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Mini Review

The Evolution of Antivenom and its Use in the Treatment of Snake Bite

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Abstract

Perhaps the first account of immunization to snakebite in the west is the Greek historical record of king Mithridates, who is said to have immunized himself for protection. This procedure is still called mithridazation but is useless in most viper bite situations and can be considered medically unsound (though some recent accounts advocate it). In the immunized state the ''memory'' of the antigen resides in the body in special cells, so that when a challenge occurs (the presence of antigen), the body is capable of mounting a physiological response and in the span of 10 to 14 days can produce sufficient antibody to provide a resistance. In a snakebite, the reverse is actually true. The ''challenge'' consists of the maximal dose of the ''antigen'' and the disease falls full on the organism before significant ''antibody response'' is possible, According to the literature the first genuine antivenom was produced in 1895 by leon Charles Albert Calmette. His paper ''Contribution a etude des venins, des toxins, et des serums antitoxiques.'', describes the production of monovalent antiserum to the Indian combra, Naja naja, Calmette was a French scientist working in the Indochina branch of the Pasteur Institute, who did this work after a visit to Paris in which he witnessed the work of Pasteur, who at the time, was interested in the work of Edward Jenner and its Jenner with whom I would like to begin.

INTRODUCTION

The wok of Edward Jenner and Vaccination (1800)

It is the work of Jenner which represents the foundation of immunology and is one of the first truly medical investigations conducted in the modern way complete with a ''clinical trail''. It began with his curiosity about what was then a local superstition, the notion that those who had suffered from cowpox were immune to the ravages of smallpox. In fact, it is recorded that his curiousity was aroused by a remark he heard a dairymaid make. She said, ''I shall never have smallpox, for I have had cowpox. I shall never have an ugly pockmarked face.'' It was at that point that jenner, reasoning in the manner later described by Szent Gyorgyi A (''Discovery is seeing what everyone has seen, and thinking what nobody has thought.'') concluded that cowpox might not only be protective for the individual, but that this protection could be transmitted to other individuals inoculated with cowpox material. This he showed to be true by removing some material from a pustule on the hand of Sarah Nelms (May 14,1796) and using it to inoculate eight year old, James Phipps, who became mildly ill. Two months later in July, he inoculated the boy again, this time with fresh material taken from a smallpox lesion (Here is the clinical trial). No disease was produced. From this he concluded that protection was complete. This process called ''vaccination'', from the latin for ''cow'' (vacca), (also termed ''variolation'' , or ''inoculation'') first consisted of the practice of removing a small amount of the fresh matter taken from a wet pustule of the small pox proper and introducing under the skin of the nonimmune person. It was effective. In fact, it was so effective that George Washington made sure that all the troops that fought with him were ''variolated''. These crude means were certainly protective, those ''variolated'' were 10 times likely to contract the serious disease than those unprotected, but it was not uniformly innocuous and carried with it some danger that the patient might actually contract the serious disease, an event quality which could prove fatal.

Louis Pasteur was profoundly influenced by the work of jenner. He reasoned that the phenomenon, the conferring of immunity was physiological and universal and could be applied to other diseases. The disease he was studying at this time was chicken cholera, but when the procedure was carried out on the chickens, many died. In 1879 Pasteur was lucky enough to have an indigent assistant named Charles Chamberland whom he instructed to inoculate an experimental group of chickens with a fresh bacterial culture while he Pasteur went on vacation, But Chamberland went on vacation himself and returned only a month later. When he injected the chickens with the old month cultures, they merely became ill and were discovered to be immune to the diseases. Profiting from the success, Pasteur helped a vaccine for anthrax and in 1885 one for rabies. It was at this moment in time (1890) that Calmette met with Pasteur and resolved to form an Institue in the east (Pasteur Institute at Saigon, now Ho Chi Minh City). It was here in Indochina, using the immune sera taken horses vaccinated with snake venom (Calmettes serum) in 1894, he developed a monovalent antiserum against the venom of Naja tripudians, which seems to be in early name for Naja naja. Calmettes serum protected against the bite of a single species, and so could only be considered to be monoclonal, but that developed by vital Brazil in 1901, after his visit to Pasteur's laboratory, protected against the bites by the central and South American Crotalus and Bothrops genera and can be regarded as the first polyvalent antivenom. Antivenoms bind to and

neutralize the venom protecting against further damage, but they do not repair damage which gas already occurred and so should be administered as soon as possible after the bite, though they are of some benefit as long as venom is present. They represent greatly enriched fractions of the serum from the host in which they are prepared, but many other proteins are present as well. Since these are foreign to the snake bitten individual they may produce immunological consequences.

