



Article

Oxidative Stress-Induced Afterdepolarizations and Protein Kinase C Signaling

Yu-Dong Fei ^{1,†}, Wei Li ^{1,†}, Jian-Wen Hou ^{1,†}, Kai Guo ¹, Xiao-Meng Chen ¹, Yi-He Chen ¹, Qian Wang ¹, Xiao-Lei Xu ², Yue-Peng Wang ^{1,*} and Yi-Gang Li ^{1,*}

¹ Department of Cardiology, Xin Hua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai 200092, China; fyd6254@gmail.com (Y.-D.F.); lwcbs@163.com (W.L.); houjianwena@126.com (J.-W.H.); guokai0201@aliyun.com (K.G.); chenxmcorrine@outlook.com (X.-M.C.); cyh1726@163.com (Y.-H.C.); wangq0327@163.com (Q.W.)

² Department of Biochemistry and Molecular Biology, Division of Cardiovascular Diseases, Mayo Clinic, 200 First St SW, Rochester, MN 55905, USA; xu.xiaolei@mayo.edu

* Correspondence: ypwang555@aliyun.com (Y.-P.W.); drliyigang@outlook.com (Y.-G.L.); Tel.: +86-21-2507-7260 (Y.-G.L.); Fax: +86-21-2057-7275 (Y.-G.L.)

† These authors contributed equally to the article.

Academic Editors: Yi-Han Chen and Jianmin Cui

Received: 1 February 2017; Accepted: 14 March 2017; Published: 30 March 2017

Abstract: Background: Hydrogen peroxide (H₂O₂)-induced oxidative stress has been demonstrated to induce afterdepolarizations and triggered activities in isolated myocytes, but the underlying mechanisms remain not fully understood. We aimed to explore whether protein kinase C (PKC) activation plays an important role in oxidative stress-induced afterdepolarizations. Methods: Action potentials and ion currents of isolated rabbit cardiomyocytes were recorded using the patch clamp technique. H₂O₂ (1 mM) was perfused to induce oxidative stress and the specific classical PKC inhibitor, Gö 6983 (1 μM), was applied to test the involvement of PKC. Results: H₂O₂ perfusion prolonged the action potential duration and induced afterdepolarizations. Pretreatment with Gö 6983 prevented the emergence of H₂O₂-induced afterdepolarizations. Additional application of Gö 6983 with H₂O₂ effectively suppressed H₂O₂-induced afterdepolarizations. H₂O₂ increased the late sodium current (I_{Na,L}) ($n = 7, p < 0.01$) and the L-type calcium current (I_{Ca,L}) ($n = 5, p < 0.01$), which were significantly reversed by Gö 6983 ($p < 0.01$). H₂O₂ also increased the transient outward potassium current (I_{to}) ($n = 6, p < 0.05$). However, Gö 6983 showed little effect on H₂O₂-induced enhancement of I_{to}. Conclusions: H₂O₂ induced afterdepolarizations via the activation of PKC and the enhancement of I_{Ca,L} and I_{Na,L}. These results provide evidence of a link between oxidative stress, PKC activation and afterdepolarizations.

Keywords: oxidative stress; afterdepolarization; triggered activity; protein kinase C; arrhythmia

1. Introduction

Reactive oxygen species (ROS) play different roles in physiological and pathological conditions. Under pathological conditions, such as heart failure (HF) [1] and ischemia/reperfusion [2], elevated ROS levels can predispose the heart to arrhythmias. Previous studies evidenced hydrogen peroxide (H₂O₂)-induced oxidative stress [3] and it is known that H₂O₂ can affect various ion channels, such as the L-type calcium currents (I_{Ca,L}) [4,5], transient outward potassium currents (I_{to}) [6,7], late sodium currents (I_{Na,L}) [8,9], and further induce electrical abnormalities including early afterdepolarizations (EADs), delayed afterdepolarizations (DADs) and triggered activities (TAs) [3,8].

Protein kinase C (PKC), a group of serine/threonine protein kinase enzymes, has been identified as an important regulator of a variety of cardiac responses including myocardial fibrosis, cardiomyocyte

hypertrophy and inflammation, which are closely associated with heart failure and ischemia [10–12]. PKC levels have been found to be significantly elevated in both HF animal models and in patients with HF [13,14], and cardioprotective effects were observed by selective inhibition of PKC isozymes [11,15]. Moreover, PKC is acknowledged to be closely associated with various ion channels and transporters [5]. PKC activation has been shown to facilitate the induction of ventricular fibrillation, which can be reversed by PKC inhibition [16].

Accumulating evidence suggests that increased oxidative stress could serve as an important arrhythmogenic factor in diseased hearts. ROS can persistently activate PKC via direct oxidative modification [17] and increasing the cellular level of inositol-1,4,5-trisphosphate and diacylglycerol [18]. Up until now, there was only scant report on the role of PKC in oxidative stress-induced afterdepolarizations and its ionic mechanisms. In the present study, we tested the hypothesis that oxidative stress may activate PKC and enhance arrhythmogenic activity in cardiomyocytes, and focused on the following issues: (1) whether PKC signaling was involved in oxidative stress-induced afterdepolarizations; (2) the underlying ionic mechanisms. Our study showed that PKC activation plays an important role in oxidative stress-induced afterdepolarizations, and augmentation of $I_{Ca,L}$ and $I_{Na,L}$ seems to be the underlying mechanism.

2. Results

2.1. H_2O_2 -Induced Afterdepolarizations in Adult Rabbit Ventricular Myocytes

Action potentials (APs) were recorded from isolated rabbit ventricular myocytes using the whole-cell current clamp mode. To elicit EADs stably, the myocytes were paced at basic cycle lengths of 6 s [3]. After the action potential duration (APD) and morphology reached steady state, cells were perfused with bath solution containing 1 mM H_2O_2 .

As shown in Figure 1, H_2O_2 perfusion prolonged APD gradually and induced afterdepolarizations and TAs after approximately 7 min, while control experiments in parallel (without H_2O_2) showed that no electrical abnormalities occurred for up to 15 min ($n = 9$). APD 90 was prolonged from 276.7 ± 77.4 to 585.0 ± 65.0 ms ($n = 9$, $p < 0.01$) at 5 min of application of 1 mM H_2O_2 . Examples of afterdepolarizations and TAs are shown in Figure 1C.

