Toxicon:X 7 (2020) 100054

Contents lists available at ScienceDirect

Toxicon: X

journal homepage: www.journals.elsevier.com/toxicon-x

Serum IgE against galactose-alpha-1,3-galactose is common in Laotian patients with snakebite envenoming but not the major trigger for early anaphylactic reactions to antivenom

Joerg Blessmann [a,b,*], Soulaphap Hanlodsomphou [b], Bounlom Santisouk [b], Khamla Choumlivong [b], Somphet Soukhaphouvong [c], Phankham Chanthilat [d], Knut Brockow [e], Tilo Biedermann [e]

[a] Department of Infectious Disease Epidemiology, Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany
[b] Setthatirath Hospital, Vientiane, Laos
[c] Savannakhet Provincial Hospital, Savannakhet Province, Laos
[d] Vientiane Provincial Hospital, Vientiane Province, Laos
[e] Department of Dermatology and Allergy Biederstein, School of Medicine, Technical University of Munich, Munich, Germany

ARTICLE INFO

Keywords:
Snakebite
Galactose-alpha-1,3-galactose
Anaphylaxis
Snake antivenom
Laos

ABSTRACT

Snake antivenom is the only specific treatment for snakebite envenoming, but life-threatening anaphylaxis is a severe side effect and drawback for the use of these typically mammalian serum products. The present study investigates the hypotheses whether serum IgE antibodies against the epitope galactose-alpha-1,3-galactose (α-gal) located on the heavy chain of non-primate mammalian antibodies are a possible cause for hypersensitivity reactions to snake antivenom. Serum samples from 55 patients with snakebite envenoming were obtained before administration of snake antivenom and tested for serum IgE (sIgE) against α-gal and total IgE. Early anaphylactic reactions (EARs) during the first 3 h after antivenom administration were classified into mild, moderate or severe and correlated with the presence of sIgE against α-gal. Fifteen (27%) out of 55 patients (37 male, 18 female, median 34 years, range 9–90 years) developed EARs after antivenom administration. Eleven, three and one patients had mild, moderate and severe EARs, respectively. Serum IgE against α-gal was detected in 17 patients (31%); in five (33%) out of 15 patients with EARs and in 12 (30%) out of 40 patients without EAR (Odds Ratio = 1.2; 95%-confidence interval: 0.3–4.2) with no correlation to severity. Although the prevalence of serum IgE against α-gal was high in the study population, very high levels of total IgE in the majority of patients question their clinical relevance and rather indicate unspecific sIgE binding instead of allergy. Lack of correlation between α-gal sIgE and EARs together with significantly increased total IgE levels suggest that sIgE against α-gal is not the major trigger for hypersensitivity reactions against snake antivenom.

1. Introduction

Snake antivenom containing mammalian immunoglobulins is the only specific treatment for snakebite envenoming in order to neutralize snake venom toxins and mitigate the progression of their toxic effects. Unfortunately, hypersensitivity reactions and sometimes life-threatening anaphylaxis occur in a significant number of cases during the first 3 h after administration of these heterologous antibodies. The rate of these early anaphylactic reactions (EARs) against snake antivenom varies depending on the product and region between 3% and 88% and usually poor physicochemical quality is blamed for it (De Silva HA et al., 2015; Prabhakar et al., 2014). However, the rate of EARs against the same horse-derived $F(ab')_2$ antivenom from Queen Saovabha Memorial Institute (QSMI), Bangkok, Thailand was much higher (53%) in a rural population in Savannakhet province in Lao People's Democratic Republic (Lao PDR) studied between 2013 and 2015 than in an urban population (3.5%) in Bangkok, Thailand, investigated between 1997 and 2006, indicating that host factors may play a role as well (Vongphoumy et al., 2016; Thiansookon and Rojnuckarin, 2008). However, the comparability of the studies is rather limited. Data on

* Corresponding author. Department of Infectious Disease Epidemiology, Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany.
E-mail address: blessmann@bnitm.de (J. Blessmann).

https://doi.org/10.1016/j.toxcx.2020.100054
Received 25 May 2020; Received in revised form 30 June 2020; Accepted 22 July 2020
Available online 28 July 2020

