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Original Article

A guinea pig model of Ciwujia Injection-induced anaphylaxis for allergic substance screening

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ABSTRACT

Objective: Though especially efficient for cardiovascular and cerebrovascular diseases treatment, many serious anaphylactic diseases could be induced by Ciwujia Injection (CWJI). However, study of the mechanism and detection of allergies have been investigated by the unknown sources of allergenic substances. In this study, a guinea pig model which could mimic the symptoms of anaphylactic reactions induced by Ciwujia Injection (CWJI) was modeled and used to screen the allergenic substance of CWJI.

Methods: Guinea pigs were sensitized three times every other day with CWJI and excited 14 d after the last sensitization administration. Then, the histamine, trypsin, IL-4 and IFN-γ levels, and the Annexin V positive rate of peritoneal mast cells (PMC) were detected, the numbers of B lymphocyte and the pathological changes were also analyzed to verify the guinea pig allergy model, PCA test and IgE antibody levels were determined to study the mechanism.

Results: The levels of total IgE, histamine, and trypsin were significantly increased after CWJI sensitization, IL-4 level was elevated, Annexin V positive of PMC cell rate, local skin reactions, and declined IFN-γ were observed after excitation. Histological examination showed that mild pathological changes in lungs were found.

Conclusion: This guinea pig model may provide a powerful tool to study the mechanism in CWJI induced anaphylaxis and screen the allergic source of CWJI.

1. Introduction

Ciwujia Injection (CWJI) is a modern preparation consisting of the alcohol water extracts of the herb of *Acanthopanax senticosus* (Rupr. Et Maximl) Harms (Ciwujia in Chinese). It has been widely used to treat cardiovascular and cerebrovascular diseases for many years in China and showed excellent clinical curative effect (Fan et al., 2016; Fu et al., 2015).

Unfortunately, some serious anaphylactic reactions occur when CWJI are used to treat patients. It has been reported that among 521 cases of CWJI ADR (adverse drug reaction), 72.3% are III to IV grade, and the grade I case was 13.1% of total ADR cases (Hu & Shang, 2010). Recently, the cases of CWJI induced anaphylactic disease showed an increasing trend, which had harmed public health (Wang et al., 2009).

A suitable animal model should be closely related to the disease, which is not only similar to the clinical and functional characteristics of the particular disease but also can simulate the underlying mechanism involved. Guinea pig is a highly IgE-responsive animal and a good model for allergic diseases. It has been widely used to study allergic reactions caused by drugs, food protein extracts, and other allergens (Knippels et al., 2003; (Opoku, Darko, & Newman, 2017; Zhou, Zhang, & Sun, 2018). There are no reliable guinea pig allergic models, so the pathogenesis is poorly understood, and the diagnosis and allergic substance screening for CWJI was restricted.

Anaphylaxis of CWJI can be life-threatening in severe cases. At present, there is no report on establishing reliable guinea pig allergy model of CWJI in China. In this study, different doses of CWJI were injected subcutaneously into sensitized guinea pigs, to explore the pathogenesis of guinea pigs allergic to CWJI and provide a good animal model for the allergic substances screening of CWJI.

2. Materials and methods

2.1. Animals and materials

Guinea pigs (half male and half female, 12-week-old) were purchased from the animal centre of Heilongjiang University of

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Chinese Medicine (Harbin, China). Guinea pigs were acclimatized for at least 5 d before the study. They were kept under SPF laboratory conditions and received the institute's grain based open formula diet and tap water. All animal experimental procedures of this study were conducted according to ethical guidelines for the care of laboratory animals. CWJI was provided by Heilongjiang Zhenbaodao Pharmaceutical Co., Ltd. (Harbin, China, batch number 20160601, 20160702, and 20160903). Heparin sodium, Evan's Blue and ovalbumin (OVA) were purchased from Sigma (St. Louis, MO). The guinea pig total IgE ELISA kits, IL-4 and IFN-γ ELISA kits were provided by Rapid Bio Lab (Calabasas, California, USA), and the primers and templates were designed by Saituo Bio Lab.

