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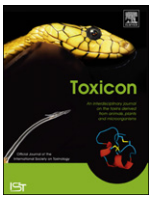

Role of the animal model on the pharmacokinetics of equine-derived antivenoms

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ABSTRACT

Antivenom pharmacokinetics has been studied in heterologous models in which the animal species used as immunoglobulin source is different from that used as recipient. In these models, after intravenous administration of antivenom, the plasma concentration of immunoglobulins shows a rapid initial declining-phase followed by a slower terminal-phase, which has been associated with antivenom distribution and elimination, respectively. We have compared pharmacokinetic parameters for equine-derived antivenom in homologous (horse) and heterologous (cow) models. It was found that the maximum concentration is lower in cows than in horses. Additionally, the steady-state distribution volume is higher in cows as compared to horses. On the other hand, models were not different in the time required to reach the maximum concentration, the area under the concentration/time curve, the half-life of decay during the slowest phase, the systemic clearance and the mean residence time. Similar results were obtained in a rabbit model, in which the pharmacokinetics was also affected by passive immunization of rabbits with anti-equine IgG. We conclude that, in addition to other physiological differences (e.g. cardiac frequency, plasmatic volume, glomerular filtration rate, etc.) between animal models, the ability to remove foreign immunoglobulins might influence the way in which the plasma concentration of antivenom decreases over time, thereby distorting the pharmacokinetic predictions based on non-compartmental models.

1. Introduction

Venoms are mixtures of toxic proteins developed by some animals for prey capture and defense against predators (Wigger et al., 2002; Aird, 2002; Nisani and Hayes, 2011). Each venom component has physicochemical characteristics that determine their ability to access different body compartments (Ismail et al., 1998; El Hafny et al., 2002) and to exert toxicity in specific tissue targets. The complex set of local and systemic alterations induced by venom components characterize envenomations by poisonous animals (Pinto et al., 2010; Khattabi et al., 2011; Otero-Patiño et al., 2012).

Antivenoms are formulations of immunoglobulins or immunoglobulin fragments, purified from plasma of animals immunized with venoms (Gutiérrez et al., 2011). Currently, antivenom administration is the recommended medical practice to treat envenomations caused by animals such as snakes (WHO, 2010), scorpions (Bahloul et al., in press), spiders (Offerman et al., 2011; Pauli et al., 2006) and caterpillars (Caovilla and Guardão, 2004), among others. From a clinical point of view, the success of

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antivenom immunotherapy depends on several factors such as envenomation severity (França et al., 2003), time between envenomation and antivenom administration (Thomas et al., 1998), antivenom potency and specificity (Sellahewa et al., 1995; Visser et al., 2008), and antivenom dose (Jorge et al., 1995).

Antivenom effectiveness also depends on the ability of antibodies to reach compartments in which venom components are distributed (Ismail and Abd-Elsalam, 1998) or to favor the redistribution of toxins from tissue compartments to the central compartment (Pépin-Covatta et al., 1996; Rivière et al., 1997; Calderón-Aranda et al., 1999; Bon, 2003). Therefore, in order to understand the mechanism by which antivenoms work and achieve their therapeutic effect, the pharmacokinetics of the molecules used as active substance (e.g. whole IgG, $F(ab')_2$ or Fab fragments) plays a determinant role and, therefore, has been widely studied (Gutiérrez et al., 2003).

Usually, antivenom pharmacokinetics is studied in models in which the animal species used as recipient is different from that used as immunoglobulin source. For example, pharmacokinetics of equine-derived antivenoms has been investigated in rabbits, mice, rats and humans (Pépin-Covatta et al., 1996; Ismail and Abd-Elsalam, 1998; Ismail et al., 1998; Bazin-Redureau et al., 1998; Quesada et al., 2006; Vázquez et al., 2005, 2010a; Sevcik et al., 2007). In these models, it has been demonstrated that, rapidly after intravenous administration, the plasma concentration of heterologous antibodies reaches its maximum concentration. Afterwards, antivenom concentration decreases bi-exponentially, with a rapid initial declining-phase followed by a slower terminal-phase.