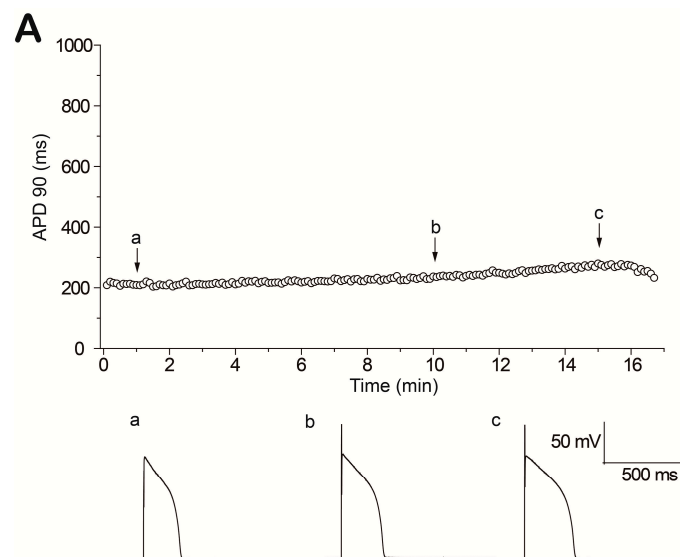


Figure 1. Cont.

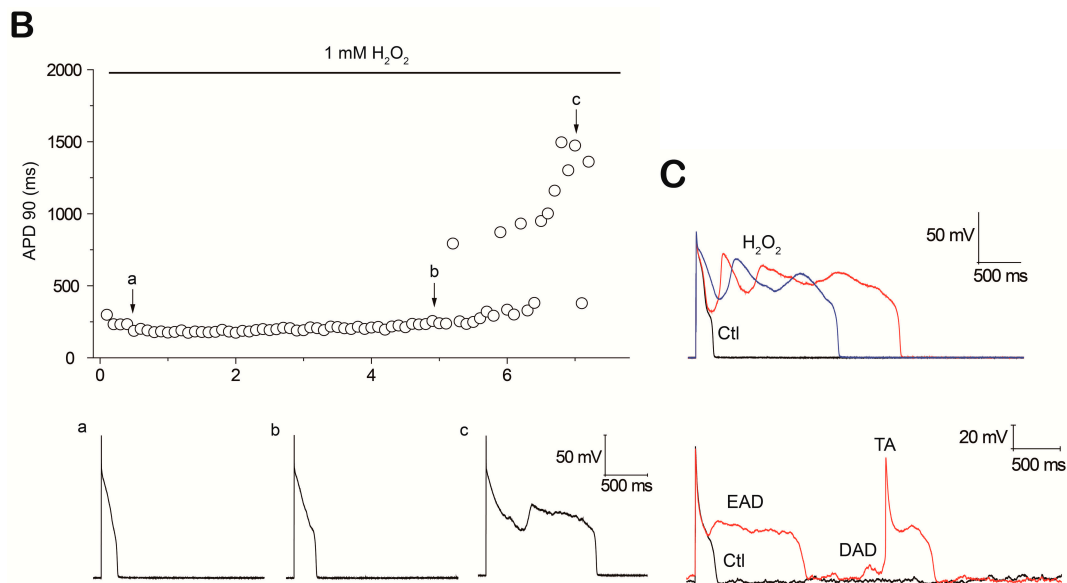


Figure 1. Afterdepolarizations induced by H₂O₂ perfusion. (A) Action potentials (APs) were elicited consecutively at basic cycle lengths of 6 s and values of action potential durations (APD) 90 were plotted over time. APD 90 was consecutively recorded from a cell perfused with standard Tyrode solution for over 15 min. APs at 1 min (a), 10 min (b), and 15 min (c) are shown below. No early afterdepolarizations (EADs), delayed afterdepolarizations (DADs) or triggered activities (TAs) occurred; (B) H₂O₂ (1 mM) was perfused continuously as indicated by the horizontal bar. APs at the beginning of the perfusion (a), and after perfusion with H₂O₂ for 5 min (b) and 7 min (c) are shown below; (C) Examples of afterdepolarizations and TAs during H₂O₂ exposure, including multiple oscillatory EADs (above), and different electrical abnormalities in a pacing cycle (below).

2.2. The Role of PKC Signaling in H₂O₂-Induced Afterdepolarizations

Next we tested whether PKC activation was involved in H₂O₂-induced afterdepolarizations by using the specific classical PKC inhibitor Gö 6983. Unlike those myocytes consistently presenting EADs approximately 7 min after exposure to 1 mM H₂O₂ (Figure 1A), pretreatment with Gö 6983 (1 μM) prevented the emergence of H₂O₂-induced EADs for up to 15 min (Figure 2A). As shown in Figure 2B, the incidence of EADs induced by H₂O₂ was significantly reduced by pretreatment with Gö 6983 (100% vs. 0%, $n = 8$). To further confirm the effect of PKC inhibition on H₂O₂-induced EADs, we applied another widely used selective PKC inhibitor, Bisindolylmaleimide (BIM). As expected, pretreatment with BIM (1 μM) prevented the emergence of H₂O₂-induced afterdepolarizations in six of six ventricular myocytes (Figure S1A).

In another series of experiments, after EADs were induced by H₂O₂ perfusion, myocytes were perfused with bath solution containing both Gö 6983 and H₂O₂. Gö 6983 effectively suppressed H₂O₂-induced EADs, DADs and TAs in five out of five myocytes. Five consecutive APs under control conditions, in the presence of H₂O₂ and after the addition of Gö 6983, are shown in Figure 3A. Values of APD 90 are plotted over time in Figure 3B. In another group of myocytes, BIM was applied after EADs were induced by H₂O₂ perfusion, and BIM also effectively reversed EADs in five of five myocytes (Figure S1B).

