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CGX1037 is a novel PKC isoform delta selective inhibitor in platelets

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Abstract

Platelets upon activation change their shape, aggregate and secrete alpha and dense granule contents among which ADP acts as a feedback activator. Different Protein Kinase C (PKC) isoforms have specific non-redundant roles in mediating platelet responses including secretion and thrombus formation. Murine platelets lacking specific PKC isoforms have been used to evaluate the isoform specific functions. Novel PKC isoform δ has been shown to play an important role in some pathological processes. Lack of specific inhibitors for PKC δ has restricted analysis of its role in various cells. The current study was carried out to evaluate a novel small molecule PKC δ inhibitor, CGX1037 in platelets. Platelet aggregation, dense granule secretion and western blotting experiments were performed to evaluate CGX1037. In human platelets, CGX1037 inhibited PAR4-mediated phosphorylation on PKD2, a PKC δ -specific substrate. Pretreatment of human or murine platelets with CGX1037 inhibited PAR4-mediated dense granule secretion whereas it potentiated GPVI-mediated dense granule secretion similar to the responses observed in murine platelets lacking PKC δ . Furthermore, pre-treatment of platelets from PKC $\delta^{-/-}$ mice with CGX1037 had no significant additive effect on platelet responses suggesting the specificity of CGX1037. Hence, we show that CGX1037 is a selective small molecule inhibitor of PKC δ in platelets.

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Declaration of interest

Dr. Swindle is the CEO of CompleGen, Inc. (Seattle, WA), which produces the new PKC δ inhibitor CGX1037. CompleGen, Inc. provided the product to Dr. Kunapuli's laboratory at no charge. The planning, conduct, and reporting of the research were performed by Dr. Kunapuli and the members of his laboratory. Data analysis was performed by Dr. Kunapuli with no input or oversight from CompleGen, Inc. The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

Keywords

Protein kinase C δ ; CGX1037; protein kinase D2; protease activated receptor-4; GPVI; platelet dense granule secretion

Introduction

Platelet activation plays an important role in regulating not only hemostasis but also thrombus formation which when unregulated leads to pathological thrombosis [1]. Members of the Protein kinase C (PKC) family have been implicated in platelet activation and degranulation [2]. The PKC family is a highly conserved group of serine/threonine kinases comprised of 11 isoforms. All PKCs share a conserved carboxy-terminal kinase domain linked by a variable hinge region to the amino-terminal regulatory domain, which shows considerable divergence across isoforms [3]. PKCs are classified based on structural arrangement, which controls mode of activation [3–5]. The classical/conventional PKCs (cPKC; PKC α , β I, β II and γ) are activated by calcium and the lipid second messenger, diacylglycerol (DAG). The novel PKCs (nPKC; δ , ϵ , θ and η isoforms) are activated by DAG but not calcium. The atypical PKCs (aPKC; ι , λ , ζ isoforms) are sensitive to phosphatidylserine.

PKC isoforms α , β , δ , θ , η , ϵ , and ζ have been identified in platelets. The use of pan-PKC inhibitors such as GF 109203X, Ro-318220, calphostin C and staurosporine and non-classical PKC inhibitors such as G06976 and LY333531 have identified a role for PKCs in platelet activation including aggregation and secretion induced by various agonists such as collagen, thrombin and ADP [2, 6–9]. However, the lack of availability of isoform specific inhibitors has restricted the understanding of the role of individual PKC isoforms in platelets.

Recent studies using rottlerin and a PKC δ TAT peptide antagonist and platelets from PKC $\delta^{-/-}$ mice have identified PKC δ as a positive regulator of PAR-mediated granule release and a negative regulator of GPVI-mediated granule release [10–13]. While rottlerin was initially identified as a PKC δ isoform specific inhibitor [14], subsequent studies have shown it to be a non-specific inhibitor [15–19], which can also act as a mitochondrial uncoupler [20, 21]. The PKC δ isoform specific RACK antagonistic peptide has been used to inhibit the activity of PKC δ in various cell types including platelets [22]. However, peptides are unstable and often susceptible to degradation and rapid elimination from the blood circulation [23]. Hence, there is a need for developing isoform specific PKC inhibitors that can be used in platelets to better understand the role of these isoforms and be able to use them *in vivo* to inhibit PKCs. In this study, we evaluated the effect of a newly described small molecule PKC δ inhibitor, CGX1037 on platelet function. We demonstrate that this inhibitor elicited similar effects on human platelets as seen in PKC δ -deficient murine platelets indicating that CGX1037 is a PKC δ selective inhibitor.

