The evaluation and optimization of animal model for anaphylactoid reaction induced by injections

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Summary

Background: **Recent research indicates that injections inducing unwanted anaphylactoid reactions occur frequently in a clinical setting. In this paper, we explored anaphylactoid reactions trends in animal models following ginsenosides injections.**

Methods: **Our anaphylactoid animal model was optimized by comparing reactions between BN rats, SD rats, guinea pigs and ICR mice to first intravenous exposure to standard compounds including ovalbumin (OVA), tannic acid (TA), Tween 80 (T80), bovine serum albumin (BSA) and Compound 48/80 (C48/80), Shengmai injection (SMI) and Xuesaitong injection (XSTI) which contains ginsenosides, respectively. The anaphylactoid symptoms were documented and the plasma levels of histamine were assessed. Subsequently, the IgE levels and total complement activity (CH50) were determined to further explore the mechanisms underlying the observed anaphylactoid reactions on the optimized animal model.**

Results: **We observed that BN rats and guinea pigs exhibited particularly exacerbated symptoms after administration of OVA, T80, TA, SMI and XSTI. Regarding histamine levels, we observed that BN rats were more sensitive to TA and XSTI, guinea pigs were more sensitive to OVA, T80 and SMI, and SD rats were more sensitive to C48/80. According to both anaphylactoid symptom scores and histamine secretion rates, BN rats, in particular, were found to be more sensitive to OVA, T80, TA, SMI and XSTI. Noteworthy however, the four rodents showed significantly weaker anaphylactoid reactions after administration of BSA.**

Conclusion: **BN rats were more suitable for comprehensive evaluation of anaphylatoid reactions following injections; both IgE levels and CH50 could be used as auxiliary mediators for the assessment of anaphylactoid reactions.** ***(Asian Pac J Allergy Immunol 2015;33:330-8)***

Keywords: *injections, anaphylatoid reaction, animal model, adverse reaction, BN rats*

Introduction

Owing to their rapid action and high bioavailability, injections of Chinese patent medicine such as Shengmai injection (SMI) and Xuesaitong injection (XSTI) are increasingly used has an adjunct therapy for clinical treatment. Unfortunately, serious anaphylactoid reactions are increasingly reported in patients receiving their first adminstration[1], positioning Chinese patent medicine as potential public health concern in China.[2] Anaphylactoid reaction, also called pseudo-allergic or idiosyncratic reaction, whose symptoms conformed to Coombs and Gell's Type I category, are actually not initiated or mediated by pre-existing IgE antibodies and are thought to represent up to 77% of all immune-mediated immediate hypersensitivity reactions.[3] Anaphylactoid reactions, which resemble in their clinical presentation generalized anaphylaxis, involve various mechanisms immediately after first exposure to antigens.[4] A variety of agents, including Tween 80 (T80), iodinated compound, vitamin k1 injection, SMI, XSTI, have been reported to give rise to anaphylactoid reactions following initial intravenous administration.[3,5] Despite its potential clinical and public health implications, there have been no universal and reliable animal model optimized for the evaluation of the anaphylactoid potential of injections. One of the reasons underlying the lack of reliable animal model availability is the complexity

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of the immunology mechanisms [1,6], which impedes our ability to control for injection quality. So far animal models[7-9] that were used to study drug allergies included rats, guinea pigs, mice, cats, dogs, pigs and cynomolgus monkeys, and research[10] showed that these different models showed dissimilar susceptibility to various compounds. This suggest that animal models could be subjected to diverse types of anaphylactoid reaction in response to different agents and thus that it is critical to optimize the drug/model system used before clinical interpretation. For instance, current research focus on anaphylactoid reactions triggered by solution additives of injections[5] or injections themselves, some of which include complex ingredients that couldn't directly explain the generation of anaphylactoid reactions.[11-12]

