Title: EQUINE RABIES IMMUNOGLOBULIN: AN INDISPENSIBE IMMUNOTHERAPY

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Keywords Anti rabies serum, Equine Rabies Immune Globulin, Human rabies Immune Globulin

Abstract Human RIG represents the gold standard in the Post Exposure treatment of rabies, but HRIG is a very expensive, available only in limited quantities and that too in industrial countries. Its high cost makes it virtually unaffordable in developing countries. In India the cost of single treatment with HRIG is equivalent to 6 months wages of an Indian Labourer.

Review Article

Equine Rabies Immunoglobulin: an indispensable immunotherapy

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Summary

Human RIG represents the gold standard in the Post Exposure treatment of rabies, but HRIG is very expensive, available only in limited quantities and that too in industrialized countries. Its high cost makes it virtually unaffordable in developing countries. In India, the cost of single treatment with HRIG is equivalent to 6 months wages of an Indian laborer. But, the modern bio-engineered heterologous serum (ERIG) has come out as a safe and affordable alternative to HRIG. The proper management of the disease, supplemented with ERIG can save many more lives which may be lost just because of improper and unaffordable post exposure management of this dreadful disease.

Key Words: Antirabies serum, Equine Rabies Immune Globulin, Human Rabies Immune Globulin

Introduction

Although rabies is to be considered as a disease that should be controlled on a priority basis, unfortunately it remains as a neglected disease in majority of the countries particularly in Asia[1]. This disease is caused by a virus of the family *Rhabdoviridae* belonging to the genus *Lyssavírus* and affects warm-blooded animals, resulting in acute encephalitis with fatal outcome. The susceptibility to rabies infection depends upon the virus strain, genetic make up of the host, concentration of neurotransmitter receptors at the site of bite, inoculum size and very importantly proximity of the bite to the central nervous system of the host. Rabies has been a continuing problem in several countries across the globe and posed a challenge in front of researchers[2].

The seriousness of the disease can be estimated by the fact that WHO has cosponsored the "first World Rabies Day (WRD), along with the Alliance for Rabies Control (ARC) and Centre for Disease Control and Prevention, Atlanta (CDC), on 8 September 2007, with the aim of dispersing awareness about the impact of rabies, its easy prevention and to control and eliminate the disease in animals as well as humans. **Over 393,000 people in 74 countries participated and over 54 million people across the world were educated through the various activities performed on the first World Rabies Day. The next World Rabies Day was organized on 28 September 2009 across the world[3]. In the current year also, 28th September will be celebrated as World Rabies Day.**

Epidemiology: Public health impact of rabies

Rabies has a worldwide distribution with the exception of Oceania and Antarctica continents, which are luckily free from it[4,5]. Although nearly 50 counties are free from rabies nearly 100 countries across the world are the victims of this deadly virus.

Rabies has been reported to take **55,000** lives annually across the world of which 31,000 deaths occurs only in Asia. Rural population contributes about 90% of this total mortality[6]. The main affected population among humans is children and young adults. The deaths from rabies are likely to be grossly under-reported in a number of enzootic countries, particularly in the youngest age groups. Approximately 10 million people receive post-exposure prophylaxis annually and is estimated to prevent nearly 330 000 deaths in Asia and Africa. Statistics indices projects that rabies is globally responsible for the total loss of 1.74 million disability-adjusted years (DALY) each year of which 996,000 cases are contributed by the Asian countries[7]. In Asia, out of around 2.5 billion people exposed to the rabies infection every year; antirabic treatment is availed by only 8 million people. The annual global expenditure for rabies prevention is, by conservative estimates, >US$ 1 billion. Although Public awareness for wildlife and domestic animals, availability of vaccines (for pre-exposure treatment) and antiserum (for post exposure treatment) have remarkably resulted in decline in the incidences of the disease. But, the frequency of post exposure prophylaxis is expected to rise dramatically as all countries are willing to replace Neural Tissue Vaccines with modern, safe and highly potent Cell Culture Vaccines.

Rabies in India

Rabies is a major health problem in India too and is responsible for extensive morbidity and mortality. Human cases of rabies are reported from all over the country, with the exception of Andaman & Nicobar and Lakshadweep Islands. It is estimated that in India, every 30 minutes a life

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is taken away by rabies. The Association for the Prevention and Control of Rabies in India (APCRI) reported in 2004 that 20,565 humans died in a year from Rabies in India. The annual incidence of animal bites was estimated to be 17.5 million per year and the majority of the victims are the children younger than 15 years.

