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Rabies virus protection issues and therapy

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Abstract

Rabies remains a serious and usually fatal disease in many countries. The World Health Organization (WHO) estimates that approximately 10 million people worldwide require medical treatment against rabies each year after being exposed to an animal suspected of having rabies. In the United States of America (USA), there are close to 40,000 post-exposure prophylaxis treatments administrated each year, which represents about 100 million dollars in costs for treatment, health care, education and prevention. Current rabies exposure immune prophylaxis includes a new treatment product tested in Phase 2 and 3 clinical trials in Israel. This product is now sold in 10 countries and should soon be available in the USA. Results of comparison clinical trials show it to be as efficacious as other human immunoglobulin G (IgG) products. In animals, there have been no documented cases in North America of rabies in vaccinated, truly immunized dogs and cats for two decades, although the disease still exists among wildlife and feral companion animal species. While most pet dogs are vaccinated for rabies, fewer cats have historically been vaccinated until recent laws have required it. The Rabies Challenge Fund (RCF) research studies are now at years 6 and 7 post-vaccination, and the initial challenge phase results showed the vaccinates to be protected from rabies.

Abbreviations: AE: Adverse Events; CNS: Central Nervous System; CVB: Center for Veterinary Biologics; ELISA: Enzyme-Linked Immunosorbent Assay; CFR: Code of Federal Regulations; IgG: Immunoglobulin G; MLV: Modified Live Virus; RCF: Rabies Challenge Fund; RVNA: Rabies Virus Neutralizing Titer; RFFIT: Rapid Fluorescent Focus Inhibition Test; USA: United States of America; USDA: United States Department of Agriculture; WHO: World Health Organization.

Introduction

Countless animals have been vaccinated routinely and repeatedly for rabies and the other common serious infectious viral and bacterial diseases, without obvious untoward effects [1-4]. But, veterinarians still need to be aware of the potential for adverse events (AE) and determine what constitutes "acceptable" harm [1-3].

Vaccines typically contain immunologic adjuvants which act to accelerate, prolong, or enhance antigen-specific immune responses when used together with specific vaccine antigens [5,6]. While vaccine adjuvants are incorporated into vaccines to enhance their immunogenicity, this increases the risk of autoimmune and inflammatory adverse events following vaccination [5]. For the killed vaccines available for human and veterinary use, potent adjuvants are included to produce a more sustained humoral immune response and compete favorably with the longer protection typically afforded by modified-live virus (MLV) products. But, these adjuvants may also induce adverse effects [1,5-17].

Discussion

Although killed or inactivated products make up about 15% of the veterinary biologicals used, they have been associated with 85% of the post-vaccination reactions, mainly because of the acute adverse responses induced by the adjuvants used in companion animal, wildlife and livestock species [1,10,16-18]. Several years ago, an "all-killed" combination vaccine for dogs was marketed, but some users encountered minor problems with discoloration and local reactions at the injection site, and the product was withdrawn. Ringworm and chlamydia vaccines introduced for use in cats are advertised as having the safety advantage of a killed product [1]. This debate about the relative merits and safety of killed versus MLV vaccines has been ongoing, and was hotly debated in a comparison of the risks, costs, and convenience of killed versus modified live human polio vaccines [19]. Documented AE from the adjuvants used in human vaccines, especially those containing aluminum and thimerosal (mercury salt), continue to appear in the literature [11-16].

Adverse events associated with vaccine adjuvants

Adjuvants have been used safely in human and veterinary medicine for decades, especially those containing aluminum salts, monophosphoryl lipid A, and squalene in animal vaccines [20-27]. In 2012, investigators from China determined that four botanical polysaccharide preparations, namely *Astragalus*, *Echinacea*, wolfberry and kelp, acted as immunopotentiators/adjuvants in mice and dogs when added to veterinary rabies vaccines [28]. Nevertheless, as cited above, adjuvants can also produce numerous AE.

Experimental studies have shown that simultaneous administration of even two-three adjuvants can overcome genetic resistance to autoimmunity [15]. Because vaccines are viewed as inherently safe and non-toxic, toxicity studies are often excluded from their regulatory safety assessment. Children are especially at risk being more vulnerable to toxicity than adults; and they are regularly exposed to more vaccine adjuvants than adults. Adjuvants impact the central nervous system at all levels and can do so by changing gene expression [13]. Further, it is now known that the neuro-immune axis, heavily targeted by adjuvants, plays a key role in brain development and immune function [12,13,15,24].