Materials and methods

Approval for this study was obtained from the Institutional Review Board of Temple University (Philadelphia, PA), and mice were used for physiological measurements using the protocol approved by the Institutional Animal Care and Use Committee (IACUC).

Reagents

CGX1037 was from Complegen, Inc. (Seattle, WA). Apyrase (type VII) and acetylsalicylic acid were obtained from Sigma (St Louis, MO). PGE₁ was purchased from Enzo Life Sciences (Plymouth Meeting, PA). AYPGKF was custom synthesized at Invitrogen (Carlsbad, CA). Collagen-related peptide (CRP) was purchased from Dr Richard Farndale (University of Cambridge). Halt protease and phosphatase inhibitor cocktail is purchased from Thermo Scientific (Rockford, IL). Total PKC δ , PKD2 phospho Ser744/748 (recognizes equivalent serines on PKD2) and β -actin antibodies were obtained from Cell Signaling Technologies (Beverly, MA). β 3 integrin antibody is from Santa Cruz Biotechnology (Dallas, TX). All the other reagents were of reagent grade and de-ionized water was used throughout.

Animals

PKC $\delta^{-/-}$ (C57/BL6 background) mice were a generous gift from Dr Keiko Nakayama (Division of Developmental Genetics, Tohoku University Graduate School of Medicine). Age-matched wild-type (WT) C57/BL6 littermates were used as controls.

Human platelet preparation

Whole blood was drawn from healthy human volunteers into tubes containing one-sixth volume of ACD (2.5 g of sodium citrate, 1.5 g of citric acid, 2 g of glucose in 100 ml of deionized water). Blood was centrifuged (Eppendorf 5810 R centrifuge) at $230 \times g$ for 20 min at room temperature to obtain platelet-rich plasma (PRP). PRP was incubated with 1 mM aspirin for 30 min at 37 °C. The PRP was then centrifuged for 10 min at $980 \times g$ at room temperature to pellet the platelets. Platelets were resuspended in Tyrode's buffer pH 7.4 (138 mM NaCl, 2.7 mM KCl, 1 mM MgCl₂, 3 mM NaH₂PO₄, 5 mM glucose and 10 mM HEPES) containing 0.2 U/ml apyrase. Platelets were counted using the Hemavet (Drew Scientific Inc., Dallas, TX) and concentration of cells was adjusted to 2×10^8 platelets/ml. Platelet samples used in all the experiments were treated with aspirin and apyrase.

Murine platelet preparation

Blood was collected from ketamine-anesthetized mice by cardiac puncture into syringes containing 3.8 % sodium citrate as anticoagulant. The whole blood was centrifuged (IEC Micromax Centrifuge, International Equipment Components, CA) at $100 \times g$ for 10 min to isolate the PRP. Prostaglandin E1 (1 μ M) was added to PRP. Platelets were centrifuged at $400 \times g$ for 10 min, and the pellet was resuspended in Tyrode's buffer (pH 7.4) containing 0.2 U/ml apyrase.

Aggregometry

Aggregation of 500 μ l of washed platelets was analyzed using a lumi-aggregometer (Chrono-log Corp., Havertown, PA). Aggregation was measured using light transmission under stirring conditions (900 rpm) at 37 °C. Each sample was allowed to aggregate for the indicated time. The chart recorder (Kipp and Zonen, Bohemia, NY) was set for 0.2 mms^{-1} .

Measurement of platelet secretion

Platelet secretion was determined by measuring the release of ATP using the Dupont Instruments luminescence biometer reagent. In experiments where inhibitors were used, the platelet sample was incubated with the inhibitors for 5 min at 37 °C prior to the addition of agonists. The secretion was subsequently measured.

Preparation of platelet membranes

Washed human platelets (2×10^9 platelets/ml) were stimulated with AYPGKF and equal volumes of tyrodes solution containing inhibitor cocktail was added. The platelets were subjected to multiple freeze-thaw cycles to lyse the cells. The lysate was centrifuged at $1500 \times g/10$ min at 4 °C and the supernatants were subjected to ultracentrifugation ($100\,000 \times g/30$ min at 4 °C). After centrifugation, the pellet was washed and re-suspended in 1% TritonX-100. The dissolved pellet was centrifuged at $15\,000 \times g/10$ min at 4 °C. To the supernatant, equal volumes of sample buffer (2M Tris, 10 % by volume glycerol, 10 % SDS, 0.5 % bromophenol blue, 1 mM dithiothreitol (DTT)) was added and boiled for 5 min.