Moreover, only behavioral symptoms or body indicators measured on the animal model are usually used to evaluate anaphylactoid reaction. A suitable animal model however, should not only reproduce the clinical and functional characteristics of the specific disease but also mimic the basic mechanism involved.[3] Therefore an excellent animal model should not only be sensitive to well-known generic compounds such as C48/80, but also reveal the potential allergenicity of injections composed of complex components. Mediator measurement combined with symptom monitoring was the primary procedure for studying anaphylactoid reactions.[5] As noted, histamine is a typical mediator that causes various pathophysiologic events in acute allergic reactions.[13] Previous studies showed that XSTI can induce anphylactoid reaction through stimulating mast cells or RBL-2H3 cells to release histamine.[14] In this context, we used ovalbumin (OVA), tannic acid (TA), Tween 80 (T80), bovine serum albumin (BSA) and Compound 48/80 (C48/80), as well as SMI and XSTI to induce anaphylactoid reactions on BN rats, SD rats, guinea pigs and ICR mice following intravenous injection. The sensitivities of animals to these compounds were evaluated through behavioral symptom scores combined with the secretion rates of histamine, and characteristics of the sensitivities of animals to standard compounds were probed and strived to find the common part. Furthermore, the mechanisms of anaphylactoid reaction on the optimized animal model were explored and the experimental data were presented as a procedure of evaluation of anaphylactoid of injections containing ginsenosides.

Methods

Animals and materials

Inbred BN rats (male, 200±20 g, 10 weeks old), outbred SD rats (male, 200±20 g, 10 weeks old), outbred guinea pigs (male, 400±20 g, 10 weeks old) and outbred ICR mice (male, 20±2 g, 5 weeks old) were purchased from WeiTongLiHua Co. (Beijing, China) and used in this study. The animals were kept under SPF laboratory conditions (Centre for Animal Experiment of Liaoning University of Traditional Chinese Medicine, Shenyang, China) and received a standard laboratory diet and filtered tap water ad libitum. All animal procedures were approved by Liaoning Provincial Animal Welfare and Care Guideline. The animals were acclimated to the laboratory environment for 1 week before starting the experiment.

Tween 80 was provided by Well Chemical Co. (Nanjing, China). Ovalbumin, Bovine serum albumin and Compound 48/80 were purchased from Sigma (St Louis, MO, USA). Tannic acid was provided by benchmark Chemical Reagent Co. (Tianjin, China). Shengmai Injection was provided by Taihang pharmaceutical Co. (Shanxi, China); Xuesaitong Injection was provided by Zhenbaodao Pharmaceutical Co. (Heilongjiang, China). Histamine ELISA Kit was purchased from IBL International (Germany), IgE ELISA Kit was purchased from Jiancheng biology Co. (Nanjing, China).

Anaphylactoid study of BN rats, SD rats, guinea pigs and ICR mice

According to the maximum injection volume of animals and the results of preliminary experiment,[14] all the animals were randomly divided into 8 groups, taking BN rats as an example: control group (5 mL/kg saline), OVA (5, 20 mL/kg) groups, 1% TA (2.5, 5 mL/kg) groups, BSA (2.5, 5 mL/kg) groups; 10% T80 (5, 15 mL/kg) groups; C48/80 (1.25, 2.5 mL/kg) groups, SMI (5.5, 20 mL/kg) groups, XSTI (0.75, 3 mL/kg) groups. The drug dosage used for BN rats were adapted to the other three animals on the basis of the body surface area. Subsequently, the corresponding test substances (prepared with saline and filtered through 0.22 μm pore size sterile filters) were administered intravenously each animals of each group as a single exposure within a time period of 20 seconds. The anaphylactoid symptoms were observed 30 minutes after the injection. Symptoms were evaluated by using a scoring system which was described by Li[15] and the immune toxicity of traditional Chinese medicine, natural medicine

(allergic, light allergic reaction) technology guidelines for research as follows: 1) 0: no abnormal reaction. 2) 1: trembling, scratching and rubbing around the nose. 3) 2: sneezing, coughing, puffiness around the eyes and the mouth, shortness of breath. 4) 3: dyspnea, wheezing, unsteady gait, cyanosis around the mouth and the tail, myasthenia of limbs, convulsions, spasm, rotation, tidal breathing. 5) 4: death.