2.2. Sensitization process

Guinea pigs received a subcutaneous administration of 5.6 mL/kg or 2.8 mL/kg CWJI. Control group were given physiological saline instead. Repeat the same process every day later for three times. Blood was collected 14 d after the last administration and serum samples were prepared and stored at −80 °C.

2.3. Total IgE level in serum

The total IgE concentrations in the serum samples were determined by total IgE ELISA kit. One way ANOVA and student's *t*-test were used to analyze differences between the groups. A value of $P < 0.05$ was considered as statistical significance.

2.4. Passive cutaneous anaphylaxis (PCA) test

Serum samples (100 μL) from sensitized guinea pig were diluted two fold in physiological saline and then injected intradermally to shaved sides of guinea pig. After 48 h, sera transferred guinea pigs were excitated by injection of 1% Evan's Blue containing 5.6 mL/kg CWJI. The animals were sacrificed and the blue spot with a diameter under the skin was recorded 30 min later. After the sensitized serum was heated at 56 °C for 3 h, the same operations were repeated (Mehlhop, Van De, & Goldberg, 1997).

2.5. Mast cell degranulation test

The standard incubation was conducted in 100 μL of Tyrode's buffer A (Hepes 2.383 g, NaCl 7.605 g, KCl 0.373 g, $CaCl_2{\cdot}6H_2O$ 0.307 g, MgCl 20.095 g, glucosum anhydricum 10.08 g, calf serum 1 mL) containing mast cells. The polled cells were incubated 4 h with antiserums, stimulated by indicated amounts of antigens, OVA, CWJI, and physiological saline. After incubated for 10 min at 37 °C, the cells were placed on ice, and then centrifuged at 150 g for 5 min at 4 °C. Degranulation of mast cell was observed and analyzed by optical microscope and transmission electron microscope. The Annexin V positive cell rate was detected by flow cytometry.

2.6. Histamine and trypsin levels

According to the "sensitization process" step, 30 min after excitation, centrifuged at 1000 g for 10 min, plasma was collected, and histamine and trypsin levels were measured using an ELISA kit.

2.7. HE of lung tissue

Lung tissue of sensitized guinea pigs was collected 1 d after CWJI excitation and fixed in neutral buffered formaldehyde. 5 μm of sliced tissues sections were stained with hematoxylin and eosin (HE) for optical microscopy.

2.8. Cytokine production and B lymphocyte numbers in blood

IL-4 and IFN-γ levels were measured with ELISA kits, number of B lymphocyte was measured with full automatic biochemical analyzer, One way ANOVA and student's *t*-test were used to analyze differences between the groups. A value of $P < 0.05$ was considered as statistical significance.

3. Results

3.1. Determination of total IgE level in serum

The total IgE level is the international "gold standard" for type I allergic reactions. IgE mediates type I allergic antibodies, which are extremely low in physiological conditions. The determination of total IgE in serum is of great significance for finding allergens and identifying the type of allergic reaction. It can preliminarily determine the type I allergic reaction of CWJI. Therefore, the determination of IgE content is selected as an indicator for judging type I allergic reaction. Total IgE levels of (43.91 ± 7.36) μg/mL were significantly increased in animals sensitized with CWJI compared to control group [(23.65 ± 5.96) ng/mL, $P < 0.01$, $n = 6$]. The results indicated that the IgE antibody concentration was related to the sensitization dose of CWJI (Table 1).

3.2. PCA reactions

The PCA experiment was carried out in the presence or elimination of IgE by heating to confirm the specific IgE of the allergic reaction induced by CWJI. The serum of CWJI sensitized guinea pigs caused positive reactions, and the mean diameter of the blue spot was (4.09 ± 0.17) mm (Fig. 1) and the positive reaction rate was 67%, while heat induced IgE antibody inactivation negative reactions were observed and shown in Table 2, as shown in Fig. 1, after passive injection of CWJI antiserum and excitated by injection of 1% Evan's Blue containing CWJI, the dye exudates into the skin to form a clear blue spot, which is consistent with the results of positive drug OVA. These results indicated that IgE was the reactive antibody that induced anaphylaxis of CWJI.

