REVIEW ARTICLE

PHARMACODYNAMICS AND PHARMACOKINETICS OF SNAKE ANTIVENOM

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Abstract

Intact or fractionated immunoglobulins are used as snake antivenom to treat snake envenomation. Intravenously administered antivenom binds with snake toxins in the circulation and neutralizes the toxins. Binding of antivenom to venom in the central compartment prevents the distribution of venom to the peripheral tissues and enhances the elimination of venom. Reduction of antivenom concentration in the central compartment is due to both distribution to the peripheral tissues and elimination. Pharmacokinetics of snake antivenom varies among the three different types of immunoglobulins, namely whole IgG, $F(ab')_2$ and Fab. Pharmacokinetics of $F(ab')_2$ antivenom is best described by two compartment model with zero order input and linear elimination kinetics. Fab and $F(ab')_2$ antivenom with smaller molecular masses have a larger volume of distribution than whole IgG antivenom. A biphasic decline of intravenously administered whole IgG and $F(ab')_2$ antivenom has been observed.

Key words: snake antivenom, pharmacodynamics, pharmacokinetics, venom, toxins

Snake envenomation

Snake envenomation is a neglected tropical disease resulting from venom injected by a venomous snake into human. Snakebites usually occur under accidental circumstances, hence are described as an accidental animal attack. Venom is a highly complex toxic secretion produced at the venom glands of venomous snakes[1]. Venom is injected into human through specifically evolved teeth known as fangs which are connected to the venom gland by a duct system[2]. Snake venom is injected into human commonly by subcutaneous, or deep intramuscular routes, and rarely via direct the intravenous route[1,2]. The composition of snake venom is highly complex and diverse[1]. As a result of its complexity, a wide range of clinical manifestations are evident in snake envenomation, due to the alteration of routine physiology and biochemistry[1,2]. Some types of snake venom initiates various clinical effects at the site of the bite, varying from local pain, swelling, blistering and local tissue necrosis, which may results in amputations[3]. Others produce systemic effects of snake venom including coagulopathy, neurotoxicity,

renal injury, rhabdomyolysis, cardiotoxicity and hypovolaemic shock[1,2].

Few snake families are considered as highly venomous in the world. Venom from the family Elapidae (cobras, kraits, Australian venomous snakes, coral snakes and sea snakes) induces neurotoxicity leading to neuromuscular paralysis. Viper (true vipers and pit vipers) venom predominantly affects the coagulation cascade, skeletal muscles and kidneys[4,5]. Snakes which belong into the subfamily Atractaspidinae (burrowing asps) and several subfamilies of rear fanged Colubrid snakes are capable of injecting venom leading to fatal envenomation[2].

Snakes are abundant in warmer climates due to their ectothermic nature[5]. Hence, snakebites and envenomation are common in tropical agricultural countries[6,7]. People seek treatment for snake envenomation from hospitals where western medical treatment is available and as well as from indigenous medical physicians. Estimation of the exact snake envenomation burden is difficult due to unavailability of data on victims seeking indigenous medical care and many other factors[8,9].

Snake envenomation is an important cause of morbidity and mortality in tropical and subtropical countries[6,10,11]. A majority of envenomations are reported in sub-Saharan Africa, south and South-East Asia, Papua New Guinea and some parts of Latin America[11,12,13]. At least 1.8 - 2.7 million people are exposed to snakebites each year, resulting in 81,000 - 138,000 annual deaths[6,7,10,11,12,13]. A minimum of 46,000 annual snake envenomation deaths occur in India[13]. Most of the victims are around 10-40 years of age and this comprises the most economically productive community in the rural agricultural world[14]. A high case fatality rate is reported in children less than 5 years of age. The highest case fatality rate for snakebites in India is reported at 5-14

years of age due to exposure to snakes while engaging in agricultural activities[15].

Snake antivenom

Antivenom is considered as the mainstay of treatment for snake envenoming[1,2,7]. Antivenom consists of polyclonal antibodies to toxins in snake venoms[16,17]. As venom is a mixture of various toxins, antivenom is composed of immunoglobulins which are polyclonal in nature[17]. They may be complete immunoglobulin (IgG) molecules or fractionated immunoglobulins such as F(ab')$_2$ or Fab[18] (Figure 1). Antibodies in antivenom are produced in animals, commonly horse, sheep, goats and rabbits, that are injected with repeated small doses of snake venom[7]. The polyclonal nature of antivenom results in neutralization of multiple toxins in venom[18,19]. Monovalent antivenom is raised against a single species of snakes, while polyvalent snake antivenom is produced by immunizing the host animal with more than one species of snake venom[19].