EARs were collected prospectively in Laos and retrospectively in Thailand, different batches of antivenom were used and the mode of administration was not standardized. Currently, the vast majority of antivenom-induced anaphylactic reactions are considered to be non-IgE-mediated hypersensitivity reactions. However, IgE-mediated anaphylaxis after sensitization to snake antivenom or other horse-derived serum products in the past is another possible cause. Actually, the exact mechanism is not yet known and EARs are most likely based on several underlying mechanisms (Leon et al., 2013; Stone et al., 2013). Recently IgE antibodies against the oligosaccharide galactose-alpha-1,3-galactose (α-gal), located on the heavy chain of non-primate mammalian immunoglobulins has been associated with two distinct forms of anaphylaxis, (i) immediate onset anaphylaxis to intravenous cetuximab and (ii) delayed onset anaphylaxis 3–6 h after ingestion of mammalian meat (Berg et al., 2014; Fischer et al., 2016; Steinke et al., 2015). Cetuximab is a chimeric mouse-human IgG_1 monoclonal antibody against the epidermal growth factor receptor, approved for use in colorectal cancer and squamous-cell carcinoma of the head and neck. Allergic reactions against Cetuximab are caused by serum IgE (sIgE) antibodies against α-gal, located on the antigen binding fragment (Chung et al., 2008). Tick bites induce the production of sIgE against α-gal (Commins et al., 2011). Therefore, prevalence of sIgE against α-gal is high in farmers and forestry workers and it is also expected to be high in snakebite victims, who usually belong to this occupational group (Fischer et al., 2017b; Gonzales-Quintela et al., 2014).

The α-gal epitope is present on horse-derived IgG in polyclonal IgG preparations of snake antivenoms and recently the reactivity of sIgE in individuals with α-gal allergy to red meat has been demonstrated against different antivenoms including monovalent green pit viper and Malayan pit viper antivenom from QSMI, Bangkok, Thailand, using basophil activation tests (Fischer et al., 2017a). The present study investigates the hypothesis whether sIgE against α-gal is responsible for hypersensitivity reactions in Laotian patients treated with snake antivenom from QSMI.

2. Methods

Between May and November 2018, 58 patients of all ages with snakebite envenoming were included into the study in three different hospitals, namely Savannakhet Provincial Hospital, Vientiane Provincial Hospital and Setthatirath Hospital located in the capital Vientiane, Lao PDR, after indication for antivenom administration had been established. Prior to antivenom administration serum samples were taken from each patient and frozen at −20 °C. In January 2019 they were tested for specific IgE against α-gal using an ImmunoCAP containing bovine thyroglobulin and for total IgE (Thermo Fisher Scientific, Uppsala, Sweden) at the department of Dermatology and Allergy Biederstein, School of Medicine, Technical University of Munich, Germany. The test was considered negative at sIgE concentration of <0.35 KU_A/l. During the first 3 h after administration of antivenom all patients were followed for occurrence of early anaphylactic reactions (EARs). EARs were classified as mild, moderate and severe reactions according to Brown's classification (Brown, 2014). Mild EARs were only skin reactions, like urticaria or erythema with or without angioedema. Moderate EARs were respiratory distress with decreasing peripheral oxygen saturation (SpO_2) not less than 92%, chest and throat tightness, wheezing, nausea, vomiting and diarrhoea. Severe EARs were cyanosis with SpO_2 < 92%, arterial hypotension with systolic blood pressure <90 mmHg and/or loss of consciousness. Monovalent and polyvalent horse-derived $F(ab')_2$ snake antivenom from QSMI in Bangkok, Thailand was used for treatment of envenoming.

The Chi-square test was applied to calculate the Odds Ratio of the data set. Comparisons between groups were made using the Mann-Whitney *U* test. The study was primarily explorative without application of sample size calculation.

2.1. Ethical statement

The study was approved by the National Ethics Committee for Health Research at the Lao Tropical and Public Health Institute (Lao TPHI) in Vientiane, Lao PDR (No. 038) in April 2018. Written informed consent was obtained from each patient or their legal representative.