Use of Hyper immune serum in the post Exposure Prophylaxis

Though the treatment of this deadly disease of great public health problem, started around 124 years ago with Louis Pasteur but still rabies is a public health problem in almost all the countries, which are victims of rabies. The disease may be treated in the pre as well as post exposure period to the virus. The vaccines are the means of protection in the pre-exposure period and are recommended for veterinarians, laboratory technicians; people involved in research and the personnel that work with animals, because of having risk to the exposure to virus[5, 8, 9].

On the other side, post-exposure prophylaxis is indicated for people accidentally exposed to the virus. It consists of vaccination against rabies or a combination of the vaccine with hyper immune serum. The use of hyper immune serum is intended to permit the neutralisation of the virus before it penetrates the peripheral nerves and to provide enough time for the immune response to develop[10].

Historical perspective to the use of Hyper-immune serum

In 1945, Habel demonstrated that the vaccine must be administered along with the serum for obtaining better results in preventing post-exposure causality in rabbits[11]. In 1950, the World Health Organization recommended the use of anti-rabies serum[12]. At present hyper immune serum was considered coadjutants in rabies prophylaxis and became part of most of the anti-rabies treatment services in the world[13, 14].

However, if prophylactic serum is given after one week of the initiation of vaccination, then probably the serum therapy loses its importance, as unnecessarily it would suppress the natural antibody formation response in the host body[12].

Types of Immunoglobulins

Different from the vaccine that supplies the antigen for the body to produce the antibodies necessary, the serum is the ready antibody. There are three classes of rabies biologicals commercialy available for passive immunization: human rabies immunoglobulin (HRIG), equine rabies immunoglobulin (ERIG), and highly purified F(ab´)2 products produced from ERIG.

Human Rabies Immunoglobulin (HRIG): Human rabies immunoglobulin (HRIG) produced under good manufacturing practices is virtually devoid of serious adverse reactions. The earliest attempt to prepare a rabies immune globulin of human origin has been reported by *Habel*[15]. Later on the HRIG was raised in different variants by different researchers like *Anderson and Sgouris*[16], **Winkler *et al*[17], Sikes**[18] and **Cabasso *et al***[19].

Human rabies immunoglobulin is a liquid or freeze-dried preparation for passive immunization, containing immunoglobulin, mainly IgG. It is obtained from the plasma of donors immunized by repeated rabies vaccination. These preparations contain specific rabies antibodies for neutralizing the rabies virus. **The use of homologous immunoglobulins for human post-exposure treatment virtually eliminates the risk of anaphylaxis and serum sickness associated with heterologous serum products.** The dose of the human rabies immunoglobulin (HRIG) is 20 IU per kg body weight (maximum 1500 IU). HRIG does not require any prior sensitivity testing. HRIG preparation is available in concentration of 150 IU per ml. Half of the calculated dose is administered by intra muscular injection and half by infiltration around the wound[21].

Constraints to the use of HRIG

Homologous HRIG no doubt has an obvious advantage over the heterologous ERIG as the administration of the former minimizes the risk of anaphylaxis or serum sickness. Although, human RIG represents the gold standard concerning passive immunization for the treatment of rabies, but it is very expensive, is available only in limited quantities and available mainly in industrialized countries. In India, the cost of single treatment with HRIG is approximately Rs. 15,000/-, which is equivalent to 6 months wages of an Indian laborer and makes it almost unaffordable[21].

Heterologous antirabies Immunoglobulin

Utilisation of anti-rabies serum of equine origin (ERIG) for prophylaxis in humans was acceptable in the medical practice only a few decades ago, because at the time of its inception ERIG induces important reactions in the recepients[22 & 23] has suffered from bad publicity due to fear of inducing anaphylaxis and serum sickness reactions[23-24].

Although the frequency of serum related reactions is relatively low and is generally not fatal, it is always recommended that serum administration should take place only in the hospitals equipped with proper facilities for control of eventual anaphylactic reactions[25].

Anaphylaxis reactions occur typically in the individuals who are second time receiving the serum therapy, the first serum administration is responsible for inducing sensitization[26]. The serum reaction is manifested by fever, urticaria, arthalgia, lymphadenopathy, proteinuria and

peripheral neuropathy. The other common symptoms in sensitive individuals are pain at the site of serum infiltration, rashes at site of infiltration and urticarial rash. Even after negative skin test, 1 - 6% of the patients may develop adverse reactions to heterologous serum administration[27].

