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# Comparative abilities of IgG and F(ab')$_2$ monovalent antivenoms to neutralize lethality, phospholipase $A_2$, and coagulant activities induced by *Daboia siamensis* venom and their anticomplementary activity

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**ABSTRACT**: The ability of monovalent IgG and F(ab')$_2$ antivenoms to neutralize lethality, phospholipase $A_2$, and coagulant activities induced by *Daboia siamensis* venom was studied. Both antivenoms were produced from the same batch of hyperimmune horse plasma and were adjusted to the same potency against the lethal effect of *D. siamensis* venom in experiments involving preincubation of venom and antivenom. Intact neutralization experiments involving independent injection of venom and antivenoms showed that the F(ab')$_2$ antivenom was slightly more effective. Significant differences in favour of F(ab')$_2$ antivenom were observed with respect to neutralization of phospholipase $A_2$ and coagulant activities. Both IgG and F(ab')$_2$ antivenoms were able to activate human complement in vitro. IgG antivenom had a significantly higher anticomplementary activity than F(ab')$_2$ antivenom.

**KEYWORDS**: equine monovalent antivenoms, $LD_{50}$, $ED_{50}$, neutralization, clotting time

## INTRODUCTION

Antivenoms are used regularly worldwide for the treatment of severe snake-bite envenomations[1,2]. Over the past decade, the availability and quality of snake antivenoms has become an important issue of concern, especially in the rural tropics where the burden of disease is greatest[3]. There seems to be a strong belief that if antivenoms manufactured with good standard will be effective for most snake bites. The methods currently used to prepare these antivenoms have not advanced much from the method originally developed by Pope[4–6]. Either a monovalent or polyvalent antivenom can be produced from the hyperimmunization of a variety of animals, and is used in the form of whole immunoglobulins or digested with pepsin to produce the F(ab')$_2$ fraction. However, most manufacturers produce F(ab')$_2$ antivenoms in order to reduce anaphylactic reactions[7].

Many studies have been conducted to compare the effectiveness of IgG and F(ab')$_2$ antivenoms to neutralize systemic and local venom effects. Because of a small molecular weight, by pharmacokinetic studies F(ab')$_2$ fragments have shown a larger volume of distribution and a faster tissue compartment absorption rate than whole IgG antivenoms[8]. These results support the notion that F(ab')$_2$ antivenoms are more effective than IgG antivenoms at neutralizing venom in tissue. However, some studies show that IgG and F(ab')$_2$ antivenoms do not differ in their abilities to neutralize the systemic and local effects of *Bothrops asper* venom[9–14]. It has been shown by in vitro methods that F(ab')$_2$ antivenoms have higher anticomplementary activity than IgG precipitated by caprylic acid and thus decreasing the risk of early adverse reactions occurring after antivenom treatment[14,15]. Regarding side effects and safety of treatment it is important to study a variety of characteristics of the IgG and F(ab')$_2$ antivenoms.

IgG and F(ab')$_2$ antivenoms in the previous studies were polyvalent antivenoms. Most studies emphasized the neutralization of lethality and local effects; haemorrhagic, oedema-forming, and myotoxic activities of venom. In contrast to previous studies, here we consider the neutralization of lethality, coagulant, and phospholipase $A_2$ ($PLA_2$) activities of *D. siamensis* venom by equine monovalent IgG and F(ab')$_2$ *D. siamensis* antivenoms. The $PLA_2$, Daboiatoxin is the key lethal component of *D. siamensis* venom. $PLA_2$ is a multifunctional enzyme that catalyses the hydrolysis of the fatty acid ester bond at the position 2 of 1,2-diacyl-sn-3-phosphoglycerides to produce free

fatty acids and lysophospholipids[16,17]. Therefore $PLA_2$ can cause local cell- and tissue-damage, as well as systemic effects in snake bite victims. Antivenom neutralization of $PLA_2$, an important component of the venom in the pathophysiology of snake envenoming[16,18,19], deserves attention. Additionally, the anticomplementary activities of equine monovalent IgG and $F(ab')_2$ antivenoms were studied.