Thirty minutes after injection, the animals were anesthetized by pentobarbital and 2 volume of blood samples were collected into chilled tubes containing EDTA-K_2 from abdominal aorta then centrifuged at 3000 × g (10 min, 4℃) to obtain plasma which was stored at -80℃ until subsequent analysis. Plasma histamine levels were determined following manufacturer instructions. The following formula[2] was used to calculate the secretion rates of histamine: (%) = (Ceg-Cnc)/Cnc*100%, where Ceg represents the histamine level of the experimental groups and Cnc represents the average histamine level of the control group. The symptom scores range <1, >1, >2 and >3, and secretion rates of histamine also were categorized as <30%, >30%, >50%, >100%, the value A, a combined measure of anaphylactoid symptom scores and histamine secretion rates was used for final results evaluation as follows: if symptom score were <1 and secretion rates of histamine <30% the A score was 0 points; if symptom score was >1 and secretion rates of histamine >30% the A score was 1 points; >2 and >50% recorded as 2 points, >3 or >100% recorded as 3 points. As a result the value A included four levels: <1, >1, >3, >5. If value A <1, the result would be determined as 'Negative', value A >1, the result would be determined as 'Suspected', value A >3, the result would be determined as 'Positive', value A >5, the result would be determined as 'Strong positive'.

Determination of plasma IgE levels

Plasma IgE levels of BN rats were determined after the first administration of each compounds with rat IgE ELISA-kit following manufacturer instructions.

Determination of CH50

A CH50 assay was performed as described by Lee et al.[16] The CH50 of each sample was determined according to the following formula: CH50 (U/μL) = ODeg*k_1/(ODpc*k_2), where ODeg and ODpc represent the absorbance rate of the experimental group and positive control group, respectively; k_1 and k_2 represent the dilution factor of each sample and the volume of plasma.

Statistical analysis

Data were analyzed using SPSS software (version 19.0). The measurement data were presented as mean±SD. One-way ANOVA and Student's *t*-test were used to compare the difference in scores between groups. Pearson's χ2-test was used for analyzing the category data. Statistical significance was considered reached when P <0.05).

Results

Anaphylactoid symptoms of BN rats, SD rats, guinea pigs and ICR mice

All BN rats (100%) showed positive reactions after beingchallenged with 10% T80 (15 mL/kg), C48/80 (1.25 mL/kg), C48/80 (2.5 mL/kg), 1% TA (5 mL/kg), XSTI (3 mL/kg) respectively, and 66.7% BN rats showed positive reactions with the dose of 20 mL/kg SMI or OVA. Only 50% BN rats showed positive reactions after challenging with 10% Tween 80 (5 mL/kg) or 1% TA (2.5 mL/kg), while no BN rats demonstrated positive response to others compounds (Table 1).

All SD rats (100%) showed positive reactions after being challenged with C48/80 (2.5 mL/kg), and 66.7% SD rats showed positive reactions in the dose of 15 mL/kg 10% T80. Only 50% SD rats showed positive reactions after being challenged with OVA (20 mL/kg) or XSTI (3 mL/kg), while no SD rats demonstrated positive response to others compounds (Table 2).

All guinea pigs (100%) showed positive reactions after challenging with 1% TA (4.4 mL/kg), C48/80 (1.1 mL/kg), C48/80 (2.2 mL/kg), and 66.7% guinea pigs showed positive reactions with the dose of 4.4 mL/kg 10% T80 or 2.6 mL/kg XSTI, while only 16.7% guinea pigs showed positive reactions after being challenged with OVA (4.4 mL/kg), 10%T80 (2.2 mL/kg), BSA (4.4 mL/kg) or SMI (4.8 mL/kg). No guinea pigs demonstrated positive response to others compounds (Table 3).

All ICR mice (100%) showed positive reactions after being challenged with C48/80 (20 mL/kg), and 50% ICR mice showed positive reactions with the dose of 20mL/kg 10% T80, while only 37.5% ICR mice showed positive reactions after being challenged with XSTI (4 mL/kg), 25% ICR mice showed positive reactions after being challenged with 1% TA (20 mL/kg). No ICR mice demonstrated positive response to others compounds (Table 4).

