MODIFIED α-GALACTOSYL CERAMIDE ANALOGS FOR STIMULATING iNKT CELLS: A PHARMACOLOGICAL CHARACTERIZATION

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CD1 proteins are a family of highly conserved antigen-presenting molecules (APM) that bind and present a variety of lipids and glycolipids to T cells. CD1d, a member of this protein family, functions as APM for the T cell receptors (TCR) of invariant natural killer T (iNKT) cells. iNKT cells are a unique subset of T lymphocytes sharing characteristics with NK cells and exerting a wide range of functions through the production of large amounts of cytokines (i.e., IL-4, INF-γ, IL-2) upon TCR triggering. A glycolipid antigen isolated from marine sponge alpha-galactosyl ceramide (alpha-GalCer) and its synthetic homologue (KRN7000) are recognized by iNKT in the context of CD1d, and structure-activity relationship (SAR) studies have demonstrated the importance for their activity of both the lipid and sugar structures.
The aim of this study was to synthesize and characterize new alpha-GalCer-related compounds. We have synthesized some glycolipid analogs with different lipid moiety structures: alpha-GalCer (**1a**), bearing a C-22 fatty acid, and two structurally related compounds (**1b** and **1c**), with a shorter branched and an aromatic chain, respectively. The biological activities of these compounds were determined by an hybridoma-based antigen presentation assay. CD1d-expressing antigen presenting cells (APC), CD1d-transfected promyelocytic cells (THP-1), were pre-incubated (2 h) with increasing concentrations (10^{-7} – 10^{-5} g/ml) of the different compounds before the addition of alpha-GalCer-specific iNKT cell hybridomas (FF13). After 48 h, IL-2 production was measured by standard ELISA methods (BD PharMingen, Canada).
Results show that all compounds tested were effective in inducing cytokine (IL-2) release in a concentration-dependent manner. Among compounds **1a** possesses a greater efficacy and potency than the others. The EC50 calculated value for this compound was 5.2 x 10^{-7} g/ml. The maximum effects calculated were: 302.6 ± 25.7 pg/ml for **1a**, 238.4 ± 0.8 pg/ml for **1b** and 236.0 ± 3.9 pg/ml for **1c**.
From these results, it seems reasonable to suppose that the presence of shorter and sterically demanding alkyl chains confers reduced biological activities to compounds. Experiments are in progress in our laboratories for studying if these new analogues can induce polarization of iNKT cells for selective Th1 or Th2 cytokine production.