## MATERIALS AND METHODS

### Venom, antivenom, and animals

Lyophilized *Daboia siamensis* venom and IgG and $F(ab')_2$ equine monovalent antivenoms were provided by the Snake Farm and the Production Department of Queen Saovabha Memorial Institute (QSMI). The raw horse hyperimmune plasma against *D. siamensis* venom was divided into two parts. Part one was whole IgG antivenoms produced by fractionating plasma with caprylic acid precipitation. The another part was $F(ab')_2$ antivenoms produced by pepsin digestion and followed with caprylic acid precipitation[20]. The venom solution and reconstituted antivenoms were stored at 4 °C throughout the duration of the study. All mice used in the experiments were Swiss albino, strain ICR, weighing between 18 and 20 g from the Horse Farm and Animal Breeding Centre, Thai Red Cross Society. All experiments in animals were approved by the Ethical committee of Animal Affair of QSMI, Thai Red Cross Society (Protocol No. 1/2010, 12 October 2010).

### Neutralization studies

*Neutralization of lethality: preincubation type experiments:* The neutralizing capacity of antivenoms were determined against 2 $LD_{50iv}$s of *D. siamensis* venom in five mice for each test dilution of antivenoms. Four various dilutions of antivenoms were tested. The test solutions were preincubated with venom at 37 °C for 30 min. Aliquots of 0.2 ml of the mixtures, containing 2 $LD_{50iv}$s (7.42 µg) of *D. siamensis* venom, were injected through the caudal vein of mice. Controls included PBS alone, venom alone, and antivenom alone. Deaths were recorded over 24 h. The 50% effective dose ($ED_{50}$) was calculated by the method of Theakston and Reid[21]. Neutralization was expressed as mg of venom neutralized by 1 ml of antivenom.

*Neutralization of lethality: independent injection of venom and antivenom type experiments:* The independent injection of venom and antivenom type experiment was performed in which antivenoms were intravenously injected after envenomation[22]. Four groups of five mice received 81.2 µg of *D. siamensis* venom (corresponding to 3 $LD_{50ip}$s) by intraperitoneal injection. Three groups of mice were intravenously injected with 0.2 ml of 3 $ED_{50}$s antivenoms at various time intervals (0, 10, and 30 min) after envenomation. Number of deaths and time of death were recorded. The control group consisted of animals injected with venom alone.

*Neutralization of phospholipase $A_2$ activity:* The phospholipase $A_2$ activity of *D. siamensis* venom was determined by modified indirect haemolytic activity[23,24]. Briefly, 0.3 ml of packed human erythrocytes washed four times with saline solution, 0.3 ml egg yolk diluted 1:4 in PBS, and 0.25 ml of 0.01 M $CaCl_2$ were added to 25 ml of 0.8% agarose dissolved in PBS, pH 8.1. The mixture was cooled to 50 °C, and then poured into a plastic plate (135 × 80 mm) and allowed to congeal at room temperature. The 3.0 mm diameter wells were filled with 10 µl of venom solution in PBS. Five various dilutions of venom were tested. The plates were incubated at 37 °C for 20 h. The corresponding concentration of venom resulting in a 20 mm diameter haemolytic halo was 100% haemolytic activity used for neutralization test. This venom concentration was incubated at 37 °C for 30 min with four various dilutions of antivenoms and 10 µl aliquote of each mixture was placed on each well. Each mixture was assayed in triplicate. The plates were incubated at 37 °C for 20 h. The average diameter of haemolytic halo was used to calculate the percentage of inhibition of haemolytic activity. The neutralization activity of antivenom was expressed as effective dose 50%, defined as the amount of antivenom (µl) that inhibited 50% of haemolytic activity. 10 µl of PBS solution and 10 µl of antivenom were tested as controls.

