

Contribution of serum IL-4 and IgE to the early prediction of horse serum allergies in guinea pigs

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Background: Horse serum (HS) was a strong allergen for xenogeneic animals. However, the methods of test for allergen didn't authorized in Pharmacopeia of the United States, European Pharmacopeia, Japanese Pharmacopeia and British Pharmacopeia. Thus, new methods of test for allergen are required to control drug allergy.

Objective: To propose a new method for detecting horse serum induced allergic reactions of guinea pigs earlier.

Methods: Guinea pigs were sensitized successively by injecting different concentration of HS intravenously once a day for three times, serum level of IL-4 and total IgE were detected by method of enzyme linked immunosorbent assay (ELISA) before guinea pigs were challenged by injecting HS intravenously only once, and the results were compared with routine method of sensitization by injecting HS intraperitoneally every other day for three times.

Results: Serum level of IL-4 and total IgE increased significantly before guinea pigs were challenged, either in day 8 after intravenous sensitization (10%HS, 0.5 mL) or in day 14 and day 21 after intraperitoneal sensitization (10%HS, 0.5 mL), and allergic reactions occurred in all guinea pigs after challenged by injecting HS (10%, 1.0 mL) only once.

Conclusion: It provides a new way to predict whether HS or other drugs can provoke allergic reactions earlier by detecting the serum level of IL-4 and total IgE in d8 after intravenous sensitization, this has a good application value in drug emergency test.

Key words: IL-4; Total IgE; Horse serum; Allergic reactions; Detection

INTRODUCTION

The incidence rate of allergic reactions induced by drug injections, showed an increasing trend in recent years [1, 2]. However, the methods of test for allergic reactions were not authorized in Pharmacopeia of the United States (USP, 32nd

edition), European Pharmacopeia (EP, 7th edition), Japanese Pharmacopeia (JP, 15th edition) and British Pharmacopeia (BP, 2010 edition). The currently used method authorized in Chinese Pharmacopeia (2010 edition, ChP 2010) is test for allergen with guinea pigs, the main limitations of this method are their relative

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Received : May 4, 2012

Accepted: September 30, 2012

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long examination period (about 2-3 weeks) and more subjective assessment index (including hair-pricking, shivering, retching, etc.) [3].

Horse serum (HS) was a strong allergen for xenogeneic animals. In this study, we will propose a new method for detecting horse serum induced allergic reactions of guinea pigs earlier by enzyme-link immunosorbent assay (ELISA), try to provide a new way to forecast whether horse serum and other drugs can provoke allergic reactions, the results are also compared with the routine method of test for allergen authorized in ChP 2010.

MATERIALS AND METHODS

Horse serum (HS, lot No.090318, specification: 100 mL/bottle) was supplied by Rueite biological technology Co., Ltd (Guangzhou city of China), Albumin from chicken egg white (AFC, lot No.048K0671) was purchased from Sigma. Cerebroprotein hydrolysate injection (CHI, lot No.0807040, specification: 2 mL/ampoule) was produced by Sida Pharmaceutical Co., Ltd (Hainan province of China). Normal Saline (N.S, lot No.08070204, specification: 100 mL/bottle) was produced by Yusi Pharmaceutical Co., Ltd (Kunming city of China). The kit for determination of serum IL-4 and total IgE were purchased from Jingmei biological engineering Co., Ltd (Shenzhen city of China). Elx-808 microplate reader was purchased from Bio-tek Company of USA.

Time-effect relationship of allergic reactions provoked by 10%HS

48 guinea pigs were randomly divided into 6 groups (Blank group and challenged group in d6, d7, d8, d9, d10), 8 guinea pigs per group, half male and half female. 8 guinea pigs were used as blank group with no treatment, other 40 guinea pigs were sensitized successively by injecting HS (10%, 0.5 mL each) intravenously once a day for three times (d0, d1, d2), venous blood were collected respectively on d6, d7, d8, d9 and d10 before 8 guinea pigs in same group were challenged. After that, each guinea pig in same group was challenged once by injecting HS (10%, 1.0 mL each) intravenously, allergic reactions were checked and recorded. Serum IL-4 and total IgE (including blank group) were detected in a month by ELISA method.

