Enhancement of FasL-induced cytotoxicity by activation of tumor-specific cytotoxic T lymphocytes with novel ceramide analogs

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Abstract

Ceramide is found to induce Fas signaling pathway of human colon carcinoma cells by tumor specific cytotoxic T-lymphocytes (CTLs) to lyse tumor cells. Cancer cells are often insensitive to Fas ligand (FasL) and evade cell death. We know that ceramide metabolite, sphingosine 1-phosphate (S1P) acts to induce cell survival. We hypothesized that L-threonine based ceramide analogs would inhibit an enzyme, ceramidase at greater affinity compared to using L-serine which is most common starting material. We have synthesized 20 ceramide analogs suggested by rational design. At the molecular level, these novel ceramide analogs were observed to increase Fas-induced activation of caspase 8, which is an essential initiator caspase of the Fas receptor death-inducing signaling complex (DISC).

Introduction

Fas signaling pathway is one of two effector mechanism used by CTLs to lyse tumor cells. Ligation onto its receptor, FasR, on sensitized cells induce programmed cell death, or apoptosis (O'Connell 1996). However, Fas signaling is often down regulated in human colon carcinoma. Advanced colon cancer cell will decrease expression of FasR and some which express FasR, are insensitive to FasL. We know that ceramide can induce cell death and its metabolite, S1P exert opposing function (Morales et al 2007). Many L-serine based ceramide analogs are synthesized to inhibit ceramidase to stop ceramide metabolism. However, we thought that L-thereonine is a better substrate for ceramide analog based on the medicinal chemistry phenomenon of "magic methyl" effect which affects affinity of compound to its substrate.

Objective

- To synthesize ceramide analogs or A-CDase (*ASAH2*) inhibitors using L-threonine as starting material
- To test if in these novel compounds would be effective in modulating sensitivity of Fas-induced apoptosis in colon carcinoma cells by CTLs

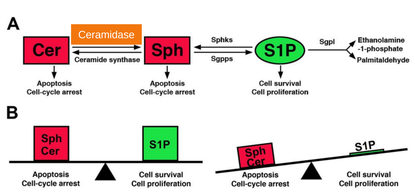


Figure 1: (A) Shows metabolic pathway of ceramide. Ceramide and sphingolipid will halt cell-cycle and induce apoptosis, while S1P will induce cell growth. (B) Displays analogy in which apoptosis will be induced if we successfully inhibit ceramidase or sphingolipid kinase by ceramide analogs.

Synthesis of Novel Inhibitors

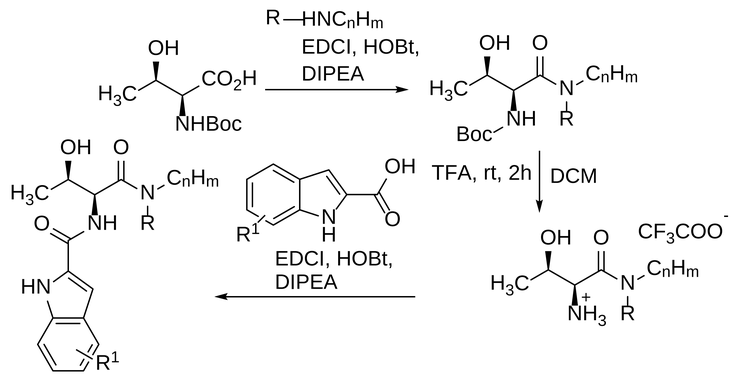
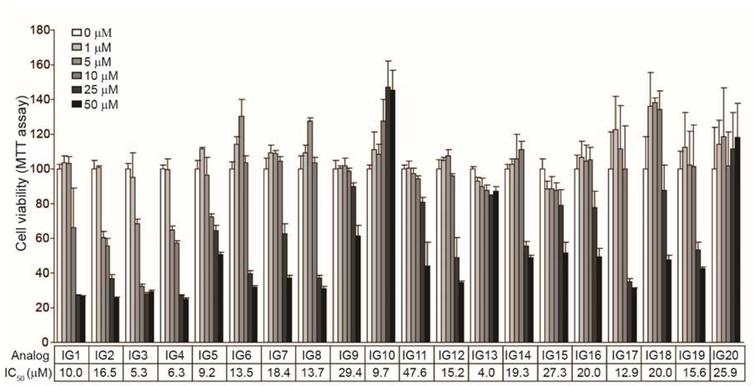


Figure 2: Scheme for synthesizing ceramide analogs (Niiro et al 2004, Gududuru et al 2004, Chang et al 2002, Joshi et al 2011, Kavanagh et al 2008)

1) Library of substituted indolyl derivatives of ceramide analogs were synthesized using N-Boc protected L-threonine
2) After removing the N-Boc group, ceramide analogs were then conjugated with various primary and secondary amines using EDCI as coupling reagents

Results from AU Cancer Center



Analog	IG1	IG2	IG3	IG4	IG5	IG6	IG7	IG8	IG9	IG10	IG11	IG12	IG13	IG14	IG15	IG16	IG17	IG18	IG19	IG20
IC_{50} (μM)	10.0	16.5	5.3	6.3	9.2	13.5	18.4	13.7	29.4	9.7	47.6	15.2	4.0	19.3	27.3	20.0	12.9	20.0	15.6	25.9

Figure 4: Cytotoxicity of all 20 novel ceramide analogs..

- Some novel ceramide analogs exhibited potent activity in human colon carcinoma
- These novel compounds were effective in activating tumor specific CTLs to induce cell apoptosis
- Activated CTLs induced Fas signaling pathway, which is seen by activation of caspase 8

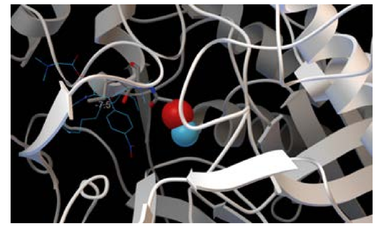


Figure 3. Docking of the novel ceramide analogs to the homology model of human N-CDase (ASAH2, isoform a).

Discussion

Novel ceramide analogs were successful in inhibiting ceramide metabolic pathway to reduce the concentration of anti-apoptotic protein, S1P. Furthermore, tumor-specific CTLs were activated to enhance cancer cell apoptosis. Cleavage (activation) of caspase 8 were shown in western blotting done by AU Cancer Center, which indicates these compounds were successful in modulating sensitivity of Fas function in colon cancer cells by CTLs.

Conclusion

Therefore, we have successfully synthesized novel ceramide analogs which exhibited potent activity in sensitizing FasL-induced apoptosis of human colon carcinoma cells by CTLs. Activation of caspase 8 in human colon carcinoma by FasL were conformed through western blotting. These L-threonine based novel ceramide analogs has potential to become adjunct agents in CTL-based colon cancer immunotherapy.

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Reference

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