3. Results

Fifty-eight patients were recruited between May and November 2018, including four patients from whom serum samples and follow up data of 2014 were available. Three patients were excluded, because serum samples had been obtained after antivenom administration. Characteristics and laboratory results of 55 patients included into the study are outlined in Table 1. Twenty-three patients were recruited at Vientiane Provincial Hospital, 16 at Savannakhet Provincial Hospital and 16 at Setthatirath Hospital. The majority of study participants were male (n = 37, 67%). The median age was 34 years (range 9–90). All snakebite victims lived in rural areas and were engaged in agricultural and/or forestry work. Monovalent Malayan pit viper, green pit viper and cobra antivenom was given to 30, 13 and one patient(s) and polyvalent haematotoxic and neurotoxic antivenom to six and five patients, respectively. Serum IgE (sIgE) concentration against α-gal was >0.35 KU_A/l in 17 (31%) individuals. Two patients had sIgE concentrations between 0.35 and 0.69 KU_A/l, 13 patients between 0.70 and 3.5 KU_A/l and two patients between 3.5 and 17.5 KU_A/l. Fifteen (27%) out of 55 patients developed EARs after antivenom administration. Eleven, three and one patient had mild, moderate and severe EARs, respectively. Serum IgE concentration was >0.35 in 5 (33%) out of 15 patients with EARs and in 12 (30%) out of 40 patients without EARs (Odds Ratio = 1.2; 95%-confidence interval: 0.3–4.2). Three out of eleven patients with mild EARs and two out of four patients with moderate to severe EARs had sIgE against α-gal. Of those two patients with highest sIgE, one developed a moderate EAR. Eleven out of 44 patients (25%), who received monovalent antivenom and four out of 11 patients (36%) who received polyvalent antivenom developed EARs (p = 0.22). In the majority of patients total serum IgE (tIgE) levels were very high (median: 2097 kU/l; range 109–19,315). They were >300 kU/l in all 17 patients with positive sIgE against α-gal (median 5068 kU/l; range: 391–19315

Table 1
Characteristics and laboratory results of 55 study patients.

	n = 55 (%)
Gender male	37 (67)
female	18 (33)
Age (years) median, range	34, 9–90
EARs total	15 (27)
mild	11
moderate	3
severe	1
EARs after monovalent antivenom (n = 44)	11
EARs after polyvalent antivenom (n = 11)	4
Serum IgE against α-gal	
negative (< 0.35 KU_A/l)	38 (69)
positive (≥ 0.35 KU_A/l)	17 (31)
0.35–0.69 KU_A/l	2
0.70–3.50 KU_A/l	13
3.51–17.5 KU_A/l	2
	Median (range)
tIgE < 100 kU/l (n = 6)	62 (5–87)
> 100 kU/l (n = 49)	2097 (109–19,315)
tIgE in patients with positive sIgE (n = 17)	5068 (391–19,315)
tIgE in patients with negative sIgE (n = 38)	1120 (5–18,948)
sIgE/tIgE (%) in patients with EARs (n = 15)	0.017 (0.002–0.268)
sIgE/tIgE (%) in patients without EARs (n = 40)	0.015 (0.001–2.46)
sIgE/tIgE (%) in patients with positive sIgE (n = 17)	0.028 (0.004–0.392)
sIgE/tIgE (%) in patients with negative sIgE (n = 38)	0.011 (0.001–2.46)

Abbreviations: tIgE = total IgE, sIgE = serum IgE against α-gal, EARs = early anaphylactic reactions.

kU/l) and significantly higher ($p < 0.001$) as compared to those with negative α-gal sIgE (median 1120 kU/l; range: 5–18948). In contrast, the sIgE/tIgE ratio (%) was not different between those with anaphylaxis (n = 15; median: 0.017%; range 0.002–0.268) as compared to those without anaphylaxis (n = 40; median: 0.015%; range 0.001–2.46) and in those with positive sIgE against α-gal (n = 17; median: 0.028%; range 0.004–0.392) as compared to those with negative sIgE against α-gal (n = 38; median: 0.011%, range 0.001–2.46).

4. Discussion

The oligosaccharide α-gal is found on the heavy chain of the antigen binding fragment of mammalian IgG antibodies and is a possible target of IgE-mediated reactivity to antivenoms, containing mammalian full IgG or $F(ab)_2$/Fab antibodies (Fischer et al., 2017a).