Dose-response relationship of allergic reactions provoked by different concentration of HS

48 guinea pigs were randomly divided into 6 groups

(concentration of HS was 0, 1.0%, 2.5%, 5.0%, 10%, 20%), half male and half female. 8 guinea pigs in same group were sensitized successively by injecting HS (different concentration corresponds to different groups, 0.5 mL each) intravenously once a day for three times (d0, d1, d2), venous blood were collected respectively on d8 before 8 guinea pigs in same group were challenged. After that, each guinea pig in same group was challenged once by injecting corresponding concentration of HS (1.0 mL each) intravenously, allergic reactions were checked and recorded. Serum IL-4 and total IgE were detected.

Allergic reactions provoked by different drugs (intravenous sensitization)

32 guinea pigs were randomly divided into 4 groups (N.S, HS, CHI, AFC), half male and half female. HS or AFC was dissolved in N.S with final concentration of 10% or 2 mg/mL, respectively. 8 guinea pigs in one group were sensitized successively by injecting corresponding drugs (0.5 mL each) intravenously once a day for three times (d0, d1, d2), venous blood were collected respectively on d8 before 8 guinea pigs in same group were challenged. After that, each guinea pig in same group was challenged once by injecting corresponding drugs (1.0 mL each) intravenously, allergic reactions were checked and recorded. Serum IL-4 and total IgE were detected.

Allergic reactions provoked by different drugs (intraperitoneal sensitization) [3]

48 guinea pigs were randomly divided into 4 groups (N.S, HS, CHI, AFC), half male and half female. HS or AFC was dissolved in N.S with final concentration of 10% or 2 mg/mL, respectively. 12 guinea pigs of the same big group were sensitized by injecting corresponding drugs intraperitoneally (0.5 mL each) every other day for three times (d0, d2, d4), venous blood were collected respectively on d14 and d21 before 6 guinea pigs in each small group were challenged in that day. After that, each guinea pig in each small group was challenged once by injecting corresponding drugs (1.0 mL each) intravenously, allergic reactions were checked and recorded. Serum IL-4 and total IgE were detected.

Detection of serum level of IL-4 and total IgE by ELISA method

Venous blood were centrifuged (3000 r/min for 15 min), serum was isolated and transferred into another tube and stored under -30°C condition, content of serum IL-4 and total IgE were detected

in a month with ELISA method followed by the instructions manual.

Judgment of allergic reactions of guinea pigs authorized in ChP2010

Allergic reactions of guinea pigs were checked within 30 minutes after challenged by injecting the same drug intravenously. Two or more types of phenomena such as hair-pricking, shivering, retching, continuous sneezing for three times, continuous cough for three times, purpura and dyspnea; or one type of phenomena such as incontinence, instability of gait or fall down steps, twitching, shock or dying, is considered as allergic reactions induced by positive drugs.

Data processing and analyzing

Data of ELISA detection were presented as $\bar{x} \pm s$. All data in this paper were analyzed by SPSS 13.0. T test was used for comparison with two sets of data, and single factor ANOVA was used for comparison with three or more sets of data.

RESULTS

Time-effect relationship between IL-4 and total IgE with allergic reactions provoked by 10%HS

After sensitized successively by injecting HS (10%, 0.5 mL each) intravenously for three times (d0, d1, d2), no guinea pigs showed allergic reactions after challenged with HS (10%, 1.0 mL each) in d6, while 5 of 8 guinea pigs showed allergic reactions after challenged in d7, and all guinea pigs showed allergic reactions after challenged in d8, d9 and d10. The onset time of allergic reactions was at about 5 minutes after challenged with HS.

The data of contents of serum IL-4 and total IgE were showed in Table 1. Compared with blank group, contents of serum IL-4 and total IgE didn't increase in d6 group ($p > 0.05$), while increased obviously in d7 ($p < 0.05$) and d8 ($p < 0.01$), and still maintained in a high level in d9 and d10 ($p < 0.01$).