3.3. Mast cell degranulation test

The PMC were incubated 4 h with antiserums, stimulated by indicated amounts of CWJI and physiological saline. The Annexin V positive rate increased as the histamine release rate and tryptase activity was increased. The Annexin V positive rate of PMC treated with CWJI extraction was (49.42 ± 4.63)%, which was remarkably higher than that of control group (8.75 ± 0.74)% ($P < 0.05$). As shown in Fig. 2, the results revealed that mast cell degranulation was one of mechanism of anaphylactoid resulted from CWJI.

Table 1
Total IgE level in serum.

Groups	Dosages	Total IgE level/(μg·mL^{-1})
Control	–	23.65 ± 5.96
OVA	1 mg/mL	73.91 ± 3.82**
CWJI (20160601)	2.8 mL/kg	24.61 ± 4.95
	5.6 mL/kg	30.05 ± 5.76
CWJI (20160702)	2.8 mL/kg	33.48 ± 6.23
	5.6 mL/kg	43.91 ± 7.36**
CWJI (20160903)	2.8 mL/kg	27.30 ± 5.93
	5.6 mL/kg	34.11 ± 8.03

** $P < 0.01$ *vs* control group.

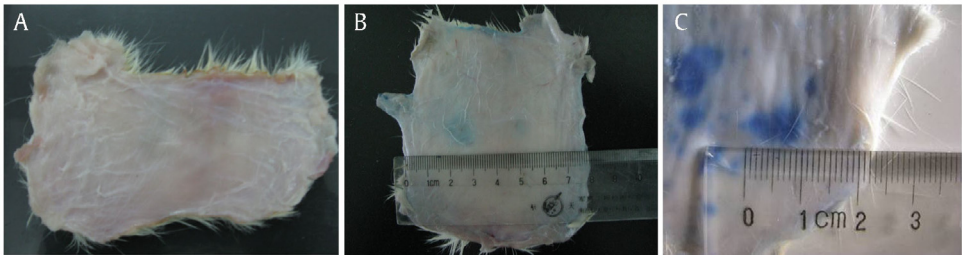

Fig. 1. PCA results of (A) control group, (B) OVA group and (C) CWJI group (20160702, 5.6 mL/kg).

Table 2
PCA test after injection of immunization or heat-inactivated serum (mean ± SD, $n = 6$).

Groups	Sensitized dose	Heated serum	Diameter/mm	Positive reaction/%
Control	—		1.24 ± 0.25	0
OVA	1 mg/mL		10.55 ± 1.75*	100*
CWJI (20160601)	2.8 mL/kg	—	0	0
	5.6 mL/kg	—	2.03 ± 0.11	0
CWJI (20160702)	2.8 mL/kg	—	2.10 ± 0.67	0
	5.6 mL/kg	—	4.09 ± 0.17*	67*
CWJI (20160903)	2.8 mL/kg	—	0	0
	5.6 mL/kg	—	2.11 ± 0.45	0

* $P < 0.05$ *vs* control group.

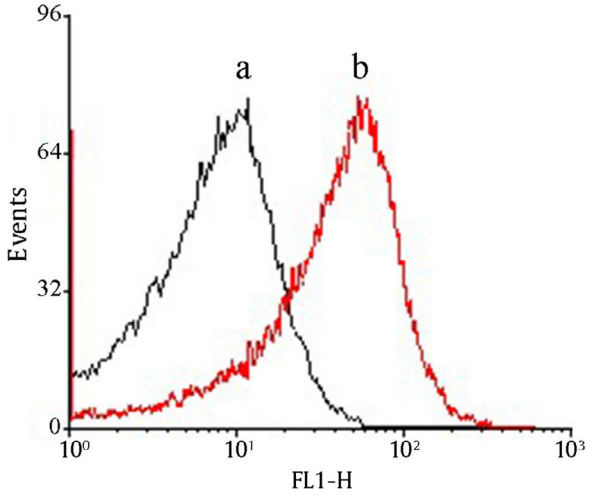

Fig. 2. Annexin V positive rate of PMC in (a) control group and (b) CWJI (20160702) group detected by flow cytometry.