There are three major types of snake antivenom used in the world; whole IgG, F(ab')$_2$ and Fab[18](Figure 1). Whole IgG antivenom contains intact immunoglobulin G molecules (size 150 kDa) purified by ammonium sulphate or caprylic acid precipitation during manufacturing process[20], F(ab')$_2$ antivenom is produced by pepsin digestion ofwhole IgG and is about 100 kDa in size[21]. Fab antivenom is the
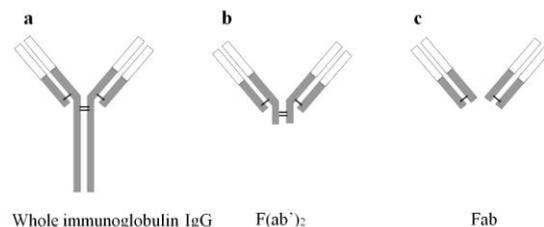

Figure 1: Different types of antibodies use as snake antivenom; a. whole IgG, b. F(ab')$_2$ and c. Fab.

smallest sized antivenom with nearly 50 kDa. It is prepared by papain digestion of whole immunoglobulins[18]. These three different types of antivenom have different pharmacodynamic and pharmacokinetic properties, which influence their motility, determining their ability to reach the tissue targets and their duration of action (presence in the central compartment)[18]. These three different types of antivenom also have varying degrees of adverse effects. The dose of antivenom, repetition of doses and the treatment regimen depend on molecular size and various other pharmacodynamic and pharmacokinetic characteristics of the antivenom[3].

Early administration of antivenom is essential to facilitate binding of antivenom to venom (toxins), before their distribution to target tissues forming irreversible toxicity (e.g. pre-synaptic neurotoxicity). Antivenom should be administered intravenously, as local or intramuscular administration delays entry to the systemic circulation rapidly[22].

Administration of foreign proteins (most commonly horse), is the basis of hypersensitivity reactions and other reactions such as pyrogenic reactions following antivenom treatment[23,24]. Reactions to antivenom are classified as mild, moderate and severe[25]. Delayed reactions can occur and are referred to as serum sickness[26,27]. The effectiveness of prophylactic medications for antivenom reactions is controversial due to lack of properly designed studies[28]. However, a recent randomized controlled trial reveals that the reduction of immediate reaction to antivenom is observed with administration of adrenaline prior to antivenom[29]. Furthermore, another study has demonstrated the inefficiency of hydrocortisone in preventing reactions to antivenom[30].

Pharmacodynamics of snake antivenom

Neutralization of venom by antivenom occurs as a result of blockade of venom toxin interaction with target tissues. Blockade of the active site (pharmacological site) of the toxin by antivenom prevents the interaction between toxin and the target tissue[18] (Figure 2).

Blockade could occur by various direct and indirect molecular mechanisms. Pharmacological site of the toxin acts as an epitope which is recognized by the paratopes of antibodies in snake antivenom[18]. Paratopes of the antibodies directly bind and occupy the pharmacological site of toxin and neutralizes the toxin (Figure 3a). Secondly, antibodies could bind to epitopes situated in the vicinity of pharmacological site of toxins and neutralization is achieved by steric hindrance with consequent inability to reach the target tissue site (Figure 3b). Antivenom also can bind an epitope at a distance from the pharmacological site of toxins, and hence conformational changes
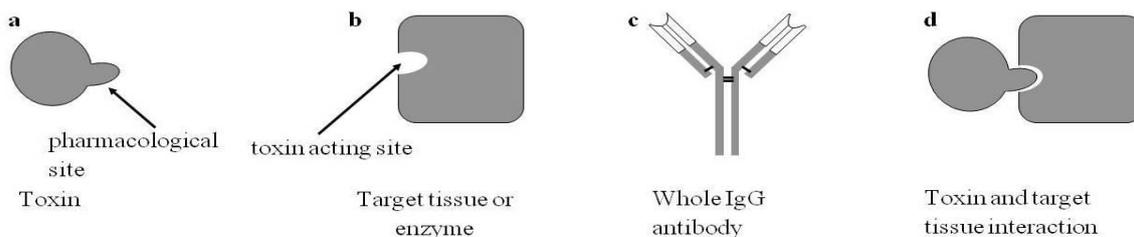

Figure 2: a. Snake venom toxin, **b**. target tissue, **c**. whole IgG antibodies in snake antivenom, **d**. toxin and target tissue interaction

in toxins lead to decreased affinity to their tissue targets (Figure 3c). Antibody induced reversion of the bondage of toxins and target tissue receptors has been described based on the conformational changes in toxins due to antivenom[31].

