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Chapter 8

ANTIVENOMS

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ABSTRACT

Close to five million snake bites and scorpion stings are recorded every year in the world, mostly in Africa, Asia and Latin America. Majority of victims require therapeutic sera to prevent death, amputation or severe neurological disorders. Antivenoms are homologous or heterologous preparations of intact or fragmented immunoglobulin G used for treatment of humans or animals suffering from severe envenoming from the bites and stings of venomous animals. Generally speaking antivenom is a term used for serum that is commercially produced to neutralize the effects of envenomations. Artificial passive immunization is used when it is necessary to protect life of a patient at very short notice like in life threatening envenomations. Readymade antibodies in the form of antivenom have been used as antidote for over a century. 90% of commercial therapeutic sera worldwide are of equine origin because of certain advantages such as: ease in handling; large volume of blood/plasma can be collected at periodic intervals; well established and validated purification process known for over a hundred years; and horses' sensitivity to venoms/toxins giving excellent immuno-conversion. Antivenom is available in two formulations viz. liquid form and lyophilized form, with each having their own advantages and disadvantages beyond the cost of manufacturing. For manufacturing of an antivenom, the host animals are hyperimmunised by injecting gradually increasing doses of venoms over a period of 4 to 6 months. Once the animals attain a certain level of antibodies against the venoms, blood/plasma collection starts. The plasma containing specific immunoglobulins is further processed by enzyme digestion and chemical separation to obtain purified immunoglobulin fraction. The bulk serum is tested for safety and potency in mice and then containerized for distribution. Effectiveness of antivenom depends on administration of adequate dose, promptness of administration after bite, specificity of antibodies to venom components, proper storage of finished product in prescribed storage conditions and its use within prescribed shelf life period.

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It should be remembered that antivenom does not reverse all effects of venom. For example, local tissue damage poorly responds to antivenom. Some of the reasons why antivenom may not give desired results could be if it does not cover the right antigenic mix, inadequate dosing not matching the severity of envenomation, late administration done after setting in of irreversible changes, incorrect diagnosis, inappropriate route and use of poor quality/deteriorated antivenom.

Antivenom being derived from animals is heterologous to humans and hence can produce either early or late reactions due to activation of immune system. It is also a partially purified product retaining some portion of unwanted proteins which further contributes to reactogenicity. Adverse reactions to antivenom can be managed with medication; hence it should not be withheld from needy person because of the associated risks. The antivenom manufacturing process and its challenges, limitations of antivenom treatment and future trends are discussed in detail in the article.

Keywords: antivenom manufacturing, quality, safety, efficacy, limitations and challenges

INTRODUCTION

It is speculated that close to five million snake bites are recorded every year in the world - mostly in Africa, Asia, Latin America and Oceania with mortality of 125,000 each year. These are all hospital based figures and could be underestimated (Chippaux, 1998). Snake bites are a major neglected tropical disease affecting predominantly poor people living in rural settings (Gutierrez et al., 2011). Apart from bite-related deaths, there are many survivors with long term disabilities due to necrotic effects of venom – such as amputations, chronic ulceration, osteomyelitis, chronic kidney failure, and neurological sequelae. Similarly scorpions are next to snakes in causing fatalities from envenoming in many parts of the world (Theakston et al., 2003). Snake venom not only has toxic actions but also possess antigenic properties and this immunogenic characteristic of snake venom has been the basis of producing antibodies to be used as antivenom and parenteral administration of animal origin antivenom has been the cornerstone of snake bite therapy since over a century now (Bon, 1996).

Antivenoms are preparations of either whole or fragmented Immunoglobulin G used for treatment of humans or animals suffering from severe envenoming from the bites or stings of venomous animals. Generally speaking, antivenom is a term used for anti-serum that is commercially produced to neutralize the effects of envenomations. Artificial passive immunization is used when it is necessary to protect life of a patient at very short notice like in life threatening envenomations. Antivenin or Antivenene are synonyms of antivenom.

