Title of Project:

Tight junctional integrity in models of celiac disease and gut ischemia.

Principal Investigator/Program Director:
Margaret T. Weis, Ph.D.
Associate Professor
Department of Biomedical Sciences
School of Pharmacy
Texas Tech University Health Sciences Center
806-356-4650 (voice)
806-356-4643 (fax)

PI Signature

Administrative Official signing for TTUHSC:
Jason Fryer
Office of Sponsored Projects
Texas Tech University Health Sciences Center
3601 4th Street, Mail Stop 6271
Lubbock, Texas 79430
(806) 743-2985 (voice)
806-743-2976 (fax)
jason.fryer@ttuhsc.edu

Administrative Official Signature

Project Period:
4/01/15 to 6/30/15

Indirect Costs:
Indirect costs have been computed at 26% of direct costs

Total Costs:
Direct and indirect costs for consulting services and any supplies will be billed to AkPharma at the completion of this project.

Progress Reports:
At completion of the project, a progress report shall be prepared by the PI, and submitted to AkPharma Inc.

Other:
All intellectual property arising from this study is and shall remain the property of AkPharma Inc.

This study has been assigned AkPharma's Purchase Order No. 03122015. All invoices to be mailed to: PO Box 111, Pleasantville, NJ 08232

Purpose: To complete a series of follow-up experiments measuring the effect of calcium glycerophosphate (CGP) on transepithelial permeability of Caco-2 cells.

Rationale: Our previous study showed that CGP significantly reduced transepithelial permeability during hypoxia, measured by both transepithelial electrical resistance (TEER) and mannitol flux in a concentration dependent manner, with and EC50 in the range of 10 μM. The glycerophosphate used in these studies was a mixture of 95% α-glycerophosphate and 5% β-glycerophosphate. While there are many literature references to glycerophosphate as a phosphatase inhibitor, virtually all of these reports used the β -isomer. If the β-isomer is indeed more active than the α -isomer, then the potency of glycerophosphate to decrease transepithelial permeability has been underestimated by our earlier experiments.

Methods: Caco-2 cells were grown in transwells as described earlier (Datta and Weis). Transepithelial resistance was measured daily until a stable value, in the range of 600 – 700 $m\Omega/cm^2$, was attained.
Both TEER and mannitol flux were measured, in the presence and absence of β – glycerophosphate, in a hypoxia chamber, adjusted to 1%O_2, 5% CO_2, and the balance N_2.

Results: As anticipated, hypoxia decreased TEER and increased mannitol flux. The rates of TEER loss and mannitol flux over the first 4 hours of hypoxia are shown in Table 1. Since control mannitol flux differed significantly between the two experimental series, the data are also expressed as a percent of control. Both α-GP and β-GP reduced the rate of TEER loss and mannitol flux, indicating that both isoforms are effective in maintaining epithelial integrity during hypoxia.

An attempt was made to calculate IC50s for both TEER and mannitol (Table 2). Although there appear to be considerable differences between the two isoforms, these values are deceptive. In fact, there is considerable overlap between the 95% confidence limits for the IC50s, indicating that no reliable conclusions can be made about the relative potencies of the two isoforms.

Conclusions: Both α-GP and β-GP have efficacy to limit hypoxia induced loss of epithelial integrity in the Caco-2 model of intestinal permeability. While no reliable conclusions can be made regarding the IC50 of either isoform, examination of the data in Table 1 will show that β-GP is at least as potent as α-GP. Furthermore, α-GP was used as the calcium salt while β-GP was used as the disodium salt, showing the effect of glycerophosphate is independent of the accompanying cation.

Table 1. The effect of hypoxia on the rate of Trans Epithelial Electrical Resistance (TEER) loss and Mannitol Flux during the first four hours of hypoxia.

Treatment (Hypoxia)	**Rate of TEER Loss % per hour**		**Rate of Mannitol Flux dpm[a] per hour (% of control)**	
	α-GP	β-GP	α-GP	β-GP
Control	22 ± 1.66	18.4 ± 1.73	240 ± 28 (100%)	53 ± 7 (100%)
1 mM CGP	10.6 ± 1.49*	13.0 ±1.79*	69 ± 16* (28.9%)	15 ± 2 (28.9%)*
100 µM CGP	14.8 ± 1.74*	12.8 ± 0.94*	140 ± 29* (58.3%)	23 ± 3 (43.6%)*
10 µM CGP	17.3 ± 2.73*	14.1 ± 1.01*	123 ± 25* (51.2%)	23 ±2 (42.1%)*
1 µM CGP	14.5 ± 2.01*	18 ± 1.45	164 ± 34* (68.3%)	30 ±3 (56.0%)*

Glycerophosphate (GP) treatment reduced both parameters in a dose-dependent fashion. Each value represents the results of three independent experiments. dpm[a] represents disintegrations per unit time.
*= $p < 0.01$ compared to hypoxia alone.

Table 2: The IC50 was estimated for each isoform for the rate of TEER loss and mannitol flux over the first 4 hours of hypoxia.

IC50	α-GP	β-GP
TEER	1.21 mM	1.85 µM
Mannitol Flux	2.8 µM	230 µM

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