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Biological activities of phosphodiesterase from *Crotalus durissus terrificus* venom

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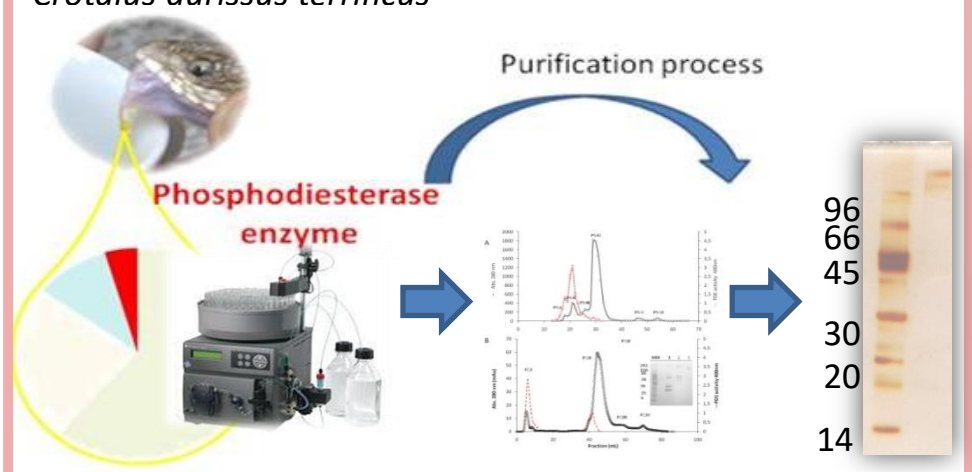
Introduction

Phosphodiesterases (PDEs) are an enzymes family that hydrolyze phosphodiester bonds sequentially from the 3´ terminus of polynucleotides to produce 5'-mononucleotides. Historically, snake venom PDEs have been widely used in sequencing and structural studies of nucleic acids. In contrast, the potential pharmacological activities of these enzymes are poorly understood and their role in envenomation remains unclear.

Previosuly, we isolated and preliminary characterized a PDE from *C. d. terrificus* (CDT) venom (CDT-PDE).

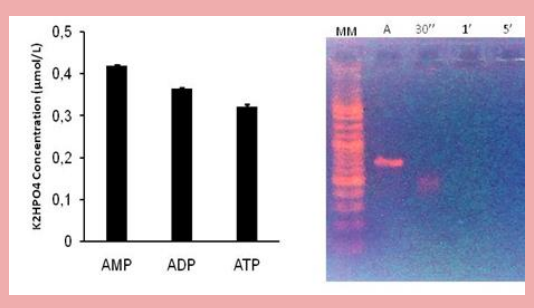
Crotalus durissus terrificus



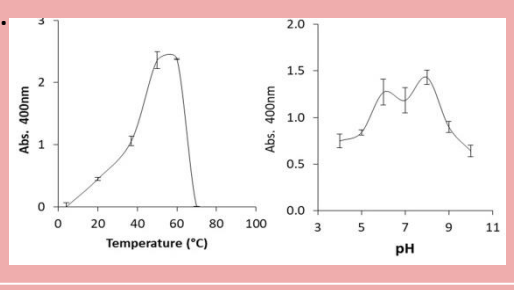
Two isoforms of CDT-PDE were isolated by two chromatographic steps with a molecular mass of ~ 100 kDa.

Enzymatic activity

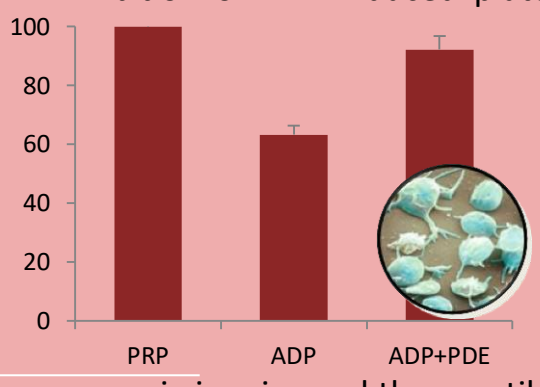
- The previous results showed that CDT-PDE hydrolyze swiftly different substrate AMP > ADP > ATP or DNA.