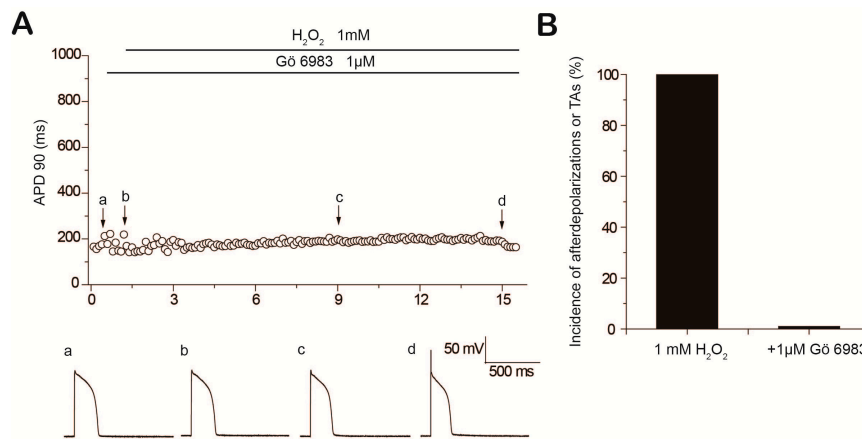


Figure 2. Prevention of H₂O₂-induced early afterdepolarizations (EADs) by the protein kinase C inhibitor Gö 6983. (A) Time course of action potential duration (APD) 90 in a myocyte treated with Gö 6983 before exposure to 1 mM H₂O₂. Action potentials under control conditions (a), in the presence of Gö 6983 (b); after perfusion of H₂O₂ for 8 min (c) and 14 min (d) are shown below; (B) Incidence of EADs, delayed afterdepolarizations (DADs) or triggered activities (TAs) in the presence of H₂O₂ and pretreated with Gö 6983.

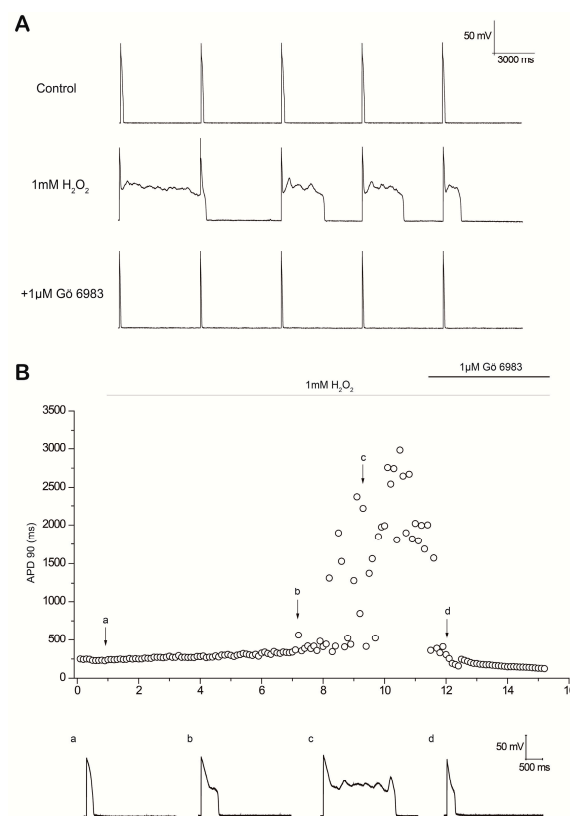


Figure 3. Suppression of H₂O₂-induced early afterdepolarizations (EADs) by the PKC inhibitor Gö 6983. (A) Gö 6983 completely suppressed all H₂O₂-induced EADs and significantly shortened action potential duration (APD). The representative five consecutive action potentials (APs) are shown in each period; (B) Time course of APD 90 in a myocyte treated with Gö 6983 after EADs were induced by H₂O₂. APs under control conditions (a), after perfusion with H₂O₂ for 6 min (b) and 8 min (c), and after application of Gö 6983 (d) are shown below.

2.3. PKC Mediates $I_{Ca,L}$ Enhancement in H_2O_2 -Induced Afterdepolarizations

The effects of H_2O_2 and the PKC inhibitor on the major membrane currents were analyzed using a voltage clamp. The representative current-voltage traces of $I_{Ca,L}$ are displayed in Figure 4A, and the averaged I-V curves ($n = 5$) are shown in Figure 4B. Our results show that 1 mM H_2O_2 significantly enhanced $I_{Ca,L}$, which was effectively attenuated by Gö 6983. The peak amplitudes of $I_{Ca,L}$ at +10 mV were measured for data analysis. As shown in Figure 4C, $I_{Ca,L}$ was elevated from -6.11 ± 0.47 to -8.82 ± 0.61 pA/pF after H_2O_2 perfusion ($p < 0.01$, control vs. H_2O_2 , $n = 5$), and decreased to -6.21 ± 0.45 pA/pF after the addition of Gö 6983 ($p < 0.01$, H_2O_2 vs. Gö 6983, $n = 5$). In the control experiments, the run-down of $I_{Ca,L}$ was less than 5% [19].

