

Antivenoms in Snake Envenoming: Are they Safe?

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Abstract

Snake antivenoms are the only definitive management of snake envenoming. Parenteral administration of antivenom mitigates the toxic effects due to snake venom components. However, these benefits come with additional risk of antivenom reactions. The morbidity and mortality of antivenom reactions largely go unnoticed due to lack of awareness and many times these are wrongly attributed to effects of snake venoms. Depending upon the duration between antivenom administration and onset of clinical manifestations, World Health Organization (WHO) has classified these reactions into three types; namely i) early anaphylactic reactions: occur within 10-180 minutes after antivenom infusion, ii) pyrogenic (endotoxic) reactions: develop within 1-2 hours after initiation of treatment, and iii) late reactions: usually develop 1-12 (mean 7) days after treatment. The conjunctival or skin hypersensitivity tests are not only unreliable but can also be sensitizing to antivenom reactions, and hence, not recommended by WHO.

The majority of early anaphylactic reactions are non-IgE mediated owing to anticomplementary activity of antivenom and few reactions are attributed to IgE mediated release of anaphylotoxins namely histamines, leukotriens etc. Contamination of antivenom with endotoxins during manufacturing process leads to development of pyrogenic reactions. Late antivenom reactions are the result of production of IgM or IgG antibodies in patients towards antivenom proteins, which ultimately cause formation of immune complexes. The deposition of these immune complexes throughout body leads to manifestations of late reactions.

There should be a vigilant approach towards prediction and prevention of antivenom reactions for a better quality of health care.

Introduction

Snake envenoming and the associated morbidity and mortality is one of the important public health problems in rural tropical areas like Southeast Asia, sub-Saharan Africa, Latin America. Snake bite is a neglected, environmental and occupational disease, which primarily affects agricultural workers and children [1-4]. It has been estimated that around 20,000 to 94,000 people die of snake bite each year [5]. A high proportion of snake envenoming usually land up with permanent tissue damage along with high socioeconomic impact. However, the true epidemiological assessment is practically very difficult, mainly attributed to the under reporting of snake bite cases; especially in the rural areas.

Antivenom serum is the most effective treatment for management of snake bite envenomation. However, antivenom usage remains a very risky job. Many times, deaths due to antivenom reactions are wrongly attributed to envenomation. The high incidence and unpredictability of antivenom reactions pose a challenging scenario for the physicians while dealing with snake envenoming. These antivenom reactions vary from mild nausea to severe, life threatening anaphylaxis. Unfortunately, sensitivity tests are unreliable and have no predictive value for early antivenom reactions [6]. Henceforth, ensuring the safety of the patients receiving antivenom has a very high priority. Prophylactic use of antihistamines, corticosteroids and adrenaline has been tried in many clinical studies to evaluate their role in prevention

of antivenom reactions, but there remains a genuine uncertainty regarding their effective and safe usage.

There are wide range of hypotheses and explanations for occurrence of antivenom reactions but conclusive evidences on their exact mechanisms are still missing. The present article deals with basic understanding of snake antivenom and associated antivenom reactions.

The Anti-Venom

Parental administration of antivenom; the only specific antidote to snake venom is the cornerstone in management of snake bites [7]. The antivenom for snake envenoming was introduced by Albert Calmette in 1895 and was quickly accepted without formal clinical trials. More than a century later, antivenoms are considered as essential drugs.

Antivenoms are derived from immunoglobulins, obtained and purified from the plasma of animals immunised with snake venoms. The toxins present in venoms, which are responsible for manifestations of envenoming are neutralized by the antivenom immunoglobulins [8].

An accurate selection of snake venoms is critical for the production of antivenoms that have capacity to cover the majority of cases of envenoming in a given geographical region, territory or country. As the composition of snake venoms is very complex and a high inter-

species and intra-species variation has been documented, production of antigenic mixture to be used for antivenom production is a challenging task for manufacturers [9]. The selection of most suitable snake venom for production of antivenom is based on the geographical area of interest, locally prevalent species of snakes and variability in the composition of snake venoms within the desired territory.

Monospecific vs. polyspecific antivenoms

There are two types of antivenom available namely, monospecific and polyspecific.

Monospecific antivenoms

These antivenoms are intended for use in envenoming due to a single species of snake or a few closely related species whose venoms show clinically effective cross-neutralization [7]. It is practically possible only when there is a very high prevalence of a single species of snakes in the desired region, but most of the countries are inhabited by more than one medically relevant species of snakes, where use of polyspecific antivenoms is highly recommended.