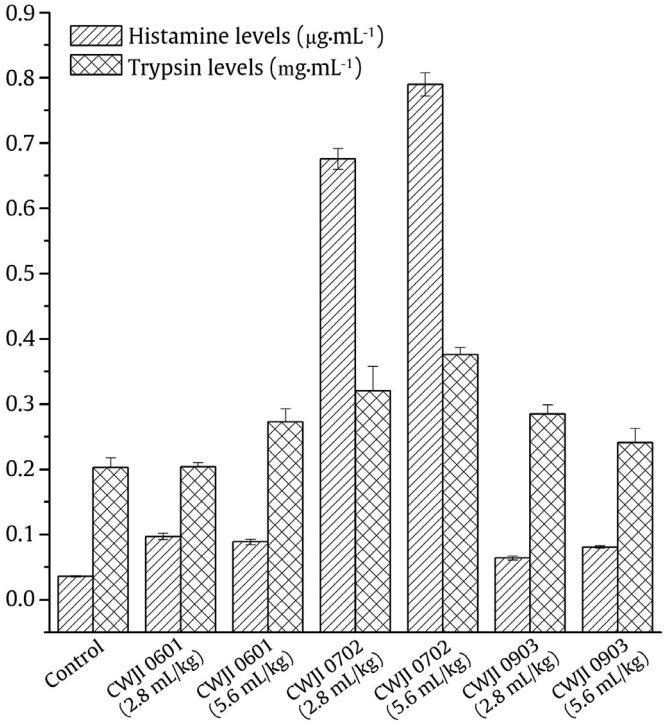

Fig. 3. Histamine and trypsin levels in plasma of different groups.

3.4. Histamine, trypsin levels, and histological examinations

Histamine and tryptase are downstream release substances of allergic reactions, and the degree of PMC degranulation is measured by measuring their levels to reflect the degree of anaphylaxis. Plasma histamine and trypsin levels were significantly elevated in CWJI (20160702) sensitized animals compared to control group (Fig. 3). These results indicated that histamine and trypsin were the main mediators of anaphylaxis in this guinea pig model. Histological examinations showed that guinea pig lungs of CWJI sensitized were slightly inflamed, and mild eosinophil infiltrations around the blood vessels was appear (Fig. 4).

3.5. Numbers of B lymphocyte in blood

B lymphocyte in the lamina propria of the respiratory tract and digestive tract mucosa are the main cells for the synthesis of IgE antibodies. Therefore, the number of B lymphocyte indirectly reflects the degree of anaphylaxis. The numbers of B lymphocyte in OVA sensitized animals (0.51 ± 0.14, 10^9/L) were significantly increased when compared to control group (0.10 ± 0.02, 10^9/L). However, there was no significance difference in the numbers of B lymphocyte between CWJI sensitized groups and control group (Table 3).

3.6. Cytokine production

Cytokines are a large class of proteins or small molecule polypeptides that transmit information between cells, with immunomodulatory and effector functions. IL-4 is produced by antigen or mitogen-stimulated CD4+ T cells, and activated mast cells

