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Research Article

Allergic Reactions of Guinea Pigs Induced by Several Chinese Herbal Injections

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Abstract

In order to explore the feasibility to predict allergic reactions induced by several Chinese herbal injections (CHIs) earlier, guinea pigs were sensitized by injecting different CHIs for three times, serum IL-4 and IgE were detected by method of enzyme linked immunosorbent assay (ELISA) before challenged once by injecting the same drug intravenously. The results were compared with that of routine method of intraperitoneal sensitization. We found the contents of serum IL-4 and IgE increased significantly before guinea pigs were challenged, either in eighth day (d8) after intravenous sensitization or in d14 and d21 after intraperitoneal sensitization by *Qingkailing* injection or *Xiangdan* injection. Allergic reactions of guinea pigs occurred after challenged by the same drug, while not seen in *Chuankezhi* injection. It suggests that CHIs-induced allergic reactions can be predicted earlier by detecting serum IL-4 and IgE in d8 after sensitization, the examination period is reduced by 1-2 weeks compared with the routine method. It has a good application value in drug emergency test.

Keywords: IL-4; IgE; Chinese herbal injections; Allergic reaction; Guinea pigs

Introduction

Type I allergy induced by several Chinese herbal injections (CHIs), showed an increasing trend recently [7,2,4]. This dangerous type of allergic reactions was commonly thought to be mediated by IgE antibodies [1,9,3,5]. Some studies showed that IL-4 and IL-13 were responsible for resulting in production of allergen-specific IgE antibodies [11,13,10]. Thus, anti-IgE and anti-IL-4 therapy had been used as a new strategy in allergy [6,16,15].

The criterion of test for allergen had not been employed in current Pharmacopeia of United States, European Pharmacopeia, Japanese Pharmacopeia and British Pharmacopeia. Although the current method of test for allergen with guinea pigs was employed in Chinese Pharmacopeia (Ch.P.), the main limitation lied with its long examination period (2-3 weeks) and subjective indexes of assessment (including hair-pricking, shivering, retching and so on), which limited its application in drug emergency test for allergen. It was necessary to find new methods for early prediction of allergic reactions induced by CHIs. In this study, we try to propose the biomarkers of serum IL-4 and IgE to predict allergic reactions induced by CHIs.

Materials and Methods

Drugs and reagents

Qingkailing injection (lot No.090118-6, specification: 10 ml/bottle; Jilin, China). *Xiangdan* injection (lot No.080524, specification: 10 ml/bottle; Zhejiang, China). *Chuankezhi* injection (lot No. 20071102, specification: 2.0 ml/bottle; Guangdong, China). All these CHIs above mentioned were produced according to the requirements of GMP and Ch.P.. Horse serum (lot No.090318, specification: 100 ml/bottle) was provided by Guangzhou Rueite Biological Technology Co., Ltd of China. Normal Saline (N.S, lot No.09121103, specification: 100 ml/bottle) was provided by Kunming Yusi Pharma. Co., Ltd of China, The kit for determination of serum IL-4 and total IgE were purchased from Shenzhen Jingmei Biological Engineering Co., Ltd of China. Elx-808 microplate reader was purchased from Bio-tek Company of USA.

Experimental animals

Hartley guinea pigs (qualified number is 2008A0026, half male and half female) were purchased from medical experimental animal center of Guangdong province, with an average weight of 280-320 g and similar age. The guinea pigs were bred and housed in environmentally controlled rooms with 12 h light/dark cycle and allowed free access to food and water. Experiments followed the criteria of the National Research Council for the care and use of laboratory animals in research and guidelines by the Animal Ethics Committee of Guangdong Institute for Food and Drug Control, respectively.

Determination of serum IL-4 and total IgE by ELISA method

Venous blood were centrifuged at 1200 *g* for 15 min, serum was isolated and transferred into another tube and stored under -30°C condition, contents of serum IL-4 and total IgE were detected in a month with ELISA method followed by the instructions manual.