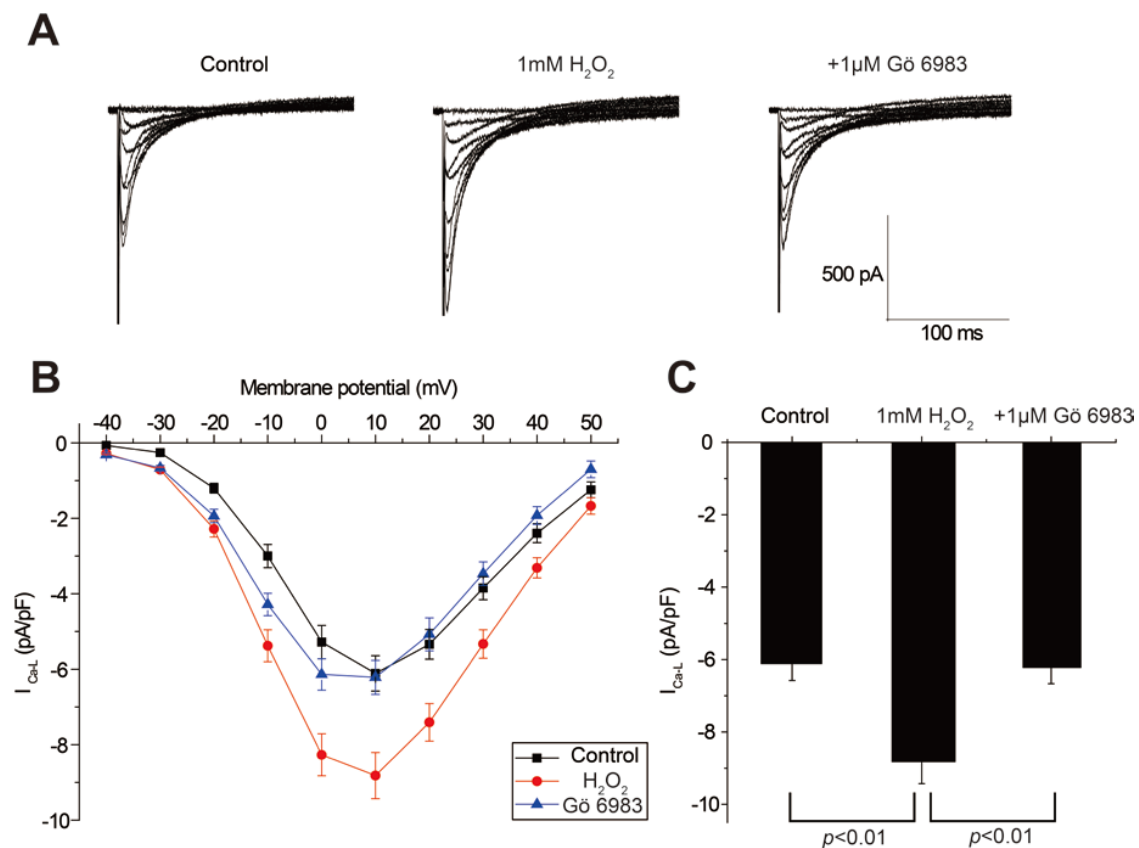


Figure 4. Effects of Gö 6983 on H_2O_2 -induced L-type calcium current ($I_{Ca,L}$) elevation. Representative current-voltage traces (A) and the averaged current-voltage curves (B) showed that H_2O_2 significantly augmented $I_{Ca,L}$, which was reversed by Gö 6983. Summary histogram (C) of $I_{Ca,L}$ at +10 mV under control condition, in the presence of 1 mM H_2O_2 alone or plus 1 μ M Gö 6983.

2.4. PKC Mediates $I_{Na,L}$ Augmentation in H_2O_2 -Induced Afterdepolarizations

$I_{Na,L}$ plays a key role in H_2O_2 -induced afterdepolarizations [19–21]. $I_{Na,L}$ curves averaged by five replicated depolarizing voltage steps from -120 to -30 mV of one representative cell are shown in Figure 5A. The amplitudes of $I_{Na,L}$ were measured at 200 ms and are summarized in Figure 5B. Results showed that $I_{Na,L}$ was elevated from -0.09 ± 0.01 to -0.23 ± 0.02 pA/pF ($p < 0.01$, control vs. H_2O_2 , $n = 7$) and attenuated by 1 μ M Gö 6983 to -0.11 ± 0.02 pA/pF ($p < 0.01$, H_2O_2 vs. Gö 6983, $n = 7$).

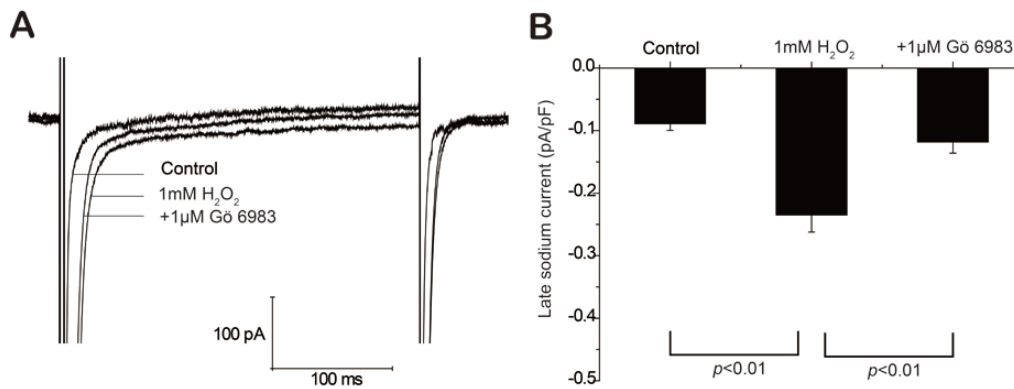


Figure 5. Effects of Gö 6983 on H₂O₂-induced late sodium current ($I_{Na,L}$) elevation. Representative current-voltage traces (A) and summary data of $I_{Na,L}$ at -30 mV under control condition, in the presence of 1 mM H₂O₂ alone or plus 1 μ M Gö 6983 (B) are shown.

2.5. Less Involvement of I_{t0} in PKC Signaling in H₂O₂-Induced Afterdepolarizations

The effects of H₂O₂ and the PKC inhibitor on I_{t0} were also analyzed. The representative current-voltage traces of I_{t0} are displayed in Figure 6A, and the averaged I-V curves ($n = 6$) are shown in Figure 6B. The peak amplitudes of I_{t0} at $+50$ mV were measured and are summarized in Figure 6C. I_{t0} was significantly elevated from 3.07 ± 0.62 to 3.88 ± 0.50 pA/pF after H₂O₂ perfusion ($p < 0.05$, control vs. H₂O₂, $n = 6$), and decreased to 3.58 ± 0.46 pA/pF after the addition of Gö 6983 ($p > 0.05$, H₂O₂ vs. Gö 6983, $n = 6$).

