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SPECIAL ARTICLE

Controlling Rotavirus-associated diarrhea: Could single-domain antibody fragments make the difference?

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Abstract Group A Rotavirus (RVA) remains a leading cause of severe diarrhea and child mortality. The variable domain of camelid heavy chain antibodies (VHH) display potent antigen-binding capacity, have low production costs and are suitable for oral therapies. Two sets of anti-RVA VHHs have been developed: ARP1-ARP3; 2KD1-3B2. Here, we explore the potential of both sets as a prevention strategy complementary to vaccination and a treatment option against RVA-associated diarrhea in endangered populations. Both sets have been expressed in multiple production systems, showing extensive neutralizing capacity against strains of RVA *in vitro*. They were also tested in the neonatal mouse model with various degrees of success in preventing or treating RVA-induced diarrhea. Interestingly, mitigation of the symptoms was also achieved with freeze-dried ARP1, so that it could be applied in areas where cold chains are difficult to maintain. 3B2 was tested in a pre-clinical trial involving gnotobiotic piglets where it conferred complete protection against RVA-induced diarrhea. ARP1 was used in the first clinical trial for anti-RVA VHHs, successfully reducing stool output in infants with RVA diarrhea, with no detected side effects.

PALABRAS CLAVE
Rotavirus;
Diarreas neonatales;
VHH;
Porción variable de anticuerpos de cadena pesada;
Nanoanticuerpos

Hacia el control de la diarrea por rotavirus A: ¿podrían los nanoanticuerpos VHH marcar la diferencia?

Resumen Los rotavirus del grupo A (RVA) constituyen la principal causa de diarrea grave y mortalidad infantil. La porción variable de los anticuerpos de cadena pesada derivados de camélidos presentan una amplia capacidad de unión antigénica (reconocen sitios antigénicos no accesibles a los anticuerpos tradicionales, con elevada afinidad) tienen bajos costos de producción y resultan ideales para las terapias orales. A la fecha, se desarrollaron 2 pares de nanoanticuerpos VHH

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contra RVA: ARP1-ARP3 y 2KD1-3B2. En este trabajo, exploramos el potencial de ambos grupos de nanoanticuerpos como estrategias de prevención complementarias a la vacunación y como una opción de tratamiento frente a la diarrea asociada a RVA en poblaciones de riesgo. Ambos pares de nanoanticuerpos fueron expresados en diferentes sistemas de producción y mostraron amplia capacidad neutralizante contra diversas cepas de RVA *in vitro*. También fueron usados en el modelo de ratón lactante, en el que evidenciaron distintos grados de éxito en la prevención o el tratamiento de la diarrea inducida por RVA. Es interesante destacar que la mitigación de los síntomas también se logró con ARP1 liofilizado y conservado, por lo que podría ser utilizado en áreas donde es difícil mantener la cadena de frío. Asimismo, 3B2 fue testeado en una prueba preclínica utilizando como modelo al cerdo gnotobiótico, al cual confirió completa protección contra la diarrea inducida por RVA. ARP1 fue usado en la primera prueba clínica de nanoanticuerpos VHH contra RVA, donde redujo significativamente las deposiciones en pacientes pediátricos con diarrea positivos para RVA, sin evidenciar ninguna reacción adversa.

Introduction

Group A Rotavirus (RVA) is the main source of severe diarrhea in young children, accounting for approximately 453,000 deaths every year, most of which occur in developing countries[56]. Globally, strains of four G-P combinations are responsible for about 90 % of all RVA-associated diarrhea cases: G1[P8], G2[P4], G3[P8] and G4[P8][46]. Although a similar distribution is found in Latin America, G9[P8] plays an important epidemiological role in this region, where the recent emergence of G12 strains has also been reported[13,33].

Prevention of RVA-associated diarrhea: where do we stand today?

Two vaccines, a single-strain attenuated human RVA (G1[P8]) vaccine (ROTARIX, GlaxoSmithKline Biologicals), and a multi-strain bovine-human reassortant RVA (G1-G4, P1A)[8] vaccine (RotaTeq, Merck), are available and have shown high efficacy in preventing severe RVA gastroenteritis in industrialized[14,43,63,64] and some developing countries[3,18,43]. However, studies show that RVA vaccines have significantly lower efficacy in countries with limited infrastructure and resources, where the burden of RVA-associated diarrhea is usually highest[34,71]. There are diverse factors that affect the performance of oral vaccines in impoverished settings including nutritional aspects such as malnutrition and zinc deficiency, the presence of competing enteropathogens, mucosal alterations in the gut due to persistent enteropathy and high levels of maternal antibodies (Abs) in breast milk[5]. Recently, several studies have determined that prenatal vitamin A deficiency alters the innate immune response against RVA vaccination[31,65]. Furthermore, children affected by severe combined immunodeficiency have suffered vaccine-acquired RVA infections and diarrhea[40].

Future efforts should focus on optimizing the efficacy, safety, accessibility and delivery of vaccines among high risk populations[30] as well as on developing new passive immunization strategies that could serve as a complement or alternative to vaccination. Recently, a new oral live attenuated RVA (G9 P[11]) vaccine (ROTAVAC) developed in India has passed clinical phase III[9]. ROTAVAC vaccine is cheaper than existing vaccines (1 USD per dose *versus* 15 USD for other commercial vaccines) and its licensure would substantially improve the access of developing countries to RVA vaccination[9].

Treatment strategies for RVA-associated diarrhea

Treatment strategies against RVA are non-specific and largely symptom-based. Clinical management of RVA-associated diarrhea is based on preventing dehydration through oral rehydration salts (ORS) administration, zinc supplementation, and continued feeding[68]. Several attempts to develop a specific treatment have been made, including the administration of RVA-specific bovine colostrum[49], monoclonal Abs[11] and egg yolk polyclonal immunoglobulin (Ig) YAbs[47,50,61], probiotics[25,41,72], drugs[42,45,57] and natural herbal compounds[6,32]. Some of these studies showed efficacy in reducing diarrhea duration or fluid loss, but none of these treatments was adopted as a standardized procedure against RVA-associated diarrhea. Additionally, passive immunization strategies, including the use of animal colostrum or IgYAbs, have raised concern about possible allergic reactions and the presence of adventitious viruses.

A new paradigm: camelid single-chain antibody fragments (VHHs)

In 1993, Hamers-Casterman *et al.*[27] discovered an IgG-like material in the serum of the camel (*Camelius dromedarius*). These molecules, which are present in all species of camelid, were composed of heavy-chain dimers and devoid of light chains, but had an extensive antigen-binding repertoire[27] (Fig. 1). The variable domain of these heavy chain antibodies, known as VHH, consists of only one polypeptide chain and therefore may be cloned and expressed as a soluble protein constituting monoclonal recombinant antibody