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A REVIEW: RECENT APPROACHES IN TREATMENT OF RABIES
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Abstract

Rabies is an acute viral disease caused by a rhabdovirus which causes encephalomyelitis in virtually all the warm blooded animals including man. An acute, viral, infectious, communicable disease of the central nervous system characterized by central nervous system (CNS) irritation, paralysis, and death. Rabies is a viral infection transmitted in the saliva of infected mammals. There are several tests that are necessary to diagnose rabies before death in humans. Rabies postexposure prophylaxis, which is highly effective if given promptly, includes wound cleansing, immunization with a modern cell-culture vaccine, and administration of human rabies immunoglobulin (HRIG)

Key words: Rabies Disease, Rabies Virus, Advancement in Rabies Vaccine, Rabies Vaccine, Recent Approaches in Treatment of Rabies.

Introduction

Rabies is an acute viral disease caused by a rhabdovirus which causes encephalomyelitis in virtually all the warm blooded animals including man. The causative agent is found in domestic and wild animals, and is transmitted to other animals and to humans through close contacts with their saliva (i.e. bites, scratches, licks on broken skin and mucous membranes).^[1] An acute, viral, infectious, communicable disease of the central nervous system characterized by central nervous system (CNS) irritation, paralysis, and death. Rabies is an important zoonotic infection in which man is dead end of the infection and hence does not play any role in its spread to new hosts. In most of the developing countries, dogs are the principle reservoirs of

rabies (canine rabies) whereas sylvatic rabies involving animals such as foxes, racoons and coyotes are principle reservoirs of this disease in developed countries. Rabies is a zoonotic viral disease which occurs in both domestic and wild animals. The virus is transmitted to other animals and humans through close contact with saliva from infected animals (i.e. bites, scratches, licks on broken skin and mucous membranes). Once symptoms of the disease develop, rabies is fatal to both animals and humans. ^[2,3] Rabies is a viral infection transmitted in the saliva of infected mammals. The virus enters the central nervous system of the host, causing an encephalomyelitis that is almost always fatal. Although all species of mammals are susceptible to rabies virus infection, only a few species are important as reservoirs for the disease in nature. Rabies primarily attacks the nervous system and causes an encephalitis. The first symptoms of rabies are usually non-specific and suggest involvement of the respiratory, gastrointestinal and/or central nervous systems. In the acute stage, signs of hyperactivity (furious rabies) or paralysis (dumb rabies) predominate. Rabies may also spread through exposure to infected domestic farm animals, groundhogs, weasels and other wild carnivores. The rabies virus, present in the saliva of an infected animal, is usually spread by a bite or scratch that punctures the victim's skin. If antibody or immunogenic vaccine is administered promptly, the virus can be prevented from invading the central nervous system. In the United States, several distinct rabies virus variants have been identified in terrestrial mammals, including major terrestrial reservoirs in raccoons, skunks, foxes, and coyotes. In addition to the terrestrial reservoirs for rabies, several species of insectivorous bats also serve as reservoirs for the disease. Wildlife is the most important potential source of infection for both humans and domestic animals in the United States. Reducing the risk of rabies in domestic animals and limiting contact with wild animals are central to the prevention of human rabies.

Materials and Methods

Vaccination of all domestic dogs, cats, and ferrets coupled with the systematic removal of stray animals that are at risk of exposure to rabid wildlife, are basic elements of a rabies control program. States, indigenously acquired rabies among humans has declined markedly in recent years. The decline is, in part, due to vaccination and animal control programs begun in the 1940s that have practically eliminated the domestic dog as a reservoir of rabies and also to the development of effective human rabies vaccines and rabies immune globulin. During 1980-2004, a total of 56 cases of human rabies were reported in the United States (including