The results of the present study showed a high prevalence of sIgE against α-gal in 17 out of 55 (31%) snakebite patients recruited at three hospitals in Lao PDR. All snakebite victims worked in agriculture and/or forestry, where they had been exposed to tick bites, which induce sIgE formation against α-gal possibly explaining the finding (Commins et al., 2011). Although tick bites were not explicitly recorded, the presence of at least 22 ixodid tick species in Laos, representing six genera and the documented occurrence of tick-borne rickettsial disease, suggest that tick bites are very common among the rural population (Vongphayloth et al., 2016; Mayxay et al., 2013). A similar high prevalence of sIgE against α-gal is found in forestry workers and hunters in the United States of America and in Germany (Berg et al., 2014; Fischer et al., 2017b). The present data didn't show any correlation between the rate of early anaphylactic reactions (EARs) and elevated sIgE against α-gal. However, determination of tIgE showed very high values with a median value of 2097 kU/l. In addition, tIgE was significantly higher in patients with positive sIgE against α-gal as compared to those without, whereas the sIgE/tIgE ratio was similar indicating clinically irrelevant very low affinity sIgE or even unspecific binding in the assay for sIgE. A high ratio of >0.75% has been proposed for correct discrimination of patients with α-gal allergy from controls in a South African population with a high prevalence of reported red meat allergy, which was, however, reached only in one of our patients who did not have anaphylaxis (Mabelane et al., 2018). Parasitic infestation in Laotian people is high, particularly with Opisthorchis viverrini and hookworms which is one possible explanation for the extremely high levels of total IgE (Somphou et al., 2011).

The present EAR rate of 27% is significantly lower compared to a rate of 53% observed in Savannakhet Province, Lao PDR, between 2013 and 2015 using a different batch of antivenom of the same manufacturer. It indicates that product quality and/or different modes of administration might affect EAR rate. There was no significant difference in the occurrence of EAR between monovalent and polyvalent antivenom, but figures are too low to draw any meaningful conclusions. The limitation of this study is the low number of patients with EARs, particularly with moderate and severe EARs. However, the comparable distribution of specific serum IgE concentration rates between EAR-groups together with very high levels of total IgE suggest that sIgE against α-gal in the tested individuals appear not to be responsible for the observed hypersensitivity reactions against snake antivenom.

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Joerg Blessmann: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Project administration, Data curation, Writing - original draft, Writing - review & editing, Supervision, Funding acquisition. **Soulaphap Hanlodsomphou:** Investigation, Data curation, Writing - review & editing, Supervision. **Bounlom Santisouk:** Investigation, Data curation, Writing - review & editing. **Khamla Choumlivong:** Investigation, Project administration, Writing - review & editing. **Somphet Soukhaphouvong:** Investigation, Data curation, Writing - review & editing. **Phankham Chanthilat:** Investigation, Data curation, Writing - review & editing. **Knut Brockow:** Conceptualization, Methodology, Formal analysis, Investigation, Resources, Data curation, Writing - review & editing, Supervision, Funding acquisition. **Tilo Biedermann:** Conceptualization, Methodology, Formal analysis, Writing - review & editing, Supervision, Funding acquisition.

Acknowledgments

We would like to thank all nurses, laboratory technicians and medical doctors of Savannakhet, Vientiane and Setthatirath Hospital for their valuable support of this study. We are grateful to the laboratory staff at the department of Dermatology and Allergy Biederstein in Munich, for serum sample testing.

This work was supported by Else Kröner-Fresenius Stiftung, Bad Homburg, Germany (Grant Number: 2016_HA68) and by grants from the Deutsche Forschungsgemeinschaft, German Research Foundation (BI696/10–1, SFB 1335 P17, SFB1371 P06).

References

Berg, E.A., Platts-Mills, T.A.E., Commins, S.P., 2014. Drug allergens and food – the cetuximab and galactose- α-1,3-galactose story. Ann. Allergy Asthma Immunol. 112 (2), 97–101. https://doi.org/10.1016/j.anai.2013.11.014.

Brown, S.G.A., 2004. Clinical features and severity grading of anaphylaxis. J. Allergy Clin. Immunol. 114 (2), 371–376.

Chung, C.H., Mirakhur, B., Chan, E., Le, Q.T., Berlin, J., Morse, M., Platt-Mills, T.A.E., et al., 2008. Cetuximab-induced anaphylaxis and IgE specific for galactose- α-1,3-galactose. N. Engl. J. Med. 358 (11), 1109–1117.

Commins, S.P., James, H.R., Kelly, L.A., Pochan, S.L., Workman, L.J., Perzanowski, M.S., et al., 2011. The relevance of tick bites to the production of IgE antibodies to the mammalian oligosaccharide galactose-α-1,3-galactose. J. Allergy Clin. Immunol. 127, 1286–1293. https://doi.org/10.1016/j.jaci.2011.02.019.