Detection of allergic reactions provoked by CHIs (intravenous sensitization)

Forty guinea pigs were randomly divided into five groups (N.S, 10% horse serum, *Qingkailing* injection, *Xiangdan* injection, *Chuankezhi* injection), half male and half female. Horse serum was dissolved in N.S with final concentration of 10% (v/v), other CHIs were applied respectively without dilution. Eight 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 at the same time on d8

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before eight guinea pigs in same group were challenged. After that, each guinea pig was challenged once by injecting the same drug (1.0 ml each) intravenously; allergic reactions were checked and recorded. Serum IL-4 and total IgE were detected.

Detection of allergic reactions induced by CHIs (intraperitoneal sensitization)

Ninety guinea pigs were randomly divided into five groups (N.S, 10% horse serum, *Qingkailing* injection, *Xiangdan* injection, *Chuankezhi* injection), half male and half female. Horse serum was dissolved in N.S with final concentration of 10% (v/v), other CHIs were applied respectively without dilution. Eighteen 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 at the same time on d8, d14 and d21 before six guinea pigs in each small group were challenged in that day. After that, six guinea pigs in each small group were challenged once by injecting the same drug (1.0 ml each) intravenously; allergic reactions were checked and recorded. Serum IL-4 and total IgE were detected.

Data processing and analyzing

Data of ELISA detection were presented as mean ± S.D. All data in this paper were analyzed by SPSS 18.0. *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

Results of CHIs induced allergic reactions (intravenous sensitization)

After sensitized successively by injecting different drugs intravenously for three times (d0, d1, d2), no guinea pigs showed allergic reactions in groups of N.S and *Chuankezhi* injection after challenged in d8, and all guinea pigs showed allergic reactions in groups of 10% horse serum, *Qingkailing* injection, *Xiangdan* injection in d8 (Table 1), the onset time of allergic reactions was at about 5 min after challenged.

The data of serum IL-4 and total IgE were shown in Table 2. Compared with the N.S group, except group of *Chuankezhi* injection ($P>0.05$), contents of serum IL-4 and total IgE increased significantly ($P<0.01$) in other three groups (10% horse serum, *Qingkailing* injection, *Xiangdan* injection).

Results of CHIs induced allergic reactions (intraperitoneal sensitization)

After sensitized by injecting different drugs intraperitoneally once every other day for three times (d0, d2, d4), no guinea pigs showed allergic reactions after challenged in d8. However, except groups of N.S and *Chuankezhi* injection, guinea pigs of other three groups (10% horse serum, *Qingkailing* injection, *Xiangdan* injection) showed allergic reactions after challenged with the same drug in d14 and d21 (Table 3), the onset time of allergic reactions was at about 5 min after challenged.

The data of serum IL-4 and total IgE were shown in Table 4. Compared with N.S group, contents of serum IL-4 and total IgE of all groups didn't increase in d8 ($P>0.05$). However, except group of *Chuankezhi* injection ($P>0.05$), serum IL-4 and total IgE of other three groups (10% horse serum, *Qingkailing* injection, *Xiangdan* injection) increased significantly in d14 and d21 ($P<0.01$).

Discussion

The most common adverse reaction of Chinese herbal injections is allergic reaction [8,21,4], however, the examination period of routine method of test for allergen was so long (2-3 weeks) after first intraperitoneal sensitization, which limited its application in drug emergency test. Here we proposed a new method to predict drug allergy through intravenous sensitization, and practiced in three batches of CHIs.

Qingkailing injection is a well-known composite formula of traditional Chinese medicine and may cause allergic reactions in clinical practice, which contains seven major bioactive components of adenosine, geniposide, chlorogenic acid, baicalin, ursodeoxycholic acid, cholic acid, and hyodeoxycholic acid [20]. In our previous study, chlorogenic acid has been confirmed as an allergen in guinea pigs [18]. *Xiangdan* injection contains *Salvlae miltiorrhizae radix et rhizoma* and *Dalberglae odoriferae lignum*, which may also induce allergic reaction in clinic practice [17]. *Chuankezhi* injection contains *Epimedii folium* and *Morindae officinalis radix*, there were no reports about its effect of allergic reaction in clinic application [19].