two in Georgia- during 1991 and 2000, respectively.)Among the 55 cases for which rabies-virus variants were obtained, 35 (64%) were associated with insectivorous bats, most commonly the silver-haired and eastern pipistrelle bats. More than half (57%) of these human cases occurred during August-November, coincident with a seasonal increase in prevalence of rabid bats detected in the United States. Despite the substantial number of cases of human rabies attributable to bat exposure, the importance of these exposures is often overlooked or under-estimated. In many of these cases, the bat bite was presumably not recognized nor the risk of rabies appreciated in order to seek appropriate medical attention. Human rabies is a completely preventable disease if the risk of acquisition is appreciated and appropriate rabies postexposure prophylaxis (consisting of wound care as well as both active and passive immunization) is obtained. Because rabies is a fatal disease, the goal of public health (in coordination with the medical community) is, first, to prevent human exposure to rabies by education and, second, to prevent the disease by administering rabies postexposure prophylaxis (PEP) if exposure occurs. Ten of thousands of people are successfully treated each year after being bitten by an animal that may have rabies. ^[4]

Causative Agent

Rabies virus belongs to the family Rhabdoviridae and genus Lyssavirus (Lyssa: Greek: rabies). The genus was at first divided into four serotypes (1–4) by antigenic cross-reactivity with sera and monoclonal antibodies, which correspond to the following species: serotype 1, rabies virus (RABV); 2, Lagos bat virus (LBV); 3, Mokola virus (MOKV); and 4, Duvenhage virus (DUVV). Further isolations of new bat lyssaviruses in Europe, then Australia and the progress in genetic characterisation of several genes (N, P, and G) supported the delineation of seven genotypes (1–7), confirming and expanding the antigenic data (Table 1): 1, RABV; 2, LBV; 3, MOKV; 4, DUVV; 5, European bat lyssavirus 1 (EBLV-1); 6, European bat lyssavirus 2 (EBLV-2); and 7, Australian bat lyssavirus (ABLV). Within each genotype, sublineages correspond to variants circulating in specific geographical regions and/or animal hosts. The genotypes further segregate in two phylogroups including genotypes 1, 4, 5, 6 and 7 (phylogroup I); and 2 and 3 (phylogroup II). Viruses of each phylogroup differ in their biological properties (pathogenicity, induction of apoptosis, cell receptor recognition, etc.) ^[5]

Recent Approaches In Treatment Of Rabies

Research considerations for the 21st century

Basic research

Molecular, genetic and epidemiological characterization of new isolates.

New isolates are being reported more frequently from different parts of the world. Scientists participating in the discovery of new lyssaviruses should be encouraged to act promptly to characterize these isolates. Antigenic, genetic and epidemiological methods have been developed and many lyssavirus sequences are now available in public databases for comparison with new isolates and for phylogenetic analysis. In addition, molecular tools, including restriction fragment length polymorphism and genotype-specific primers, have been developed for rapid screening and classification of new isolates. It is of particular importance to verify if rabies biologicals, such as vaccines and antibodies, protect against newly isolated viruses.^[44]

Biologicals

After the advent of cell-culture-based vaccines in the 1970s, no major advances have occurred in the development of new human rabies biologicals, as far as commercial realization is concerned. Of several options for future paradigm shifts, the technology available via reverse genetics opens powerful arenas to use negative-stranded RNA viruses as cloning and expression vectors.

Additionally, newer, safer and more effective recombinant viruses, for example focused upon adenoviruses, as well as DNA and plant-based vaccines, should continue to receive greater attention. In all cases, the use of genetically engineered rabies vaccines should comply with national and international biosafety guidelines. Assuming continued emergence of new lyssaviruses, especially in bats, the need of a broader protection spectrum of rabies vaccines is needed.

For example, DNA vaccines with plasmids expressing chimeric G protein(s) made from the fusion of two halves (part) of G originating from different genotypes have induced in mice antibodies with a wider spectrum of neutralization against various lyssaviruses.

These chimeric G proteins could be used to prepare anti-lyssavirus vaccines. In addition, insertion of foreign epitopes/antigens within the lyssavirus G protein was also demonstrated in mice and offers perspectives to

prepare a multivalent vaccine (Activation of innate immune responses by novel vaccine carriers and adjuvants and their protection when used for post-exposure prophylaxis should be further elucidated.

The opportunity to combine basic rabies parenteral or oral vaccination with concomitant immunocontraception for dogs and other carnivores is also of value. Similarly, pragmatic methods other than population reduction for the control of rabies in vampire bats should be investigated.