Polyspecific antivenoms

The polyspecific antivenoms are produced by immunizing an animal with venoms of more than one species of snakes of high medical relevance to the concerned geographic area. Another methods of production includes i) immunizing individual animals with venom of a single species and then mixing the various hyper immune plasmas for fractionation and ii) mixing appropriate quantities of relevant purified antivenoms before formulation [7].

These polyspecific antivenoms should be promoted whenever feasible technically, as they offer clinical advantages like better usefulness than monospecific antivenoms. Their use reduces the need for identification of snakes prior to initiation of antivenom therapy and simplicity in logistics provides great advantages [7].

Antivenom production

There are at least 45 manufacturers of antivenom worldwide. For the production of anti-snake venom, usually horses are preferred for immunization with the venoms from either single or multiple snake species; although other animals like sheep's, donkeys, camels, hen, goats and monkeys can also be used for the same purpose [10-19]. After the immunization, the plasma of animals is subjected to fractionation and extraction of immunoglobulin substances. These can be a) intact IgG, isolated using either ammonium sulphate or caprylic acid [20]. b) F(ab')₂ fragments, with pepsin digestion and ammonium sulphate or caprylic acid fractionation [21,22] ; and c) Fab fragments, prepared from papain digestion and ammonium sulphate fractionation [23].

There are two dosage preparations of antivenom available: i) liquid and ii) freeze dried. Improved stability (5 hrs with freeze dried vs. 2-3 hrs with liquid) is the main advantage of freeze dried preparation; which is of vital importance where cold chain is improperly maintained.

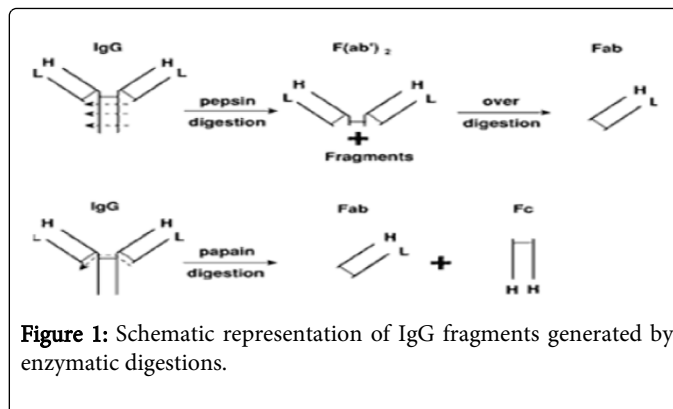


Figure 1: Schematic representation of IgG fragments generated by enzymatic digestions.

Screening production animals for adventitious agents
IgG concentrations [(NH ₄) ₂ SO ₄ /NaSO ₄ precipitation]
Enzyme digestion (pepsin ? F(ab') ₂ ; papain ? Fab)
Caprylic acid stabilization
Ion exchange (removes Fc)
Affinity purification (concentrates venom-specific IgG)
Pasteurisation (10 h at 60°C)
Endotoxin exclusion (to not more than 0.5 u/kg/dose)
Lyophilisation

Table 1: Steps in antivenom production [24].

Antivenom Reactions

As per World Health Organization (WHO) guidelines on the management of snake bites, it has been documented that usually more than 10% of patients who receive antivenom suffer from antivenom reactions [25]. Graph 1 represents the data on incidence of antivenom reactions documented in various clinical trials worldwide, which ranges from 3% to as high as 88%; along with type of active substance used in antivenom [26]. This kind of wide range in the antivenom reactions demonstrates high heterogeneity in product safety profile of antivenom.

These antivenom reactions are broadly classified by World Health Organization based upon the time lapse between antivenom infusion and onset of clinical manifestations as i) Early anaphylactic reactions; ii) pyrogenic (endotoxin) reactions; and iii) Late (serum sickness type) reactions [25].

Early anaphylactic reactions

These reactions usually occur within 10-180 minutes after antivenom administration. These include itching, urticaria, dry cough, fever, nausea, vomiting, abdominal colic, tachycardia, diarrhoea. Some patients may also develop severe anaphylactic reactions like hypotension, angio-oedma and bronchospasm [25]. These can be manifestations of two different pathogenic mechanisms like either IgE mediated reactions or non-IgE mediated reactions. Adrenaline intramuscularly into upper lateral thigh is the mainstay of treatment.