Dose-response relationship between IL-4 and total IgE with allergic reactions provoked by different concentration of HS

After sensitized successively by injecting different concentration of HS (0.5 mL each) intravenously for three times (d0, d1, d2), no guinea pigs showed allergic reactions after challenged by N.S and 2.5%HS (1.0 mL each) in d8, while 5 of 8 guinea pigs showed allergic reactions after challenged by injecting 5.0%HS, and all guinea pigs showed allergic reactions after challenged by injecting 10% or higher concentration of HS. The onset time of allergic reactions was at about 5 minutes after challenged.

The data of contents of serum IL-4 and total IgE were showed in Table 2. Compared with blank group, contents of serum IL-4 and total IgE provoked by HS didn't increase in concentration of 2.5% group ($p > 0.05$), while increased obviously in concentration of 5.0% ($p < 0.05$) and 10% or higher concentration groups ($p < 0.01$).

Relationship between IL-4 and total IgE with allergic reactions provoked by different drugs (intravenous sensitization)

After sensitized successively by injecting different drugs (0.5 mL each) intravenously for three times (d0, d1, d2), no guinea pigs showed allergic reactions in groups of N.S and CHI after challenged in d8, and all guinea pigs showed allergic reactions in groups of 10%HS and AFC (Table 3). The onset time of allergic reactions was at about 5 minutes after challenged.

The data of contents of serum IL-4 and total IgE were showed in Table 4. Compared with the N.S group, contents of serum IL-4 and total IgE increased significantly ($p < 0.01$) in d8 in groups of 10%HS and AFC, while not in CHI group ($p > 0.05$).

Relationship between IL-4 and total IgE with allergic reactions provoked by different drugs (intraperitoneal sensitization)

After sensitized by injecting different drugs intraperitoneally every other day for three times (d0, d2, d4), except groups of N.S and CHI, guinea pigs in groups of 10%HS or AFC showed allergic

Table 1. Time-effect relationship of serum level of IL-4 and total IgE provoked by 10% horse serum ($\bar{x} \pm s$, $n = 8$)

Subjects (Unit)	Contents of IL-4 and total IgE before challenged in different time					
	Blank	d6	d7	d8	d9	d10
IL-4 (ng/L)	92.4 \pm 11.8	96.2 \pm 15.8	104.1 \pm 11.4 [†]	142.5 \pm 19.2 ^{††}	202.3 \pm 32.0 ^{††}	218.5 \pm 26.7 ^{††}
Total IgE (IU/mL)	43.3 \pm 6.3	46.3 \pm 7.1	51.1 \pm 8.0 [†]	109.9 \pm 15.2 ^{††}	138.1 \pm 15.4 ^{††}	147.0 \pm 18.8 ^{††}

vs. Blank group of respective index: [†] $p < 0.05$, ^{††} $p < 0.01$.

Table 2. Dose-effect relationship of serum level of IL-4 and total IgE provoked by different concentration of horse serum in d8 ($\bar{x} \pm s$, $n = 8$)

Subjects (Unit)	Contents of IL-4 and total IgE provoked by different concentration of horse serum					
	0	1%	2.5%	5%	10%	20%
IL-4 (ng/L)	93.8 \pm 14.1	97.2 \pm 14.1	99.6 \pm 16.2	110.2 \pm 16.5 [†]	135.2 \pm 19.3 ^{††}	148.3 \pm 22.4 ^{††}
Total IgE (IU/mL)	44.1 \pm 6.3	47.2 \pm 7.9	47.8 \pm 10.1	61.3 \pm 10.1 [†]	86.2 \pm 12.3 ^{††}	101.2 \pm 16.4 ^{††}

vs. Blank group of respective index: [†] $p < 0.05$, ^{††} $p < 0.01$.