*Neutralization of coagulant activity:* Fifty microlitres of $2LD_{50i.v.}$s venom was incubated at 37 °C for 30 min with various amount of antivenom and 0.01 M Tris buffer, pH 7.3 (containing 0.15 M NaCl). The total reaction volume of 100 µl and 100 µl of 0.05 M $CaCl_2$ were added to 100 µl of plasma. All tubes were placed in the 37 °C water bath and the clotting times recorded[25]. As control, the venom ($2LD_{50i.v.}$s) incubated with Tris buffer, without antivenom, was done in the same condition. The normal clotting time was recorded when plasma alone was incubated with Tris buffer and $CaCl_2$. Neutralization was expressed as the amount (µl) of antivenom at which clotting time was increased to normal clotting time. The clotting times at each amount of either IgG or $F(ab')_2$ antivenom were also compared.

**Table 1** Lethal effect neutralization of *D. siamensis* venom by antivenoms in experiment type involving independent injection of venom and antivenoms.

| Delay in antivenom administration (min) | Lethality (dead mice / total injected mice) | | Delayed death (delayed dead mice / total dead mice)[†] | |
|---|---|---|---|---|
| | IgG antivenom | $F(ab')_2$ antivenom | IgG antivenom | $F(ab')_2$ antivenom |
| 0 | 5/5 | 3/5 | 4/5 | 2/3 |
| 10 | 5/5 | 4/5 | 3/5 | 2/4 |
| 30 | 5/5 | 4/5 | 1/5 | 1/4 |

[†] Delayed dead mice means mice died later than the control group. Control mice injected with 3 $LD_{50ip}$s alone, and not receiving antivenom, died at $65.2 \pm 3.5$ min (mean $\pm$ SD; $n = 5$).

### Anticomplementary activity

After three times washing with phosphate buffer saline, sheep erythrocytes were resuspended in the same buffer and sensitized by rabbit anti-sheep erythrocyte serum (haemolysin). Seven dilutions of 1 ml antivenoms (either IgG or $F(ab')_2$) in PBS were incubated for 1 h at 37 °C with 0.25 ml fresh human serum diluted 1:5 with PBS. Then 0.25 ml of the sensitized erythrocyte suspension was added to each tube and the tubes were incubated for 1.5 h at 37 °C. The amount of 1.0 ml of cold PBS was added to each mixture in order to stop the reaction, and then each tube was centrifuged at 700$g$ for 10 min. The corresponding absorbances at 540 nm were recorded[26]. The results were expressed as percentage of remaining complement activity. Controls included incubation of human serum with PBS, in the absence of antivenom (100% complement activity), and incubation of antivenom dilutions with sensitized erythrocytes.

### Statistical analysis

Results were expressed as mean $\pm$ SD. The mean values of two experimental groups was determined the significance of the differences by the Paired $t$-test.

## RESULTS

### Characteristics of antivenoms: IgG and $F(ab')_2$

After fractionation process, both antivenoms had a minimum potency of 0.6 mg venom/ml antivenom when reconstituted to 10 ml in saline solution. They passed the quality requirements. A band of 100 kDa was observed in a preparation of $F(ab')_2$ fragments antivenom. While a preparation of IgG antivenom presenting one band of 150 kDa was obtained (results not shown).

### Lethality neutralization: Determination of $ED_{50}$

In preincubation type experiments, both the IgG and $F(ab')_2$ antivenoms were able to neutralize the lethality of 2 $LD_{50iv}$s *D. siamensis* venom when injected into the caudal vein of mice over a 24 h period. $ED_{50}$ were determined to be 12.4 mg venom neutralized per ml antivenom (95% confidence interval = 11.8–12.9 mg venom/ml antivenom) and 12.4 mg venom/ml antivenom (95% confidence interval = 12.0–12.7 mg venom/ml antivenom) for 10 ml reconstituted IgG and $F(ab')_2$ antivenoms, respectively.