Western blotting

Platelets were stimulated with agonists and the reaction was stopped by the addition of 6M perchloric acid (to precipitate proteins). Samples were kept on ice and then centrifuged. Sample buffer (2M Tris, 10 % by volume glycerol, 10 % SDS, 0.5 % bromophenol blue, 1 mM dithiothreitol (DTT)) was added to the pellet and boiled for 5 min. Proteins were separated by 8 % SDS-polyacrylamide gel electrophoresis and transferred onto nitrocellulose membranes. Membranes were blocked by incubation with Odyssey blocking buffer (Licor) for 1 h at room temperature. Membranes were probed overnight at 4 °C with the desired primary antibody and then washed 3 times with Tris buffered saline-Tween 20 (TBS-T). The membranes were then incubated with infrared dye-labeled secondary antibodies for 60 min at room temperature and washed 3 times with TBS-T. Membranes were developed using the Odyssey imaging system (Licor).

Statistical analysis

Western blot data and secretion data were compiled from at least three independent experimental results. The results were quantified and expressed as means $\pm$ SEM. Statistical significance was tested by Student's *t*-test. P value < 0.05 was considered statistically significant.

Results

Identification of PKC δ selective inhibitor

CGX1037, a selective inhibitor of PKC δ was identified using the XenoGene discovery platform. This platform employs strains of *Saccharomyces cerevisiae* dependent upon the expression and function of a human gene and gene product for growth (United States Patent: 6998261, Functional Gene array in Yeast). Arrays of these yeast strains, each complemented by a different human gene are used to screen chemical libraries to identify compounds that selectively inhibit growth of a single yeast strain expressing a particular human gene [24–26]. For this study, the members of the novel class of human PKCs (PKC δ , PKC θ , PKC ϵ and PKC η) replaced the essential PKC1 gene of *S. cerevisiae*. These novel PKC dependent strains were then used to identify compounds that selectively inhibited growth of PKC δ -dependent yeast. One such compound, CGX1037 potently inhibited growth of the PKC δ dependent yeast (IC₅₀ = 0.02 μ M) while exhibiting 150 to 250 fold less activity against yeast dependent upon other novel PKCs (Table I). Since PKC δ is a novel PKC, we compared the effect of CGX1037 on other known novel class of PKC isoforms such as PKC θ , PKC ϵ and PKC η , which are closely related to PKC δ . In addition to PKCs, we also tested the effect of CGX1037 on 3 other kinases, 3-phosphoinositide dependent protein kinase 1 (PDK-1), serum/glucocorticoid regulated kinase 1 (SGK) and v-akt murine thymoma viral oncogene homolog 3 (AKT-3) and showed that the IC₅₀ value of CGX1037 towards PKC δ is much lower compared to its effect on other kinases.

CGX1037 inhibits PAR-mediated dense granule secretion whereas it potentiates GPVI-mediated dense granule secretion

Previous work from our lab has shown that PKC δ differentially regulates GPVI- and PAR4-mediated platelet functional responses [8, 12]. Hence we tested if the identified inhibitor, CGX1037 inhibits PKC δ in platelets by measuring platelet functional responses. We performed a dose–response study of the inhibitor at various concentrations. We pre-incubated washed human platelets with various concentrations of the inhibitor and measured PAR4 and GPVI-mediated platelet aggregation and secretion and compared with the responses from PKC δ -deficient murine platelets. As shown in Figure 1(A) and (B), CGX1037 significantly potentiated CRP-induced human platelet secretion at 2.5 μ M concentration but inhibits at 30 μ M concentration. Similarly, we also used PAR4 agonist and compared the responses with vehicle control. From Figure 2(A) and (B), pretreatment with 2.5 μ M CGX1037 significantly inhibited AYPGKF-induced human platelet secretion whereas 30 μ M CGX1037 potentiated PAR4-mediated secretion. These results agree with our previous findings [12] that in PKC δ null murine platelets (Figure 3A), GPVI-mediated platelet dense granule secretion is potentiated whereas PAR-mediated dense granule secretion is inhibited as shown in Figure 3(B) and (C). Since the inhibitory effects of 2.5 μ M CGX1037 (same dose for GPVI and PAR agonists) in human platelets are similar to those observed in PKC δ deficient murine platelets (Figure 3), we conclude that CGX1037 efficiently inhibits PKC δ at 2.5 μ M concentration in platelets. However, at higher concentrations, the inhibitor seems to exhibit non-specific effects.

