PKC Agonist Exposure Sufficient to Activate T Cells In Vivo also Causes Coagulopathy

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Background

• Activation of latent HIV reservoir is part of a strategy for HIV cure as it should enable the elimination of infected cells by immune mediated clearance mechanisms and facilitate long-term remission or cure.1
• Protein kinase C (PKC) agonists, such as Prostratin and Brystatin, are highly effective at activating latent HIV.2
• DAG lactones are a class of small molecules that activate PKCs3
• Effective use of PKC agonists is limited by their severe toxicity, with a mechanism not clearly elucidated.4,5

Objectives

• To elucidate the primary mechanism of toxicity of the novel small molecule PKC agonist, C-232A.
• To develop a predictive in vitro screening platform to inform on potential toxicity of future PKC agonists.

Methods

• PKC translocation by small molecule agonists was assessed by fluorescent microscopy of GFP-tagged PKC in A549 cells and by immunoimurization endogenous PKC in Jurkat cells.
• Ex vivo activation of HIV transcription by qPCR and CD69 expression in HIV-infected donor T cells was measured by flow cytometry and CD69 expression.
• Platelet activation is a critical safety liability associated with PKC agonists.
• Investigational toxicology endpoints were assessed including flow cytometry of GFP-labeled PKC in A549 cells and by fluorescent microscopy of GFP-labeled PKC-θ in Jurkat cells and by an LC-MS/MS method.

Results (Cont’d)

Figure 3. Structure of the novel small molecule PKC agonist C-232A.

Table 1. C-232A induces selected cytokine and chemokines in rhesus after IV administration.

Table 2. Concentrations of selected cytokines and chemokines in plasma of both vehicle control and C-232A groups 4 h after IV administration.

Figure 4. Dose escalation PK/PD study of C-232A by IV infusion in rhesus macaques results in both T cell activation and severe toxicity.

Figure 5. Abnormal hematology and coagulation parameters in rhesus macaques administered C-232A indicate platelet activation, aggregation and excessive clotting.

Figure 6. IV infusion of C-232A at 1 mg/kg manifests in hemorrhage and thrombosis across multiple organs in the rhesus macaque, a hallmark of disseminated intravascular coagulation (DIC).

Figure 6. In vivo platform in whole blood predicts C-232A and Prostratin platelet toxicity relative to pharmacodynamic potency.

Figure 7. Certain PKC isoforms are expressed in platelets.

Conclusions

• The primary mechanism of C-232A toxicity in rhesus macaques, as well as rats (data not shown), is mediated by platelet activation, aggregation and coagulopathy. The in vitro data presented here, as well as published in vivo data6, suggests a similar toxic mechanism of action for Prostratin.
• Platelet activation is a critical safety liability associated with PKC agonists and should be carefully monitored in any preclinical or clinical studies. The in vitro screening tools presented here should inform on the platelet activation potential of PKC agonists prior to in vivo studies.

References

4. Tel: (650) 235-3764
5. 333 Lakeside Drive
6. AIDS Res Hum Retroviruses

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