Table 1. Evaluation of anaphylactoid reaction in BN rats

Group	Dose (mL/kg)	Judgment of the typical symptom score						Positive reaction (%)	Plasma histamine levels (ng/mL)	Secretion rate of histamine (%)	A#	Evaluation result
		0	1	2	3	4	Mean					
Control	5	6	0	0	0	0	0	0	111.41±31.65	-	-	Negative
OVA	5	6	0	0	0	0	0	0	126.76±7.95	13.77±7.13	0	Negative
	20	2	2	1	0	1	1.33±1.51	66.7*	265.88±54.38**	138.63±48.80	4	Positive
10%T80	5	3	3	0	0	0	0.50±0.55	50*	143.94±7.04*	29.19±6.32	0	Negative
	15	0	0	0	2	4	3.67±0.52	100**	189.72±12.03**	70.28±10.80	5	Positive
BSA	2.5	6	0	0	0	0	0	0	116.37±10.29	4.44±9.23	0	Negative
	5	5	1	0	0	0	0.17±0.41	16.7	130.54±9.92	17.16±8.90	0	Negative
C48/80	1.25	0	0	1	5	0	2.83±0.41	100**	291.73±11.26**	161.83±10.11	5	Positive
	2.5	0	0	0	4	2	3.67±0.52	100**	343.89±20.75**	208.65±18.62	6	Strong positive
1%TA	2. 5	3	3	0	0	0	0.50±0.55	50*	147.22±14.79	32.14±13.27	1	Negative
	5	0	0	0	4	2	3.67±0.52	100**	210.52±13.88**	88.95±12.46	5	Positive
SMI	5.5	6	0	0	0	0	0	0	117.75±9.75	5.68±8.75	0	Negative
	20	2	4	0	0	0	0.67±0.52	66.7*	230.52±40.51**	106.89±36.36	3	Suspected
XSTI	0.75	5	1	0	0	0	0.17±0.41	16.7	135.84±6.15	21.92±5.52	0	Negative
	3	0	0	0	2	4	3.67±0.52	100**	309.80±29.95**	178.05±26.88	6	Strong positive

*P <0.05, **P <0.01 compared with control group. A# mean the A value.

Histamine measurements of BN rats, SD rats, guinea pigs and ICR mice

In BN rats, OVA (20 mL/kg), 10% T80 (5, 15 mL/kg), C48/80 (1.25 mL/kg), C48/80 (2.5 mL/kg), 1% TA (5 mL/kg), SMI (20 mL/kg) and XSTI (3 mL/kg) groups showed a significant increase in histamines levels compared to the control group (P <0.01, P <0.05) (Table 1)

In SD rats, OVA (20 mL/kg), 10% T80 (15 mL/kg), C48/80 (1.25 mL/kg), C48/80 (2.5 mL/kg), SMI (20 mL/kg) and XSTI (3 mL/kg) groups showed a significant increase of histamines levels compared to the control group (P <0.01) (Table 2).

In guinea pigs, OVA (4.4 mL/kg), 10% T80 (4.4 mL/kg), C48/80 (1.1 mL/kg), C48/80 (2.2 mL/kg), 1% TA (4.4 mL/kg) and XSTI (2.6 mL/kg) groups showed a significant increase of histamines levels compared to the control group (P <0.01, P <0.05) (Table 3).

In ICR mice, 10% T80 (20 mL/kg), C48/80 (3.5 mL/kg), C48/80 (20 mL/kg) and XSTI (4 mL/kg) groups showed a significant increase of histamines levels compared to the control group (P <0.01) (Table 4).