Historical Perspective of Production of Equine Rabies Immunoglobulin (ERIG)

Development of hyper immune serum destined to passive immunization against Rabies; occurred simultaneously with the anti rabies vaccine[28]. Babes and Cerchez[29] first observed the efficacy of rabies immunoglobulin. In 1950, WHO recommended the use of anti rabies serum due to the high number of studies performed since 1889[30].The purified equine-based rabies antiserum (ERIG) possesses specific activity of neutralizing the rabies virus[31,32].

In India, both public and private sectors are engaged in manufacturing the anti rabies serum. Out of the total 16 million vials /year global market demand, 5-6 million vials/year are required in India. Antirabies serum in India was first prepared in 1903 by Central Research Institute Kasauli **(earlier known as Pasteur Institute of India Kasauli)** a Government of India concern[33]. Haffkine Biopharmaceuticals Corporation Ltd. Mumbai; Vins Bioproducts, Hyderabad; Cadila, Pharmaceutical, Ahmedabad; Bharat Serum & Vaccines Ltd, Thane and Serum Institute of India, Pune are among the other leading Government and private manufacturers in the trade. ERIG has been prepared in the past using a combination of inactivated and fixed (live) strains of rabies virus[34]. Fuenzalida and Palacios[35] developed a better method for hyper immunization of horses. The animals were given a series of subcutaneous injection of anti rabies vaccine in increasing concentration followed by simultaneous subcutaneous, intra-peritoneal and intradermal injections of pure virus in Freund's incomplete adjuvant. The horses were bled 20 days after completion of 48 days immunization schedule[34]. It consists of a solution of purified immunoglobulin raised in horses and mules and is obtained from the serum of these hyperimmunized animals.

A brief introduction to the production of ERIG

ERIG is prepared using various immunogenic preparations, consisting usually of a combination of inactivated and fixed strain of rabies virus[34]. The animals are given a series of injections of the vaccine. All the injections are given subcutaneously into the lateral aspect of the neck. The standard immunization schedule lasts for about 15 weeks and the first manufacture bleeding is done two weeks later. The blood is collected, centrifuged and the serum without erythrocytes is collected for preparation of the product that contains the antibody.

Immunization of equine with cultured rabies antigen was also tried by different agencies for anti rabies serum production; using two-types of antigens *i.e.,* live and inactivated, both prepared from VP strain of rabies virus cultured on BHK-S13 cells and maintained by weekly passage. First stage involved immunization with inactivated antigen followed by a second stage using live antigen in a volume of inoculums as large as 100 ml, until a satisfactory titre was achieved[34].

A therapeutic antirabies immunoglobulin preparation of equine origin for human use was produced at the Queen Saovable Memorial Institute, Bangkok, Thailand by immunizing horses with a purified Vero cell rabies vaccine[36].

ERIG: Recent Developments

Anti-rabies serum, routinely used nowadays, consists of a solution of purified immunoglobulins obtained from the serum of hyper immunised horses inoculated with rabies virus. Purification techniques can be used to reduce the risk of sensitisation to ERIG. Their objective is to maximize the specific activity and to minimize the allergenic substances in the product. The purification of immunoglobulins from human plasma is carried out according to the technique of Cohn et al, based on the selective precipitation of proteins by chilled ethanol. This technique has been adapted for purifying heterologous immunoglobulins.

Safer production of equine serum is recommended to eliminate or minimize the risk of anaphylactic type hypersensitivity and serum sickness reactions. With the advancement in technology, now a days the anti rabies serum preparations are purified by specific enzyme treatment, Ammonium sulfate fractionation and thermo coagulation[37]. It minimizes the foreign protein burden, which results in reducing the incidence of hypersensitivity therefore making the treatment more safe and efficient[38-39]. The incidence of hypersensitivity reactions during the early 1990s, were reported to be as high as 40 % in the individuals receiving anti rabies serum therapy whereas nowadays with the availability of more refined ERIG, the anaphylactic reactions occurs are very few and this frequency reported in the recent past is 1:40,000 patients[40]. A recent study also supports this fact, when prophylactic treatment with ERIG was given to 33 individuals; none of them developed any type of hypersensitivity or serum sickness reactions mediated by immunocomplexes[5].

Barriers to the post exposure treatment of the disease

It was also proved that the mortality resultant from severe bites is reduced tenfold if the serum is administered associated with vaccine[41,42]. But, the common factors

contributing to the disease seems to be the lack of awareness among the public and health authorities; non-availability, short supply and high cost of modern vaccines and immunoglobulin, and lack of keen interest of the Government authorities in controlling the canine rabies on war footing basis.