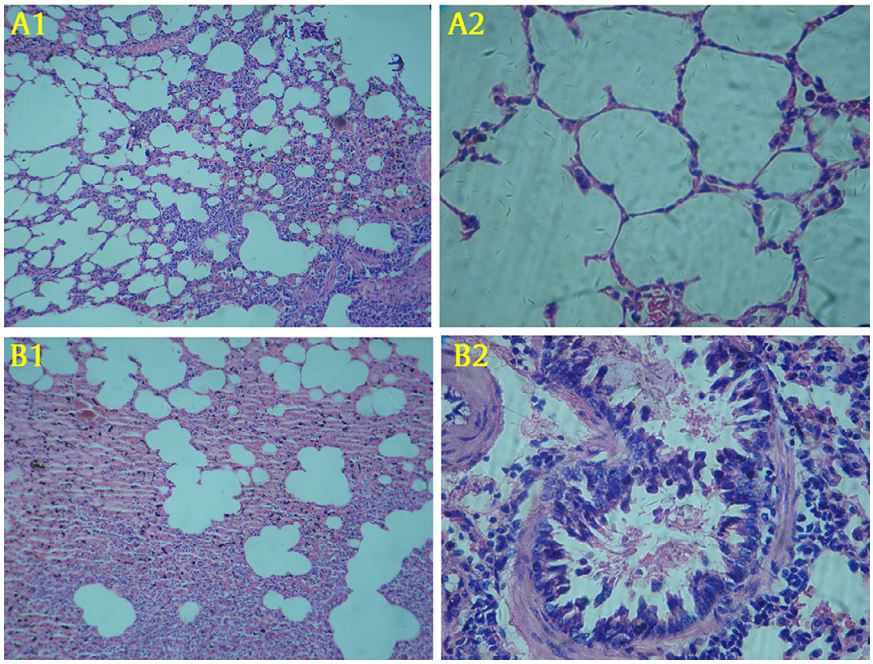

Fig. 4. Histological changes in lungs from control group (A1: × 100; A2: × 400) and CWJI (20160702, 5.6 mL/kg) sensitized group (B1: × 100; B2: × 400) of guinea pigs after CWJI excitation.

Table 3
Numbers of B lymphocyte in blood of different groups (mean±SD).

Groups	Dosages	B lymphocyte numbers/($10^9 \cdot L^{-1}$)
Control	–	0.10 ± 0.02
OVA	1 mg/mL	0.51 ± 0.14**
CWJI (20160601)	2.8 mL/kg	0.08 ± 0.02
	5.6 mL/kg	0.07 ± 0.02
CWJI (20160702)	2.8 mL/kg	0.10 ± 0.02
	5.6 mL/kg	0.11 ± 0.03
CWJI (20160903)	2.8 mL/kg	0.13 ± 0.04
	5.6 mL/kg	0.11 ± 0.03

** $P < 0.01$ *vs* control group.

can also produce IL-4. IFN-γ inhibits the proliferation of TH2 cells, thus inhibiting humoral immune function. IFN-γ not only inhibits the production of IL-4 by TH2-type cells, but also inhibits the action of IL-4 on B lymphocyte, in particular, inhibits the production of IgE by B lymphocyte. The production of cytokines in CWJI, OVA and control group were examined respectively. IL-4 levels of CWJI (20160702) were significantly higher than control group ($P < 0.05$). IFN-γ levels of CWJI (20160702) were significantly lower than control group ($P < 0.05$) (Table 4).

Table 4
Cytokine levels of different groups.

Groups	Dosages	IL-4/($\mu g \cdot mL^{-1}$)	INF-r/($pg \cdot mL^{-1}$)
Control	–	3.58 ± 0.53	16.18 ± 2.31
OVA	1 mg/mL	6.16 ± 0.77	12.11 ± 1.26
CWJI (20160601)	5.6 mL/kg	3.75 ± 0.65	17.45 ± 3.85
	2.8 mL/kg	3.62 ± 0.56	16.54 ± 2.94
CWJI (20160702)*	5.6 mL/kg	4.35 ± 0.59	10.10 ± 1.57
	2.8 mL/kg	3.91 ± 0.63	13.11 ± 1.67
CWJI (20160903)	5.6 mL/kg	3.96 ± 0.59	13.38 ± 2.73
	2.8 mL/kg	3.80 ± 0.89	12.43 ± 1.87

* $P < 0.05$ *vs* control group serum.

4. Discussion

Allergic reaction is mediated by IgE antibodies, which induce acute generalized urticaria. Allergic reactions to β-lactams are the most common cause of adverse drug reaction mediated by IgE antibodies. However, IgE antibodies are not always necessary to activate the release of mediators from mast cells and induce acute urticarias. Some drugs, such as opiates or codeine, act directly on mast cells, induce an exacerbation of chronic urticaria by a pharmacological mechanism involving the arachidonic acid metabolism (Woessner & Simon, 2013; Katrina & Mealey, 2013). Additionally, angioedema is a well-known complication of angiotensin-converting enzyme inhibitors by its action on bradykinin, which is a potent vasodilator agent. Topical drugs, such as antibiotics, disinfectants, or anesthetics, may cause urticaria, which sometimes progresses to generalize urticaria.