The manufacture of a very effective polyclonal antivenom to the pit vipers of the Unites States by Wyeth laboratories is well described in the volume entitled ''Venoms'' (AAAS publication number 44, 1956 Washington DC edited by Eleanor E Buckley (Wyeth laboratories) and Nand or Porges (US Department of Agriculture). Of special interest are the two chapters ''Development of a multivalent antivenin for the family Crotalidae'' (Criley BR, Wyeth laboratories, pages 373-380) and ''Standardization of Polyvalent antivenin. Gingrich C and Hohennadel (Wyeth Laboratories, pages 381-385). The first makes the point that the inclusion of many antigens (venoms) may be unnecessary (and even detrimental), if they do not contribute to the amelioration of the bite effects. In their work this group repeatedly tested the neutralizing potency of the preparation against the presence or absence of each venomous contribution before settling on the final antigen mixture, (*C. atrox, C. adamanteus, C. terrificus*, and *B.atrox*), and enormous amount of excellent work. The second describes the method for antivenom standardization used to develop a product which contains ''the basic antigens of all crotaline venoms''. Here they make an interesting rationalization of the fact the initial injections of their venom cocktail into the horses produced severe necrosis, ''Because of the natural environment and feeding habits of poisonous snakes, their mouth are always heavily contaminated with bacteria; it is inevitable that the venom from these creatures will be infected.''

Therefore in all subsequent work the solutions of venom were first incubated for 24 hours at 37°C in 0.5% concentration of formalin ''in order to destroy bacterial contaminants'' before injection. After the adoption of this procedure no further casualties occurred that could be attributed to injection of the antigen. ''Local reactions were milder only occasionally were abscesses or necrotic lesions observed.'' This generalization/speculation is not true, but their precaution produced a material that did less damage yet was still antigenic. Without realizing it they adopted the same method as that developed in 1923 by Alexander Glenny to inactivate tetanus toxin, they created a ''toxoid''. (As a matter of fact, we have investigated this phenomenon and found that the mouths of healthy snakes are remarkably free of bacteria that might be implicated in the production of disease).

With the development of effective antivenom it became necessary to review the methods of its administration. Many of the early preparations were ineffective in preventing both the lethal effect and the local necrosis after intraperitoneal or local infiltration of the injured tissue (Minton 1954). Consequently in our laboratory the new Wyeth antivenom was tested specifically not only against the lethal effect of the snake venom in the mouse, rabbit, and dog but also the locally necrotic one. We found that the local damage, previously attributed to bacterial contamination was not affected in the slightest by the administration of large quantities tetanus or gas gangrene antitoxins given in the same way. Obviously there was no reason to believe that all the venom proteins including enzymes which produce hemolysis, cytolysis or hemorrhage available polyvalent antivenom (Wyeth) labeled with radioiodine (I-131) we found that after intramuscular, supra scapular, intraperitoneal or intravenous administration (all venues mentioned in the literature), the antivenom accumulates at the site of the venom injection. When given prior to the administration of venom it drastically reduces the local effect. It was our finding that the most rapid accumulation of antivenom occurred in the bite area after intravenous administration of the serum with little or no difference between administration by the venous or arterial route. While substantial levels are developed after intramuscular injection of the antivenom, its release into the blood vascular compartment from that site is of more sustained nature and takes much longer to reach site of venom injection. The Wyeth preparation was made, as was common for several biological at that time, gas gangrene, tetanus antitoxin, preparations at the time, in an equnine host. This often produced a sensitivity to horse products that could manifest itself in a variety of ways from frank anaphylaxis to lingering allergy. Despite the fact that these were easily managed by epinephrine and/or corticosteroid, these side effects sounded the death knell for the Wyeth product. Finally, because of litigation, they were forced to employ the services of a physician fulltime to do nothing but appear in court to explain them, Wyeth decided that the continued manufacture of the antivenom was unprofitable and ceased its manufacture.