HISTORY

Readymade antibodies in the form of antiserum are raised in animals and have been used as antidote for over a century. Inspired by the success of demonstration of antitoxic properties of serum of animals immunized by Emil Behring with diphtheria and Shibasaburo Kitasato with tetanus bacteria, Albert Calmette in 1894 reported antitoxic properties of serum of rabbits immunized with cobra venom. He further produced first cobra antivenom in donkeys

and in 1894, his antivenom saved life of his own snake collector. In 1895, his horse derived antivenom was already in use in India and Viet Nam. Albert Calmette is thus regarded as pioneer of snake bite immunotherapy. Many scientists soon followed him and started antivenom manufacturing in other countries: Vital Brazil in Brazil, Frank Tidswell in Australia and Haffkine in India.

Initially, the crude serum separated from clotted blood of hyper-immunized horses was used as antivenom. However, due to high incidence of reactions associated with earlier therapy, whole or fractionated immunoglobulin is now used as antivenom. The purification methods have undergone refinement resulting in high margin of safety (WHO, 2010).

TYPES OF ANTIVENOMS

Antivenoms can be classified into different types depending on their intended use, source (host animal used), specificity, composition (type of active substance) and presentation.

1. Intended Use

Human beings can have confrontation with many venomous animals like land and sea snakes, scorpions, bees and wasps, spiders, fishes, ticks, venomous frogs and mammals like platypus and occasionally there could be unfortunate incidences of bites/stings by these venomous animals resulting in envenomations of varying degrees from mild to very severe sometimes leading to death. Antivenoms have been prepared as an antidote against venoms of some of these animals and few examples of antivenoms are as follows:

- Snake antivenom
- Scorpion antivenom
- Jellyfish antivenom
- Spider antivenom
- Stonefish antivenom
- Tick antivenom
- Bee antivenom

2. Source (Host Animal Species Used)

Majority of commercial therapeutic sera worldwide are heterologous ones, being of equine origin. Horses are preferred host animals because of certain advantages such as: ease in handling, comparatively large volume of blood/plasma can be collected at periodic intervals, well established and validated plasma purification process, and their sensitivity for venoms/toxins giving excellent immuno-conversion with smaller doses. Although homologous antisera against tetanus and rabies are available, no homologous antivenom is available. Other host animals like sheep, goat and rabbits have been used for production for

commercial sera. Some other animals such as camel and chicken have been tried in experimental feasibility studies; however, no commercial use is reported so far.

3. Specificity

Antivenoms could be mono-specific (raised against venom of single snake species) or poly-specific (raised against venom of two or more snake species).

Advantage of mono-specific antivenom is that it can be more potent as it contains high proportion of antibodies per dose and hence a smaller dose may be required. However, for using this type of antivenom exact identification of offending snake species is essential for administration of correct antivenom. In some countries venom detection kits are available which can help in identification of offending snake species. Disadvantage of mono-specific antivenom is that its use is limited to those geographical areas where target snake species is prevalent and hence distribution can be a problem.

Advantage of poly-specific antivenom is that it is applicable to wider geographical areas, identification of biting snake is not important and it is convenient for production and distribution. However, a possible disadvantage could be that its specificity can be lesser against each venom per dose and hence larger dose may be required. Similarities in antigenic nature of venoms of related species used in production of poly-specific antivenom broadens the spectrum of para-specific neutralization ability of antivenom due to synergistic actions (Theakston et al., 2003).

4. Composition of Antivenom (Type of Active Substance Present)

Different methods for purification of the active ingredient (specific immunoglobulin fraction) from plasma have been described in detail (WHO, 2010). It is important that the fractionation process used should not affect the neutralization capacity of antivenom and at the same time should give sufficiently high yields and purity.

Whole IgG Antivenom

Two major procedures for purification of whole IgG antivenoms have been used commercially. First procedure is salting out in which two different salt concentrations of either ammonium sulfate or sodium sulfate are used in acidic conditions to remove unwanted proteins from plasma. However, this method had a drawback of leaving high percentage of contaminant proteins compromising the safety, and the yields are low. In second procedure, caprylic (octanoic) acid is used for precipitating out unwanted proteins. This method is more popular for commercial production because of higher yields and purity. Whole IgG molecule has a molecular weight of about 150 kDa, and has no membrane permeability due to large molecular size. It can cause compliment activation. However, it has a long half-life in blood (about 2-4 days) and high avidity.