The results from another series of experiments which antivenoms were administered after envenomation are shown in Table 1. After intraperitoneal injection of three $LD_{50ip}$s of venom, all the mice died at $65.2 \pm 3.5$ min. When 3 $ED_{50}$s of $F(ab')_2$ antivenom were administered by the i.v. route immediately, 10, or 30 min after envenomation, neutralization was partial. It showed different pattern for IgG antivenom. All the mice died although three $ED_{50}$s of IgG were immediately administered (Table 1).

### Phospholipase A2 neutralization activity

The concentration of venom that produced a 20 mm diameter haemolytic halo in the erythrocyte egg yolk agarose gel plate was determined to be 4.88 mg venom/ml PBS, pH 8.1. When either IgG or $F(ab')_2$ antivenom was incubated with this venom concentration, the neutralization of phospholipase activity were observed. The amount of antivenoms that inhibited 50% of haemolytic activity was 183 and 106 µl for IgG and $F(ab')_2$ antivenoms, respectively. (Fig. 1).

### Coagulant neutralization activity

Human plasma from the donor at the National Blood Bank, Thai Red Cross Society with added Tris buffer had a clotting time of $7.6 \pm 0.2$ min, whereas human plasma with 7.42 µg of *D. siamensis* venom had a clotting time of 30 s. Both antivenoms were gradually effective in neutralizing coagulation activity of *D. siamensis* venom when 1, 2, 4, and 6 µl were added (Table 2). The amount of antivenoms, indicated by plotting the amount of antivenom-clotting time curve

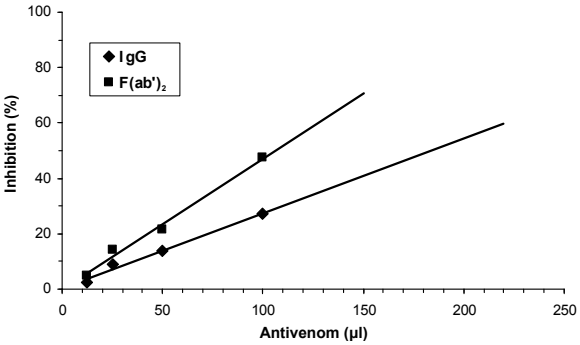


**Fig. 1** Neutralization of phospholipase $A_2$ activity of *D. siamensis* venom by IgG or F(ab')$_2$ antivenoms. The venom concentration resulting in 20 mm diameter haemolytic halo on erythrocyte egg yolk agarose gel plates was incubated at 37 °C, 30 min with varying dilutions of antivenom. The amount of antivenom that inhibited 50% of haemolytic activity (resulting in 10 mm diameter of haemolytic halo) was the neutralization activity.

**Table 2** Coagulant activity neutralization of *D. siamensis* venom by antivenoms in experiments with in vitro test.

| Amount of antivenom (µl) | Clotting time (min)[a] | |
|---|---|---|
| | IgG antivenom | F(ab')$_2$ antivenom |
| 1* | $1.17 \pm 0.26$ | $1.50 \pm 0.01$ |
| 2* | $1.25 \pm 0.27$ | $2.33 \pm 0.20$ |
| 4* | $3.17 \pm 0.52$ | $4.25 \pm 0.27$ |
| 6* | $8.42 \pm 0.74$ | $8.00 \pm 0.45$ |
| Normal Plasma | $7.58 \pm 0.20$ | |
| Control venom ($2LD_{50}$s) | $0.50 \pm 0.01$ | |

[a] Values correspond to mean $\pm$ standard deviation; $n = 6$.
* Significant difference when comparing that amount of IgG and F(ab')$_2$ ($p < 0.05$).

(not shown), that were used for returning clotting time of human plasma in the presence of venom to normal clotting time were 5.4 and 5.8 µl for IgG and F(ab')$_2$, respectively. F(ab')$_2$ antivenom showed significantly higher neutralizing ability than IgG at the amount of 1, 2, and 4 µl of antivenom ($p < 0.05$).