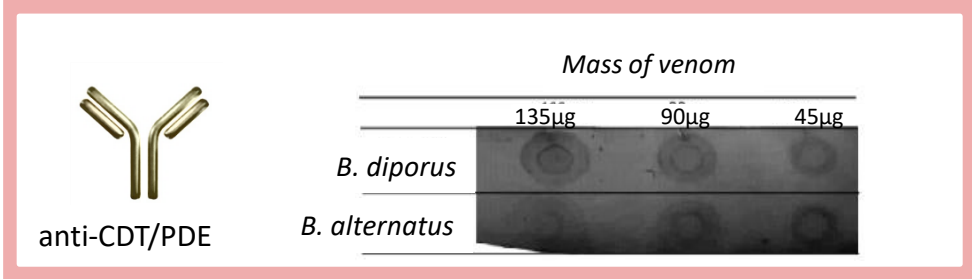
- The highest enzymatic activity was observed at 37 -60 °C and pH 6-8.5 and.



- CDT-PDE also shown inhibition of ADP-induced platelet aggregation



- CDT-PDE also was immunogenic in mice and there antibodies cross-recognized PDE from botrops venoms.



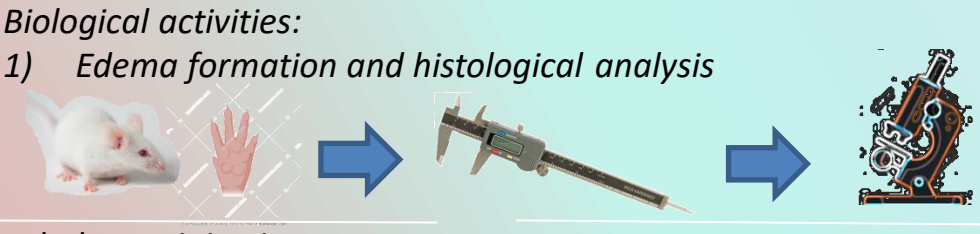
Dot blot analysis showed not only the reactivity with *C.d.terrificus* venom but also cross reactivity with *B. alternatus* and *B. diporus* venom.

The subjects of the present investigation were evaluated the edema-forming activity and locomotory behavior induced by CDT-PDE.

Material y methods

Biological activities:

1) Edema formation and histological analysis

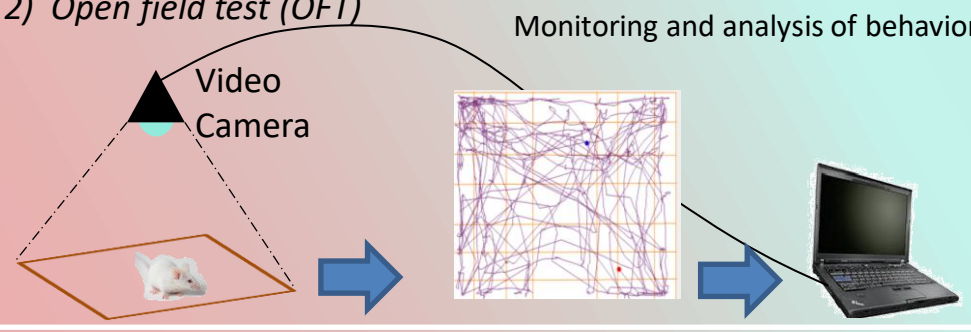


subplantar injection :

- *CDT-PDE (*1 µg*)*
- *CDT-PDE + ADP* (50 nmol)
- *ADENOSINE* (50 nmol)
- *ADP*
- *PBS (phosphate saline solution)*