Table 3. Test for allergic reactions of guinea pigs after challenged by different drugs in d8 (method of intravenous sensitization)

Groups	Positive/negative allergic reactions of guinea pigs
Normal saline	8 negative
Horse serum	8 positive
Cerebroprotein hydrolysate injection	8 negative
Albumin from chicken egg white	8 positive

Table 4. Contents of serum level of IL-4 and total IgE provoked by different drugs before challenged in d8 (method of intravenous sensitization; $\bar{x} \pm s$, $n = 8$)

Groups	IL-4/ng/L	Total IgE/IU/mL
Normal saline	94.1 \pm 13.3	44.5 \pm 6.3
Horse serum	149.2 \pm 21.4 [*]	118.3 \pm 19.8 [*]
Cerebroprotein hydrolysate injection	93.5 \pm 10.1	45.8 \pm 6.3
Albumin from chicken egg white	168.6 \pm 29.3 [*]	128.4 \pm 20.1 [*]

vs. Normal saline group of respective index: ^{*} $p < 0.01$.

reactions after challenged by injecting 10%HS or AFC once in d14 and d21 (Table 5).

The data of contents of serum IL-4 and total IgE were showed in Table 6. Compared with N.S group, contents of serum IL-4 and total IgE in 10%HS or AFC group increased significantly in d14 and d21 ($p < 0.01$), while not in CHI group ($p > 0.05$).

DISCUSSION

Type I allergy was a dangerous type of allergic reactions induced by drugs [4]. IL-4 is a kind of cytokines produced by auxiliary T-cell, which is the most important factor for promoting production of IgE, and the role of IgE in type I allergy has been confirmed in many studies [5-8].

In this study, we proposed a new method of detecting allergic reactions of guinea pigs after sensitized successively by injecting drugs intravenously once a day for three times, and detected

the content of serum level of IL-4 and total IgE in d8 after first sensitization (before challenged by same drugs). We found the contents of IL-4 and total IgE of guinea pigs increased significantly after sensitization in d8 before challenged by injecting 10%HS intravenously, however which would be firstly detected in d14 and d21 by method of intraperitoneal sensitization (according to authorized method of ChP 2010). We speculated that the mode of intravenous sensitization could stimulate auxiliary T-cells quickly to produce IL-4 and promote the synthesizing of IgE, thus provoke allergic reactions earlier. 10% or higher concentration of HS provoked obvious allergic reactions by both methods of sensitization, the results confirmed that HS should be a strong allergen.

This study also indicated that the index of serum IL-4 and total IgE could be used for rapid detection of allergic reactions induced by 10%HS or other drugs. Compared with the method of test for allergen authorized in ChP 2010, the examination period of this proposed method was reduced by 1-2 weeks, and the index

Table 5. Test for allergic reactions after challenged by different drugs in different time (method of intraperitoneal sensitization)

Groups	Allergic reactions of guinea pigs challenged in different time	
	d14	d21
Normal saline	6 negative	6 negative
Horse serum	6 positive	6 positive
Cerebroprotein hydrolysate injection	6 negative	6 negative
Albumin from chicken egg white	6 positive	6 positive

Table 6. Content of serum IL-4 and total IgE provoked by different drugs before guinea pigs were challenged in different time (method of intraperitoneal sensitization; $\bar{x} \pm s$, n = 6)

Groups	IL-4/ng/L		Total IgE/IU/mL	
	d14	d21	d14	d21
Normal saline	94.1 \pm 10.2	93.6 \pm 10.6	44.3 \pm 5.6	43.2 \pm 6.7
Horse serum	194.7 \pm 23.8*	186.1 \pm 20.9*	137.7 \pm 18.1*	133.1 \pm 18.7*
Cerebroprotein hydrolysate injection	96.4 \pm 11.2	93.4 \pm 9.3	46.2 \pm 6.5	44.9 \pm 6.4
Albumin from chicken egg white	205.8 \pm 29.4*	193.2 \pm 24.5*	146.4 \pm 17.3*	137.4 \pm 19.2*

vs. Normal saline group of respective index: * $p < 0.01$.

of serum IL-4 and total IgE are more objective than subjective phenomena of allergic reactions in guinea pigs such as hair-pricking, coughing, sneezing, continuous coughing and so on.

It provides a new way to predict whether horse serum and other drugs can provoke allergic reactions through detecting IL-4 and total IgE earlier, and this has a good application value in drug emergency test.

ACKNOWLEDGEMENTS

This study was supported by projects of drug control system of Food and Drug Administration of Guangdong Province (NO. ZA20081208 and NO. YJ 201036).

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