Fragmented IgG Antivenom

F(ab')2. This is the most widely used method for commercial production of antivenom which was originally developed by Pope et al. (1939) and now used with different modifications. In this method, whole IgG molecule is cleaved to bivalent F(ab')2 fragments using pepsin under acidic conditions, and the FC fragment is removed by thermocoagulation. After this step, the unwanted proteins are precipitated out using either salt or caprylic acid and the solution is subjected to tangential flow filtration to remove residual salts/caprylic acid and low molecular weight proteins followed by step of concentration. F(ab')2 molecule has a molecular weight of about 100 kDa, and, as a result, no membrane permeability. F(ab')2 has half-life in blood and avidity similar to that of whole IgG.

Fab's. In this method, whole IgG molecule is digested using papain to yield Fab' fragments. This method has been used for antivenom production from sheep plasma. Fab' molecule has a molecular weight of about 50 kDa, it is the least immunogenic amongst all IgG forms, it has good membrane permeability due to smaller molecular size, and it does not cause complement activation. However, it has a very short half-life in blood (about 4-24 hours) and poor avidity.

Due to differences in molecular mass, the pharmacokinetics of the heterologous IgG molecule and its fractions is different. Fab' fragments distribute very fast, readily reach extravascular compartments, and rapidly eliminated by renal route. In contrast IgG and F(ab')2 are not eliminated by renal route and have much more prolonged action. IgG and F(ab')2 have two binding sites (as compared to one for Fab') and hence are able to form large and stable complexes or precipitates with antigen. For complete neutralization of venom biotoxins, maintaining high concentration of specific antivenom antibodies in circulation for many hours is essential and hence IgG and F(ab')2 antivenoms are more effective and popular (WHO, 2010).

5. Formulation

Commercial antivenom is available in two formulations viz. liquid form and lyophilized form each having their own advantages and disadvantages besides difference in cost of manufacturing. The liquid antivenom requires continuous cold chain (2 to 8°C) during transportation and storage and usually has shelf life of 2-3 years whereas the lyophilized antivenom can be stored at ambient temperatures and have a longer shelf life of at least 4 years. However, as the lyophilization process involves additional costs in production, the price is higher than that of liquid. Lyophilized products are better suited for the regions where cold chain conditions for storage and distribution of liquid products can't be ensured.

ANTIVENOM MANUFACTURING PROCESS

Manufacturing process of antivenoms is unique and totally different from that for most of the biological products like vaccines or other biochemical entities. In a typical antivenom manufacturing process, the host animals are hyper-immunized by injecting gradually increasing sub-lethal doses of venoms over a period of 4 to 6 months. Once the animals attain

a certain level of antibody titer against the venoms, blood is collected periodically in a sterile container having anticoagulant. The plasma containing specific immunoglobulins is separated and subjected to enzyme digestion and chemical separation to yield specific antibody fraction. This is further formulated using excipients and preservatives and, after quality tests, containerized to be used as antivenom.

As the basic raw material required for manufacturing is animal derived, its manufacturing process has some peculiarities:

Long gestation period for initial start - The host animals can only be immunized slowly over a period of time to avoid deleterious effects of venoms hence it takes anywhere between 8 to 12 months from the induction of animals to get actual finished product in hand for sale.

No immediate scaling up of production is possible - Basic raw material used in manufacturing is blood/plasma which can be collected periodically in proportion to body weight thus production cannot be increased suddenly unless more productive animals are available. Therefore production levels have to be planned well in advance by allocating adequate number of host animals.

Delicate balancing - The most challenging aspect of antivenom manufacturing is to strike delicate balance between animal health and productivity apart from addressing to variations at different stages of production - such as difference in venom composition, difference in immune response between individual animals, difference in neutralization titers reached against each venom in poly-specific antivenom, variations in yield/recovery between each batch/lot, etc. – while ensuring consistent product quality. Overall, antivenom manufacturing is a technologically challenging task.

