control group) which served as positive control, the solution was red and transparent, the bottom of the tube was clear without red blood cell residues.

The upper solution of 1#-5# tubes (experimental group) and negative control group did not showed the color of the injection, and precipitated erythrocytes were found at the bottom of 1#-5# tubes, indicating that the injection did not cause any hemolysis in vitro. These results indicated that multivitamin injection could be used for intravenous injection and did not cause hemolysis *in vitro*.

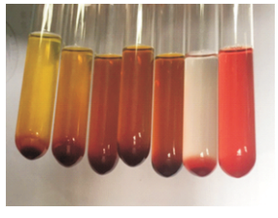

Figure 1: Result of hemolysis test for 4h (1#-7# tubes from left to right)

Intravenous irritation assessment

After administration the rabbit ear-border vein was observed by naked-eye, itwas not found the hyperemia swelling or erythema phenomenon at injection site in both experimental group and saline control group. The appearance and histopathology examination of rabbit ear vein after intravenous injection was showed in Figure 2.

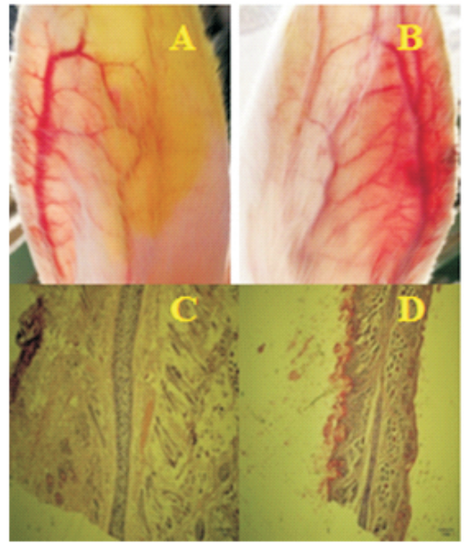

Figure 2: The appearance examination of rabbit ear vein, experimental group (A), normal saline (B) and histopathology examination of rabbit ear vein, experimental group (C), normal saline (D)

It indicated that multivitamin injection had not cause obvious irritation. There was no obvious edema and vascular congestion and fester or other irritation changes in both of experimental group and saline control group. No inflammatory phenomena were found in both groups. The results showed multivitamin injection did not cause obvious intravenous irritation and it could be used for intravenous injection.

Paw licking test

The average and total licking time within 30 minutes were shown in Table 2. It was found that the licking time of experimental group was higher than that of the control group. Multivitamin injection had certain irritation to the subcutaneous tissue because the first licking time of experimental group was earlier than that of control group, indicating that multivitamin injection can induce some irritation to the subcutaneous tissue of rats.

Table 2: Results of rat paw lick test

Group of mice	First time licking (min)		Total time licking (S)		The average time licking (S)			
	Control groups	Experimental group	Control groups	Experimental group	Contrl groups	Experimental group	Contrl groups	Experimental group
1	7.1	19.5			1	36		
2	18.3	11.1			5	72		
3	28.5	12.5	20.9	12.4	4	56	5.6	54.6
4	26.4	11.4			2	38		
5	24.1	7.5			16	71		

In this paper, polyoxyl 40 hydrogenated castor oil (RH40) was used as a solubilizer for fat soluble vitamins A, D, E, and K, essential oils and other water-insoluble drugs, RH40 occurs as a white to yellowish, semisolid paste at 20 °C that liquefies at 30 °C. It has a very faint characteristic odor and is almost tasteless in aqueous solution. The HLB value of this non-ionic solubilizer was 14-16 with melting point of about 30 °C. Solutions of RH40 in aqueous alcohols and purely aqueous solutions are also stable.

RH40 are used in a variety of parenteral pharmaceutical formulations. RH40 was demonstrated as a nontoxic and nonirritant material in acute toxicity tests in animals . However, there are reports of cardiovascular changes and nephrotoxicity in various species of animals .

Conclusion

In this study multivitamin injection was successfully prepared and the safety was investigated. Safety assessments showed that multivitamin injection did not cause any hemolysis in vitro and no obvious intravenous irritation and subcutaneous tissue irritation were found in paw licking test. In conclusion, multivitamin injection as a new drug delivery system for clinical parenteral application.

Conflicts of interest

The authors declare no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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