### Anticomplementary activity of antivenoms

IgG and F(ab')$_2$ antivenoms showed anticomplementary activity, measured by the consumption of human complement when various dilutions of the antivenoms were incubated with fresh human serum (Fig. 2). IgG antivenom showed a higher anticomplementary activity than F(ab')$_2$ antivenom (Fig. 2).

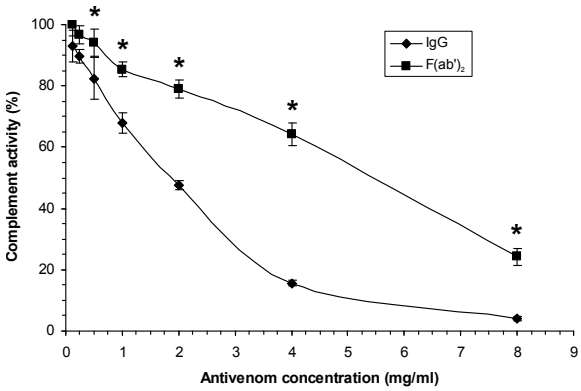


**Fig. 2** Anticomplementary activity of IgG or F(ab')$_2$ antivenoms. Seven dilutions of 1 ml antivenoms were incubated with 0.25 ml fresh diluted (1:5) human serum. Then, a suspension of sensitized sheep erythrocytes (0.25 ml) was added and the extent of haemolysis determined by recording the absorbance of supernatants at 540 nm. Complement activity is reported as percentage, taking as 100% the complement activity of human serum in absence of antivenom. Results are presented as mean $\pm$ SD ($n = 3$). (*) Significant difference when comparing amount of IgG and F(ab')$_2$ ($p < 0.05$).

## DISCUSSION

Nowadays, most antivenoms are either partially purified immunoglobulin (IgG) or F(ab')$_2$ depending on the method of refinement (extraction with caprylic acid or traditional digestion with pepsin). This has raised the question which is preferred between IgG or F(ab')$_2$ antivenoms. Some studies using *Bothrops asper* venom show that IgG and F(ab')$_2$ antivenoms do not differ in their abilities to neutralize venom with respect to systemic and local effects[9,10]. However, as an antivenom producer, it is important to produce the most effective antivenoms with minimal side effects. Our present studies were performed to compare the differences in *D. siamensis* IgG and F(ab')$_2$ antivenoms produced by QSMI.

The preincubation type assays carried out in mice have shown that IgG and F(ab')$_2$ antivenoms do not differ in their ability to neutralize $2LD_{50iv}$s of *D. siamensis* venom. Similar results were observed by Morais et al[9] and León et al[10,14] when the ability of IgG and F(ab')$_2$ antivenoms to neutralize the lethal activity of *Bothrops* species venoms were compared. The pharmacokinetic evidence showed that F(ab')$_2$ has a large volume of distribution and reach the tissue compartment faster[8,27–29]. The finding suggested that F(ab')$_2$ antivenom might be more effective than IgG antivenom in neutralizing lethal toxicity of venoms.

This pharmacokinetic characteristics may explain the results of our experiments with less lethality using $F(ab')_2$ antivenom indicating better neutralization shown in Table 1. It was observed that lethality induced by *D. siamensis* venom were partially neutralized if $F(ab')_2$ antivenom was administered immediately or 10 min after envenomation. Under the experimental conditions, the different results are considered to be due to pharmacokinetic parameters and not to variations in $ED_{50}$.

To confirm the differences in neutralizing ability of both antivenoms, neutralization of $PLA_2$, coagulation activities (Fig. 1 and Table 2) in *D. siamensis* venom, anticomplement activity (Fig. 2) of IgG and $F(ab')_2$ antivenoms were tested. Effective dose of $F(ab')_2$ in neutralizing $PLA_2$ activity was significantly lower than IgG. This is consistent with neutralization of lethality tested by independent injection of venom and antivenom which could reflect the better binding of small $F(ab')_2$ molecule to the binding site of $PLA_2$ molecule than the larger IgG molecule. The same explanation goes to the better neutralization of coagulant activity of $F(ab')_2$ over IgG antivenoms.