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The most common AE following routine vaccination in humans affect the central nervous system (CNS) [16]. These phenomena were classically attributed to the effects of the vaccine antigens. However, more recently, concerns have focused on the widespread use of aluminum and mercury-containing compounds in the vaccines given to humans and animals [5,6,11-17].

Studies using animal models and in human patients have indicated that these metals can inflict both immune and inflammatory responses, defined since 2011 as the autoimmune syndrome induced by adjuvants (ASIA syndrome) [11]. Presently, it includes four conditions that share similar signs and symptoms and result from hyperactive immune responses: siliconosis, macrophagic myofasciitis, Gulf war syndrome, and post-vaccination phenomena [7,11,14]. The common denominator was the triggering effect of the adjuvants, in combination with other environmental factors and genetic predisposition [1,2] When combined, these factors cause the failure of self-tolerance, which equates to autoimmunity [2].

The AE associated with administration of adjuvanted vaccines in humans are both neurological and neuropsychiatric [8,10-15]. For example, aluminum nanoparticles recently have been shown to possess a unique capacity to cross the blood-brain and blood cerebrospinal fluid barriers, thereby inciting harmful immune inflammatory responses in neural tissues [16]. The authors suggest that these findings could explain why vaccines appear to have a predilection for affecting the CNS. To date, however, these authors comment that the pharmaceutical industry and drug regulatory agencies assert that the concentrations of aluminum and mercury used in vaccines do not represent a health hazard [16,17].

Proving the ASIA concept was accomplished with experimental animal models including those for: rheumatoid arthritis, systemic lupus erythematosus, autoimmune thyroid disease, anti-phospholipid syndrome, and myocarditis. The reported neurotoxicity affects learning, memory, cognition, speech, increases seizure propensity, and alters behavior by increasing anxiety, insomnia, dementia and confusion [24]. Animal models are now widely used to understand the mechanisms, etiology and pathogenesis of these diseases; and results could help promote development of new diagnostic, predictive and therapeutic methods [7,11,14,16,17,27].

Effects of aluminum and mercury

Exposure to aluminum and mercury is widespread in nature and our lives. These metals are found in many sources of drinking water, as a food additive especially in processed "fast" convenience foods, in many cosmetics, field, lawn and garden fertilizers and herbicides, and in pharmaceuticals including vaccines; they thus can accumulate in the bodies of humans and most, if not all, species. They not only are neurotoxins, but also are immunotoxic, genotoxic, pro-oxidant, and pro-inflammatory [6-17]. Further, they are recognized to be endocrine disrupters, depress glucose metabolism, and interfere with calcium homeostasis, and mitochondrial and other biochemical pathways [16,17].

Mercury is another commonly acknowledged trigger of ASIA syndrome [14]. Use of mercury compounds has been widespread in medicine, despite its known toxicity. Mercury and other heavy metals mainly affect the body in two ways: via toxic and immunological reactions – which cause hypersensitivity or autoimmunity. Studies show that these metals, can be a risk factor for the development of various autoimmune diseases, such as autoimmune thyroiditis, multiple sclerosis, and kidney disease, and nonspecific symptoms such as chronic fatigue and myalgia. Animal studies have shown that mercury, aluminum, nickel, chromium, silver and gold, can be either non-toxic or induce severe diseases, such as skin disease or autoimmunity, which depends upon the individual animal's genotype. Endocrine status and the presence of chronic infections are factors that might predispose to the risk of sensitization [7,11,14].

The type of allergy induced by metals is delayed-type hypersensitivity and manifests often as a contact dermatitis. Thimerosal (merthiolate), like nickel, is one of the most frequent allergens in children and adolescents, and in companion animals vaccinated for rabies [1,2,7,11,14]. These metals exert both specific and non-specific effects contributing to the ASIA syndrome [14].

Vaxjo is a newly published, web-based vaccine adjuvant database [29]. Basic vaccine information stored includes: adjuvant name, components, structure, appearance, storage, preparation, safety, function. Currently over 100 vaccine adjuvants have been annotated in Vaxjo, and they have been used in over 380 vaccines produced against over 81 pathogens, cancers, or allergies.

