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Systemic and local myotoxicity induced by snake venom group II phospholipases A_2: Comparison between crotoxin, crotoxin B and a Lys49 PLA_2 homologue ☆

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Abstract

The patterns of myotoxicity induced in mice by crotoxin, crotoxin B and a Lys49 phospholipase A_2 (PLA_2) homologue were compared. Lys49 PLA_2-induced local myotoxicity is reflected by creatine kinase (CK) loss in injected gastrocnemius muscle, and by a profile of CK increase in plasma characterized by a rapid increment and drop after intramuscular injection, and by a lack of CK increase in plasma after intravenous injection. In contrast, crotoxin and crotoxin B, which induce local and systemic myotoxicity, provoked a more prolonged increment in plasma CK activity upon intramuscular injection, and induced increments in plasma CK after intravenous injection. The three toxins promoted a similar extent of local myotoxicity, assessed by the loss of CK in injected gastrocnemius. A method for the quantitative assessment of the ability of toxins to induce systemic myotoxicity is proposed, based on the estimation of the ratio between the area under the curve in the plasma CK activity (total myotoxicity) to the loss of CK in injected gastrocnemius (local myotoxicity). The highest ratio corresponded to crotoxin, and the lowest corresponded to Lys49 PLA_2, the former being a systemic myotoxin and the latter a local myotoxin. Neutralization by antivenoms also differed between the toxins: a drastic reduction in plasma CK, with very poor neutralization of local CK loss, was achieved in the case of crotoxin B when antivenom was injected intravenously, whereas no neutralization was achieved in the case of Lys49 PLA_2. When tested in undifferentiated myoblasts in culture, Lys49 PLA_2 induced cytotoxicity, whereas crotoxin and crotoxin B did not, evidencing that the latter are devoid of widespread cytolytic activity. Molecular modeling analysis showed that Lys49 PLA_2 has a conspicuous cationic face, which is likely to interact with diverse membranes. In contrast, crotoxin B, despite its overall basic p*I*, has a lower density of positively charged residues at this molecular region. It is suggested that Lys49 PLA_2s homologues interact, through this cationic face, with many different cell types, thus lacking specificity for muscle cells. In contrast, crotoxin B

☆ *Ethical statement*: This manuscript presents an experimental study performed following the standard procedures of scientific ethics, including the use and care of experimental animals.

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has a more selective interaction with targets in the muscle cell membrane. This selectivity might be the basis for the ability of crotoxin and crotoxin B to induce systemic myotoxicity.

Keywords: Myonecrosis; Group II phospholipases A_2; Snake venoms; Creatine kinase; Systemic myotoxicity

1. Introduction

Acute muscle damage, myonecrosis, is a common finding in envenomations inflicted by snakebites (Warrell, 1996). Venom-induced myotoxicity occurs in two clinical patterns: (a) local myotoxicity, characteristic of viperid snakebites, which affects predominantly the muscles located in the vicinity of the region where venom is injected (Milani et al., 1997), and (b) systemic myotoxicity, rhabdomyolysis, which causes widespread muscle damage associated with generalized myalgia, large increments in the plasma activity of muscle-derived enzymes, such as creatine kinase (CK), and myoglobinuria. Systemic myotoxicity is characteristic of envenomations by sea snakes, various terrestrial elapids and some viperid species, such as the South American subspecies of the rattlesnake *Crotalus durissus* (Azevedo-Marques et al., 1987; Warrell, 1996; White, 1995).

The most abundant myotoxic components in snake venoms are phospholipases A_2 (PLA_2s), some of which induce local myotoxicity whereas others inflict systemic muscle damage (Gutiérrez and Ownby, 2003). The terms 'general myotoxin' or 'myoglobinuric myotoxin' were proposed for myotoxins that act systemically and induce rhabdomyolysis, whereas the term 'local myotoxin' was suggested for locally acting PLA_2s (Gopalakrishnakone et al., 1997). Group II myotoxic PLA_2s inducing local myotoxicity comprise Asp49, catalytically active variants (Kaiser et al., 1990; Pereira et al., 1998), and a subgroup of PLA_2 homologues that present substitutions in key positions, especially in residue 49. Most of them are Lys49 variants lacking enzymatic activity, but still being able to exert myotoxicity by a catalytically independent mechanism (Ward et al., 2002; Lomonte et al., 2003). On the other hand, some viperid class II PLA_2s induce both local and systemic myotoxicity. An example of a systemic myotoxin with documented clinical impact is crotoxin, from the venom of South American subspecies of *C. durissus* (Gopalakrishnakone et al., 1984). Crotoxin is an heterodimeric complex, held by non-covalent bonds, constituted by a moderately toxic PLA_2 subunit, crotoxin B, and a catalytically inactive and non-toxic smaller subunit, crotoxin A or crotapotin (Bouchier et al., 1991; Bon, 1997).

In order to further understand the differences between local and systemic group II myotoxic PLA_2s, in the present work we compared a local myotoxic Lys49 PLA_2 homologue, from the venom of *Bothrops alternatus*, with crotoxin and its PLA_2 subunit crotoxin B, which are systemic myotoxins from the venom of *C. d. collilineatus*. The comparison included (a) the kinetics of CK activity in plasma; (b) the efficacy of antivenoms to neutralize local and systemic myotoxicity; (c) the effects on skeletal muscle myoblasts in culture; and (d) the structural features that might determine the ability of these proteins to induce local or systemic myotoxicity.

2. Materials and methods

2.1. Toxins

Crotoxin and crotoxin B were isolated from the venom of the rattlesnake *C. d. collilineatus* (Ponce-Soto et al., 2007b). Lys49 PLA_2 homologue was isolated from the venom of *B. alternatus*, as described previously (Ponce-Soto et al., 2007a). The complete amino acid sequences of crotoxin B, the PLA_2 subunit of crotoxin, and of the Lys49 PLA_2 homologue have been described (Ponce-Soto et al., 2007a, b). The isoform of crotoxin B used in this study has ~90% identity with the sequence of crotoxin B from the subspecies *Crotalus durissus terrificus* (Ponce-Soto et al., 2007b). Homogeneity of the preparations was demonstrated by SDS-PAGE and reverse-phase HPLC (Ponce-Soto et al., 2007a, b).

2.2. Experimental animals

Mice of the strain CD-1, weighing between 18 and 20 g, were used throughout the study. Animals were maintained on a 12:12 h light:dark cycle and received food and water *ad libitum*. All experiments