The A value (symptoms plus histamine measurements) of BN rats, SD rats, guinea pigs and ICR mice

The A value of BN rats in C48/80 (2.5 mL/kg) and XSTI (3 mL/kg) groups was determined as strong positive. The A value of BN rats in OVA (20 mL/kg), 10% T80 (15 mL/kg), C48/80 (1.25 mL/kg) and 1% TA (5 mL/kg) groups was determined as positive. The A value in SMI (20 mL/kg) group was determined as suspected, while the others groups were characterized by a negative A score (Table 1).

The A value of SD rats in C48/80 (2.5 mL/kg) group was determined as strong positive, while in OVA (20 mL/kg), 10% T80 (15 mL/kg), C48/80 (1.25 mL/kg), SMI (20 mL/kg) and XSTI (3 mL/kg) groups the A value was determined as suspected. The others groups were characterized by a negative A score (Table 2).

The A value of guinea pigs in C48/80 (2.2 mL/kg) group was determined as strong positive, while in C48/80 (1.1 mL/kg) and XSTI (2.6 mL/kg) groups the A value was determined as positive. In the 1% TA (4.4 mL/kg) group, the A value was determined as suspected while the others groups were characterized by a negative A score (Table 3).

Table 2. Evaluation of anaphylactoid reaction in SD rats

Group	Dose (mL/kg)	Judgment of the typical symptom score						Positive reaction (%)	Plasma histamine levels (ng/mL)	Secretion rate of histamine (%)	A#	Evaluation result
		0	1	2	3	4	Mean					
Control	5	6	0	0	0	0	0	0	114.89±4.81	-	-	Negative
OVA	5	6	0	0	0	0	0	0	126.05±9.47	9.72±8.24	0	Negative
	20	3	3	0	0	0	0.50±0.55	50*	364.61±41.17**	217.36±35.83	3	Suspected
10%T80	5	6	0	0	0	0	0	0	117.72±8.12	2.50±7.07	0	Negative
	15	2	1	2	1	0	1.33±1.21	66.7*	184.87±28.49**	60.92±24.80	3	Suspected
BSA	2.5	6	0	0	0	0	0	0	115.96±18.53	0.93±16.13	0	Negative
	5	6	0	0	0	0	0	0	123.73±33.08	7.69±28.80	0	Negative
C48/80	1.25	6	0	0	0	0	0	0	379.11±36.51**	229.99±31.78	3	Suspected
	2.5	0	0	0	4	2	3.33±0.51	100**	431.07±27.36**	275.22±23.82	6	Strong positive
1%TA	2. 5	6	0	0	0	0	0	0	115.42±6.70	0.47±5.83	0	Negative
	5	6	0	0	0	0	0	0	131.70±20.84	14.63±18.14	0	Negative
SMI	5.5	6	0	0	0	0	0	0	114.34±7.47	-0.47±6.51	0	Negative
	20	4	2	0	0	0	0.33±0.52	33.3	226.54±16.63**	97.18±14.48	3	Suspected
XSTI	0.75	5	1	0	0	0	0.17±0.41	16.7	114.44±10.39	-0.38±9.05	0	Negative
	3	3	2	1	0	0	0.67±0.82	50*	210.98±39.82**	83.65±34.66	2	Suspected

*P <0.05, **P <0.01 compared with control group. A# mean the A value.

The A value of ICR mice in C48/80 (20 mL/kg) group was determined as strong positive, while in 10% T80 (20 mL/kg) group it was determined as suspected. The others groups were characterized by a negative A score (Table 4).

Anaphylactoid study of BN rats, SD rats, guinea pigs and ICR mice in lower and higher equivalent dose

According to our system score, BN rats in lower doses were more sensitive to TA and C48/80, Guinea pigs were more sensitive to OVA, T80 and C48/80; according to measures of histamine secretion rate, BN rats were more sensitive to TA and XSTI, Guinea pigs were more sensitive to OVA, T80, C48/80 and SMI; according to the A value, BN rats were more sensitive to TA and C48/80 while Guinea pigs were more sensitive to T80 and C48/80. (Figure 1)

According to our system score, BN rats in higher doses were more sensitive to OVA, T80, TA, SMI and XSTI and Guinea pigs were more sensitive to C48/80; according to measures of histamine secretion rate, BN rats were more sensitive to TA and SMI while Guinea pigs were more sensitive to C48/80 and XSTI; according to the A value, BN rats were more sensitive to OVA, T80, TA, SMI and XSTI, while all animals were sensitive to C48/80. (Figure 1)

Plasma IgE levels and CH50 of BN rats

IgE levels in treatment groups were found to be significantly increased (P <0.01, P <0.05) compared to the control after challenges with OVA (20 mL/kg), C48/80(2.5 mL/kg), 1%TA (2.5, 5 mL/kg) and SMI (20 mL/kg) (Table 5).