Distribution (e.g. the passage of antibodies from the central to the peripheral or tissue compartment) and elimination are processes which occur simultaneously. However, since the antivenom concentration/time curve can be explained through bi-compartmental models (Pépin-Covatta et al., 1996; Rivière et al., 1997; Quesada et al., 2006), it has been assumed that the rapid initial declining-phase is mostly influenced by antivenom distribution and that the slower terminal-phase is mostly explained by antivenom elimination.

Considering that immunoglobulins are immunogenic molecules (unlike low molecular mass drugs), it has been suggested that the decrease in the plasma concentration of antivenom over time could be affected not only by the rate of antibody extravasation, but also by immunologically-mediated removal of heterologous immunoglobulins (Scherrmann, 1994; Bazin-Redureau et al., 1998; Lobo et al., 2004). Using a triexponential model, Vázquez et al. demonstrated the effect that the immunoglobulin immunogenicity has on the pharmacokinetics of antivenoms at 120 h after administration (Vázquez et al., 2010b). However, the analysis of the effect of the natural immune response on the pharmacokinetics of antivenoms in the first hours after antivenom administration is pending.

In order to study how the physiological characteristics of the animal model used as recipient affects the antivenom pharmacokinetics, we compared the pharmacokinetic parameters for an equine-derived formulation in horses and cows. The role of the ability of immune mechanisms to remove foreign immunoglobulins was addressed by reproducing the observed differences between homologous (horses) and heterologous (cows) models in a rabbit model in which the immunoglobulin heterology was accentuated by the passive immunization of animals with anti-equine IgG.

2. Materials and methods

2.1. Antivenom

Antivenom was produced at Instituto Clodomiro Picado (batch 4240108POLQ). This formulation is made of whole immunoglobulins purified by caprylic acid precipitation (Rojas et al., 1994) from the plasma of horses immunized with venoms of the snakes *Bothrops asper*, *Crotalus simus* and *Lachesis stenophrys* (Angulo et al., 1997). The antivenom was formulated to attain a concentration of antivenom antibodies of 17 mg/mL, with 8.5 g/L NaCl, 2.5 g/dL phenol, and pH 7.2.

2.2. Antivenom administration and sampling of blood

This study was performed using non-envenomed animals. An intravenous bolus of undiluted antivenom was administered to four Criollo horses (300–350 kg body weight) and four Jersey cows (200–250 kg body weight), at a dose (D) of 6.8 mg/kg. Similarly, the antivenom was administered to two groups of four New Zealand rabbits (2.5–3.5 kg body weight) at a dose of 51 mg/kg. In the first rabbit group, the animals received the antivenom alone. In the other one, immediately before antivenom administration, animals were passively immunized by the intravenous administration of rabbit anti-equine IgG sera (dose of 0.8 mL/kg). In all animals, antivenom injection was performed in less than one minute. Blood samples were collected at 0, 1, 5, 15, 30, 60, 180, 360, 1440, 2880, 10,080, 12,960, 20,160, 30,240 and 40,320 min after administration of the antivenom. After allowing blood to clot at 20–22 °C, serum was separated by centrifugation and stored at −20 °C until use. All procedures used in this study were approved by the Institutional Committee for the Care and Use of Laboratory Animals (CICUA) of Universidad de Costa Rica and met the International Guiding Principles for Biomedical Research Involving Animals (CIOMS, 1985).

2.3. Determination of the plasmatic concentration of antivenom antibodies

To determine equine antivenom antibodies in horse and cow sera, polystyrene plates (Costar 9017, Corning INC., Acton, MA) were coated overnight at 20–22 °C with 100 μL of a solution of *B. asper* snake venom (3 μg/100 μL). After washing the plates five times with PBS, 100 μL of serum samples diluted 1:1000 in PBS–2% bovine serum albumin (BSA), were added and the plates were incubated for 1 h at 20–22 °C. Then, microplates were washed five times with PBS. Later, 100 μL of rabbit anti-equine IgG conjugated with peroxidase, diluted 1:1000 with PBS–2% BSA were added to each well. Again, microplates were incubated for 1 h at 20–22 °C. After a final washing step, color was developed by

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