Besides vaccines, rabies immunoglobulin is a critical part of human rabies postexposure prophylaxis, particularly after severe and multiple bites to the face by rabid carnivores.

In addition to standard laboratory potency tests that determine the relative concentration of rabies virus neutralizing antibodies per unit volume, some measure of expected efficacy is desirable. Reproducible animal models should be developed to assess the effectiveness of various immunoglobulins after rabies virus infection.

The in vivo half-life of antibody preparations in relevant target tissues should be established for new immunoglobulin preparations. Levels of antibodies needed for successful passive immunization and their duration should be established. Immunoglobulin preparations that may have to be given repeatedly need to be tested for potential interference with active immunization. Animal models may be useful to generate data needed for the assessment of suitable immunoglobulins or alternatives, such as monoclonal antibodies, in modern rabies prophylaxis. The current NIH test for vaccine potency is fraught with difficulties, and more appropriate methods to assess the relative antigenic content are desirable.

Treatment

At least five patients that had all received either pre- or post-exposure prophylaxis developed clinical signs of rabies and subsequently recovered.

In 2004, a teenager in Wisconsin, USA, bitten by a bat became the first person to recover from the disease after experimental therapy that included a drug-induced coma, with no use of rabies biologicals.

In keeping with recent communications on the palliative treatment of human rabies victims, new research on antiviral drugs, focused on negative-stranded RNA viruses, should be supported. Current research on short interfering RNA should be expanded to include the lyssaviruses.

Combined with realistic animal models, a holistic approach should entail rapid intra vitam diagnostics, basic patient care, vaccination, administration of immunoglobulins, cytokines, etc. Insights gleaned from pathobiological studies can be used in the design of additional approaches in the future.

Epidemiology

Recent observations suggest that bats are important lyssavirus reservoirs, and the virus variants associated with Chiroptera may occasionally spill over into other mammals, with the potential for adaptation and establishment.

It is particularly disturbing that there is sometimes no evidence of direct exposure in human cases of infection associated with bats. New research should focus on the epidemiology of bat lyssaviruses and potential pathogenic mechanisms.

No recent comprehensive studies exist using relevant hosts and viruses, or alternative routes and unusual settings.^[45]

Pathobiology

Lyssaviruses naturally infect neurones resulting in dysfunction and death. Further fundamental studies are required to understand the molecular basis of rabies pathobiology in neurones and other relevant tissues.

The diversity of lyssavirus genotypes offers opportunities to address these questions through their differential pathogenicity in cell and animal models.

Recently, organs (including kidneys and liver) were transplanted from a misdiagnosed patient to other humans and caused rabies.

The wide distribution of virus throughout the body may compel us to revise our views about rabies transmission. Rather than discourage organ donation or transplantation, such scenarios should be viewed as important opportunities to review current practices to determine if there are ways to enhance the safety of transplant procedures without having an impact on organ supply, as well as to raise questions about basic rabies pathogenesis.

For example, the likely mechanism of infection during transplantation remains unclear. Similarly, the effects of patient immune suppression on disease development are not predictable.

Prevention and rapid screening for recognition of transplant-transmitted infections may be improved in various ways, including the development of appropriate animal models to study the process of overall pathobiology.

There is a need to investigate in experimental animals at what stage of infection the virus is present in organs other than the CNS. Clinicians treating human rabies cases should be encouraged to obtain, in the course of the disease, samples of secretions, blood and other body fluids and tissues for testing for the presence of rabies virus.

New vaccines, immunoglobulins, cytokines and antivirals should be used in an experimental setting in preparation for any future suspected cases that may arise if a organ recipient has received an implant from a donor who was later found by screening or diagnostic follow-up to have been infected with rabies virus.

Operational research for canine rabies control

Operational research should be conducted to remove or alleviate the main constraints and obstacles to canine rabies control programmes, which, as outlined below, include a lack of visibility, coordination, infrastructure, dog population management and community awareness.