The IgE mediated reactions

These are attributed to the antivenom, occurring due to previous exposure to animal immunoglobulins to the patients. There is production of IgE antibodies against antivenom proteins. These immunoglobulins; upon administration of antivenom, interact with high affinity IgE receptor i.e. FcεRI located on basophils and mast cells, induce cell degranulation leading to release of mediators of anaphylaxis e.g. histamine, leukotrienes, platelet activating factors. They cause vasodilation and increased vascular permeability producing the clinical manifestations of anaphylaxis [26-48]. Another source for early antivenom reactions is traces of antibiotics in the antivenom. Horses and other animals which are utilized in the synthesis of antivenom are generally treated with antibiotics for their infections. These antibiotics can make their way to the antivenom, which triggers the immunoglobulin (IgE) mediated response.

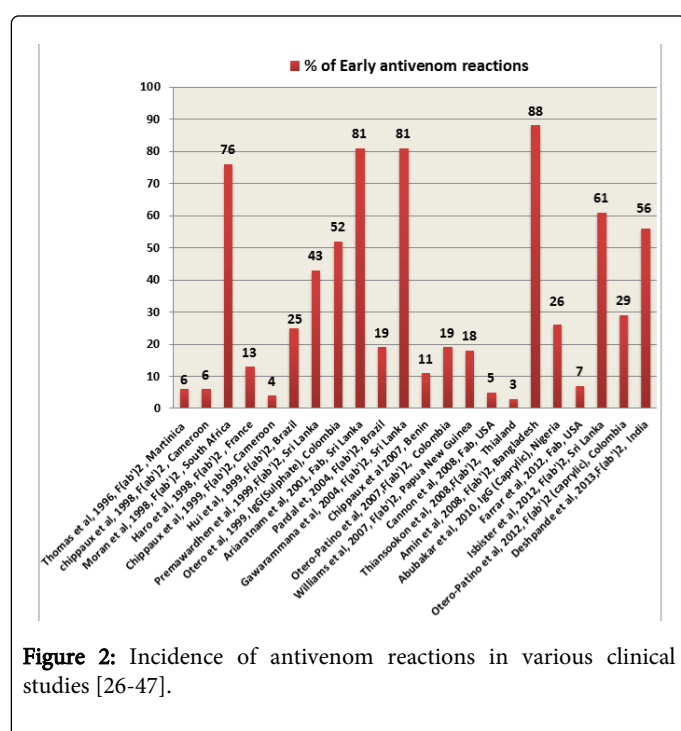


Figure 2: Incidence of antivenom reactions in various clinical studies [26-47].

Non-IgE mediated reactions

The majority of the early antivenom reactions are non IgE mediated. These reactions occur de novo with no any history of previous antivenom administration in the patients [39,46,49]. Thus, it rules out the possible role of conjunctival or intra-dermal hypersensitivity testing for prediction of early antivenom reactions. WHO has not recommended the use of such tests prior to infusion of antivenom, stating that these tests are not only non-productive to predict reactions, but they also delay the onset treatment which is of paramount importance in the management of snake bites [25]. In addition these sensitivity tests can in them be sensitizing to early antivenom reactions, which defeats their very purpose.

It is hypothesised that the antivenom anticomplementary activity (ACA) and heterophilic antibodies present in antivenom plays a crucial role in the development of non IgE mediated reactions [50,51].

Anticomplementary activity of antivenom and its role in antivenom reactions

Sutherland (1977) proposed a possible role of ACA of antivenoms in early anaphylactic reactions [50]. It is the most accepted explanation for pathogenesis of non-IgE reactions [51]. Thus to reduce incidence of early non-IgE mediated reactions, few steps are proposed which includes;

i) Reduction in the total load of protein administered: There is a correlation between total proteins and ACA of antivenom [52] and hence the goal of reducing total proteins and thereby decreasing the incidence of anaphylactic reactions can be achieved by utilization of more purified products; [22] increasing the antibody titres in immunized horses, which produces antivenoms with low protein and high potency [53] and; preventing antivenom overdosing [34].