Antibodies bind with toxins in the circulation and make large molecular weight venom-antivenom complexes which entrap the toxins in the central circulation [3]. Entrapment of toxins in central circulation limits the distribution of toxins to peripheral organs. Whole IgG molecules or $F(ab')_2$ fragments in antivenom facilitates formation of multivalent immuno-complexes, and such complexes may be removed by phagocytic cells in reticular-endothelial system[18]. Formation of multivalent immune-complexes is not seen with Fab antivenom[18]. Binding of antibodies to toxins occurs in the central compartment, plasma, interstitial fluid or after the toxins bind to their tissues targets. Therefore, neutralization effects of antivenom occur in free toxins as well as tissue bound toxins.

Some studies have demonstrated that antibodies in snake antivenom are able to reverse the binding of α-neurotoxins already bound to cholinergic receptors in the neuromuscular junction[32,33,34,35]. Antibodies with high affinity are less likely to dissociate from venom-antivenom complexes and exhibit high possibility of removing toxins already bound to their tissue targets[18]. Such efficient antibodies promote the redistribution of toxins from the tissue compartment to the central compartment, the circulation.

Although, it is vital to assess the neutralization of pharmacologically relevant effects of venom by antivenom, most antivenom manufactures only assess the lethality neutralization abilities of antivenom. The World Health Organization recommendations for antivenom manufacturing include assessment of neutralization of myotoxic effects, coagulopathic, haemorrhagic , necrotizing, oedema forming and defibrillating effects of venom by antivenom[36].

The clinical effectiveness of snake antivenom depends on the ability of antibodies or fragments to bind, reverse, redistribute or eliminate the toxins at various tissue spaces in the body. This is not applicable to toxins active
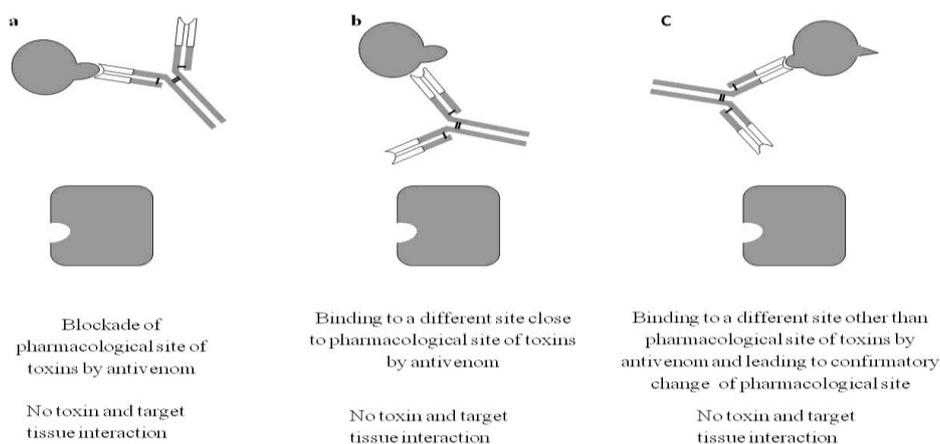

Figure 3: Mode of action of snake antivenom preventing the interaction between pharmacological active site of toxin and target tissue; **a.** blockade of pharmacological site of toxin by antivenom, **b. antivenom** binding to a different site close to pharmacological site of toxins, **c.** antivenom binding to a different site other than pharmacological site of toxins and leading to confirmatory change of pharmacological site.

intracellularly[18].