Rabies: a priority in national health policy

In rabies-endemic countries with a high number of rabies deaths per 100 000

Inhabitants, ways and means to bring rabies to the priority level it deserves as part of the national health policy should be identified. Rabies should be listed as an important health problem in countries with high reported or estimated numbers of human rabies deaths and providing large number of post-exposure prophylaxis annually.

Coordinated national rabies programme

In most countries, several ministries deal with rabies. Generally the ministry of health is responsible for the prevention of rabies in humans and the ministry of agriculture is in charge of rabies control in animals. The ministry of local government and/or the ministries of commerce, industry or science and technologies are involved in rabies vaccine production and imports, dog population management and dog immunization. NGOs and animal rights and welfare groups also have a stake in rabies control and often these groups and organizations act independently and in tandem. The interaction and collaboration between the veterinary and

public health departments is minimal or non-existent at all levels in most countries, resulting in unproductive use of resources.

A central coordinating body or mechanism should be established to ensure that the efforts for rabies control are cohesive and give satisfactory results.^[46]

A New Flavor-Coated Sachet Bait For Delivering Oral Rabies Vaccine To Raccoons And Coyotes

- (1) attractive to the target species,
- (2) could be distributed by aircraft,
- (3) as effective (or more so) than the currently used fish meal polymer bait, and
- (4) could be produced in large numbers by automated procedures and could be purchased by user groups at substantially lower cost.

New Rabies Vaccine May Require Only A Single Shot, Not Six.

A replication-deficient rabies virus vaccine that lacks a key gene called the matrix (M) gene induced a rapid and efficient anti-rabies immune response in mice and non-human primates, according to James McGettigan, Ph.D., assistant professor of Microbiology and Immunology at Jefferson Medical College of Thomas Jefferson University.

"The M gene is one of the central genes of the rabies virus, and its absence inhibits the virus from completing its life cycle," Dr. McGettigan said. "The virus in the vaccine infects cells and induces an immune response, but the virus is deficient in spreading."

The immune response induced with this process is so substantial that only one inoculation may be sufficient enough, according to Dr. McGettigan. In addition, the vaccine appears to be efficient in both pre-exposure and post-exposure settings.

Oral Rabies Vaccine (Orv) Bait Uptake By Captive Striped Skunks

Vaccine ingestion seemed more likely if VCs were directly coated with the bait matrix. To make baits attractive to skunks and to ensure puncture of the VC, modifications to current baits should consider a smaller size, a meat-flavored matrix, a slightly pressurized VC, and a direct coating of matrix on the VC.

Rabies Challenge of Captive Striped Skunks (*Mephitis mephitis*) following Oral Administration of a Live Vaccinia-Vectored Rabies Vaccine

The skunks that did not survive failed to seroconvert following vaccination. None of the skunks that accepted multiple doses of the vaccine offered in coated sachets survived challenge, nor were rabies virus-neutralizing antibodies (VNAs) detected in the sera. Likewise, none of the five skunks ingesting a single sachet developed VNA against rabies. However, in this group one skunk did survive rabies challenge.

This preliminary study showed that the vaccinia-vectored oral rabies vaccine Raboral V-RGH, as formulated for use in raccoons, is capable of protecting a percentage of skunks against rabies. However, although the fishmeal-coated sachets were readily consumed, subsequent challenge of these animals revealed poor vaccine delivery efficiency.

Evaluation of Baits For Delivery Of Oral Rabies Vaccine To Dogs In Guatemala

All baits included a plastic sachet that contained a placebo vaccine (water). Bait trials were conducted February–April, 2002, at five sites using 261 dogs. Bait acceptance ranged from 50.0% to 87.1%, and the combined proportion of sachets either swallowed or punctured ranged from 23.1% to 83.9%. The four bait types with the highest acceptance by dogs were the wax-coated sachet coated with poultry oil and poultry meal (87.1%), the dog meal polymer coated with poultry oil and poultry meal (82.8%), the fish meal polymer coated with poultry oil and poultry meal (77.4%), and the chicken head bait (77.8%). These four bait types were accepted most often as determined both by consumption and combined proportion of sachets swallowed or punctured ($P < 0.0001$). Future trials should demonstrate efficacy of oral rabies vaccination in Guatemala based on the use of selected bait matrices and the poultry oil/poultry meal attractant.