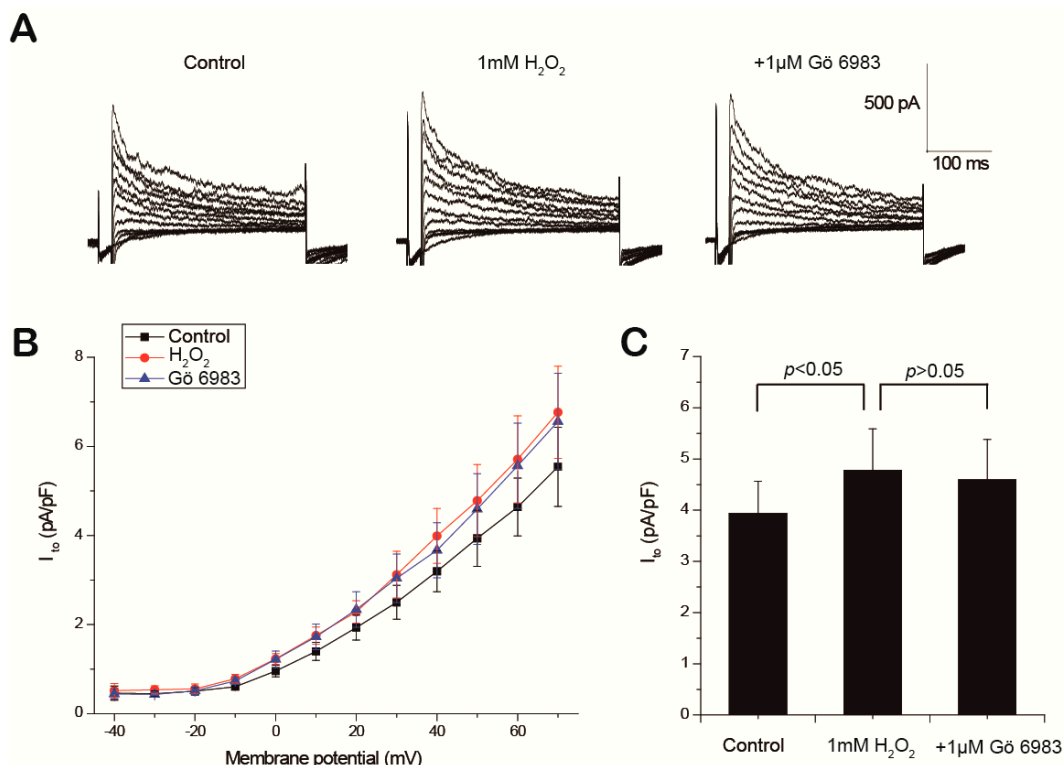


Figure 6. Effects of Gö 6983 on H₂O₂-induced transient outward potassium current (I_{t0}) elevation. Representative current-voltage traces (A), the averaged current-voltage curves of I_{t0} (B) and summary histogram of I_{t0} at $+50$ mV (C) under control condition, in the presence of 1 mM H₂O₂ alone or plus 1 μ M Gö 6983 are shown.

3. Discussion

The present study provided the following novel findings: (1) the specific classical PKC inhibitor Gö 6983 both prevented and reversed the emergence of H₂O₂-induced afterdepolarizations effectively, indicating PKC activation mediates oxidative stress-induced afterdepolarizations; (2) PKC inhibition by Gö 6983 attenuated H₂O₂-induced enhancement of I_{Ca,L} and I_{Na,L}, but not I_{to}.

PKC, a classical second messenger, is acknowledged to be involved in several pathological progressions including hypertrophy, fibrosis, inflammation, ischemic preconditioning and atherosclerosis. Thus, PKC serves as a potential therapeutic target for heart failure, hypertension and coronary heart diseases [10,22]. Previous studies have investigated the effect of PKC activation by endothelin-1 and a PKC agonist on arrhythmogenesis [16,23]. However, the relationship between PKC and arrhythmias in different pathological conditions, including oxidative stress, remains unclear.

Oxidative stress is involved in a variety of cardiovascular diseases, including heart failure and ischemic heart disease. Cardiac arrhythmias are prevalent in patients with these conditions. Afterdepolarization-mediated TAs play an important role in ROS-induced arrhythmias [24], but the underlying molecular mechanisms remain not fully elucidated. It is well known that classical PKC isozymes are activated by Ca²⁺ and diacylglycerol [25]. When inactive, the regulatory domain of PKC is bound to the catalytic domain, inhibiting the enzyme activity, and when PKC is activated, this autoinhibition is released [26]. It is noteworthy that, besides Ca²⁺ and diacylglycerol, mild oxidative stress can activate PKC by selectively oxidizing the regulatory domain [17]. These findings motivated us to explore the role of PKC signaling in oxidative stress-induced afterdepolarizations, and the present study provided direct evidence showing that PKC is indeed involved. In order to confirm that the results were not the side effects of PKC inhibitors, two widely used selective PKC inhibitors (Gö 6983 and BIM) were both tested, and the results demonstrated that it was the PKC inhibitory effect that affected H₂O₂-induced afterdepolarizations.

H₂O₂ may affect I_{Ca,L}, I_{Na,L} and I_{to} via direct oxidative modification, intracellular signaling pathways or transcription and translation regulations [5]. Our results show that acute application of H₂O₂ is capable of enhancing these major membrane currents significantly. In our study, adding the PKC inhibitor along with H₂O₂ for several minutes effectively reversed the H₂O₂-induced alterations on I_{Ca,L} and I_{Na,L}, suggesting the mechanistic role of PKC on the H₂O₂-induced intracellular signaling cascade. However, acute application of the PKC inhibitor failed to reverse the enhancement of I_{to}, indicating a minor role of I_{to} on H₂O₂-induced effects.

When activated, PKC can phosphorylate downstream proteins including the L-type Ca²⁺ channel [27] and voltage-gated Na⁺ channel [28], resulting in I_{Ca,L} facilitation and I_{Na,L} augmentation. Previous studies have demonstrated that the elevation of not only I_{Na,L} but also I_{Ca,L} was involved in the ROS-induced afterdepolarization formation [3]; thus, elevation of I_{Na,L} can reduce the repolarization reserve, while activation of I_{Ca,L} can generate EAD upstroke. It is known that augmentation of I_{Ca,L} can increase Ca²⁺ influx and enhance cellular Ca²⁺ loading. In addition, APD prolongation can also enhance cellular Ca²⁺ loading, and further promotes PKC activation. In our study, the inhibition of PKC activation significantly reversed H₂O₂-induced enhancement of both I_{Ca,L} and I_{Na,L}, these might result in reduced cellular Ca²⁺ loading by attenuating Ca²⁺ influx via I_{Ca,L} and shortened APD.

The results collectively suggested that PKC signaling plays a central role in oxidative stress-induced afterdepolarizations, although activation of other kinases such as protein kinase A and Ca/calmodulin-dependent protein kinase II (CaMKII) cannot be ruled out, and it is beyond the present study protocol. Previous studies have demonstrated that CaMKII activation played a role in H₂O₂-induced afterdepolarizations [3]. As PKC and CaMKII are both kinases that can be activated by the oxidative modification and elevated Ca²⁺ level, they may share similar ionic mechanisms in oxidative stress-induced afterdepolarizations. Another study demonstrated similar inhibitory effects on intracellular Ca²⁺-induced I_{Na,L} by both inhibitors of PKC and CaMKII [29]. This evidence together with our results indicates that PKC activation is essential in oxidative stress-induced afterdepolarizations.

It has already been demonstrated that ROS levels are elevated in a number of cardiovascular diseases and predispose the heart to arrhythmias. Free radical scavengers were supposed to be a potential therapy strategy for alleviating arrhythmias [30], but this proposal was found to be not effective due to some limitations [31]. Thus, downstream second messengers, such as PKC, may be potential novel therapeutic targets for arrhythmias. Regulators of PKC are already in clinical trials for different indications [32–36], and systemic delivery of activators and inhibitors of PKC has been proven to be well tolerated [32,33]. Future studies are warranted to test the safety and feasibility of PKC inhibitors to treat arrhythmias in experimental settings and in patients.