CGX1037 inhibits PKD2 phosphorylation but not PKC δ translocation in platelets

Previous studies from our lab also identified Protein kinase D2 (PKD2) as a specific substrate for PKC δ but not PKC θ or PKC ϵ by using murine platelets lacking specific novel PKC isoforms [10]. Konopatskaya et al also showed that PKD2 regulates platelet secretion and thrombus formation [27]. Since we showed from Figures 1 and 2 that CGX1037 inhibits PKC δ at 2.5 μ M concentration, we used this concentration to check if phosphorylation on PKD2 is inhibited by CGX1037 in human platelets. Human platelets were pre-incubated with CGX1037 and were stimulated with the PAR4 agonist, AYPGKF and probed for PKD2 phosphorylation. As shown in Figure 4(A) and (B), AYPGKF-induced PKD2 phosphorylation was significantly inhibited with CGX1037 indicating that CGX1037 inhibits PKC δ in platelets.

PKC isoforms translocate to the membrane by virtue of its ability to bind to diacylglycerol (formed during agonist stimulation) and further are positioned close to their substrates by receptors for activated C kinases (RACKs) [28]. Since CGX1037 inhibited PKC δ -mediated PKD2 phosphorylation downstream of PAR4, we evaluated whether CGX1037 also inhibits PKC δ translocation downstream of PAR4 in platelets. Washed human platelets were pre-incubated with either DMSO or CGX1037, stimulated with AYPGKF and platelet membranes and cytosolic fractions were prepared. As shown in Figure 4(C) and (D), CGX1037 did not inhibit AYPGKF-induced PKC δ translocation suggesting that CGX1037 inhibition of PKC δ is not by inhibiting its translocation in platelets. β 3-integrin is used as a loading control and it was observed only in platelet membrane fractions. Note that PKC δ translocation is not 100%.

Effect of CGX1037 in murine platelets

We have shown that CGX1037 inhibits PKC δ in human platelets. To check if CGX1037 also inhibits PKC δ in murine platelets, we pre-incubated murine platelets with 2.5 μ M CGX1037 and evaluated for PAR4- and GPVI-mediated platelet responses. As shown in Figure 5(A) and (B), CGX1037 inhibited AYPGKF-induced platelet responses whereas it potentiated CRP-induced platelet responses similar to the responses observed in PKC δ -deficient murine platelets (Figure 3). This suggests that CGX1037 inhibits PKC δ in both human and murine platelets.

Specificity of the PKC δ inhibitor, CGX1037

Using Xenogene platform, we have identified CGX1037 as a PKC δ inhibitor. As specificity is an important issue in developing a small molecule inhibitor, we tested the specificity of CGX1037 by using it in PKC δ -deficient murine platelets and determined whether the inhibitor had any additional effect on platelet functional responses compared to those of PKC δ -deficient murine platelets treated with vehicle control. These PKC δ -deficient murine platelets were pre-incubated with vehicle (DMSO) or CGX1037 prior to stimulation with AYPGKF and dense granule secretion was determined. As shown in the secretion tracings (Figure 6A and C) and its quantitation (Figure 6B and D), PAR4-mediated dense granule secretion induced by low and high concentrations of AYPGKF in PKC δ -deficient murine platelets pre-incubated with CGX1037 was not significantly different from the secretion responses seen in PKC δ -deficient platelets pre-incubated with vehicle suggesting that

CGX1037 does not have any significant additional effects other than inhibiting PKC δ in platelets.

Discussion

As platelet activation plays an important role in mediating hemostasis and thrombosis, identifying molecules responsible for regulating platelet function is important for treating not only platelet disorders but also other cardiovascular ailments that involve platelets in their pathology. The PKC family of isoforms regulates multiple signaling pathways involved in platelet activation, degranulation [2, 29] and thrombus formation [30, 31]. PKC is one such protein that regulates platelet activation downstream of both GPCR and tyrosine-kinase linked receptors. The importance of the PKC isoforms have been elucidated either by using inhibitors targeting the proteins or by using murine platelets obtained from mice deficient in specific PKC isoforms [10, 12, 32–34]. While mouse models provide important insight into the role of specific PKC isotypes, genetic differences do exist and may not accurately reflect human responses to specific agonists. Thus, it is essential to verify PKC isoform-specific involvement in human platelets. In addition, results obtained from knockout mice studies are difficult to interpret due to the compensatory effects. As human platelets are devoid of nuclei and are incapable of being manipulated at molecular level, using inhibitors to target specific proteins is the best available method to study the role of various molecules in human platelets. The activity of different PKC isoforms is context sensitive and these kinases can be positive or negative regulators of signaling pathways. This contextual-dependency of PKC function often makes it difficult to determine the precise roles of PKC isoforms in normal and aberrant cellular processes. The development of selective activators and inhibitors has greatly improved our understanding of PKC isoform function in specific cells and tissues, and in various disease conditions [35, 36].