In this study, a guinea pig model was developed to mimic the symptoms of anaphylactic reactions induced by CWJI. Previous studies have shown that CWJI could cause systemic reactions in guinea pig. However, they did not study their effects on specific organs and did not further study the relevant mechanisms (Yu, 2008). In this experiment, local anaphylactic reactions and histological damage of the respiratory system were examined. In addition, IgE antibodies (total IgE and specific IgE) and cytokine production were also determined. This guinea pig model is the first systematic intact animal model that mimics the allergic reaction induced by CWJI, the symptoms and mechanism were explored simultaneously. The experimental results showed that guinea pigs could be used to determine CWJI-induced allergic reactions. Guinea pig is a highly IgE response strain that responds severely to CWJI and has immune and allergy symptoms.

There was a positive correlation between IgE and CWJI doses. In addition, the severity of systemic and local anaphylactic reactions was related to the CWJI dose. Therefore, high doses of CWJI tended to produce more IgE, causing a more intense reaction. However, histamine levels did not show the similar trend. The

reason may be that histamine was metabolized due to its short half-life in plasma (Cheng, 2009). The cause of this phenomenon may also be related to previous studies in which lower antigen doses caused more severe reactions due to tolerance (Liu, Zheng, & Yang, 2013).

Most anaphylactic reactions to CWJI are thought to be immediate hypersensitivity, occurring within 24 h of administration 14 d later. The role of IgE is critical in immediate hypersensitivity reactions. Although some studies have found no increase in total IgE in certain allergic diseases (Carlos, Eduardo & Antonio, 2016), in clinical diagnosis, total IgE levels have been widely used as diagnostic parameters (Catal, Topal, & Selimoglu, 2015). This study confirmed the relationship between total IgE levels and allergic reactions, indicating that total IgE can be used as a potential predictor of CWJI-induced allergic reactions. We also found a significant increase in specific IgE in the serum of CWJI-sensitized animals.

By heating inactivation of the immune serum, the PCA response is eliminated, the result strongly suggested the role of IgE in the guinea pig model. These results showed that CWJI-induced anaphylaxis was mediated with IgE, and high doses of CWJI can cause positive reactions as OVA. The pharmacological effects of CWJI are mainly concentrated in circulatory systems, in which the level of histamine is significantly increased. Eosinophil infiltration in the lung samples lead to inflammation of the respiratory system. It suggests that the respiratory system may be another target organ when CWJI causes an allergic reaction. However, these lesions were small compared to respiratory damage caused by allergens because allergens recorded significant respiratory symptoms and significant pathological changes. (Arts and Bloksma, 2003). This might be explained as follows: components of CWJI are small molecular compounds. As a result, the skin, but not the respiratory and gastrointestinal systems, is an easy target organ for anaphylactic reactions (Merk, Baron, & Neis, 2007; Dong et al., 2007). In clinical diagnosis and therapy, we should take more attention to the skin rash caused by CWJI. The production of cytokines is considered to be a general feature of allergic reactions (Jongyota, Wigraipat, & Nontapa, 2008; Selgrade, Boykin, & Haykal-Coates, 2006). In the present study, the levels of IL-4 (Th2 prone) and IFN-γ (Th1 prone) in serum of CWJI sensitized guinea pigs were measured to investigate their associated molecular events. CWJI could stimulate the production of type 2 cytokines with higher levels of IL-4 but lower levels of IFN-γ. These data were consistent with the increasing trend of serum IgE, which suggested that the anaphylaxis caused by CWJI might be mediated by Th_2 cells. (Li, Gao, Wang, & Liu, 2010).

5. Conclusion

In summary, the guinea pig model exhibits the same immune parameters (IgE, cytokines) as the CWJI caused allergic reactions in clinical practice. In addition, this model mimics some physiological indicators such as histamine and trypsin levels and some pathological changes in lungs. Therefore, this guinea pig model provides a useful tool for exploring the treatment and mechanism of CWJI caused anaphylaxis.

Conflict of interest

The authors declare no conflicts of interest.

Acknowledgments

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