Using C. Elegans as Model Organism to Study the Mode of Action of a Natural Toxin, Psymberin

Cheng-Yang Wu, Ph. D.
The University of Texas Southwestern Medical Center, 2011
Graduate School of Biomedical Sciences
Supervising Professor: Michael G. Roth, Ph. D.

Date Available: 12/12/2013
Summer 2011
Biological Chemistry
Bibliography pp. 94-103

Keywords: psymberin; pederin family; forward genetic screen; C. elegans; mode of action; translation inhibitor; SAR

Psymberin is an extremely potent cytotoxin isolated from the marine sponges Psammocinia and Ircinia ramose. Several cancer cell lines are sensitive to psymberin, including breast, melanoma and colon cancer cell lines. Psymberin is the only member of the pederin natural product family that contains a dihydroisocoumarin side chain. The cytotoxicities of psymberin in various human tumor cell lines are between sub-nanomolar to nanomolar IC50. Like pederin, the first member of this natural product family, psymberin and mycalamide A inhibit translation in vivo and in vitro. This inhibition by psymberin is 40 to 100 fold more potent than cycloheximide, which inhibits >90% translation at 100 micromolar in vivo. In a SAR study, both the cytotoxicity of psymberin and psymberin-induced translation inhibition were attenuated by substituting the psymberin side chain with the pederin side chain. However, the attenuation of cytotoxicity was relatively greater than of translation. The stereo configuration and both side chains of psymberin are required for both inhibition of translation and cytotoxicity. The result of the SAR study suggests that additional bioactivity is contained in psymberin. Psymberin is at best a poor substrate for small molecule pumps in the cell.

Two separate forward genetics screens in C. elegans isolated seven independent psymberin-resistant mutants. In each the mutation was a C361T point mutation in the rpl-41 gene that changes Pro65 to Leu65 in the protein coding sequence. The psymberin-resistant mutant strain DA2312 is resistant to psymberin only. This mutation did not appear to cause weaker binding of psymberin to the ribosome, but must allow translation to continue with the toxin bound.

There are additional modes of actions of psymberin compared to mycalamide A. The endogenous protein level of LC3, an autophagy marker, is decreased faster with psymberin treatment than mycalamide A. In HT-29 cells, psymberin is capable of synergizing TNFa-induced necrotic cell death more efficiently than mycalamide A. The results from SAR study and from study of the psymberin-specific mutation in C. elegans suggest that psymberin may induce fast cell death through multiple pathways, including translation inhibition, apoptosis and necrosis. The structural uniqueness of psymberin has functional consequences suggesting that the mode of action of psymberin on the ribosome is different from other members of the pederin family.