In summary for aluminum, as stated by Tomljenovic and Shaw [16]:

"All the clinical and experimental evidence collected thus far identifies at least three main risks associated with aluminum (sic) in vaccines:

1) It can persist in the body (up to 8-11 years following vaccination);
2) It can trigger pathological immunological responses;
3) It can make its way into the CNS where it can drive further deleterious immuno-inflammatory processes resulting in brain inflammation and long-term neural dysfunction."

Other issues with rabies vaccines

Vaccination of pet and research dogs with polyvalent vaccines containing rabies virus or rabies vaccine alone was shown to induce production of antithyroglobulin autoantibodies, a provocative and important finding with implications for the subsequent development of hypothyroidism [26].

Post-vaccinal polyneuropathy is a recognized entity associated occasionally with the use mostly of canine distemper and rabies vaccines, and the ovine bluetongue virus vaccines, but any vaccine could presumably be implicated [2,9,10, 27,30-33]. This can result in various clinical signs including muscular atrophy, inhibition or interruption of neuronal control of tissue and organ function, muscular excitation, incoordination and weakness, as well as seizures [2,10,31].

Killed virus vaccines like those for rabies virus or ovine bluetongue virus, can trigger immediate and delayed adverse vaccine reactions [1,2,10,14,18,30-34]. While there may be immediate hypersensitivity reactions, other acute events tend to occur 24-72 hours or up to a week afterwards, and as long as 45 days later in the case of more delayed reactions. Documented reactions in the above citations include: behavioral aggression and separation anxiety, destruction and shredding of clothing and bedding; obsessive behavior, barking, fearfulness, self-mutilation, tail chewing; pica, with eating wood, stones, earth, and feces; seizures and epilepsy; fibrosarcomas at the injection site; and autoimmune diseases such as those affecting bone marrow and blood cells, joints, eyes, skin, kidney, liver, bowel, and CNS [1-3,18].

Based upon experience in the USA, rabies vaccines are the most common group of AE reported to the United States Department of Agriculture (USDA) Center for Veterinary Biologics (CVB) [32-33]. Currently, 14 rabies vaccines are labeled for use in dogs, but only two do not contain the thimerosal (mercury) adjuvant/preservative. These vaccines meet the standard requirements of USDA Title 9, Code of Federal Regulations (CFR), which requires that the vaccine provide protection of equal to or greater than 88% when comparing vaccinated animals to controls [30].

All rabies vaccines are evaluated prior to licensure, but not all safety concerns may be seen, because of an insufficient number of animals for low frequency events, insufficient duration of observation, sensitivities of subpopulations (e.g., breed, reproductive status, and unintended species), or interactions with concomitantly administered products [18].

Despite the serious under-reporting of vaccine AE, the Report cited above [32], states that between April 1, 2004 and March 31, 2007, nearly 10,000 adverse event reports (all animal species) were received by manufacturers of rabies vaccines. Approximately 65% of the manufacturer's reports involved dogs. This 2008 Report further states that "Rabies vaccines are the most common group of biological products identified in adverse event reports received by the CVB." During the 3-year period covered in this report, the CVB received 246 AE reports for dogs in which a rabies vaccine was identified as one of the products administered [32].

More facts about rabies and rabies titers

If a person or animal is bitten by a dog, cat or ferret, the animal causing the bite should be observed for 10 days. If the animal remains healthy, then one can be assured that there was no rabies virus in the saliva at the time of the bite. Whether that observation occurs at home or at a clinic should not be determined by vaccine status. Remember also that even in areas where terrestrial rabies is not active, that rabies in bats is seen nationwide [35,36].

A review of rabies challenge-studies indicates that there is a positive correlation between rabies virus neutralizing antibody (RVNA) titers and the level of protection after virus challenge. Pre-exposure vaccination coupled with a RVNA titer at or above 0.5 IU/mL indicates greater assurance of protection than does the animal's current vaccination status [35].

Because we may not know if an animal has been exposed to rabies virus, the KSU Rabies Diagnostic Laboratory [35] recommends that rabies titers be done routinely for dogs and cats. To provide the individual with best and safest medicine, a yearly rabies titer would make sure the pet has protection from unknown exposures. The circulating rabies neutralizing antibody level does not last the lifetime of the pet. In vaccine trials, as the titer falls below 0.5 IU/ml the risk of contracting rabies after challenge goes up. Thus, when rabies titers drop below 0.5 IU/ml, giving a rabies booster is the prudent, safe decision [35,36].