QUALITY OF ANTIVENOM

Antivenom is an important lifesaving biological product, therefore its quality and safety are very important. Both of these parameters depend on the control of source materials as well as on the subsequent Good Manufacturing Practices (GMP) that have a bearing on the biological activity of the end-product. Quality of venom and production of good quality plasma is of paramount importance in antivenom production as these two are the basic starting materials for the production process.

(A) Venom

It is a modified form of saliva. It is a mixture of many different proteins and peptides having toxic/non-toxic and enzymatic activities. Primary function of venom is for obtaining food by immobilizing and killing of prey (Bücherl et al., 1968). Snake venoms are not single toxins but cocktail of many components: enzymes, polynucleotides, toxins, non-toxin proteins, carbohydrates, metals, lipids, free amino acids, nucleotides and biogenic amines (Bawaskar, 2004). Venom also helps in digestion process by pre-digestion of body tissues of prey. The action of venom is the combined effect of all components present in the venom. Venom possesses diverse pharmacological and clinical properties like neurotoxicity, myotoxicity, cardiotoxicity, coagulant and hemostatic properties, edema induction,

hemorrhagic properties, and direct action on vital organs. However, snake venoms exhibit marked variation in their extent of induction of toxic effects (Shashidharamurthy et al., 2002).

Venom variation. The debate about the clinical efficacy of snake antivenoms and the degree of variation in venom composition began soon after production of the first antivenoms in the late nineteenth century (Fry et al., 2003). Variation in venom composition and biochemical and pharmacological properties has been studied for a long time and has been very well documented (Warrel, 1989; Daltry et al., 1996; Fry et al., 2002).

Clinicians have often noted that envenomation by same species of snake does not always generate same symptoms in victims, and therefore although antivenoms can be a valuable aid in snake bite therapy they cannot always be equally effective.

The major reasons for the variation in snake venom composition are as follows:

- Interspecies variation due to phylogeny;
- Intraspecies variation influenced by environmental factors like habitat, climate (geographic origin of snake), season, age, sex and prey preference.

The ubiquity of venom variation in snakes pose special problems for the manufacture of antivenom because intraspecies variation in venom composition of a given snake species can affect the neutralization capacity of antivenoms. Therefore, in contrast with other animal-derived immunoglobulins (e.g., tetanus or rabies immunoglobulins), the design of the antigenic mixture to be used in antivenom manufacture is a critical and delicate task. Because of regional and individual variation in venom composition, it is necessary to include venom from large number of individual animals covering the entire bio-geographical distribution of particular venomous snake species (WHO, 2010).

There are variations in venom composition and antigenicity within the geographical range of a single taxonomic species as well as other causes of intra-species variation such as changes according to the age of the specimens (Fry et al., 2003; Warrell, 1997). Therefore, pooled representative samples of venoms should be prepared from snakes of different geographical origins and ages. Such venom preparations are used both to hyper-immunize animals, as part of antivenom production process, and also to provide reference venom samples for routine and/or preclinical potency assessment of antivenoms.

It is essential that venoms used for antivenom manufacture should be representative of the snake population living in the area where the antivenom is going to be used. In order to account for the variability in venom composition of an individual species (Saravia et al., 2002; Faure and Bon, 1987; Creer et al., 2003), it is imperative that the venom of an adequate number of snakes (generally not less than 20 to 50 specimens) from the same geographical location should be collected together to ensure that the antivenom is designed to treat envenoming in the region efficiently. The success of antivenom therapy depends on ability of immunoglobulins to bind and eliminate snake venom toxins present in body. Treatment outcome can vary greatly with geographical area as the venom composition and antigenic properties of toxins can be highly variable across range of given snake species (Alirol et al., 2010).

Venom is the prime raw material involved in the production of specific antivenom immunoglobulin, and a deciding factor in the production of effective antivenoms.