Exposure Time of Oral Rabies Vaccine Baits Relative To Baiting Density And Raccoon Population Density

Estimate raccoon population density within the fragmented forest, old-field, and industrial landscape at PBS; and 2) quantify the time that placebo, Merial RABORAL V-RGt vaccine baits were available to raccoons. From August through November 2002 we surveyed raccoon use of PBS along 19.3 km of paved-road transects by using a forward-looking infrared camera mounted inside a vehicle. We used Distance 3.5 software to

calculate a probability of detection function by which we estimated raccoon population density from transect data. Estimated population density on PBS decreased from August (33.4 raccoons/km²) through November (13.6 raccoons/km²), yielding a monthly mean of 24.5 raccoons/km². We also quantified exposure time for ORV baits placed by hand on five 1-km² grids on PBS from September through October. An average 82.7% (SD54.6) of baits were removed within 1 wk of placement. Given raccoon population density, estimates of bait removal and sachet condition, and assuming 22.9% nontarget take, the baiting density of 75/ km² yielded an average of 3.3 baits consumed per raccoon and the sachet perforated.^[47]

A molecular epidemiological study of rabies virus in central Ontario and western Quebec.

Recent developments in molecular techniques and DNA amplification have provided invaluable tools for virus diagnosis and typing. This technology is now being applied to various laboratory and street isolates of rabies viruses. In particular, it will help document regional evolution of rabies virus, it may improve our knowledge of the historical relationships between rabies strains from different continents and help probe the molecular diversity of the Lyssavirus genus as a whole.

Advances in Diagnosis of Rabies

New Diagnostic Technique

1. Identification of the agent

A) Shipment of samples

b) Collection of samples

Occipital foramen route for brain sampling

Retro-orbital route for brain sampling

c) Routine laboratory tests

Immunochemical identification of rabies virus antigen

i) Fluorescent antibody test

ii) Immunochemical tests

Detection of the replication of rabies virus after inoculation

i) Mouse inoculation test

ii) Cell culture test

2. Serological tests

a) Virus neutralisation test in cell culture: fluorescent antibody virus neutralisation test (a prescribed test for international trade)

b) The rapid fluorescent focus inhibition test (RFFIT) for determining rabies virus-neutralising antibody (a prescribed test for international trade)

Reference sera

Test sera

Calculation of virus-neutralising antibody titres

c) Virus neutralisation in mice

d) Enzyme-linked immunosorbent assay^[48]

Rabies Neutralizing Antibody Detection by Indirect Immunoperoxidase Serum Neutralization Assay Performed on Chicken Embryo Related Cell Line.

The aim of this study was to evaluate the indirect immunoperoxidase virus neutralization (IPVN) and mouse Neutralization test (MNT) to detect antibodies against rabies virus from vaccinated dogs and cattle. The IPVN was set up for the ability to measure 0.5 International Units/ml (IU) of antibody required by the World Health Organization and the Office International des Epizooties as the minimum response for proof of rabies immunization. IPVN was developed and standardized in chicken embryo related (CER) cell line when 141 dog and 110 cattle sera were applied by serial five-fold dilutions (1:5, 1:25, 1:125) as well as the positive and negative reference controls, all added in four adjacent wells, of 96-well microplates. A 50 µl amount of CVS32 strain dilution containing 50-200 TCID₅₀/ml was mixed to each serum dilution, and after 90 min 50 µl of 3 x 10⁵ cells/ml cell suspension added to each well.^[49] After five days of incubation, the monolayers were fixed and the IPVN test performed. The correlation coefficient between the MNT and IPVN performed in CER cells was $r = 0.9949$ for dog sera ($n = 100$) and $r = 0.9307$ for cattle sera ($n = 99$), as well as good specificity (94.7%), sensitivity (87.5%), and agreement (96.6%) were also obtained. IPVN technique can adequately

identify vaccinated and unvaccinated animals, even from low-responding vaccinated animals, with the advantage of low cost and faster than MNT standard test. ^[50]

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