The steps involved in antivenom production like pepsin digestion, salting out, and chromatographic separation makes the antivenom more pure and with lower total protein contents [21,22].

ii) Removal of Fc fragments by enzymatic digestion of immunoglobulins The fragment crystallisable region (Fc) are removed for the immunoglobulin by a method of pepsin digestion to generate F(ab')₂ fragments. The Fc region is responsible for complement activation by the classical pathway. It is widely accepted that this removal of Fc fragments does a positive impact by reducing generation of complement component C5a and thereby reducing the incidence of antivenom reactions [26]. A few manufacturers use the method of papain digestion generating monovalent.

iii) Fab neutralising fragments which showed a very low incidence of adverse reactions [54]. Reduction of IgG protein aggregates in the antivenoms- It has been postulated that protein aggregates in the antivenoms contribute to the development of early antivenom reactions. Traditionally, assessment of the percentage of proteins aggregates in the antivenom preparations has been used as a quality control test [20,22,34,55,56]. Otero et al. have shown that whole IgG antivenom produced by ammonium sulphate precipitation contain a higher extent of protein aggregates than the caprylic acid-fractionated whole IgG antivenoms, ultimately a higher incidence of antivenom reactions [34].

iv) Treatment with β-propiolactone- Treatment of immunoglobulins with β-propiolactone reduces ACA, and this can be used for decreasing incidence of early antivenom reactions [26,57].

Heterophilic antibodies in human plasma

Heterophilic antibodies, stimulated by animal contacts, vaccines or ingestion of food are present in the plasma of all people [58-60]. It has been proposed that immune complexes formed by antivenom proteins and heterophilic antibodies, may be involved in pathogenesis of non-IgE mediated reactions induced by equine derived antivenoms [61,62].

Intravenous route of administration is shown to be related to high incidence of antivenom reactions as compared to intramuscular route [63]. The antivenoms show best pharmacokinetic and pharmacodynamic profiles when given intravenously, and henceforth the choice of route of administration is intravenous one unless the antivenom is given in settings different than medical centres [25,64].

Pyrogenic (endotoxic) reactions

The pyrogenic reactions which include chills, rigors, fever, myalgia, headache, nausea, increase in heart rate and vasodilatation and a fall in blood pressure, usually develop 1-2 hours after administration of antivenom. Febrile convulsions may be precipitated in children. These

reactions are caused by pyrogen contamination during the manufacturing process. The standard line of management includes adrenaline, cooling physically, IV fluids and anti-pyretics.

Bacterial lipopolysaccharides (LPS), which are integral part of the outer cell membrane of Gram negative bacteria, are the most common pyrogens in biologically derived pharmaceuticals [65]. The serum LPS binding protein binds with LPS aggregates and transfers them to cluster of differentiation antigen 14 (CD14), which is found to be anchored to plasma membrane of monocyte and macrophages. It helps to form a complex of LPS with myeloid differentiation 2 protein (MD-2) and the Toll-like receptor 4 (TLR-4) [66,67]. It leads to production and release of cytokines like interleukins (IL-1 β , IL-6), Interferons (INF- γ) and tumour necrosis factors (TNF- α) from monocytes and macrophages; particularly in liver and lungs and thus causing the pyrogenic reactions [68].

Strict adherence to the Good Manufacturing Practices to avoid contamination of microbial products in the antivenom production is the key to decrease in the pyrogenic antivenom reactions [69].

Late (serum sickness type) reactions

These reactions usually develop 1-12 (mean 7) days after treatment. Clinical manifestations include fever, nausea, vomiting, diarrhoea, itching, recurrent urticaria, arthralgia, myalgia, lymphadenopathy, periarticular swellings, mononeuritis multiplex, proteinuria with immune complex nephritis and, rarely, encephalopathy [25]. Patients who suffer early reactions and are treated with antihistamines and corticosteroid are less likely to develop late reactions.

These reactions correspond to serum sickness; type III hypersensitivity in the Gell-Coombs classification [70]. There is IgG mediated antibody response towards the heterologous proteins in the antivenom. There is more than 100 times increase in the antibodies concentration towards the heterologous immunoglobulins [71]. The formation of soluble antigen-antibody complexes is responsible for the late reactions. These complexes recognise, activate classical pathway of complement and neutrophils which leads to the manifestations of late antivenom reactions. As there is correlation between the incidence of antivenom reactions and the total amount of heterologous proteins and hence, antivenom dosage and protein concentration are the important determinants for development of late antivenom reactions [72].