Significant post-rabies adverse reactions are an issue not only for dogs and cats, but also are of serious concern for horses, as they must be given rabies boosters annually. Many horses have incredibly high rabies blood antibody titers, and yet still must be revaccinated annually by law, and then can suffer a chronic disease state post-rabies vaccination. As a result, the KSU Rabies Diagnostic Laboratory is actively pursuing rabies titer information from horse vaccine trials. Neutralizing antibody is neutralizing antibody, no matter the species; the goal is to confirm success of the 0.5 IU/ml level in horses as well [35,36].

New data on rabies titers

Anamnestic antibody responses with current vs out-of-date rabies vaccines were studied in 74 dogs/33 cats. All animals had anti-rabies antibody titers measured by the rapid fluorescent focus inhibition test (RFFIT) of > 0.5 IU/mL, 5-15 days after a rabies booster. Dogs with out-of-date vaccine status had a higher median rabies titer increase after a rabies booster, and most (26/33) cats had titers of > 12 IU/mL, 5 -15 days after the booster.

Their findings were to give an immediate rabies booster vaccination with observation for 45 days in dogs/cats with out-of-date vaccine status, if they are exposed to rabies, as is the practice for those animals current on their rabies vaccine.

Presently, however, for out-of-date rabies cases, if exposed to a proven or suspect rabid animal, the options are either euthanasia or 6-month quarantine at the owner's expense. These new data are of obvious benefit and impact [35].

Compendium of Animal Rabies Prevention and Control, 2016

According to this National Association of State Public Health Veterinarians report [36], dogs & cats exposed to rabies that are overdue for a vaccine can now have a rabies booster shot followed by an observation period rather than be quarantined or euthanized. This effectively reduces the quarantine period from six (6) months to four (4) months for unvaccinated dogs and cats exposed to rabies. The Association also recommended national level collecting and reporting of any additional data elements on rabid domestic animals.

The best "herd health" protection against rabies in both individual animals and the population is to have all of them currently vaccinated against rabies. [Note: rabies exemptions may be approved on a case-by-case basis with written justification of the primary care veterinarian.]

New rabies immune prophylaxis

Another approach to rabies exposure immune prophylaxis comes from a new treatment product tested in Phase 2 and 3 clinical trials in Israel (KamRAB). The clinical trials were prospective, randomized, double-blind, and non-inferiority study of 118 healthy subjects. The study evaluated pharmacokinetic parameters of anti-rabies IgG levels in serum at different time points and assessed whether this immunoglobulin G (IgG) interfered with the development of self-active antibodies. In addition, safety and tolerability were assessed. The trial's primary end point measured the anti-rabies titer on day 14 as well as on additional time points for secondary end points, following drug infusion and infusion of an active vaccine as recommended by the standard-of-care.

Results of comparison clinical trials show it to be as efficacious and safe as other current IgG immunoglobulin products. This product is now sold in 10 countries and should soon be available in the USA (www.kamada.com)

Rabies challenge fund (rcf) 2016 challenge trials update (www.rabieschallengefund.org)

The USDA's new rabies challenge virus was given; 6 weeks post-challenge results met the interim goal to satisfy Title 9, CFR requirements for rabies vaccine licensing. Fifteen dogs were in the trial.

Only one of the 5 dogs vaccinated in 2007 showed protection against rabies, while 4 of 5 dogs vaccinated in 2009 (80%) were protected against the live rabies virus challenge.

Once all 5 unvaccinated control dogs showed very early clinical signs of rabies virus infection, they were humanely euthanized. As required, the surviving 5 vaccinates were observed for another 6 weeks to detect late development of clinical signs of rabies. No clinical issues occurred, so these dogs were successfully immunized and protected against rabies.

Whole blood mononuclear cells collected at post-challenge days 4 and 10 were tested by flow cytometry to determine the kinetics of immune memory cell responses. Tissue samples from the euthanized dogs underwent rabies virus detection testing. Serum samples collected at various time points throughout the study were evaluated by rabies serum virus neutralization and ELISA testing methods.

Another challenge with 15 dogs was completed to fulfill the USDA Title 9 requirements for rabies vaccine licensing and to establish a canine rabies titer standard as confirmed by challenge data. Results were similar to those of the initial trial.

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