Protein kinase C (PKC) delta activation negatively regulates phorbol 12-myristate 13-acetate (PMA) induced superoxide (SO) release in polymorphonuclear leukocytes (PMNs)

Stephanie Liu, Stephanie Percy, Samuel Johansson, Christine Adekayode, Gregory Stoner, Amelie Bottex, Jovan Adams, Robert Barsotti, Qian Chen, and Lindon H. Young

Department of Bio-Medical Sciences, Philadelphia College of Osteopathic Medicine, 4170 City Avenue, Philadelphia, PA 19131

Isolation of PMNs

Male Sprague-Dawley rats (350-400 g, Charles River), used as PMN donors, were anesthetized with 2.5% isoflurane and given a 16 ml intraperitoneal injection of 0.5% glyoxyl (Sigma Chemical) dissolved in PBS. Rats were reanesthetized with isoflurane 16-18 h later, and the PMNs were harvested by peritoneal lavage in 30 ml of 0.9% NaCl, as previously described [5,8]. Peritoneal lavage fluid was centrifuged at 200 x g for 10 min at 4°C. Then, the PMNs were washed in 20 ml PBS and centrifuged at 200 x g for 10 min at 4°C. PMNs were resuspended in 2.5 ml PBS and density was calculated. The PMNs preparation were >90% pure and >95% viable according to microscopic analysis.

Measurement of SO release from Rat PMNs

The SO release from PMNs was measured spectrophotometrically (model 260 Gilford, Nova Biotech; El Cajon, CA) by the reduction of ferricytochrome c [5,8]. The PMNs (5 x 10^6) were resuspended in 450 μl of PBS and incubated with ferricytochrome c (100 μM, Sigma Chemical) in a total volume of 900 μl PBS in the presence or absence of PKC δ activator (Myr-MRAAEDPM, MW=1130 g/mol, 2.5-20 μM, n=10-13), PKC δ inhibitor (Myr-SFNSYEGLS, MW=1326 g/mol, 1-20 μM, n=7-10) or rottlerin (MW=516 g/mol, 0.625-20 μM, n=8-12) for 15 min at 37°C in spectrophotometric cells. The PMNs were stimulated with 15 or 30 mM SO (MW=616 g/mol, n=13-26) in a final reaction volume of 1.0 ml. Absorbance at 550 nm was measured every 30 sec for up to 420 sec for PMN and the change in absorbance (SO release) from PMNs was determined relative to time 0. Cell viability among all study groups was determined by 0.3% trypan blue exclusion at the end of SO release assay. Viable cells remained unstained and non-viable cells stained blue.

Statistical Analysis

All data in the text and figures are presented as means ± S.E.M. The data were analyzed by analysis of variance using post hoc analysis with Fisher’s PLSD test. Probability values of <0.05 are considered to be statistically significant.

Conclusions and Future Research

As expected, PKC δ−/− attenuated PMN SO release up to 50%, whereas, rottlerin attenuated the PMN response up to 96% compared to untreated control PMNs. However, the PKC δ−/− only enhanced PMN SO release up to 30%. Moreover, cell viability was >95±4% in all study groups suggesting that the effects of rottlerin, PKC δ−/−, and PKC δ+ on PMN induced SO release were not related to cell viability. The data suggest that PKC δ negatively regulates PMN NADPH oxidase SO release. PKC δ−/− is relatively ineffective in regulating PMN SO release compared to rottlerin. The data suggest that inhibiting the ATP binding site of PKC δ by rottlerin may be more effective than the inhibition of translocation of PKC δ to PMN substrates such as NADPH oxidase. Preliminary data shows that superoxide dismutase (SOD) inhibited untreated PMN (30 μM, n=3) induced SO release by 95±4% and also attenuated PMN plus rottlerin (20 μM, n=3) induced SO release by 96±4%. Cell viability was 96±1% in SOD treated groups. These preliminary results suggest that the effects of rottlerin to augment the reduction of ferricytochrome c in the presence of PMN are related to the generation of SO release. Additional trials will be performed to confirm the preliminary results. Further research will be aimed at determining the phosphorylation sites of p47phox by PKC δ.

References