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Mixed galactolipid anomers accentuate apoptosis of multiple myeloma cells by inducing DNA damage

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ABSTRACT

This study describes an interesting observation that the mixture of anomeric galactolipids has synergistic effects on the growth inhibition of human multiple myeloma (MM) cells. We determine that the equivalent mixture of a pair of α- and β-galactolipids with a 14-carbon lipid chain can cause stronger poly ADP-ribose polymerase cleavage and DNA damage, producing more late apoptotic MM cells, than either anomer alone.

1. Introduction

Multiple myeloma (MM) is a neoplastic disorder that features the accumulation of abnormal plasma cells in the bone marrow, impeding the manufacture of normal blood cells. This may subsequently result in kidney damage, hypercalcemia, and bone lesions.[1,2] Clinically, the treatment of MM relies on the use of steroids, immunomodulatory drugs including thalidomide, lenalidomide, and pomalidomide, and proteasome inhibitors including bortezomib and carfilzomib.[3–5] Recently, development of histone deacetylase inhibitors[6] and alkylators[7] has provided additional therapeutic regimes for the disease. Unfortunately, MM still represents an incurable disease with the average survival of patients being six months. This implies that identification of new drug leads for MM is in urgent need.

Glycolipids are universal cell-surface components that transmit signals between cells. Because of their unique amphiphilic property, glycolipids have been widely used as non-ionic green surfactants[8] in cosmetics and as co-dopants in the fabrication of glycoliposomes for targeted drug delivery.[9] They also possess promising anticancer activities. For example, galactosylceramides derived from marine sponge can activate natural killer T cells, and have been developed as immunostimulant agents.[10] Glycoglycerolipids isolated from spinach have been shown to inhibit tumor angiogenesis and suppress DNA polymerase activity.[11] We have also described the preparation of triazolyl glycolipids with cytotoxicity toward some cancer cells.[12–14]

With continuing interest in the discovery of potential anticancer drug leads,[15–18] we report here an interesting observation that the anomeric mixture of some galactolipids shows synergistic effect on the growth inhibition of human MM cells.

2. Results and discussion

Through a series of previous studies we have determined that the length of lipid chain may impact the cytotoxicity of glycolipids;

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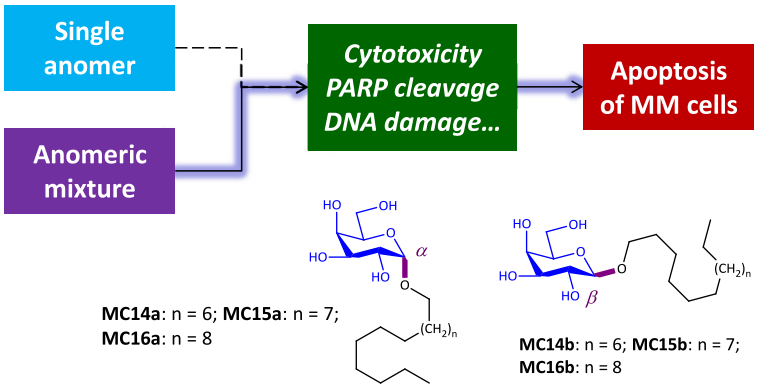

Figure 1. Schematic depiction of the anomeric synergism, and structure of the galactolipids used in this study.

those with relatively long chains (>12C) were more toxic toward cancer cells.[12,13] As a consequence, α- and β-galactolipids synthesized previously by a $BF_3 \cdot Et_2O$ promoted glycosylation reaction between lipid alcohols and peracetyl 1-*O*-D-galactoside, followed by a deacylation reaction[19,20] with relatively long carbon-chain lengths (14C, 15C, and 16C) were used (Fig. 1). The purity and structural homogeneity have been demonstrated before biological tests (see Supporting information). We have determined that the anomeric mixtures of these galactolipids showed evident synergistic effect on the growth inhibition of a human lung cancer cell line (A549).[19] In the present study we further examined the toxicity of both single and mixed anomers toward MM cells.

