Calcium Glycerophosphorylcholine (CGP) Preserves Transepithelial Integrity

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Abstract

We hypothesized that CGP would mitigate the effect of hypoxia, cytokines (IK and/or α-gliadin peptide 31-55 (αG)) the gliadin peptide fragment of celiac disease) to increase transepithelial permeability. All experiments used Caco-2 monolayers with transwell experimental resistance (TEER) of 600-700 Ω·cm². All agents were added to the lateral side of the cell monolayer. In normoxic cells, TEER increased at 0.69 ± 0.11% /hour and mannitol flux was 28 ± 4 dpm/hour. In hypoxic cells, TEER decreased 19.4 ± 1.31 % /hour and mannitol flux increased to 162 ± 27 dpm/hour. 10 µM CGP reduced hypoxia induced TEER loss to 10.5 ± 1.86 % /hour and mannitol flux to 353 ± 45 dpm/hour (p=0.001 compared to normoxia or hypoxia alone). CK stimulated TEER loss was 2.1 ± 0.31% /hour and mannitol flux was 54 ± 1 dpm/hour. 10 µM CGP reduced TEER loss to 1.4 ± 0.28% /hour, and mannitol flux to 13 ± 1 dpm /hour (p=0.001 compared to control or hypoxia alone). In separate experiments, α-GP + CK increased mannitol flux from 21.3 to 39 ± 3 dpm /hour; α-GP + CK + CGP (1 mM) group, mannitol flux was 32 ± 1 dpm /hour (p=0.05 vs α-GP + CK). In these same experiments, IFNγ induced TEER loss was 2.72 ± 0.45 % /hour in the peptide + CK group vs. 1.20 ± 0.32% /hour in the α-GP + CK + CGP protein (p=0.05 vs α-GP + CK). E-cadherin protein was 75.8% ± 6.6% of control in the α-GP + CK group, while in the α-GP + CK + CGP treated cells, E-cadherin was 101 ± 2.9% of control (p<0.02 vs α-GP + CK). In summary, CGP showed a significant time and concentration dependent effect to attenuate increased gut permeability caused by hypoxia, αG, or CK that an effect was observed even at 1µM. These factors may make it worthwhile to pursue calcium glycerophosphorylcholine as an adjunct to other therapies to prevent loss of gut epithelial integrity in hypoxia or celiac disease.

Introduction

Intestinal/secretion (IR) injury is a broad area of medical significance, and includes gut ischemia consequent to such conditions as endurance sports activity and congestive heart failure. The same impaired system activation increases synthesis of an array of cytokines, including TNFα, IL-1β and INFγ. Furthermore, there is evidence of mitigating the effect of hypoperfusion on gut permeability have focused on restoring gut vascular function. Gut epithelial integrity is significantly decreased, at least in part, on sphenogaine-1-phosphate (1P1) generation. As alkaline phosphoscatalyzes the conversion of SIP to sphenogaine and inorganic phosphate, herein we test the hypothesis that by inhibiting intestinal alkaline phosphatase, CGP might raise the SIP concentrations, thus helping to preserve intestinal integrity during ischemic insult.

Materials and Methods—Continued

All experiments were conducted on Caco-2 cells, a line of cells that express the characteristics of small intestinal enterocytes. Cells were grown on a transwell inserts in minimal essential medium (MEM) supplemented with 20% fetal calf serum. Cells were seeded at a density of 3.9 X 10⁴/cm². The tightness of the tight junctions in a transporting epithelium was evaluated by measuring the transepithelial electrical resistance (TEER) which was in the range of 600-700 Ω·cm².

Results—Continued

Sphinogaine-1-Phosphate: SIP concentrations were measured in the protein lysate of Caco-2 cells using a commercially available ELISA kit.

Results—Continued

The table represents the effect of Calcium Glycerophosphorylcholine (CGP) on α-gliadin peptide 31-55 induced Mannitol Flux. αp < 0.05 vs control. The graph represents the effect of 10µM CaGlycerophosphorylcholine on α-gliadin peptide fragment 31-55 induced Mannitol Flux.

Results—Continued

The effect of Calcium Glycerophosphorylcholine (CGP) on Sphinogaine 1 Phosphate (SIP) concentrations in the presence of cytokine.

Summary/Conclusions

• Calcium Glycerophosphorylcholine (CGP) is shown to have only a small effect on transepithelial permeability in normal conditions.

• In contrast, CGP has a significant time and concentration dependent effect to attenuate increased gut permeability caused by hypoxia, cytokine stimulation, and α-gliadin 31-55.

• CGP is also found to increase Sphinogaine 1 Phosphate (SIP) in cytokine-stimulated cells suggesting that the effect of CGP on mannitol flux may be linked to the increased SIP levels. However, the present data is insufficient to permit any firm conclusions.

• Additional research on its mechanism of action is required to further strengthen our hypothesis. So far there have not been any data reflecting this aspect of this action of calcium glycerophosphorylcholine.

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