Some toxins initiate their toxic effect sooner than later following envenomation [3,37]. Pro-coagulant and anticoagulant toxins immediately react with the clotting factors in the coagulation cascade soon after the toxins enter to the circulation[16]. Therefore, coagulopathic effects of snake venom starts immediately after envenomation and the effectiveness of antivenom for coagulopathy is less evident[38,39]. Some studies have demonstrated the limitation of effectiveness of snake antivenom for coagulopathic envenomation[37,38]. Snake venom haemorrhagic toxins also commence their toxic effect soon after the toxins reach to the circulation, as the target site is just at the vicinity of toxins. Very early administration of antivenom may prevent the development of central toxic effects such as coagulopathy and haemorrhages. Pre-synaptic neurotoxicity occurs due to irreversible destruction of microarchitecture of the pre-synaptic nerve endings[40,41]. Therefore, administration of antivenom should be done before the patient develops clinically evident pre-synaptic neurotoxicity[3]. Lack of predictors and biological markers for early detection of neurotoxicity result in antivenom being less

effective for neurotoxic envenomation. Administration of antivenom before the toxins reach the circulation is not practical in clinical settings, as no rapid venom detection facilities are available anywhere in the world[42]. The effectiveness of antivenom for myotoxicity is evident in some clinical studies[43].

Pharmacokinetics of snake antivenom

The pharmacokinetics of snake antivenom is similar to the pharmacokinetics of other intravenous medications. Antivenom is delivered to the central compartment, the circulation, as an intravenous infusion following a constant rate administration. Antivenom then distributes to other tissue compartments and organs of the body. Elimination is probably by the renal or the reticuloendothelial system and this has to be confirmed by further research evidence[18]. Reduction of antivenom concentration in the central compartment is due to both distribution and elimination processes.

The presence of snake venom in circulation (the central compartment) creates another venom-antivenom complex and leads to reduction of free antivenom concentrations
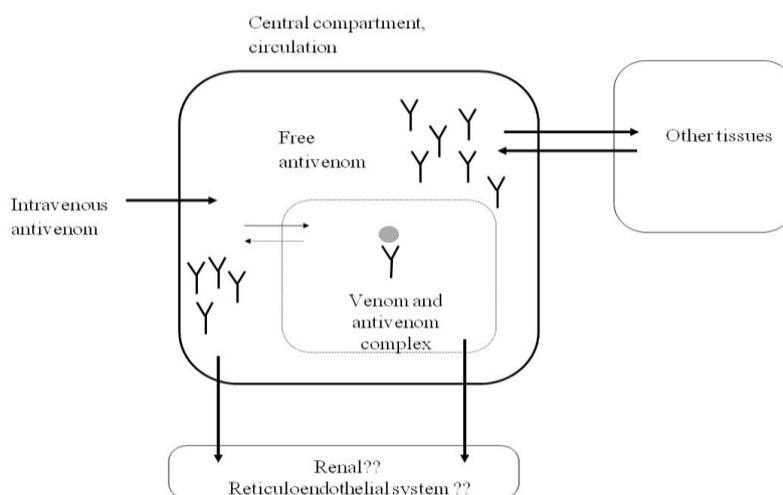

Figure 4: Distribution and elimination of snake antivenom in various tissue compartment in the body.

in the circulation. Free antivenom concentrations are commonly measured in blood by enzyme immune-sorbent assay which creates a platform for detailed studies on antivenom pharmacokinetics[44,45,46] (Figure 4).

Different types of antivenom have different pharmacokinetics due the variation of their molecular masses. Fab and $F(ab')_2$ antivenoms with smaller molecular mass have a larger volume of distribution than whole IgG[47,48,49]. Intravenously administered whole IgG and $F(ab')_2$ antivenom express biphasic decline in the circulation. After an initial rapid decrease (distribution phase) of venom in the circulation, there is a slower first order decline (terminal elimination phase)[48]. This is best described by a two compartment pharmacokinetic model as shown in (Figure 5).

Negligible concentrations of whole IgG and $F(ab')_2$ antivenom fragments have been detected in urine, suggesting that renal filtration is not the predominant route of elimination of IgG and $F(ab')_2$ antivenom[48]. However, Fab antivenom has a rapid elimination half-life compared to IgG and $F(ab')_2$ due to its molecular size[49]. Fab antivenom is rapidly filtered through the glomeruli, reabsorbed and catabolized by proximal convoluted tubular cells[50]. IgG and $F(ab')_2$ molecules having larger molecular mass are not filtered by the glomeruli resulting in a longer stay in the circulation[18]. According to mice studies IgG catabolism takes place in the gastrointestinal system and liver, while gastrointestinal system and the kidneys are responsible for the catabolism of $F(ab')_2$ [51].

Pharmacokinetics of antivenom could be altered by the presence of venom in the circulation[18,44]. Fab-venom complexes formed from high molecular weight viper toxins exhibit slower elimination than free Fab fragments. Fab-viper venom complexes are poorly eliminated by the renal route[45,52]. However this does not appear to occur with Fab fragments binding to low molecular weight toxins and Fab-Low molecular weight toxins complexes are eliminated by the renal route[53]. Mononuclear phagocyte system are involved in the process of clearance of whole IgG / $F(ab')_2$ - toxin complexes in the body[45].