Antivenoms are composed of antibodies from immunized horses. The use of heterologous proteins, horse antibodies, for treatment results in the possibility of adverse reactions. Dependent on the dose, route and speed of administration, and the quality of refinement, the reactions may vary from mild symptoms like chills, nausea, and fever, to serious problems such as bronchospasms and anaphylactic shock. Adverse reactions after antivenom administration are of clinical concern[25,30]. The occurrence of adverse reactions has been reported to range from 6% up to 87% in different type of antivenoms[31–33]. Although the cause of these reactions are unclear, complement activation by antivenom proteins has been proposed to be the cause. Complement activation has been described in patients receiving either heterologous or homologous immunoglobulins. In vitro detection of complement activation by antivenoms has been used to predict their anticomplementary activity associated with potential adverse reactions in vivo[34]. The present investigations show that both antivenoms activate human complement in vitro, although $F(ab')_2$ had lower anticomplementary activity. This would indicate that removal of the Fc fragment of equine immunoglobulins by pepsin digestion reduced, but did not eliminate, anticomplementary activity. This has been previously observed[9,14,34]. Besides anticomplementary activity, immune response against heterologous protein were performed in the previous study of León et al[14]. A single intravenous dose of either IgG or $F(ab')_2$ equine antivenoms was injected into mice. Then, the antibody response to equine immunoglobulin was tracked using ELISA technique weekly during one month. Higher anti-immunoglobulin titres against IgG were presented in mice injected with IgG than in those receiving $F(ab')_2$ antivenom when plates were coated with equine IgG. No significant differences in antibody titres were detected between the groups when plates were coated with $F(ab')_2$ antivenom. This would suggest that the presence of an intact Fc does not enhance an anti-immunoglobulin response to $F(ab')_2$ fragments. The results of anticomplementary activity and immune response to antivenoms indicate that the presence of Fc in immunoglobulins are not the predominant factor in the development of adverse reactions after receiving antivenoms. None of the animals receiving whole IgG antivenom in the study of Estrada et al[35] showed any evidence of early adverse reaction. The factors might include the physicochemical condition of the immunoglobulins, the presence of molecular aggregates, and the total load of protein administered.

In this investigation, our prepared IgG and $F(ab')_2$ antivenoms were equally effective antivenoms. They were adjusted to have the same potency and the preincubation assay was performed in test tube. The antibodies in antivenoms reacted directly with venoms without influences from the body system. The same potency of both antivenoms neutralized the same amount of venoms. In the other experiment, the situation model of snake bite was performed in mice by the independent injection of venom and antivenom type assay. The single dose of antivenom was selected for the neutralization of lethality in order to express the difference in neutralizing ability between IgG and $F(ab')_2$ antivenoms. However, the neutralization of lethality in human envenomations, the repeated doses of antivenom is needed.

The following conclusions were drawn from the study: (1) No significant difference was observed between whole IgG and $F(ab')_2$ antivenoms in their ability to neutralize lethal toxicity of *D. siamensis* venom by in vitro test. (2) $F(ab')_2$ antivenom was more effective in inhibiting $PLA_2$ and coagulation activity. (3) Intact neutralization ability (in vivo neutralization) against lethality of venom by $F(ab')_2$ antivenom is more effective than IgG antivenom. (4) $F(ab')_2$ antivenom showed a lower anticomplementary activity than IgG antivenom. The results of the present study suggest that the appropriated form of snake antivenoms should be $F(ab')_2$ fragments.