4. Materials and Methods

4.1. Solutions and Drugs

The standard Tyrode solution and Kraft-Bruhe (KB) solution were made with Milli-Q grade water. The standard Tyrode solution contained (in mM) 135 NaCl, 5.4 KCl, 0.33 NaH₂PO₄, 1.0 MgCl₂, 10 glucose, 10 HEPES, 1.8 CaCl₂ with pH adjusted to 7.4 with NaOH. The KB solution contained (in mM) 85 KOH, 50 K-glutamate, 30 KCl, 1.0 MgCl₂, 20 taurine, 0.5 Ethylene glycol bis(2-aminoethyl ether) tetraacetic acid (EGTA), 10 glucose, 10 HEPES with pH adjusted to 7.4 with KOH. Chemicals and reagents were purchased from Sigma Aldrich (St. Louis, MO, USA) unless otherwise noted. BIM was purchased from Selleckchem (Houston, TX, USA). Nifedipine, Gö 6983 and BIM were dissolved in Dimethyl Sulfoxide (DMSO) as stock solution according to the manufacturer's instructions before diluting into the superfusate solution at the final concentration. The final concentration of DMSO was less than 0.1%.

4.2. Cell Isolation

The use and care of animals conformed to the Guide for the Care and Use of Laboratory Animals of Shanghai, China and was approved by the Institutional Animal Care and Use Committee of Xinhua Hospital (Approval No.: XHEC-F-2016-009, Date: 22 March 2016). Hearts were removed from 23 adult New Zealand White rabbits (2 to 3 kg) anesthetized with intravenous pentobarbital, and were then perfused retrogradely in Langendorff apparatus at 37 °C with Ca²⁺-free Tyrode's solution containing 1.4 mg/mL collagenase (Type II, Worthington, Lakewood, NJ, USA) and 0.1 mg/mL protease (type XIV, Sigma, St. Louis, MO, USA) for 15 to 30 min. After washing out the enzyme solution, the hearts were removed from the apparatus and swirled in a culture dish in KB solution. The Ca²⁺ concentration was gradually increased to 1.8 mM, and the cells were then kept at 4 °C until use.

4.3. Patch-Clamp Methods

All experiments were performed at 35 °C to 37 °C. Myocytes were perfused in standard Tyrode solution for 5 min and then patch-clamped using the whole-cell configuration. H₂O₂ (1 mM) and Gö 6983 (1 μM) were added to the bath superfusate. For AP recordings, patch pipettes (resistance 2 to 3 MΩ) were filled with pipette solution containing (in mM) 110 K-aspartate, 30 KCl, 5 NaCl, 0.1 EGTA, 10 HEPES, 5 MgATP, 5 creatine phosphate, 0.05 cAMP, pH 7.2 with KOH. APs were elicited with 2 ms, 2–4 nA square pulses at basic cycle lengths of 6 s.

For I_{Ca,L} recording, the cells were superfused with a modified Tyrode's solution in which KCl was replaced by CsCl, and the patch pipettes (resistance 1 to 2 MΩ) were filled with an internal solution containing (in mM): 110 Cs-Aspartate, 30 CsCl, 5 NaCl, 10 HEPES, 5 MgATP, 5 creatine phosphate, 0.1 EGTA, 0.05 cAMP, pH 7.2 with CsOH. I_{Ca,L} was recorded using a series of 200 ms steps from –40 to +50 mV with increments of 10 mV from a holding potential of –80 mV prior to a 100 ms prepulse to –40 mV.

To isolate I_{Na,L}, the pipette solution contained (in mM) 110 Cs-aspartate, 30 CsCl, 10 HEPES, 0.5 EGTA, 0.2 Na₃-GTP, 5 Na₂-phosphocreatine, 5 MgATP, pH 7.2 with CsOH. The bath solution was the same as that used for I_{Ca,L}. Nifedipine (30 μM) was added to the bath solution to block calcium

currents. $I_{Na,L}$ was elicited by 300 ms pulses from -120 to -30 mV, and the amplitude was measured at 200 ms.

For transient outward potassium current (I_{to}) recording, the pipette and bath solutions were the same as those for AP recording. TTX ($10 \mu\text{M}$) and Cd^{3+} (0.3 mM) were added into the Tyrode's solution to inhibit $I_{Na,L}$ and $I_{Ca,L}$. I_{to} was elicited at basic cycle lengths of 10 s by a series of 300 ms steps from -40 to $+70$ mV with increments of 10 mV from a holding potential of -70 mV prior to a prepulse to -40 mV.

4.4. Data Acquisition and Analysis

Voltage and current signals were measured with a MultiClamp 700B patch-clamp amplifier (Axon Instruments, Sunnyvale, CA, USA) controlled by a personal computer using a Digidata 1440A acquisition board driven by pCLAMP 10 software (Molecular Devices, Sunnyvale, CA, USA). Data are presented as means \pm standard error of means unless indicated. Statistical significance was assessed using unpaired Student's t tests, and $p < 0.05$ was considered significant.

5. Conclusions

PKC activation mediates oxidative stress-induced afterdepolarizations via enhancement of $I_{Ca,L}$ and $I_{Na,L}$, and PKC might be a potential antiarrhythmic therapeutic target.

Supplementary Materials: Supplementary materials can be found at www.mdpi.com/1422-0067/18/4/688/s1.

Acknowledgments: This work was supported by National Natural Science Foundation of China grant (No. 81270258 to Yi-Gang Li, Nos. 81370257, 81670414 to Yue-Peng Wang, No. 81470497 to Jie Chen), the State Key Program of National Natural Science Foundation of China (No. 81530015 to Yi-Gang Li) and Shanghai City Committee of Science and Technology Research Projects (Nos. 12411951900, 13140903801, and 14441902502 to Yi-Gang Li).