CH50 decreased significantly (P <0.01, P <0.05) in treatment groups compared to the control groups after challenging with 10% T80 (5, 15 mL/kg), C48/80(2.5 mL/kg), 1%TA (5 mL/kg) and XSTI (3 mL/kg) (Table 5).

Discussion

In this study we found that when administrating treatment using the lower dose of OVA, 10% T80, 1% TA, BSA, SMI and XSTI, there was no obvious anaphylactoid symptom in any of the four rodent types. BN rats and guinea pigs showed little sensitivity to these antigens with relatively weak symptoms, such as trembling, scratching and rubbing around the nose which are considered non-typical symptoms; BN rats and guinea pigs also expressed typical symptoms such as dyspnea, myasthenia of the limbs et al. In the case of higher

Table 3. Evaluation of anaphylactoid reaction in guinea pigs

Group	Dose (mL/kg)	Judgment of the typical symptom score						Positive reaction（%）	Plasma histamine levels (ng/mL)	Secretion rate of histamine (%)	A#	Evaluation result
		0	1	2	3	4	Mean					
Control	5	6	0	0	0	0	0	0	5.77±0.09	-	-	Negative
OVA	2.2	6	0	0	0	0	0	0	6.79±0.40	17.66±7.03	0	Negative
	4.4	5	1	0	0	0	0.17±0.41	16.7	7.50±0.55*	30.04±9.62	0	Negative
10%T80	2.2	5	1	0	0	0	0.17±0.41	16.7	7.29±0.74	26.29±12.87	0	Negative
	4.4	2	1	3	0	0	1.16±0.98	66.7*	8.87±1.39**	53.71±24.04	3	Suspected
BSA	2.2	6	0	0	0	0	0	0	5.76±0.26	-0.14±4.46	0	Negative
	4.4	5	1	0	0	0	0.17±0.41	16.7	6.45±0.67	11.80±11.54	0	Negative
C48/80	1.1	0	0	1	5	0	2.83±0.41	100**	17.60±1.98**	205.12±34.36	5	positive
	2.2	0	0	0	0	6	4	100**	31.88±2.80**	452.60±48.49	6	Strong positive
1%TA	2.2	6	0	0	0	0	0	0	5.93±0.50	2.82±8.62	0	Negative
	4.4	0	2	3	1	0	1.83±0.75	100**	8.70±1.43**	50.75±24.70	3	Suspected
SMI	1.6	6	0	0	0	0	0	0	5.74±0.26	-0.45±4.43	0	Negative
	4.8	5	1	0	0	0	0.17±0.41	16.7	7.40±0.50	28.21±8.75	0	Negative
XSTI	0.65	6	0	0	0	0	0	0	5.84±0.28	1.29±4.80	0	Negative
	2.6	2	1	2	1	0	1.33±1.21	66.7*	26.05±3.61**	351.57±62.62	4	positive

*P <0.05, **P <0.01 compared with control group. A# mean the A value.

dose administration, all animals presented more serious anaphylactoid symptoms after intravenous injection of C48/80, in contrast, no changes were observed in the four animal models with BSA. In addition, BN rats were the most sensitive to TA and XSTI in the evaluation of symptoms, followed by guinea pigs. These observation suggest that different animal produce different immune responses upon challenge with different allergen and it was dose dependent.[17]