A successor of the Wyeth preparation is Crofab. The designers of this preparation arranged to use sheep as host animals, thinking that an ovine host might circumvent the problems associated with the development of equine antibodies. To go further we must understand the molecular nature of the antibody. As a graphic model we might imagine a clenched fist with the thumb index and middle finger extended. The fingers represent the antibody combining portions of the molecule, the index finger constituting the first antibody combining portion or fragment which is usually indicated as F(ab)1 and the middle finger representing the second antibody combining portion or fragment, F(ab)2. The thumb represents the constant portion of the antibody, F(ab)2., that portion which is characteristic of the host animal, horse or sheep in this case. These regions are separable by incubation of the molecule with an enzyme that digests protein, for example, papain. CroFab was manufactured from the antibody combing properties of only the first, or F(ab)1 fragment removed from the whole antibody molecule by digestion and fractionation. They used the venoms of the eastern diamond back rattlesnake (*Crotalus adamanteus*), the western diamondback rattlesnake (*Crotalus atrox*), the Mohave rattlesnakes (*Crotalus scutulatus*) and that of the cottonmouth (*Agkistrodon p.piscivorus*) in the antigen mix. In use this pharmaceutical appears to be effective except for the fact that the small size of the antibody combining fragment allows it to be eliminated too quickly from the body. This has resulted in the apparent cure and discharge from hospital of patients still envenomed. In these coagulopathies, problems of the circulatory system which require larger doses occur delivered over longer periods may appear subsequently. This large quantity of ovine protein though less allergenic

that horse serum may still produce some allergic response to treatment.

Subsequently more preparations designed for the treatment of snake envenomation have been developed. One of the most ell known of these is Anavip, the name in the United states of Antivipmyn (Bioclon, Mexico), which is distributed in the United States by rare disease therapeutics (Franklin, TN). It is one of the most recent medications designed to circumvent short half-life of the antivenom in the body. It utilizes the antibody combining properties of the F(ab2), a larger fragment not so easily flushed raised against the venoms of the Fer-de-lance (Bothrops asper) and the south American rattlesnake (*Crotalus durissus*) in an equine host. In use no late onset coagulopathies were observed but here again the medication is generated in an equine host with the consequent sensitivities. Boehringer Ingelheim Vetmedica produces Antivenom Crotalidae Polyvalent (ACP) which is a concentrated lyophilized preparation consisting of whole IgG molecules of equine origin. As antigens they use the eastern diamondback rattlesnake (*Crotalus durissus*) and the Fer-de-lance (*Bothrops atrox*). Hers is also unpurified liquid equine plasma called RTLR (MG Biologics, Ames IA) made from the Eastern diamondback rattlesnake (*Crotalus adamanteus*), the western diamondback rattlesnake (*Crotalus atrox*), the Prairie rattlesnake (*Crotalus viridis*), and the Mohave rattlesnake (*Crotalus scutulatus*) as antigens.

This is an outline of the present state of affairs as far as the medications presently in use for the treatment of snakebite is concerned. Despite the fact that it seems we have progressed with no progress, there is a very significant difference between the start and the end of this narration. The difference lies in the price. The ''shelf price'' of a dose of CroFab sheep derived medication presently used in the treatment of snakebite in North America is $3100. The cost to the patient can be much more. There has been no study nor probably will there ever be of the comparative efficacy of the Wyeth product and Anavip, the two equine derived antisera for the treatment of snakebite. For all present purposes, however we can assume them to be equally effective. The Wyeth product was priced at approximately $3.50 per unit dose. The cost of Anavip is $1220 per unit dose. The cost to the patient can start at $8900 may reach $14000. In mexico where Anavip/Antivipmyn is made it is priced at $100 a unit. Boyer (Personal Communication) has made a cost analysis of this and found that 70% of this increase in price is due to handling by the pharmaceutical industry and especially the hospitals. The cost of this new and improved medication used in the treatment of snakebite here in the United States may be $20,000/vial. Once again through avarice on the part of those individuals responsible for nothing more than dispensing the units of antiserum to the physician.

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