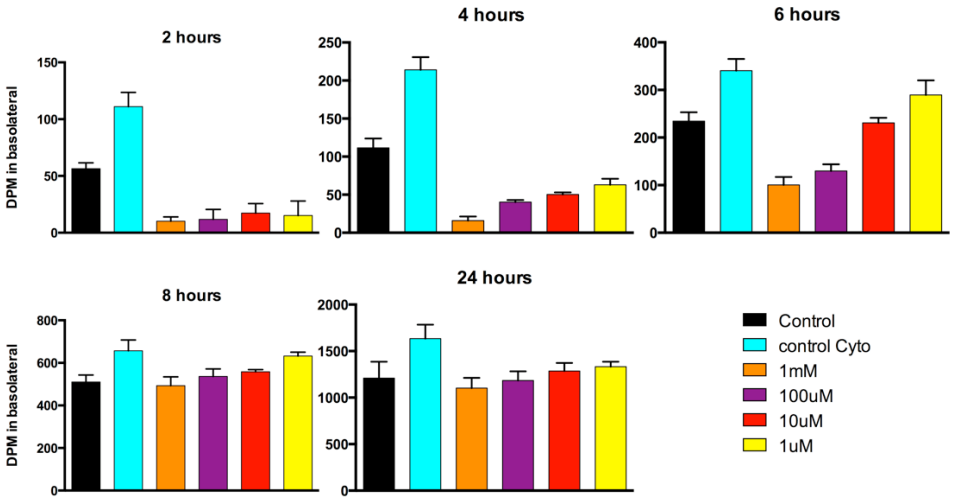

The effect of CGP on cytokine-stimulated transepithelial mannitol flux in Caco-2 cells. Cytokine treatment significantly ($p < 0.05$) stimulated transepithelial mannitol flux, an effect that was significantly ($p < 0.05$) attenuated by CGP in a concentration dependent fashion. Each treatment represents the mean of three independent experiments. Data were analyzed by 2-way ANOVA, followed by Tukey's test for multiple comparisons.

Rationale:

Cytokines are a category of small proteins that are important in cell-cell signaling. Three of the cytokines, tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-γ), and interleukin beta (IL-β) are involved in cell signaling during inflammation. Hence, treating cells with a combination of TNF-α, IFN-γ, and IL-β mimics the early phases of the inflammatory response. Our working hypothesis for these studies is that cytokine stimulation will increase transepithelial permeability in Caco-2 cells, and that the effect will be reduced by concurrent treatment with CGP.

Methods:

Cells were grown to confluence on transwell inserts, then challenged with a mixture of TNF-α, IFN-γ, and IL-β (cytomix), in the presence or absence of increasing concentrations of CGP. At the appropriate time points, apical to basolateral mannitol flux was measured. Transepithelial electrical resistance (TEER) was measured in a separate set of experiments.

Results:

As shown in Figure 6, cytomix alone increased transepithelial manitol permeability in a time-dependent fashion, and CGP delayed the permeability increase in a concentration-dependent manner.

Treatment	Rate of TEER loss	Rate of mannitol flux
	% per hour	dpm per hour
Control	0.064 ± 0.38*	28 ± 3*
Cytokine	2.1 ± 0.31	54 ± 1
Cytokine+ 1 mmol/L CGP	0.76 ± 0.24*	4 ± 1*
Cytokine+ 100 µmol/L CGP	0.87 ± 0.32*	10 ± 1*
Cytokine+ 10 µmol/L CGP	1.4 ± 0.28*	13 ± 1*
Cytokine +1 µmol/L CGP	2.4 ± 0.34	16 ± 2*

Rate of TEER loss and mannitol flux during the first four hours (mannitol flux) of 10 hours (TEER) following cytokine stimulation. CGP treatment reduced both parameters in a dose-dependent fashion. *$p < 0.001$ *vs* cytokine alone. Each value represents the results of three independent experiments. Data is expressed as mean ± SE