FDA. Guidance for Industry -- Immunotoxicology Evaluation of Investigational New Drugs (2002) pointed out that the existing methods (including ASA and PCA) for detecting the ability of large molecular substances to produce signs of anaphylactoid reaction might not be appropriate for determining the sensitizing potential of nonreactive small molecule weight drugs. Moreover, the suggestion of anaphylactoid reaction was motivated if signs of anaphylactoid reaction are observed in animal studies as well as follow-up studies. However, different animal exhibited distinct sensitivity to different compounds, as well as diverse anaphylactoid symptoms.[18] For instance, histamine level, an early diagnostic marker which had been described as a "gold standard" for acute allergic reaction assessment, was selected to evaluate drug anaphylactoid reaction in this study.[19-20] The secretion rate of histamine could complement the inadequate evaluation of anaphylatoid reaction, and eliminate the differentia of histamine of different animal itself, hence representing a reliable mediator.[2] Histamine levels in guinea pigs were particularly reactive to OVA, 10% T80, SMI; in BN rats histamine levels varied mostly following 1% TA and XSTI injection; in SD rats and guinea pigs it was after injection of C48/80 that histamine levels increased the most. Our results further suggest that the animal models used inthis study present variable degrees of histamine expression when challenged with diverse antigens. Additionally, the clinical symptoms were related to the histamine concentration in blood: no symptoms if histamine <1 ng/mL, only skin response if 1-2 ng/mL, systemic reaction if 3 ng/mL, and severe reaction if >100 ng/mL (mainly manifested as cardiovascular and respiratory symptoms (allergic shock)).[20] Therefore a suitable animal model should enable reactions that not only reproduce the clinical and functional characteristics of the specific disease but also mimic the basic mechanism involved.

Because of the limitations of symptom scores and the histamine level use to evaluate anaphylactoid reaction, we constructed the A value score which

Table 4. Evaluation of anaphylactoid reaction in ICR mice

Group	Dose (mL/kg)	Judgment of the typical symptom score						Positive reaction (%)	Plasma histamine levels (ng/mL)	Secretion rate of histamine (%)	A#	Evaluation result
		0	1	2	3	4	Mean					
Control	20	8	0	0	0	0	0	0	22.12±1.67	-	-	Negative
OVA	7	8	0	0	0	0	0	0	22.86±3.16	3.36±14.28	0	Negative
	20	8	0	0	0	0	0	0	23.75±2.19	7.40±9.91	0	Negative
10%T80	7	8	0	0	0	0	0	0	21.84±1.73	-1.26±7.83	0	Negative
	20	4	4	0	0	0	0.50±0.53	50*	37.94±5.07**	71.55±22.95	2	Suspected
BSA	7	8	0	0	0	0	0	0	21.23±1.98	-3.91±8.94	0	Negative
	20	8	0	0	0	0	0	0	23.17±1.86	4.76±8.42	0	Negative
C48/80	3.5	8	0	0	0	0	0	0	26.81±3.08**	21.21±13.91	0	Negative
	20	0	0	0	0	8	4	100**	53.83±3.39**	143.40±15.31	6	Strong positive
1%TA	7	8	0	0	0	0	0	0	21.58±1.93	-2.44±8.72	0	Negative
	20	6	2	0	0	0	0.25±0.46	25	26.80±1.40**	21.17±6.33	0	Negative
SMI	8	8	0	0	0	0	0	0	21.82±1.86	-1.36±8.41	0	Negative
	20	8	0	0	0	0	0	0	23.44±2.10	5.99±9.50	0	Negative
XSTI	1	8	0	0	0	0	0	0	21.97±1.22	-0.66±5.51	0	Negative
	4	5	3	0	0	0	0.38±0.52	37.5	26.34±3.07**	19.10±13.88	0	Negative

*P <0.05, **P <0.01 compared with control group. A# mean the A value.

combined both measures for a more comprehensive evaluation.[2] In BN rats the A value was the most sensitive to 1% TA and XSTI injections; In guinea pigs the A value was the most sensitive to 10% T80 injection while we found no difference in A value after injection of OVA, BSA, SMI and C48/80. When challenged with the maximum dose, BN rats were more sensitive to OVA and 10% T80 than other animals; SD rats were sensitive to SMI to the same extent as BN rats.