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## REFERENCES


1. White J, Warrell D, Eddleston M, Currie BJ, Whyte IM, Isbister GK (2003) Clinical toxicology—Where are we now? *J Toxicol Clin Toxicol* **41**, 263–76.
2. Warrell DA (1992) The global problem of snake bite: its prevention and treatment. In: Golpalakrishnakone P, Tan CR (eds) *Recent Advances in Toxicology Research*, I. National Univ of Singapore, Singapore, pp 121–53.
3. Lalloo DG, Theakston RD, Warrell DA (2002) The African challenge. *Lancet* **359**, 1527.
4. Pope CG (1938) Desegregation of proteins by enzymes. *Br J Exp Pathol* **19**, 245–51.
5. Pope CG (1939) The action of proteolytic enzymes on the antitoxins and proteins in immune sera, I. True digestion of the proteins. *Br J Exp Pathol* **20**, 132–49.
6. Pope CG (1939) The action of proteolytic enzymes on the antitoxins and proteins in immune sera, II. Heat denaturation after partial enzyme action. *Br J Exp Pathol* **20**, 201–12.
7. Chippaux JP, Goyffon M (1998) Venoms, antivenoms and immunotherapy. *Toxicon* **36**, 823–46.
8. Ismail M, Abd-Elsalam MA, Al-Ahaidib MS (1998) Pharmacokinetics of $^{125}$I-labelled *Walterinnesia aegyptia* venom and its specific antivenins: flash absorption and distribution of the venom and its toxin versus slow absorption and distribution of IgG, F(ab')$_2$ and F(ab) of the antivenin. *Toxicon* **36**, 93–114.
9. Morais JF, de Freitas MC, Yamaguchi IK, dos Santos MC, da Silva WD (1994) Snake antivenoms from hyperimmunized horses: comparison of the antivenom activity and biological properties of their whole IgG and F(ab')$_2$ fragments. *Toxicon* **32**, 725–34.
10. León G, Rojas G, Lomonte B, Gutiérrez JM (1997) Immunoglobulin G and F(ab')$_2$ polyvalent antivenoms do not differ in their ability to neutralize hemorrhage, edema and myonecrosis induced by *Bothrops asper* (terciopelo) snake venom. *Toxicon* **35**, 1627–37.
11. Gutiérrez JM, León G, Rojas G, Lomonte B, Rucavado A, Chaves F (1998) Neutralization of local tissue damage induced by *Bothrops asper* (terciopelo) snake venom. *Toxicon* **36**, 1529–38.
12. León G, Stiles B, Alape A, Rojas G, Gutiérrez JM (1999) Comparative study on the ability of IgG and F(ab')$_2$ antivenoms to neutralize lethal and myotoxic effects induced by *Micrurus nigrocinctus* (coral snake) venom. *Am J Trop Med Hyg* **61**, 266–71.
13. León G, Valverde JM, Rojas G, Lomonte B, Gutiérrez JM (2000) Comparative study on the ability of IgG and Fab sheep antivenoms to neutralize local hemorrhage, edema and myonecrosis induced by *Bothrops asper* (terciopelo) snake venom. *Toxicon* **38**, 233–44.
14. León G, Monge M, Rojas E, Lomonte B, Gutiérrez JM (2001) Comparison between IgG and F(ab')$_2$ polyvalent antivenoms: neutralization of systemic effects induced by *Bothrops asper* venom in mice, extravasation to muscle tissue, and potential for induction of adverse reactions. *Toxicon* **39**, 793–801.
15. Otero-Patiño R, Cardoso JLC, Higashi HG, Nunez V, Diaz A, Toro MF, Garcia ME, Sierra A, et al (1998) A randomized, blinded, comparative trial of one pepsin-digested and two whole IgG antivenoms for *Bothrops* snake bites in Uraba, Colombia. *Am J Trop Med Hyg* **58**, 183–9.
16. Kini RM (2003) Excitement ahead: structure, function and mechanism of snake venom phospholipase $A_2$ enzymes. *Toxicon* **42**, 827–40.
17. Burke JE, Dennis EA (2009) Phospholipase $A_2$ structure/function, mechanism, and signaling. *J Lipid Res* **50**, Suppl, S237–42.
18. Mebs D (1998) Phospholipases $A_2$ that show myotoxic activity. In: Bailey GS (ed) *Enzymes from Snake Venom*. Alaken, Inc. Fort Collins, Colorado, pp 451–79.
19. Francis BR,Da Silva NJ, Kaiser II (1998) A new type of toxic snake venom phospholipases $A_2$ which promotes hypotension and hemorrhage in mice. In: Bailey GS (ed) *Enzymes from Snake Venom*. Alaken, Inc. Fort Collins, Colorado, pp 481–502.
20. Rojas G, Jiménez JM, Gutiérrez JM (1994) Caprylic acid fractionation of hyperimmune horse plasma: description of a simple procedure for antivenom production. *Toxicon* **32**, 351–63.
21. Theakston RD, Reid HA (1983) Development of simple standard assay procedures for the characterization of snake venom. *Bull World Health Organ* **61**, 949–56.
22. Chanhome L, Wongtongkam N, Khow O, Pakmanee N, Omori-Satoh T, Sitprija V (1999) Genus specific neutralization of Bungarus snake venoms by Thai Red Cross banded krait antivenom. *J Nat Toxins* **8**, 135–40.
23. Habermann E, Hardt KL (1972) A sensitive and specific plate test for the quantitation of phospholipases. *Anal Biochem* **50**, 163–73.
24. Gutiérrez JM, Avila C, Rojas E, Cerdas L (1988) An alternative in vitro method for testing the potency of the polyvalent antivenom produced in Costa Rica. *Toxicon* **26**, 411–3.
25. Hall GH (1956) The coagulant action of Russell viper venom investigated by the thromboplastin generation test. *J Clin Pathol* **9**, 237–41.
26. León G, Lomonte B, Gutiérrez JM (2005) Anticomplementary activity of equine whole IgG antivenoms: comparison of three fractionation protocols. *Toxicon* **45**, 123–8.
27. Covell DG, Barbet J, Holton OD, Black CD, Parker RJ, Weinstein JN (1986) Pharmacokinetics of monoclonal immunoglobulin $G_1$, F(ab')$_2$, and Fab' in mice. *Canc Res* **46**, 3969–78.
28. Scherrmann JM (1994) Antibody treatment of toxin poisoning—recent advances. *J Toxicol Clin Toxicol* **32**,

