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Poster presentation

Vascular smooth muscle PDE5 is much more sensitive to N-alkylated zaprinast than platelet PDE5

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Background

Cyclic nucleotide phosphodiesterases (PDEs) play a key role in signal transduction downstream RCPGs by hydrolyzing specifically cyclic nucleotides. PDE is a superfamily of enzymes presently constituted of 11 gene families [1]. Among them, the PDE5 family is constituted by an unique gene with three different splice variants [2], and is characterized by its specificity for cGMP hydrolysis, the presence of cGMP-binding sites and its insensitivity to calcium-camoldulin. We have characterized PDE5 in vascular smooth muscle from human, bovine and rat aorta, and showed that zaprinast specifically inhibits PDE5 [3]. The specific inhibition of vascular PDE5 by zaprinast (1 μM) induces relaxation of rat aorta along with an increase in cGMP level [4]. Furthermore, we showed that PDE5 in platelets participates in controling aggregation [5]. The aim of the present study was to compare peptidic sequences, biochemical properties and pharmacological sensitivities of vascular PDE5 and platelet PDE5.

Materials and methods

Cytosolic fractions of smooth muscle from bovine aorta and from human platelet were submitted to DEAE-sepharose chromatography and subsequently to affinity chromatography on ECH-Sepharose 4B resin coupled with NH_2-zaprinast. Fractions containing PDE5 were submitted to Western blot and to gel electrophoresis. PDE5 bands were excised from gel and submitted to gel-digestion in order to perform MALDI-TOF analysis. PDE activity was determined by a radioenzymatic assay using ^{3}H-cGMP as a tracer [5]; Km and Vmax determinations as well as Ki were determined on purified PDE5s. N-alkylated zaprinast was synthetized by L Lebeau using as a scaffold NH_2-zaprinast.

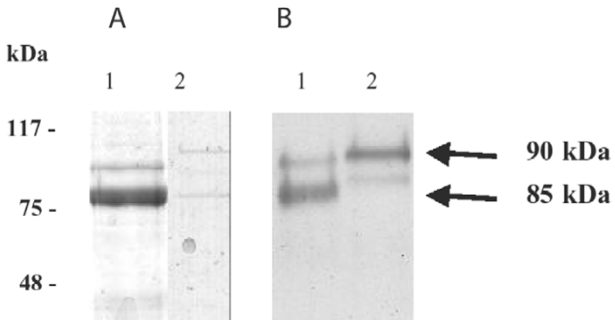

Figure 1
Comparison of purified platelet PDE5 (lanes 1)and vascular PDE5 (lanes 2) detected by Coomassie blue coloration **(A)** and by immunodetection **(B)**.

Table 1: Biochemical and pharmacological comparisons of vascular and platelet PDE5s

	Vascular PDE5	Platelet PDE5
Km (μM)	0.44 ± 0.02	0.31 ± 0.04
Vmax (μmole.min-1.mg-1)	0.90 ± 0.04	0.71 ± 0.03
	K_i values (nM)	
Compounds		
zaprinast	340	600
NH2-zaprinast	57	100
N-alkylated-zaprinast	0.032	1.8
sildenafil	2	2.5
eucilat	1,750	1,020
N-alkylated-eucilat	1,280	1,000

Results

Coomassie blue coloration of electrophoresed purified vascular smooth muscle PDE5 and platelet PDE5 (Figure 1A) shows two signals for platelet (a major band at 85-kDa and a minor band at 90-kDa; lane 1) and one signal for vascular smooth muscle (90-kDa band; lane 2). Immunodetection using a polyclonal specific antibody against bovine lung PDE5 [6] (Figure 1B) shows that platelet PDE5 is a 85-kDa protein (lane 1), whereas vascular smooth muscle PDE5 is a 90-kDa protein (lane 2).

MALDI-TOF analysis obtained for trypsic peptides from the 90-kDa Coomassie band of vascular PDE5 (Figure 1A, lane 2) allowed to identify, after Mascot Search, this vascular protein as belonging to PDE5 family when compared with bovine lung PDE5, with a significant score of 126 (significant score being > 60). A similar analysis performed on the 85-kDa band from platelet showed a 32% overlap when compared with the bovine lung PDE5 sequence. Furthermore, a score of 148 was obtained for matching platelet PDE5 sequence with human PDE5A2 sequence. Altogether, these data show that vascular smooth muscle PDE5 and human platelet PDE5 are different proteins.

Table 1 shows that platelet PDE5 differs significantly from vascular PDE5 by their submicromolar K_m values, platelet PDE5 displaying a slightly higher affinity for cGMP. Zaprinast, the conventional inhibitor of PDE5, is 2-fold more active in inhibiting vascular PDE5 than platelet PDE5. NH2-zaprinast is 6-fold more potent than zaprinast on both vascular PDE5 and platelet PDE5. N-alkylated-zaprinast preferentially inhibits vascular PDE5 with a K_i value of 32 pM, thus being 1,800-fold more potent than NH2-zaprinast, and it is 56-fold more potent in inhibiting vascular PDE5 than platelet PDE5. It should be noticed that no such a difference in potencies was observed between eucilat and alkyl-eucilat, both of them inhibiting vascular PDE5 and platelet PDE5 in the micromolar range. Furthermore, sildenafil cannot discriminate vascular PDE5 from platelet PDE5.

Conclusion

Our results show that vascular PDE5 and platelet PDE5 are different proteins which can be discriminate by N-alkylated-zaprinast and suggest that they might represent different PDE5 variants which can be selectively inhibited. These data open new therapeutic opportunities for using PDE5 inhibitor treatment in patients treated with antiaggregant.

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