PKC δ has been identified as an important regulator of platelet functional responses. PKC δ is a negative regulator of GPVI-mediated dense granule secretion and a positive regulator of PAR4-mediated dense granule secretion [8, 12]. The differential role of PKC δ downstream of GPCR and GPVI provides another evidence of this differential regulation in signaling pathways in platelets. Apart from its role in regulating platelet responses, PKC δ is implicated in various diseases. Recently, a patient with autoimmune lymphoproliferative syndrome (ALPS)-like disease was identified with a homozygous loss-of-function mutation in PKC δ resulting in chronic lymphadenopathy, splenomegaly, autoantibodies, elevated immunoglobulins and natural killer cell dysfunction suggesting the importance of normal function of PKC δ in humans [37]. On the other hand, autoantibodies against PKC δ are implicated in a subset of patients with coeliac disease [38]. Inhibition of PKC δ was also shown to reduce endotoxin- [39] or sepsis- [40]induced lung injury and aneurysm pathogenesis [41]. Hence, inhibition of PKC δ was suggested as a potential therapeutic strategy to treat abdominal aortic aneurysm (AAA) [41]. These studies suggest an important regulatory role for PKC δ in various pathological processes including thrombosis. Due to its essential role in regulating various pathologies, design and use of small molecule inhibitors specific to PKC δ is important and selective inhibition of PKC δ could contribute to treatment of certain disease states.

Therapeutically relevant inhibition of PKC δ has been problematic due to the lack of selectivity of the currently available small molecule inhibitors. PKC inhibitors available until now act on ATP binding domain of PKCs and hence they can also act on lot of similar kinases. For example, Go6976 identified as a classical PKC inhibitor was shown to inhibit Syk as well [42]. LY333531 was identified as a PKC β inhibitor, but was later shown to target both PKC α and β isoforms [43]. The high level of homology (~60% amino acid identity) among the members of the novel PKC family has made the development of isotype selective small molecule inhibitors extremely difficult. So far, PKC δ inhibitors such as rottlerin are non-specific or more specific RACK inhibitors are peptide based and hence a small molecule inhibitor would have more advantages over peptides for *in vivo* studies. Identification of such novel inhibitors as presented in this study would atleast be a first step in development of therapeutics based on PKC δ inhibition. CGX1037 was identified as a novel PKC δ inhibitor by Xenogene technology. We show that CGX1037 potentiates GPVI-mediated dense granule secretion and inhibits PAR-mediated dense granule secretion at same dose (2.5 μ M) in both human and murine platelets correlating with the known role for PKC δ in platelets. Our studies also show that this inhibitor does not have any significant additional non-specific affects in platelets. However, caution must be taken when this inhibitor is used in other cells, which may be expressing different kinases.

Different PKC inhibitors target different regions on PKCs [15]. As of now, we do not know the region of PKC δ targeted by CGX1037. However, we showed that CGX1037 did not inhibit translocation of PKC δ to the membrane in platelets. Depending on the region of PKC δ targeted by CGX1037, it is likely to inhibit specific PKC δ functions but not impose global inhibition of all PKC δ dependent processes.

Conclusions

Corroborating with the previously known role for PKC δ , CGX1037 differentially regulated platelet responses wherein it inhibited PAR-mediated dense granule secretion but potentiated GPVI-mediated dense granule secretion in human and murine platelets. CGX1037 also inhibited phosphorylation on PKD2, a PKC δ substrate in platelets. We have therefore shown that CGX1037 is a PKC δ isoform selective inhibitor in platelets. However, non-specific effects of this inhibitor could not be eliminated with these results. To partially identify any nonspecific effects of CGX1037, we measured platelet responses from PKC δ -deficient platelets pre-incubated with the inhibitor and showed that CGX1037 has no additional significant effect supporting the selectivity of CGX1037. Thus, we show that CGX1037 is a novel PKC δ isoform selective inhibitor and can be employed to study the role of PKC δ .

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Figure 1.