Population pharmacokinetics of the Indian polyvalent $F(ab')_2$ antivenom has revealed that two compartment model with constant-rate input, linear elimination kinetics and combined error model best describes the pharmacokinetics of $F(ab')_2$ antivenom.
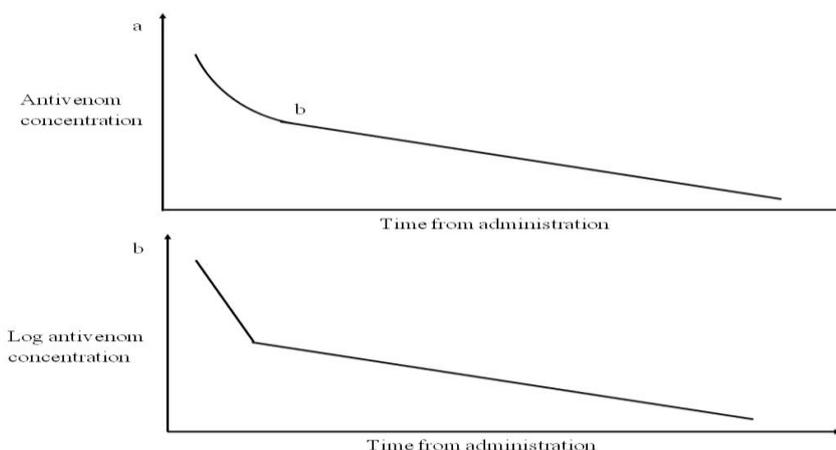

Figure 5: a. Plasma concentration versus time profile of a drug showing a two compartment model, **b.** Time profile of a two-compartment model showing log antivenom concentration versus time.

This study estimated the various pharmacokinetic parameters of F(ab')$_2$ antivenom against Russell's viper envenomation as clearance, 0.078 L/h, central compartment volume 2.2 L, inter-compartmental clearance 0.178 L/h and peripheral compartment volume 8.33 L. The median half-life of distribution was 4.6 h and half-life of elimination was 140 h[54]. This detailed population pharmacokinetic study concluded that Indian F(ab')$_2$ snake antivenom displayed biexponential disposition pharmacokinetics, with a rapid distribution half-life and more prolonged elimination half-life[54].

Relationship of pharmacodynamics and pharmacokinetics of snake antivenom in clinical use

The effectiveness of a medication depends on many factors, including the correlation of pharmacokinetics and pharmacodynamics. The presence of therapeutic concentrations of a medication at the appropriate tissue compartment is crucial to achieve the optimum effectiveness. Ideally the dose of antivenom should be administered according to the injected dose of snake venom and the severity of clinical features[55,56] although determination of the injected dose of venom is impractical in clinical settings, due to unavailability of facilities[42]. Thus, fixed estimated dose of antivenom is used as the therapeutic dose of antivenom during the treatment of envenomation[40,57,58]. The venom also could slowly be absorbed from the bite site into the circulation, especially following deep intramuscular injection. Administration of repeated doses of antivenom depends on dose of venom injected, slow reabsorption of venom into the circulation, distribution and elimination half-life of antivenom[52,59]. Frequent repeated doses of antivenom are indicted during the treatment of using Fab antivenom which exhibit rapid clearance from the circulation[49,52,60].

Conclusion

Prevention of interaction between toxins and target tissues is achieved by neutralization of toxins by antibodies in antivenom. Blockade could occur by various direct and indirect molecular mechanisms. Direct blockade of the pharmacological site of toxin, blockade of the access to the tissue targets and change of the conformation of toxin by antivenom arrest the action of toxins with target tissues.

Pharmacokinetics of antivenom play a major role in determining their effectiveness in clinical practice. Variation of molecular masses among different types of antivenom results in unique pharamcolkinetic parameters. Two compartment model with zero order input, linear elimination kinetics and a combined error model best described the pharmacokinetics of F(ab')$_2$ antivenom. Fab and F(ab')$_2$ antivenom have a larger volume of distribution than whole IgG. Depletion of intravenously administrated whole IgG and F(ab')$_2$ antivenom in the circulation follows a biphasic model.

Conflict of interest

None declared by both authors

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