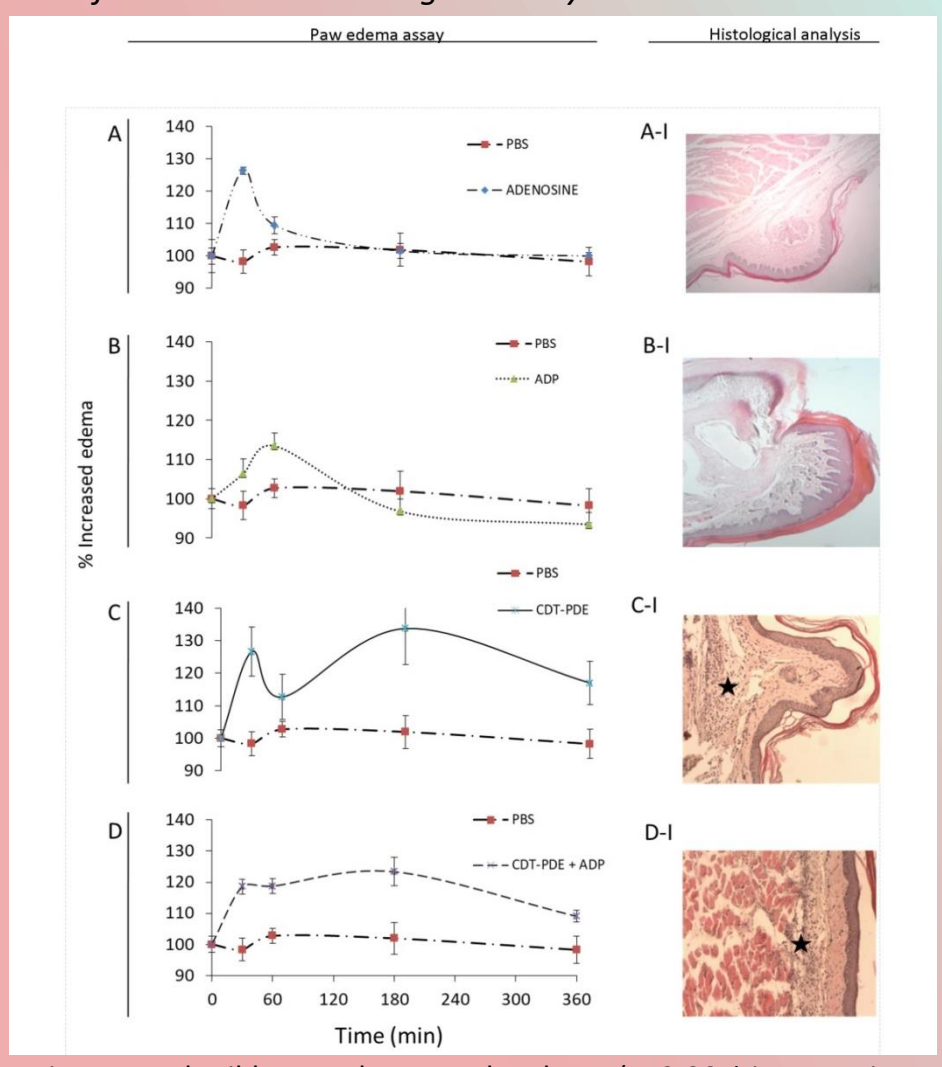
Edema was measured as the increase in paw thickness using low-pressure spring calipers at various intervals (0.5, 1, 3, and 6 h).

2) Open field test (OFT)



Result and Discussion

1) *Edema formation and histological analysis*



All agonists caused mild to moderate early edema (~12-30% increase in paw thickness) after injection that peaked within 0.5 h for adenosine and PDE, and within 1 h for ADP. Based on the extent of edema, ADP appeared to be the least active of the agonists. For adenosine and ADP, the edema returned to basal levels by 3 h post-injection. In the case of PDE, the initial peak of edema was followed a second, more prolonged response that peaked after 3 h and persisted for up to 6 h. Histological analysis of foot pads revealed no tissue alterations 6 h after injection with adenosine or ADP (Fig. 1A-I and B-I, respectively). In contrast, mice injected with PDE and PDE+ADP showed a cellular inflammatory infiltrate composed essentially of polymorphonuclear leukocytes (Fig. 1C-I and D-I, respectively).

2) Open field test	**Untreated**	**Treatment**	
		PBS	CDT-PDE
Locomotion ratio (IAC ÷ FAC)*	1.68 ± 0.36	1.90 ± 0.14	0.94 ± 0.16
Locomotor activity	Decrease	Decrease	Increase

Table 1. Locomotor activity in mice injected with PDE compared to untreated mice or mice injected with PBS. *IAC – initial distance (m) covered during the first 5 min of the OFT, FAC – final distance (m) covered during the last 5 min (10-15 min) of the OFT. The data are the mean ± SD (n=5/group).

Table 1 shows that PDE reduced the locomotor activity in the initial minutes after injection, **but this effect was transitory.**

CONCLUSION: The results of this investigation indicate that PDE in *C. d. terrificus* venom from northeastern Argentina is **edematogenic** and causes an **inflammatory infiltrate**. Further investigations are required to assess the contribution of this enzyme to the systemic manifestations associated with envenomation by this species.