MM cell lines RPMI 8226 and NCI-H929 were employed for the MTS [(3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium)] cell viability assay.[21] α-(**MC14a**, **MC15a,** and **MC16a**) and β-galactolipids (**MC14b**, **MC15b,** and **MC16b**), and their equivalent mixtures (e.g., *if the concentration of single anomers is 50 μg/mL, the mixture contains 25 μg/mL each*) were tested for their ability to inhibit the growth of the MM cell lines (Fig. 1). The half maximal inhibitory concentration (IC_{50}) values of the compounds are shown in Table 1.

We first determined that all the galactolipids with different chain lengths showed toxicity toward the MM cell lines used. The α-galactolipids were more toxic than the β-counterparts. For instance, while **MC16b** did not have cytotoxic effect on NCI-H929, **MC16a** showed moderate toxicity toward the cell line. This suggests that anomerism might impact the bioactivity of glycolipids, which is in accordance with our previous observations.[19]

Interestingly, we then determined that the mixed anomers showed improved inhibitive effects on the growth of the cell lines used (Table 1). As the combination index (CI) is a reliable tool for measuring the combination therapeutic effect of two individual bioactive compounds (CI <0.9 and CI >1.1 suggest synergism and antagonism, respectively),[22–24] we calculated the CI values of all groups of mixed anomers. As shown in Table 2, all the anomeric mixtures showed evident synergistic effects (CI = 0.15–0.41) with a low mixing concentration (17 μg/mL α- mixed with 17 μg/mL β-galactolipid) for RPMI 8826 and NCI-H929. With a relatively high mixing concentration (50 μg/mL α- mixed with 50 μg/mL β-galactolipid), the synergism became weaker. This suggests that the lower concentration favors the synergism of the two anomeric compounds, and that increase of the concentration might lead to an antagonistic effect.

Poly ADP-ribose polymerase (PARP) is a substrate of caspase and is involved in DNA repair. PARP cleavage produces PAR, which can be viewed as a sign of apoptosis.[25] Therefore, western blotting was performed to analyze the cleavage activity of PARP induced by the single anomers or anomeric mixtures with RPMI 8226 cells (Fig. 2). We determined that the anomeric mixtures caused stronger PARP cleavage than either anomer alone with two different mixing concentrations (8.5 + 8.5 or 17 + 17 μg/mL). The mixture of 14C-galactolipids (**MC14a** + **MC14b**) showed the strongest synergistic effect, which was selected for further assays.

Former literature reports have shown that glycolipid fractions isolated from Spinach could inhibit the activity of replicative DNA polymerases (pols) such as α, δ, and ε, leading to severe DNA damage.[26] Histone H2AX is a tumor suppressor that facilitates the preservation of genome integrity by being massively and quickly phosphorylated at the sites of nascent DNA double-strand breaks (DSBs) in chromatin. To investigate whether the mixed anomers could induce DNA damage, the phosphorylation of H2AX at Ser139

Table 1
Cytotoxicity of single and mixed galactolipids toward multiple myeloma cells determined by MTS

Compound	IC_{50} (μg/mL)[a]	
	RPMI 8226	H929
MC14a	15.7 ± 1.0	12.9 ± 1.1
MC14b	20.9 ± 2.2	15.2 ± 1.9
MC14a + **MC14b**[b]	15.6 ± 1.2	13.7 ± 1.3
MC15a	17.8 ± 0.6	10.8 ± 1.9
MC15b	16.7 ± 1.4	18.2 ± 3.0
MC15a + **MC15b**[b]	17.6 ± 0.2	14.5 ± 1.5
MC16a	11.8 ± 1.3	10.0 ± 0.3
MC16b	39.4 ± 1.2	>100
MC16a + **MC16b**[b]	13.3 ± 1.6	14.4 ± 1.2

[a] Values are mean of three experiments.
[b] The α- and β-galactolipids were mixed equivalently.

Table 2
Combination index (CI) of mixed galactolipids toward multiple myeloma cells

Concentration (μg/mL)		CI value[a]	
		RPMI 8226	H929
MC14a	**MC14b**		
17	17	0.41	0.29
50	50	1.24	0.84
MC15a	**MC15b**		
17	17	0.19	0.15
50	50	0.55	0.39
MC16a	**MC16b**		
17	17	0.21	0.19
50	50	0.60	0.58

[a] CI values indicate quantitatively the degree of a drug interaction. CI <0.9, CI = 0.9–1.1 and CI >1.1 indicate synergism, additive effect and antagonism, respectively.