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Human heterophilic antibodies against equine immunoglobulins: assessment of their role in the early adverse reactions to antivenom administration

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Summary The presence of human heterophilic antibodies against horse immunoglobulins (HHA-HI) was determined by ELISA in sera from healthy volunteers and from patients who received equine antivenom for therapy of snake bite envenoming. These patients were selected from two independent clinical studies: one in Colombia in which patients received antivenom constituted by whole IgG ($n = 25$); and the other in Brazil where an antivenom constituted by $F(ab')_2$ fragments was administered ($n = 31$). Results show that healthy volunteers have antibodies, mainly of the IgG class, able to react with whole equine IgG. Additionally, patients have IgG antibodies that react both with whole equine IgG and $F(ab')_2$ fragments. In both clinical studies, no significant differences were observed in the HHA-HI titres between the patients who presented early adverse (anaphylactoid) reactions and those who did not develop them. In addition, no variation in titre was observed in samples collected before and after antivenom administration. These results do not support the hypothesis that the incidence of early adverse reactions to antivenom administration correlates with the titre of HHA-HI in the serum of patients. Nevertheless, participation of these antibodies as part of a multifactorial pathogenic mechanism associated with these reactions cannot be ruled out.

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1. Introduction

Antivenoms constitute the only scientifically validated treatment for snake bite envenoming (Theakston et al., 2003). Antivenoms must have suitable characteristics of identity, effectiveness, purity and security (Good Manufacturing Practices, 1999). The safety of antivenoms is related to the potential transmission of an infectious agent (Burnouf et al., 2004) or to the development of adverse reactions. One type of adverse response to antivenom administration is related to contamination with bacterial endotoxins, thereby inducing a pyrogenic reaction characterised by chills, fever and tremors (Warrell, 1995). However, these reactions are due to the presence of bacterial contaminants and are not caused by the immunoglobulins of antivenoms.

Adverse reactions to antivenom administration can be of two main types. Early adverse reactions (EAR) usually occur during the administration of antivenom and may be either true anaphylactic reactions, which are rarely reported and correspond to type I hypersensitivity (Williams et al., 2007), or de novo anaphylactoid reactions, with an incidence that ranges between 6% and 80% (Chippaux et al., 1998; Gawarammana et al., 2004; Moran et al., 1998). Anaphylactoid reactions occur in patients who have not previously been exposed to antivenom proteins (Sutherland, 1977). These EARs are characterised by urticaria, itching, fever, tachycardia, vomiting, abdominal colic, headache, bronchospasm, hypotension and angio-oedema, although in many cases only urticaria and itching occur (Warrell, 1995). Late adverse reactions occur 5—26 days after the administration of antivenom and mostly correspond to serum sickness, i.e. type III hypersensitivity (LoVecchio et al., 2003).

The mechanisms responsible for EARs have not been conclusively demonstrated. A role has been suggested for the anticomplementary activity of antivenoms in the genesis of EARs (Sutherland, 1977). Such a mechanism has also been suggested as the basis of adverse reactions to intravenous human immunoglobulin administration owing to the formation of protein aggregates during manufacture (Barandun et al., 1962). Despite the fact that complement consumption has not been demonstrated following antivenom administration in clinical cases (Malasit et al., 1986), reduction of anticomplementary activity is achieved in the manufacture of antivenoms through processes such as pepsin digestion (Jones and Landon, 2003) and β-propiolactone treatment (León et al., 2005). However, although pepsin-digested $F(ab')_2$ antivenoms display a lower in vitro anticomplementary activity than whole IgG antivenoms (Morais et al., 1994), the incidence of EARs following administration of $F(ab')_2$ antivenoms shows striking variation (Chippaux et al., 1998; Gawarammana et al., 2004; Moran et al., 1998), suggesting that factors other than the nature of the active principle (IgG or $F(ab')_2$) play a predominant role in the onset of EARs. Furthermore, a recent clinical study comparing two IgG antivenoms with different anticomplementary activity did not show variations in the incidence of EARs (Otero et al., 2006). Therefore, mechanisms other than complement activation must be at play in the onset of these reactions.

An alternative mechanism of EARs is the formation of immune complexes between human heterophilic antibodies directed against horse immunoglobulins (HHA-HI) and the antibodies present in antivenoms (Herrera et al., 2005). The presence of HHA-HI in human serum has been described (Henning et al., 2000; Herrera et al., 2005) and a concentration of 0.017 mg/ml of such antibodies has been quantified in immunoglobulin preparations (Sevcik et al., 2008). Increased titres of these antibodies occur in patients undergoing autoimmune processes such as rheumatoid arthritis (Pope and McDuffy, 1981). Thus, equine-derived antivenom administration to patients having HHA-HI could trigger an EAR by means of a type III hypersensitivity mechanism. Similar reactions take place during the administration of plasma to IgA-deficient patients (Sandler et al., 1995) and are involved in allergies to some foods (Ayuso et al., 2000). The objective of this work was to assess whether there is a relationship between the incidence of EARs and the titre of HHA-HI in patients envenomed by snake bites in Colombia and Brazil.

2. Materials and methods

2.1. Clinical study and samples

The titre of HHA-HI was determined in the serum of eight adult healthy volunteers from Costa Rica and in the sera of victims of snake bite envenomings selected from two independent clinical studies: one in Colombia during 2003—2005, in which patients received antivenom constituted by whole IgG molecules ($n = 25$); and the other in Brazil during 2002—2004, where an antivenom constituted by $F(ab')_2$ fragments was administered ($n = 31$). For comparative purposes, the samples selected corresponded to a similar number of patients presenting EARs and patients who did not present EARs. These samples correspond to patients included in more extensive clinical trials, and the actual incidence of EARs in these studies will be published elsewhere. All participating patients, or their relatives, as well as the healthy volunteers signed the corresponding informed consent forms.

The number of antivenom vials administered was recorded and, upon antivenom administration, patients were carefully observed for the development of EARs over 24 h following the end of the infusion of the antivenom. The most common manifestations of EARs were nausea, vomiting, colic, abdominal pain, urticaria and chills. Serum samples, obtained from blood collected before antivenom administration, were obtained from all patients. The samples were identified with a code number that was blinded to the analysts who determined the titre of HHA-HI. In addition, titres were compared between samples collected before antivenom infusion and 24 h (IgG antivenom) or 12 h ($F(ab')_2$ antivenom) after antivenom administration.

2.2. Determination of HHA-HI titres

To determine the HHA-HI titres, microplates were coated with 100 μl/well of a 5 mg/dl solution of horse IgG or $F(ab')_2$ fragment, purified by caprylic acid fractionation (León et al., 1997; Rojas et al., 1994). After washing the plates five times with Tris-buffered solution (TBS) (0.05 M Tris, 0.15 M NaCl, 20 μM $ZnCl_2$, 1 mM $MgCl_2$, pH 7.4), 100 μl of human serum samples, diluted 1:1000 in TBS—2% bovine serum albumin (BSA), was added and the plates were