363–75.
29. Pepin S, Lutsch C, Grandgeorge M, Scherrmann JM (1995) Snake F(ab')$_2$ antivenom from hyperimmunized horse: pharmacokinetics following intravenous and intramuscular administrations in rabbits. *Pharmaceut Res* **12**, 1470–3.
30. Theakston RD, Warrell DA, Griffiths E (2003) Report of a WHO workshop on the standardization and control of antivenoms. *Toxicon* **41**, 541–57.
31. Malasit P, Warrell DA, Chanthavanich P, Viravan C, Mongkolsapaya J, Singhthong B, Supich C (1986) Prediction, prevention, and mechanism of early (anaphylactic) antivenom reactions in victims of snake bites. *Br Med J* **292**, 17–20.
32. Sellahewa KH, Gunawardena G, Kumararatne MP (1995) Efficacy of antivenom in the treatment of severe local envenomation by the hump-nosed viper (*Hypnale hypnale*). *Am J Trop Med Hyg* **53**, 260–2.
33. Moran NF, Newman WJ, Theakston RD, Warrell DA, Wilkinson D (1998) High incidence of early anaphylactoid reaction to SAIMR polyvalent snake antivenom. *Trans Roy Soc Trop Med Hyg* **92**, 69–70.
34. Sutherland SK (1977) Serum reactions. An analysis of commercial antivenoms and the possible role of anticomplementary activity in de-novo reactions to antivenoms and antitoxins. *Med J Aust* **1**, 613–5.
35. Estrada R, Herrera M, Segura A, Araya J, Boschini C, Gutiérrez JM, León G (2010) Intravenous administration of equine-derived whole IgG antivenom does not induce early adverse reactions in non-envenomed horses and cows. *Biologicals* **38**, 664–9.