De Silva, H.A., Ryan, N.M., de Silva, H.D., 2015. Adverse reactions to snake antivenom and their prevention and treatment. Br. J. Clin. Pharmacol. 81 (3), 446–452. https://doi.org/10.1111/bcp.12739.

Fischer, J., Yazdi, A.S., Biedermann, T., 2016. Clinical spectrum of α-gal syndrome: from immediate-type to delayed immediate-type reactions to mammalian innards and meat. Allergo J Int 25, 55–62. https://doi.org/10.1007/s40629-016-0099-z.

Fischer, J., Eberlein, B., Hilger, C., Eyer, F., Eyerich, S., Ollert, M., Biedermann, T., 2017a. Alpha-gal is a possible target of IgE -mediated reactivity to antivenom. Allergy 72, 764–771.

Fischer, J., Lupberger, E., Hebsacker, J., Blumenstock, G., Aichinger, E., Yazdi, A.S., Reick, D., Oehme, R., Biedermann, T., 2017b. Prevalence of type I sensitization to alpha-gal in forest service employees and hunters. Allergy 72, 1540–1547. https://doi.org/10.1111/all.13156.

Gonzales-Quintela, A., Dam Laursen, A.S., Vidal, C., Skaaby, T., Gude, F., Linneberg, A., 2014. IgE antibodies to alpha-gal in the general adult population: relationship with tick bites, atopy, and cat ownership. Clin. Exp. Allergy (44), 1061–1068.

Leon, L., Herrera, M., Segura, A., Villalta, M., Vargas, M., Guitierrez, J.M., 2013. Pathogenic mechanism underlying adverse reactions induced by intravenous administration of snake antivenoms. Toxicon 76, 63–76.

Mabelane, T., Basera, W., Botha, M., Thomas, H.F., Ramjith, J., Levin, M.E., 2018. Predictive values of alpha-gal IgE levels and alpha-gal IgE:Total IgE ratio and oral food challenge-proven meat allergy in a population with a high prevalence of reported red meat allergy. Pediatr. Allergy Immunol. 29, 841–849.

Mayxay, M., Castonguay-Vanier, J., Chansamouth, V., Dubot-Peres, A., Paris, D.H., Phetsouvanh, R., et al., 2013. Causes of non-malarial fever in Laos: a prospective study. Lancet Glob Health 1, e46–54.

Prabhakar, D.R., Motiram, M.V., Ghanshyam, B.C., 2014. Antivenoms in snake envenoming: are they safe? J. Clin. Toxicol. 4 (2) https://doi.org/10.4172/2161-0495.1000184.

Somphou, S., Mak, T.K., Vanmany, M., Rasphone, O., Vounatsou, P., Utzinger, J., et al., 2011. Helminth and intestinal Protozoa infections, multiparasitism and risk factors in champasack province, Lao People's democratic republic. PLoS Neglected Trop. Dis. 5 (4), e1037. Apr 12.

Steinke, J.W., Platts-Mills, T.A.E., Commins, S.P., 2015. The alpha-gal story: lessons learned from connecting the dots. J. Allergy Clin. Immunol. 135 (3), 589–596.

Stone, S.F., Isbister, G.K., Shahmy, S., Mohamed, F., Abeysinghe, C., Karunathilake, H., Ariaratnam, A., Jacoby-Alner, T.E., Cotterell, C.L., Brown, S.G.A., 2013. Immune

response to snake envenoming and treatment with antivenom: complement activation, Cytokine production and Mast cell Degranulation. PLoS Neglected Trop. Dis. 7 (7), e2326 https://doi.org/10.1371/journal.pntd.0002326.
Thiansookon, A., Rojnuckarin, P., 2008. Low incidence of early reactions to horse-derived F(ab')$_2$ antivenom for snakebites in Thailand. Acta Trop. 105, 203–205.
Vongphayloth, K., Brey, P., Robbins, R.G., Sutherland, I.W., 2016. First survey of the hard tick (Acari: ixodidae) fauna of Nakai District, Khammouane Province, Laos, and an updated checklist of the ticks of Laos. Syst. Appl. Acarol. 21 (2), 166–180.
Vongphoumy, I., Chantilat, P., Vilayvong, P., Blessmann, J., 2016. Prospective, consecutive case series of 158 snakebite patients treated at Savannakhet provincial hospital, Lao People's Democratic Republic with high incidence of anaphylactic shock to horse-derived F(ab)$_2$ antivenom. Toxicon 117, 13–21.