WHO Recommendations for Passive Immunity

Use of antirabies immune serum or globulin of equine origin in the post exposure immunization of man has become accepted in medical practice only during the past three decades[43]. WHO strongly recommends the administration of rabies immunoglobulin along with vaccine in class III exposures (single or multiple transdermal bites), scratches and contamination of mucous membrane with saliva. Presently both human rabies immunoglobulin (HRIG) and purified equine rabies immunoglobulin (ERIG) are available. But HRIG is very expensive, as it is imported and also not freely available in many countries. Whereas, ERIG is indigenously produced, less expensive and easily available. This treatment is especially important when the incubation period is anticipated to be short[43]. Although, HRIG is an ideal product with no adverse reactions, but its cost and less availability are the limiting factors.

Dosage and administration of ERIG

WHO recommended human dose of equine-based RIG as 40-IU/Kg-body wt. As much as possible, the serum should be infiltrated into and around the wound area. All the wounds should be infiltrated with RIG. If the total volume of RIG is not sufficient to infiltrate all the wounds, it can be diluted with normal saline (up to 3 times) before administration. RIG has to be given as a single dose and should not be repeated. After local infiltration, the remainder of the serum should be given intramuscularly in the gluteal region. Before administering the ERIG the instructions by manufacturer on the leaflet accompanying the antiserum must be followed. The ARS produced for use in India contains 300 IU per ml. If there was complete post exposure or pre exposure treatment in past one year with tissue culture vaccines there is no need for administration of RIG[44].

Other rabies biological products

F(ab´)2 products: F(ab´)2 fragments are obtained by cleavage of the immunoglobulin by a proteolytic enzyme, pepsin, followed by separation of the F(ab´)2 fragments from the Fc fragment. Many of the ERIGs now available are produced in this way. F(ab´)2 fragments are cleared more rapidly in vivo than intact immunoglobulins. Undesirable side-effects are rare and are similar to those listed above for ERIGs.

Rabies immune globulin (RIG) is essential for post-exposure prophylaxis but is expensive and is not widely available. A monoclonal antibody cocktail is useful for post-exposure treatment of mammals for rabies and rabies-related viruses. The cocktail is immunoreactive with both glycoprotein and nucleoprotein epitopes of rabies virus and is cross-reactive with rabies-related virus *Duvenhage* and Mokola. The specific virus neutralizing activity is higher than human or equine anti-rabies hyper-immune sera presently recommended by the World Health Organization as the post-exposure therapy of choice. Rabies virus-neutralizing human monoclonal antibodies (Mabs) were evaluated in vitro and in a Syrian hamster model as a potential future alternative.

Considering the fact that globally, approximately 10 million people each year are treated after exposure to rabies. Some 40,000 to 70,000 people die of the disease each year, mainly in Africa, China and India. Post exposure prophylaxis for severe bites requires both active immunizations, using vaccines and passive immunization in the form of rabies immunoglobulins (RIG), Zydus and WHO are developing next-generation biologicals to fight rabies. Rabies monoclonal antibodies (MAbs) are expected to be an innovative therapy and can emerge as a potent alternative. While the rabies vaccine induces active immunity, the Rabies monoclonal antibodies can be safely administered with the vaccine to provide immediate passive neutralizing activity.

Equine Rabies Immunoglobulin: A safe substitute for HRIG

Human RIG is available in confidential quantities on specific markets and is too expensive approximately five times more expensive than purified horse serum. Hence, where HRIG is not available or affordable, purified equine immunoglobulin (ERIG) or F(ab´)2 products of ERIG should be used. The adverse-reaction rate of patients receiving highly purified ERIGs has been reduced to <12%. Most of the new ERIG preparations are highly purified commercial preparations. These are potent, safe and considerably less expensive than HRIG. Moreover, the occurrence of adverse events has been significantly reduced. This product is available in around 1500 IU/ vial, each vial costing about Rs. 350-450 in India.

To conclude, in view of the high costs of rabies immunoglobulin of human origin (HRIG), heterologous (mainly equine) immunoglobulins are required for the prevention of rabies in persons who have been severely exposed (category III) to the virus. Since RIG should be administered in all category III exposures and category II exposures involving immunodeficient individuals, it should be made available at all the treatment centres in the country at an affordable price to prevent innocent deaths. If used under trained medical supervision, the use of highly purified horse immunoglobulin will save many precious lives, which are lost just because of non-availability and the

cost involved in the Post exposure treatment using homologous rabies immunoglobulin.

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