During the initial phase of late reactions, small soluble immune complexes of human IgG and antivenom proteins are formed. These complexes get distributed throughout the body and deposited in peculiar anatomical sites like synovial membranes, glomerular membranes or other endothelial basement membranes. They activate classical complement pathway leading to release of mediators of anaphylaxis viz. serotonin, histamine, leucotrienes from mast cells. Increased capillary permeability and vasodilation ensues local edema and erythema [73-75]. Neutrophils get attracted towards these complexes and try to phagocytize them, but due to their adherence to basement membranes, the phagocytosis gets defeated. It ultimately leads to release of lytic enzymes which causes the local tissue destruction [76]. As the concentration of anti-antivenom IgG gradually increases, large and insoluble immune complexes are formed which get easily removed by mononuclear phagocytic systems.

A few clinical studies have documented incidence of late antivenom reactions ranging from 10% to 56% [46,72,77]. However the true incidence cannot be estimated, as follow up rate after the treatment

varies at each centre and mostly patients do not come to regular follow up especially in rural setup. These late reactions usually respond to a 5-day course of oral antihistamine. Patients who fail to respond in 24-48 hours should be given a 5-day course of prednisolone.

Prevention of Antivenom Reactions

Prophylactic treatment with adrenaline, antihistamine, corticosteroids

In areas where snakebites are common, qualified staff and equipment are often lacking in health centres. With such limited resources, taking steps to safely reduce the risk of adverse antivenom reactions through prophylaxis is desirable. Consequently, a safe, efficacious pre-medication regimen for the prevention of potentially life-threatening anaphylactic reactions would be particularly relevant and important in the management of snakebite in those regions. Traditionally, parenteral adrenaline, hydrocortisone and antihistamines, either chlorpheniramine or promethazine, have been used for pre-medication to prevent early antivenom reactions following antivenom use with variable results. Adrenaline is the most effective treatment for management of early anaphylactic reactions by reducing bronchospasm and capillary permeability. A clinical trial by Premawardhena et al. documented decrease in incidence of antivenom reactions from 43% to 11% with the use of low dose (0.25 ml; 1:1000) adrenaline premedication [33]. However; potential side effects like intracranial bleeding, hypertension limit their usefulness. De Silva et al. studied the effect of premedication with low dose adrenaline, promethazine and hydrocortisone alone and in various combinations [78]. They concluded that premedication with promethazine resulted in 77% reduction in incidence of early antivenom reactions. Corticosteroids have a very limited role as prophylactic medication for reducing early antivenom reactions, as they take several hours for their onset of action; and by that time early anaphylactic reactions have taken their toll.

Speed of intravenous infusion

The incidence of early anaphylactic reactions is also ascribed to the rate of infusion of the antivenom [79]. It is believed that rapid infusion may lead to higher incidence of antivenom reactions and thus, common practice is to infuse the antivenom at a slower rate [28,46,78-80]. In contrast to common belief, a few clinical studies have proven that the speed of infusion does not correlate with the incidence of antivenom reactions [6,45]. A study by Abubakar et al. have shown that even a fast intravenous injection at a speed of 2mL/min of undiluted antivenoms demonstrated a acceptable incidence of anaphylactic reactions [77].

Conclusions

The benefits of antivenom in managing snake envenomings come with additional risk of antivenom reactions. These antivenom reactions are broadly classified into three types according to time interval between antivenom infusion and onset of reactions; namely 1) early anaphylactic reactions- IgE mediated and non-IgE mediated, occur within 10-180 minutes of antivenom administration; 2) pyrogenic reactions- develop within 1-2 hours, induced by presence of endotoxins and 3) late reactions- manifested in 1-12 days; initiated by immune complexes formed by antivenom proteins and IgM and IgG antibodies produced in patients receiving antivenoms. The

pathogenesis of antivenom reactions can be attributed to factors like: a) manufacturing practices; e.g. contamination with endotoxins [81] b) physicochemical properties of antivenom; e.g. purity [53], protein aggregates [82], antibiotic traces and c) immunological characteristics of antivenom e.g. anticomplementary activity [50], immunogenicity [62,83,84], presence of anti-endothelium, anti-mast cells or anti-leucocytes antibodies in antivenoms [26].

While treating snake envenomation; prediction, prevention and management of antivenom reactions hold a prime importance in modern medicine. The morbidity and mortality of antivenom reactions largely go unnoticed due to lack of awareness and these are sometimes very wrongly attributed to the snake venoms. Failure at strictly adhering to Good Manufacturing Practices, wide variations in the steps involved in production of antivenoms throughout the world, inadequate quality control, are the key determinants in antivenom reactions. There is a need to shift focus of research on aspects of antivenom reactions for their better understanding and production of antivenoms with high safety profiles ensuring a better quality of health services.

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