We found that contents of serum IL-4 and total IgE increased significantly in d8 after guinea pigs were sensitized successively by intravenous injecting of *Qingkailing* injection or *Xiangdan* injection for three times. However, serum IL-4 and total IgE could only be detected in d14 and d21 for the first time followed by routine method of intraperitoneal sensitization, while not in d8. The final results of this new method were in accordance with that of whole body animal experiments, which suggested that the indexes of IL-4 and IgE might be used as new biomarkers to predict drug allergy. And the indexes were more objective than subjective phenomena of allergic reactions. This has a good application value in drug emergency test.

Our results indicated that different mode of sensitization (path and interval of frequency) might result in different onset time of allergic reactions in guinea pigs, we suggested that the mode of successive

Table 1: 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
N.S	eight negative
Horse serum	eight positive
Qingkailing injection	eight positive
Xiangdan injection	eight positive
Chuankezhi injection	eight negative

Table 2: Contents of serum 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^{-1}	Total IgE / IU·mL^{-1}
N.S	94.1 ± 13.3	44.5 ± 6.3
Horse serum	139.6 ± 18.4**	103.2 ± 17.2**
Qingkailing injection	121.8 ± 15.1**	76.8 ± 9.1**
Xiangdan injection	122.4 ± 16.9**	84.5 ± 11.1**
Chuankezhi injection	95.2 ± 11.3	45.7 ± 7.0

vs. N.S group of respective index: **$P<0.01$

Table 3: 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		
	d8	d14	d21
N.S	six negative	six negative	six negative
Horse serum	six negative	six positive	six positive
Qingkailing injection	six negative	six positive	six positive
Xiangdan injection	six negative	six positive	six positive
Chuankezhi injection	six negative	six negative	six negative

Table 4: Contents 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^{-1}			Total IgE / IU·mL^{-1}		
	d8	d14	d21	d8	d14	d21
N.S	92.4 ± 13.7	94.1 ± 10.2	93.6 ± 10.6	45.8 ± 6.9	44.3 ± 5.6	43.2 ± 6.7
Horse serum	95.2 ± 15.3	194.7 ± 23.8**	186.1 ± 20.9**	48.2 ± 8.1	137.7 ± 18.1**	133.1 ± 18.7**
Qingkailing injection	94.3 ± 14.9	145.9 ± 17.4**	139.3 ± 16.8**	46.4 ± 7.9	94.2 ± 11.4**	93.7 ± 10.4**
Xiangdan injection	94.8 ± 16.6	149.4 ± 16.2**	137.9 ± 17.5**	47.3 ± 8.3	92.1 ± 7.9**	90.5 ± 13.4**
Chuankezhi injection	91.5 ± 14.3	95.7 ± 11.0	96.5 ± 12.1	43.8 ± 7.1	46.1 ± 6.5	42.8 ± 5.1

vs. N.S group of respective index: **P<0.01

sensitization by injecting drugs intravenously might stimulate auxiliary immune cells quickly to produce IL-4, and promoted the synthesizing of IgE earlier [1,14,16], thus allergic reactions occurred earlier (d8) than that of intraperitoneal sensitization (d14 and d21). However, more experiments involved in CHIs should be carried out to authenticate this viewpoint.

Conclusion

In this study, we proposed a new method to detect allergic reactions induced by CHIs and found that IL-4 and IgE might play important roles in prediction of drug allergy, the examination period was reduced by 1-2 weeks compared with the routine method of test for allergen in Ch.P., which suggested that serum IL-4 and IgE could be used as new biomarkers to predict allergic reactions induced by CHIs, This has a good application value in dealing with the emergency of drug allergy.

Authorship Contribution

Gui Nan Xiao conceived and designed the study and wrote the manuscript, Qing Ping Sun conducted the statistical analysis, all authors carried out the experiments.

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