BSA is a well-known 68-kDa protein implicated in some cases of food hypersensitivity reactions. BSA can be present in culture medium used for artificial insemination and severe allergic reactions have been reported.[21-22] BSA had also been identified as a minor allergen in bovine dander and serum.[23] Our study showed however that the four kinds of animals exhibited little sensitivity to BSA whether using symptoms evaluation or histamine level as evaluation standards. Ther is still some debate regarding the immune-dominance of OVA as the major egg allergen, while it has been identified as a significant allergen associated to food hypersensitivity which is also as a positive control for type I allergy.[24] All animals in our study presented significant increases of histamine levels with non-typical symptoms following injection with OVA in contrast with what we observed for BSA. We suspect BSA to display a relatively high similarity to the mouse, rat and guinea pig serum albumins as compared to OVA,[25] thus explaining the relatively marked difference between OVA and BSA induced reactions. T80 is often used as a positive control for the evaluation of anaphylaxis or anaphylactoid reaction of Beagle dogs, because it has been identified as one of the main cause of anaphylactoid reaction, which status is confirm by our results.[26] TA is an exogenous substance suggested to be capable of inhibiting allergic reactions and which might be useful for the treatment or prevention of type I allergic diseases.[27] However it is also suspected to be an anaphylactoid potential allergen[28] which can cause significant anaphylactoid reaction when used in high concentration as demonstrated in our study. C48/80 is a well-known activator of mast cells via phospholipase D and heterotrimeric GTP-binding proteins.[29] It is also known to be a potent inducer of degranulation and non-specific anaphylactoid reaction, responsible for the release of histamine.[13] XSTI could stimulate the histamine release from the degranulation of mast cells and RBL-2H3 cells.[14] To further optimize the animal models for anaphylactoid reaction, the above antigens and saponins TCMIs were selected in this

Table 5. IgE and CH50 levels in BN rats

Group	Dose (mL/kg)	Plasma IgE levels (U/mL)	CH50 (U/μL)
Control	5	1.45±0.44	4.72±0.74
OVA	5	1.94±0.14	4.15±0.45
	20	2.67±0.52**	4.01±1.12
10%T80	5	1.81±0.45	2.88±0.72*
	15	1.56±0.72	0.95±0.32**
BSA	2.5	1.71±0.47	3.79±0.22
	5	1.69±0.57	4.01±0.24
C48/80	1.25	1.50±0.49	3.37±0.58
	2.5	2.25±0.35**	2.07±0.61**
1%TA	2. 5	1.98±0.29*	3.34±0.48
	5	2.53±0.48**	0.79±0.12**
SMI	5.5	1.85±0.39	3.38±0.55
	20	2.07±0.37*	3.40±0.42
XSTI	0.75	1.56±0.41	3.37±0.53
	3	1.42±0.37	1.01±0.29**

*P <0.05, **P <0.01 compared with control group.

study for comprehensive evaluation. We found that different kinds of animals presented diverse sensitivity to different antigens. BN rats were the most sensitive to OVA, TA, T80, XSTI and SMI, followed by guinea pigs, which presented some limitations as a suitable model, including significant differences in immune-physiology as compared to other species as well as a lack of tools to study its immune system.[30]

The preliminary mechanism of anaphylactoid reaction was explored on the BN rats. We found that no regular IgE levels responses were observed with the antigens, but OVA and SMI may have a role in IgE allergy modulation. C48/80 and 1% TA may be anaphylatoxin-mediated C activation-related pseudoallergy (CARPA) and IgE plus anaphylatoxin double triggered reactions. 10% T80 and XSTI could decrease the CH50 through the consumption of complements to increase the complex of complement activation in the process of anaphylactoid reaction.[31]

In summary, BN rats were more suitable for the comprehensive evaluation of anaphylactoid reaction following injections; the IgE levels and CH50 could be used as auxiliary mediators for the evaluation of anaphylactoid reaction.

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