Author Contributions: Wei Li, Xiao-Lei Xu and Yi-Gang Li conceived and designed the experiments; Yu-Dong Fei, Jian-Wen Hou, Kai Guo, Xiao-Meng Chen, Yi-He Chen, and Qian Wang performed the experiments; Xiao-Lei Xu, Yue-Peng Wang and Yi-Gang Li analyzed the data; Yu-Dong Fei, Wei Li and Jian-Wen Hou wrote the paper.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Tsutsui, H.; Kinugawa, S.; Matsushima, S. Oxidative stress and heart failure. *Am. J. Physiol. Heart Circ. Physiol.* **2011**, *301*, H2181–H2190. [[CrossRef](#)] [[PubMed](#)]
2. Slezak, J.; Tribulova, N.; Pristacova, J.; Uhrik, B.; Thomas, T.; Khaper, N.; Kaul, N.; Singal, P.K. Hydrogen peroxide changes in ischemic and reperfused heart. Cytochemistry and biochemical and X-ray microanalysis. *Am. J. Pathol.* **1995**, *147*, 772–781. [[PubMed](#)]
3. Xie, L.H.; Chen, F.; Karagueuzian, H.S.; Weiss, J.N. Oxidative-stress-induced afterdepolarizations and calmodulin kinase II signaling. *Circ. Res.* **2009**, *104*, 79–86. [[CrossRef](#)] [[PubMed](#)]
4. Hudasek, K.; Brown, S.T.; Fearon, I.M. H_2O_2 regulates recombinant Ca^{2+} channel $\alpha 1\text{C}$ subunits but does not mediate their sensitivity to acute hypoxia. *Biochem. Biophys. Res. Commun.* **2004**, *318*, 135–141. [[CrossRef](#)] [[PubMed](#)]
5. Sag, C.M.; Wagner, S.; Maier, L.S. Role of oxidants on calcium and sodium movement in healthy and diseased cardiac myocytes. *Free Radic. Biol. Med.* **2013**, *63*, 338–349. [[CrossRef](#)] [[PubMed](#)]
6. Berube, J.; Caouette, D.; Daleau, P. Hydrogen peroxide modifies the kinetics of HERG channel expressed in a mammalian cell line. *J. Pharmacol. Exp. Ther.* **2001**, *297*, 96–102. [[PubMed](#)]
7. Zhao, Z.; Xie, Y.; Wen, H.; Xiao, D.; Allen, C.; Fefelova, N.; Dun, W.; Boyden, P.A.; Qu, Z.; Xie, L.H. Role of the transient outward potassium current in the genesis of early afterdepolarizations in cardiac cells. *Cardiovasc. Res.* **2012**, *95*, 308–316. [[CrossRef](#)] [[PubMed](#)]
8. Ward, C.A.; Giles, W.R. Ionic mechanism of the effects of hydrogen peroxide in rat ventricular myocytes. *J. Physiol.* **1997**, *500*, 631–642. [[CrossRef](#)] [[PubMed](#)]

9. Song, Y.; Shryock, J.C.; Wagner, S.; Maier, L.S.; Belardinelli, L. Blocking late sodium current reduces hydrogen peroxide-induced arrhythmogenic activity and contractile dysfunction. *J. Pharmacol. Exp. Ther.* **2006**, *318*, 214–222. [[CrossRef](#)] [[PubMed](#)]
10. Palaniyandi, S.S.; Sun, L.; Ferreira, J.C.; Mochly-Rosen, D. Protein kinase C in heart failure: A therapeutic target? *Cardiovasc. Res.* **2009**, *82*, 229–239. [[CrossRef](#)] [[PubMed](#)]
11. Boyle, A.J.; Kelly, D.J.; Zhang, Y.; Cox, A.J.; Gow, R.M.; Way, K.; Itescu, S.; Krum, H.; Gilbert, R.E. Inhibition of protein kinase C reduces left ventricular fibrosis and dysfunction following myocardial infarction. *J. Mol. Cell. Cardiol.* **2005**, *39*, 213–221. [[CrossRef](#)] [[PubMed](#)]
12. Braz, J.C.; Gregory, K.; Pathak, A.; Zhao, W.; Sahin, B.; Klevitsky, R.; Kimball, T.F.; Lorenz, J.N.; Nairn, A.C.; Liggett, S.B.; et al. PKC- α regulates cardiac contractility and propensity toward heart failure. *Nat. Med.* **2004**, *10*, 248–254. [[CrossRef](#)] [[PubMed](#)]
13. Belin, R.J.; Sumandea, M.P.; Allen, E.J.; Schoenfelt, K.; Wang, H.; Solaro, R.J.; de Tombe, P.P. Augmented protein kinase C α -induced myofilament protein phosphorylation contributes to myofilament dysfunction in experimental congestive heart failure. *Circ. Res.* **2007**, *101*, 195–204. [[CrossRef](#)] [[PubMed](#)]
14. Bowling, N.; Walsh, R.A.; Song, G.; Estridge, T.; Sandusky, G.E.; Fouts, R.L.; Mintze, K.; Pickard, T.; Roden, R.; Bristow, M.R.; et al. Increased protein kinase C activity and expression of Ca²⁺-sensitive isoforms in the failing human heart. *Circulation* **1999**, *99*, 384–391. [[CrossRef](#)] [[PubMed](#)]
15. Ferreira, J.C.; Koyanagi, T.; Palaniyandi, S.S.; Fajardo, G.; Churchill, E.N.; Budas, G.; Disatnik, M.H.; Bernstein, D.; Brum, P.C.; Mochly-Rosen, D. Pharmacological inhibition of β IIPKC is cardioprotective in late-stage hypertrophy. *J. Mol. Cell. Cardiol.* **2011**, *51*, 980–987. [[CrossRef](#)] [[PubMed](#)]
16. Aydin, O.; Becker, R.; Kraft, P.; Voss, F.; Koch, M.; Kelemen, K.; Katus, H.A.; Bauer, A. Effects of protein kinase C activation on cardiac repolarization and arrhythmogenesis in Langendorff-perfused rabbit hearts. *Europace* **2007**, *9*, 1094–1098. [[CrossRef](#)] [[PubMed](#)]
17. Gopalakrishna, R.; Anderson, W.B. Ca²⁺- and phospholipid-independent activation of protein kinase C by selective oxidative modification of the regulatory domain. *Proc. Natl. Acad. Sci. USA* **1989**, *86*, 6758–6762. [[CrossRef](#)] [[PubMed](#)]
18. Shasby, D.M.; Yorek, M.; Shasby, S.S. Exogenous oxidants initiate hydrolysis of endothelial cell inositol phospholipids. *Blood* **1988**, *72*, 491–499. [[PubMed](#)]
19. Hou, J.W.; Li, W.; Guo, K.; Chen, X.M.; Chen, Y.H.; Li, C.Y.; Zhao, B.C.; Zhao, J.; Wang, H.; Wang, Y.P.; et al. Antiarrhythmic effects and potential mechanism of WenXin KeLi in cardiac Purkinje cells. *Heart Rhythm* **2016**, *13*, 973–982. [[CrossRef](#)] [[PubMed](#)]
20. Li, W.; Wang, Y.P.; Gao, L.; Zhang, P.P.; Zhou, Q.; Xu, Q.F.; Zhou, Z.W.; Guo, K.; Chen, R.H.; Yang, H.T.; et al. Resveratrol protects rabbit ventricular myocytes against oxidative stress-induced arrhythmogenic activity and Ca²⁺ overload. *Acta Pharmacol. Sin.* **2013**, *34*, 1164–1173. [[CrossRef](#)] [[PubMed](#)]
21. Li, W.; Yu, Y.; Hou, J.W.; Zhou, Z.W.; Guo, K.; Zhang, P.P.; Wang, Z.Q.; Yan, J.H.; Sun, J.; Zhou, Q.; et al. Larger rate dependence of late sodium current in cardiac Purkinje cells: A potential link to arrhythmogenesis. *Heart Rhythm* **2017**, *14*, 422–431. [[CrossRef](#)] [[PubMed](#)]
22. Murphy, S.; Frishman, W.H. Protein kinase C in cardiac disease and as a potential therapeutic target. *Cardiol. Rev.* **2005**, *13*, 3–12. [[PubMed](#)]
23. Puglisi, J.L.; Yuan, W.; Timofeyev, V.; Myers, R.E.; Chiamvimonvat, N.; Samarel, A.M.; Bers, D.M. Phorbol ester and endothelin-1 alter functional expression of Na⁺/Ca²⁺ exchange, K⁺, and Ca²⁺ currents in cultured neonatal rat myocytes. *Am. J. Physiol. Heart Circ. Physiol.* **2011**, *300*, H617–H626. [[CrossRef](#)] [[PubMed](#)]
24. Morita, N.; Lee, J.H.; Xie, Y.; Sovari, A.; Qu, Z.; Weiss, J.N.; Karagueuzian, H.S. Suppression of re-entrant and multifocal ventricular fibrillation by the late sodium current blocker ranolazine. *J. Am. Coll. Cardiol.* **2011**, *57*, 366–375. [[CrossRef](#)] [[PubMed](#)]
25. Dorn, G.W., 2nd; Force, T. Protein kinase cascades in the regulation of cardiac hypertrophy. *J. Clin. Investig.* **2005**, *115*, 527–537. [[CrossRef](#)] [[PubMed](#)]
26. House, C.; Kemp, B.E. Protein kinase C contains a pseudosubstrate prototope in its regulatory domain. *Science* **1987**, *238*, 1726–1728. [[CrossRef](#)] [[PubMed](#)]
27. Yang, L.; Liu, G.; Zakharov, S.I.; Morrow, J.P.; Rybin, V.O.; Steinberg, S.F.; Marx, S.O. Ser1928 is a common site for Cav1.2 phosphorylation by protein kinase C isoforms. *J. Biol. Chem.* **2005**, *280*, 207–214. [[CrossRef](#)] [[PubMed](#)]