(B) Hyperimmune Plasma

Collection of good quality plasma for production of antivenom involves combination of following factors:

Good Animal Husbandry Practices

This starts with careful selection of host animals for the production considering regional availability of animals, suitability for scale of production envisaged, convenience, etc. All newly procured animals are kept in isolation (usually for 4 weeks) during which complete health screening of host animals is carried out to ensure that only healthy animals are used for production purpose. During subsequent immunization/blood harvesting phase, the animals are housed properly as per prevailing climatic conditions in such a way that environmental stress is kept to minimum and hygiene is maintained. After start of immunization animals are exposed to different venom effects and subsequent periodic blood collection predisposes animals to anemia and weight loss. It is therefore essential to give balanced feed to satisfy all nutritional requirements pertaining to the regular stress of immunization and periodic plasma protein loss. Continued veterinary surveillance for sickness and other emergencies arising out of immunization procedure is a must. Special attention is required for maintaining disease free animals for minimizing the risk of infectious contamination of plasma.

Immunization and Use of Adjuvants

Immunization is the most critical step in antivenom production for achieving higher neutralization titers using refined immunization protocols. Use of low venom dose has big effect on not only maintaining health of animals and cost of production but also on the safety and efficacy of antivenom. During immunization, efforts should be made to avoid/minimize local or generalized reactions to venom and/or adjuvants used in immunization. Because of its diverse pharmacological actions (e.g., hemotoxicity, neurotoxicity and nephrotoxicity), venom has deleterious effect on different systems and vital organs of donor animals sometimes showing generalized effects whereas venom cytotoxicity can cause local effects such as tissue necrosis and abscesses. Increasing venom doses to obtain higher titers often leads to deterioration of health, whereas static doses may give inadequate titers. Repeated exposure to high doses of venom antigen can cause immune tolerance in some host animals. For keeping venom doses at minimum and increasing immune response immune-modulators/adjuvants are used (Stills, 2005). Complete and Incomplete Freund's adjuvants and aluminum salts are commonly used adjuvants but Freund's adjuvants can cause local granulation wounds and are not entirely safe. Recently use of Montanide group of adjuvants has been reported to be superior to Freund's adjuvant and less toxic and safe in horses during commercial production of antivenom (Waghmare et al., 2009). To reduce venom toxicity, its modification/detoxification by chemical or physical methods has been tried so that higher quantities of venom can be administered to enhance immune response. However, it is reported that use of modified venom in immunization mixture gives lower neutralization compared to crude venom probably due to alterations in venom toxins in the process (WHO, 2010). Traditionally, immunization protocols were using rather large doses of venoms which had detrimental effects on health and productive life of host animals. Recently, a newer protocol using low dose, low volume and multisite injections with superior results has been described (Chotwiwatthanakun et al., 2001). Considering the importance of immunization

technique in the whole manufacturing process, all efforts should be directed towards development of such protocols that utilize minimum amount of venom along with safe and effective adjuvant to get high specific neutralization titers in short period of time.

Collection Practices for Animal Plasma

Once the donor animal attains desired neutralization titer against venom, periodic regular blood collection is carried out in relation to body weight. In earlier days, for collecting crude antivenom serum whole blood was allowed to clot. Due to continued loss of blood cells, the donor animals were more prone to weight loss and drop in hematocrit, thus reducing their productive life. To overcome this drawback, nowadays blood is collected in sterilized container having anticoagulant solution and cells are allowed to settle by gravity in cold conditions. The supernatant plasma is manually removed and used as starting material for production. The cellular components which are of no use in manufacturing are re-suspended in normal saline and returned back to same donor animal within 24 hours. The same procedure can also be carried out by using automated plasmapheresis machine which separates plasma online by centrifugation and cells are continuously returned back to animals. This method allows collection of higher volume of plasma as animal is not deprived of cellular mass as in manual operations. However, apart from the higher cost of machine and disposables, this procedure takes more time for completion and usually animals do not cooperate for a period that long. Therefore it is not a popular method in large scale commercial production units.

