Glyceollin Transport, Metabolism and Effects on P-glycoprotein Function in Caco-2 Cells
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Purpose
The overarching goal of the funded project is to understand the disposition and effects in the intestine-proper of glyceollin, a soy-derived phytoalexin possessing both anti-tumoral and anti-diabetic effects in rodents. The specific aim of the presented work was to characterize glyceollin transport and its effects on intestinal P-glycoprotein (Pgp) function in Caco-2 cells.

Methods
Caco-2 cell culture was based on standard techniques. Permeability was conducted across cells grown on collagen coated 0.4 micron PTFE supports. Glyceollin was measured using LC/MS-MS, and Pgp function assessed using rhodamine 123 (R123) as a Pgp substrate.

Results
From a 25 µM donor concentration, glyceollin permeability coefficient in the AB direction was 2.1 ± 0.15 x 10^-4 cm/s, which puts the molecule in the category of drugs that are completely absorbed in humans provided they are sufficiently soluble and not metabolized in the intestine. BA permeability was similar: 1.6 ± 0.10 x 10^-4 cm/s, suggesting a passive diffusion dominated mechanism of transport. Analysis of Caco-2 cell lysates following 3 hours of exposure to 25 µM glyceollin revealed oxidative and direct conjugative (via sulfation and glucuronidation) metabolism, thus suggesting an expectation of less than complete absorption following oral administration. Unlike the Pgp inhibitor verapamil, glyceollin did not inhibit Pgp function over the concentration range 1 to 300 µM. Based on qt-PCR, immunoblotting and measurement of Pgp activity with R123, exposure to 100 µM glyceollin for 24 hours did not alter Pgp expression and activity in Caco-2 cells. In contrast, the same concentration of rifampin increased ABCB1 derived mRNA expression and the BA permeability of R123.

Conclusion
Results indicate that glyceollin is rapidly transported, that its oral bioavailability may be limited by metabolism, including in the intestine, and that this phytoalexin does not alter intestinal Pgp function.