28. Qu, Y.; Rogers, J.C.; Tanada, T.N.; Catterall, W.A.; Scheuer, T. Phosphorylation of S1505 in the cardiac Na⁺ channel inactivation gate is required for modulation by protein kinase C. *J. Gen. Physiol.* **1996**, *108*, 375–379. [[CrossRef](#)] [[PubMed](#)]
29. Ma, J.; Luo, A.; Wu, L.; Wan, W.; Zhang, P.; Ren, Z.; Zhang, S.; Qian, C.; Shryock, J.C.; Belardinelli, L. Calmodulin kinase II and protein kinase C mediate the effect of increased intracellular calcium to augment late sodium current in rabbit ventricular myocytes. *Am. J. Physiol. Cell. Physiol.* **2012**, *302*, C1141–C1151. [[CrossRef](#)] [[PubMed](#)]
30. Xing, D.; Chaudhary, A.K.; Miller, F.J., Jr.; Martins, J.B. Free radical scavenger specifically prevents ischemic focal ventricular tachycardia. *Heart Rhythm* **2009**, *6*, 530–536. [[CrossRef](#)] [[PubMed](#)]
31. Cristofori, L.; Tavazzi, B.; Gambin, R.; Vagnozzi, R.; Vivenza, C.; Amorini, A.M.; di Pierro, D.; Fazzina, G.; Lazzarino, G. Early onset of lipid peroxidation after human traumatic brain injury: A fatal limitation for the free radical scavenger pharmacological therapy? *J. Investig. Med.* **2001**, *49*, 450–458. [[CrossRef](#)] [[PubMed](#)]
32. PKC-DMES Study Group. Effect of ruboxistaurin in patients with diabetic macular edema: Thirty-month results of the randomized PKC-DMES clinical trial. *Arch. Ophthalmol.* **2007**, *125*, 318–324.
33. PKC-DMES Study Group. The effect of ruboxistaurin on visual loss in patients with moderately severe to very severe nonproliferative diabetic retinopathy: Initial results of the Protein Kinase C β Inhibitor Diabetic Retinopathy Study (PKC-DRS) multicenter randomized clinical trial. *Diabetes* **2005**, *54*, 2188–2197.
34. Herbst, R.S.; Oh, Y.; Wagle, A.; Lahn, M. Enzastaurin, a protein kinase C β -selective inhibitor, and its potential application as an anticancer agent in lung cancer. *Clin. Cancer Res.* **2007**, *13*, 4641–4646. [[CrossRef](#)] [[PubMed](#)]
35. Bates, E.; Bode, C.; Costa, M.; Gibson, C.M.; Granger, C.; Green, C.; Grimes, K.; Harrington, R.; Huber, K.; Kleiman, N.; et al. Intracoronary KAI-9803 as an adjunct to primary percutaneous coronary intervention for acute ST-segment elevation myocardial infarction. *Circulation* **2008**, *117*, 886–896. [[PubMed](#)]
36. Advani, R.; Peethambaram, P.; Lum, B.L.; Fisher, G.A.; Hartmann, L.; Long, H.J.; Halsey, J.; Holmlund, J.T.; Dorr, A.; Sikic, B.I. A Phase II trial of aprinocarsen, an antisense oligonucleotide inhibitor of protein kinase C α , administered as a 21-day infusion to patients with advanced ovarian carcinoma. *Cancer* **2004**, *100*, 321–326. [[CrossRef](#)] [[PubMed](#)]



© 2017 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).