All efforts in plasma collection should be directed at collecting plasma without bio-burden by ensuring complete asepsis in venipuncture, use of sterilized tubes and containers, and handling and storage of the blood and plasma in properly controlled environment. This reduces the chances of bacterial contamination which contribute to pyrogens/endotoxins in plasma and also increases safety to donor animals during transfusion of cells. The principles of GMP should be strictly followed and all the practices, equipment and premises should be complying with it.

EFFECTIVENESS OF ANTIVENOM

Efficacy of antivenom is its ability to neutralize the toxic effects of venoms against which it has been produced. Effectiveness is tested by animal models as no *in vitro* validated tests are available. The test is performed using experimental rodents (usually mice in weight range of 18-20 g) with standardized and validated testing protocols. The test consists of two parts. In first part the lethality of venom defined as Median Lethal Dose (LD_{50}) is determined. Once knowing the LD_{50} value of venom, fixed LD_{50} dose of venom is mixed with varying dose of antivenom and injected to mice after incubation. Percentage survival or death after 24/48 hours within challenged animal group is analyzed using probit analysis to find out Median Effective Dose (ED_{50}) of antivenom which is able to protect 50% of injected animals. The quality of venom used for these assays and strain of animals used are very important and can give varied results due to variation in these factors.

Under clinical conditions the effectiveness of antivenom depends on number of factors:

1. Administration of adequate dose. The dose of antivenom should match the average amount of venom injected in a bite which determines the degree of envenomation. The average venom yield in different snake species has been reported (Mirtschin and Dunstan, 2006; Tumbare and Khadilkar, 2004). The therapeutic dose of antivenom should be sufficient enough to neutralize venom injected in a bite. Therefore the dose of antivenom is same in children and in adults.

2. Promptness of administration after bite. After a bite, venom enters the blood circulation and gets distributed in different organs and systems. It should be remembered that venoms cause both reversible and irreversible injuries to the tissues. Antivenom acts by binding to epitopes on venom components and thus stops venom action; however, it does not repair damaged tissues. So every effort should be made to prevent irreversible tissue damage/ changes by prompt administration of antivenom because any delay in administration results in increased dose requirement and longer duration for recovery.

3. Specificity of antibodies to venom components. Venom composition is dynamic and known to have inter- and intraspecies variation, however, venom components share some common epitopes giving para-specific neutralization of venoms of related species. Thus, when the antibodies in antivenom are specific to venom, the degree of neutralization will be better and will give quick response to therapy.

4. Proper storage of finished product in prescribed storage conditions. Depending on the stability of formulated product, antivenoms are required to be stored at prescribed storage conditions to preserve its biological activity.

Lyophilized product is stable at room temperature and does not require special storage facilities. Ideally, it should be stored in a cool place and without exposure to excessive heat.

Liquid product should be stored continuously at 2 to 8°C and does not require freezing. It should be always protected from heat.

Antivenoms should be used within prescribed shelf life period of 2-3 years for liquid preparations and 4-5 years for lyophilized product as per the shelf life indicated on the package.

It should be remembered that antivenom does not reverse all effects of venom. Coagulopathies and post-synaptic paralysis in cobra bites are generally reversible and hence responds well to antivenom therapy. Pre-synaptic and kidney damage generally does not reverse immediately after antivenom administration. In krait bites, recovery of pre-synaptic paralysis is slow as it depends on natural repair of damaged neuromuscular junction. Delayed antivenom administration after viper bites may sometimes cause kidney damage. Generally, local tissue damage is poorly responsive to antivenom.

CAN ANTIVENOM FAIL?

Presently antivenom immunoglobulins are the only specific therapeutic products for the treatment of envenoming. A fundamental difficulty associated with antivenom use, recognized since early 20th century, is the absolute requirement of specificity. The success of antivenom therapy depends on ability of immunoglobulins to bind and eliminate snake venom toxins present in body. Treatment outcome can vary greatly with geographical area as the

venom composition, and antigenic properties of toxins can be highly variable across range of given snake species (Alirol et al., 2010). However, similarities in venom toxins of closely related venomous species may provide para-specific neutralization which can tempt clinicians to use closest available antivenom, if specific antivenom is not available.