CGX1037 potentiates GPVI-mediated dense granule secretion. (A) Human platelets (2×10^8 cells/ml) pre-incubated with DMSO vehicle or 500 nM or 1.25 µM or 2.5 µM or 10 µM or 30 µM of the inhibitor, CGX1037 for 5 min at 37 °C were stimulated with 2 µg/ml of CRP. The reaction was stopped by the addition of 6M perchloric acid after 90 s. (B) ATP secretions measured from human platelet activation were quantified and expressed as mean $\pm$ SEM. Secretions measured with agonist pre-incubated with DMSO (vehicle) were considered 100% and data are reported as mean $\pm$ SEM. The aggregation and secretion tracings shown and the quantitation are representative of at least three independent experiments. “*” represents p value < 0.05 .

Figure 2.

CGX1037 inhibits PAR-mediated dense granule secretion. (A) Human platelets (2×10^8 cells/ml) pre-incubated with DMSO vehicle or 500 nM or 1.25 μM or 2.5 μM or 10 μM or 30 μM of the inhibitor, CGX1037 for 5 min at 37 °C were stimulated with 150 μM of AYPGKF. The reaction was stopped by the addition of 6M perchloric acid after 90 s. (B) ATP secretions measured from human platelet activation were quantified and expressed as mean $\pm$ SEM. Secretions measured with agonist pre-incubated with DMSO (vehicle) were considered 100% and data are reported as mean $\pm$ SEM. The aggregation and secretion tracings shown and the quantitation are representative of at least three independent experiments. “*” represents p value < 0.05 .

Figure 3.

PKCδ negatively regulates GPVI-mediated dense granule secretion whereas it positively regulates PAR-mediated dense granule secretion. (A) Washed murine platelets (1×10^8 cells/ml) from wild type and PKCδ-deficient mice were lysed, subjected to Western blotting and were probed for total PKCδ. β-actin was used as a loading control. (B) Washed WT and PKCδ null murine platelets (1×10^8 cells/ml) were stimulated with 2 μg/ml of CRP or (C) 150 μM AYPGKF and aggregation and ATP release were measured.

Figure 4.

CGX1037 inhibits PAR-mediated PKD2 phosphorylation but not PKCδ translocation. (A) Washed human platelets (2×10^8 cells/ml) were pre-incubated with DMSO (vehicle) or 2.5 μM of the inhibitor, CGX1037 for 5 min at 37 °C and then stimulated with 150 μM AYPGKF for 90 s. The samples were subjected to Western blotting and probed for phospho PKD2 ser 744/748. β-actin was used as a loading control. US = Unstimulated. (C) Washed human platelets were stimulated with 150 μM AYPGKF with or without CGX1037 for 90 s and platelet membranes and cytosolic fractions were prepared. The samples were subjected to Western blotting and probed for total PKC δ. β3 integrin was used as a loading control. US = Unstimulated. (B & D) Densitometry was performed on the blots and the data obtained were quantified and represented as mean ± SEM. The blots shown and the data quantified were representative of at least three independent experiments. “*” represents p value < 0.05.

Figure 5.

CGX1037 inhibits PKC δ in murine platelets. Washed murine platelets (1×10^8 cells/ml) from wild type mice were pre-incubated with either DMSO or 2.5 μ M CGX1037 and were stimulated with (A) 150 μ M AYPGKF or (B) 5 μ g/ml of CRP and measured for platelet aggregation and dense granule secretion. The tracings are representative of at least three independent experiments.

Figure 6.

Specificity of the PKC δ inhibitor, CGX1037. (A) Aggregation and secretion tracings of washed murine platelets (1×10^8 cells/ml) from PKC δ -deficient mice were pre-incubated with DMSO or 2.5 μ M of the inhibitor, CGX1037 for 5 min at 37 °C and then stimulated with 150 μ M AYPGKF or (C) 500 μ M AYPGKF for 90 s. (B) & (D) ATP secretions were measured, quantified and expressed as mean $\pm$ SEM. The data and quantitation are representative of at least three independent experiments.

Table 1

IC50 values for CGX1037-mediated inhibition of various human protein kinase-dependent XenoGene yeast strains.

Kinase	PKCδ	PKCθ	PKCε	PKCη	PDK1	SGK1	AKT3
CGX1037 (μM)	0.02	4	5	3	6	2	2

PDK1, 3-phosphoinositide dependent protein kinase 1; SGK, serum/glucocorticoid regulated kinase 1; AKT3, v-akt murine thymoma viral oncogene homolog 3. The IC50 values given in the table are the representative of a minimum of three studies each done in duplicate.