Only under following circumstances, antivenom can be said to have failed or was ineffective:

1. Antivenom did not cover the offending snake species;
2. Use of inadequate dose of antivenom not matching with degree of envenomation;
3. Late administration after setting in of irreversible changes;
4. Incorrect diagnosis;
5. Administration by inappropriate route;
6. Use of poor quality of antivenom;
7. Use of antivenom which is deteriorated during storage.

SAFETY OF ANTIVENOMS

Antivenom being derived from animals is heterologous to humans and hence can give either early or late reactions due to activation of immune system. It is also a partially purified product retaining some portion of unwanted proteins which further contributes to reactogenicity. The adverse events following antivenom administration have been described in detail (Morais and Massaldi, 2009; Warrell, 2010).

The total dose of protein administered is one of the main determinants of reactogenicity in antivenom therapy, the antivenom prepared using pooled venoms from different geographical regions is likely to give better neutralization against snake varieties from wider area and hence lesser dose might be required during treatment which will not only increase safety to snake bite victims but can also reduce the treatment cost and make it more affordable for poor people who are the commonest victims.

Use of 0.25 ml of 1:1000 Inj. Adrenaline, given subcutaneously before antivenom administration, reduced incidence of acute reaction to antivenom (Premawardhena et al., 1999). Adequate dilution of antivenom with saline and controlling the rate of infusion also helps in reducing reactions. Due to possibility of adverse reactions, Inj. Adrenaline should be always kept handy, before starting the dose of antivenom. Skin hypersensitivity test reveals IgE mediated Type-I hypersensitivity to horse or sheep protein. However, since the majority of early or late antivenom reactions result from direct complement activation rather than from IgE mediated hypersensitivity, skin test has limited predictability value and hence should not be used as it may delay the treatment (Warrell, 2010).

It should be remembered that adverse reactions to antivenom can be managed with medication and hence it should not be withheld from needy person because of the associated risks.

LIMITATIONS OF ANTIVENOM TREATMENT

Snake venoms of many viperids and elapids result in local tissue damage at the site of bite. Viper bites are usually associated with massive edema, blistering and hemorrhages whereas cobra bites cause necrosis. In many cases, such changes may result in permanent disabilities (Warrell et al., 1995a, b). It is usually experienced that antivenoms are effective in neutralizing systemic signs of venom biotoxins but are only partially effective against local effects of venom. The reason for this could be lower immune response of donor animals to venom toxins responsible for local tissue damage. In order to improve this, proper collection, handling, storage and preparation of venom for immunization and proper immunization protocols using appropriate adjuvants are required to be developed to elicit proper antibody response to these immunogens.

CHALLENGES

1. Low recovery and yields. Raising adequate neutralizing antibodies by appropriate immunization method while maintaining good animal health is a challenging task. However, after harvesting of plasma, the yield of immunoglobulin fractions recovered during manufacturing process is only about 50-60% which is much lower than yields in many other purification processes. Thus, in spite of putting in so much effort in animal maintenance, immunization and manufacturing a large portion of antibodies are not recovered in the currently used methods. Development of cost-effective means to achieve higher recovery/ yield remains a big challenge.

2. Opposition to use of animals for production purpose. Use of invasive methods on large animals for the production of biologicals is being objected by animal welfare activists who are insisting on reducing and ultimately discontinuing use of mammals for this purpose. Many companies are either reducing or slowly closing the production due to changes in regulatory requirements for animal use in many countries. Presently, no alternative method is available for producing antivenom and shortage of antivenom - especially in those countries having no domestic production - is likely to worsen further.

3. The need to increase antivenom effectiveness. This topic has been under debate for a long time. Venom variability has been the main reason for this debate because antivenom produced using venoms of a particular species from a particular geographical region may not completely neutralize effects of venom of the same species in other region. Such incidences have been reported from within the same country as well as from different countries. The only way to overcome this is by incorporating in the immunization mixture the venoms from that particular region where the antivenom is intended to be used. However, in many cases it is not possible due to various reasons such as non-availability of venom collection centers, lack of facilities, or regulatory prohibition. Antivenoms produced using low quality venom will have low neutralizing capacity. Hence it is absolutely essential for the production of effective and safe antivenom to establish a larger number of modern venom collection facilities for producing good quality venoms. Another reason for low effectiveness is low immunogenic fractions in the venom which do not give adequate antibody response. Generally, toxins have low molecular weight (and, therefore, low immunogenicity). Therefore, the non-toxic high

molecular weight venom proteins that are strong immunogens suppress the antibody formation against the low molecular weight toxic components of venom (Akbar et al., 2010). To overcome this challenge, different modalities such as use of newer adjuvants, improvements in immunization protocol, and identification of toxic low molecular weight venom fractions and their use in immunization can be tried.

4. Financial constraints in manufacturing. World Health Organization in its consultative meeting in 2007 has admitted that production of antisera products has declined worldwide due to economic constraints that have forced withdrawal from the market of some private manufacturers in many countries. This is mainly due to market instability and unprofitability. Due to economic reasons, there is no further investment in innovation. The challenge ahead is how to make the antivenom production commercially sustainable in the present economic and regulatory environment to maintain continued supply of this essential life-saving drug.

THE FUTURE TRENDS

The methodology of production of antivenom has not changed much after its start almost a century ago; however, the availability of modern proteomic tools in the last decade has provided interesting insights in to the venomics and antivenomics.

Development of Universal Antivenom. Antivenoms currently produced are regional as they are produced using venoms from a limited geographical area yielding antibodies specifically against it. The recent developments in venomics/antivenomics have given novel insight into the structure-function relationship for many venom toxins and provided an opportunity to develop an antivenom which will be more effective and less expensive and can be used over a large geographical area.

Use of IgY. Chicken IgY is functional equivalent to mammalian IgG. The IgY is passed from hen to the chicken embryo via the egg yolk. Egg yolk has a high concentration of chicken IgY and it is the main antibody in egg yolk. Therefore, use of chickens as replacement for mammals for antibody production has been tried wherein blood collection is replaced by extraction of specific antibodies from egg yolk. Production of IgY and its characterization and use as therapy for some bacterial and viral infections has been described (Carlander, 2002). It has been tried and tested at experimental level and has been found to be useful; however, it is not being used for commercial production of antivenom so far. IgY is preferred alternative to reduce and replace use of experimental animals, and it confirms to the Three R (Replacement, Reduction, Refinement) guiding principles. Hence it can be used as an alternative source of antibodies. In some recent studies chicken antibodies raised against snake venoms have shown to have neutralizing activity (Almeida et al., 1998; Paul et al., 2007; Manjula et al., 2008). Developments in poultry industry have made possible large scale low cost egg production, which might provide cheap source of therapeutic IgY in the future.

Monoclonal antibodies (MAbs). Efforts are going on to replace use of polyclonal antibodies by MAbs for therapeutic use, and recently MAbs have been successfully developed for various clinical applications (Nelson et al., 2010). Similarly, efforts are being taken to develop snake antivenom MAbs. Experimental development of MAbs against some snake venom toxins have been reported (Lomonte and Kahan, 1998; Tremeau et al., 1986). However, as snake venom is a complex mixture of many biotoxins, the biggest challenge is

going to be development of MAbs against each individual toxin so that a mixture of many mono-specific MAbs can be prepared as snake antivenom. Therefore it will be costly as well as difficult and would take much more time for development and licensing.

Development of herbal antivenoms. There are many herbal medicines known to have effect against the bites/stings of venomous animals and traditionally used in many countries. Some of these herbal formulations are claimed to reduce local post-bite complications also. However, no scientific study of these herbal formulations is available. Moreover, finding a suitable model to demonstrate effectiveness of herbal compounds is also a problem area as mechanism of action of these compounds might be different from that of antivenom immunoglobulins. Studies are going on to develop a herbal mixture which can be used either as first aid measure before administration of antivenom can start under medical supervision (Gomes et al., 2007) or as a combination therapy with antivenom (Alam and Gomes, 1998).

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