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Short Communication

Inhibition of activated murine T-lymphocytes by synthetic glycoglycerolipid analogues

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Abstract

Glycoglycerolipid analogues, characterized by a glycerol aglicone β-linked in position 2 to a glucose or galactose residue, and by a lipophilic C_6 acyl chain on the glycerol unit, significantly inhibit proliferation of activated T cells. The inhibitory activity displayed by such synthetic compounds is comparable to the immunosuppressive properties shown by the natural glycolipids simplexides, to which they are structurally related. Vice versa, when the acyl chain is located on the sugar unit, no immunomodulating activity is observed, suggesting a strict relationship between the activity and the location of the acyl chain.

Keywords: Glycoglycerolipids; Immunosuppressive agents; T cells; Concanavalin A

1. Introduction

Glycolipids isolated from marine organisms have recently shown interesting biological activity; in particular, they are active on the immune system of mammals, exhibiting immunostimulating or immunosuppressive properties according to their structures and their anomeric configuration [1].

However, the compounds are extracted from natural sources in very small amounts, often as mixtures of homologues, and their complex structures hardly allow their industrial production, requiring difficult, multistep syntheses.

Among the bioactive marine glycolipids an unique structure is shown by simplexides [2], a new structural kind of immunosuppressive glycolipids isolated from the sponge *Plakortis simplex*, in which a long-chain secondary alcohol (or better a mixture of homologous long-chain alcohols) is β-linked to a disaccharidic α-glucosyl-galactosyl unit.

Very recently, we synthesized [3–6] analogues of natural glycoglycerolipids, which were tested in the context of a research project aimed at evaluating their anti-tumour promoting potential [4,6,7]. Many structures were prepared and assayed and, among them, those exhibiting an acyl chain located at position 1 of the 2-*O*-β-D-gluco- or galactopyranosylglycerol skeleton, with a lipophilic acyl tail and a hydrophilic head, resemble simplexides structure and might be considered simple models of such potent immunosuppressors, in particular, 1-*O*-hexanoyl-2-*O*-β-D-glucopyranosyl-*sn*-glycerol (**1**) and 1-*O*-hexanoyl-2-*O*-β-D-galactopyranosyl-*sn*-glycerol (**2**), which have in the position 1 of glycerol a medium length fatty acid acyl chain [4,5], and that were among the compounds which exhibited the highest anti-tumour-promoting activities [7].

With the aim of improving our comprehension of the structural requirements for immunomodulating activity of glycolipids, we evaluated the immunomodulating properties of **1**–**2** and we report here the effect of compounds **1**–**2** on activated murine T-lymphocytes.

Moreover, with the aim to ascertain the influence that the acyl chain location may exert on the immunomodulating activity of these compounds, we

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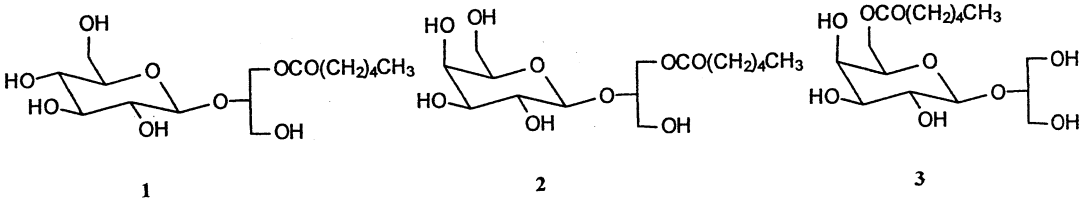
tested also 2-*O*-(6-*O*-hexanoyl-β-D-galactopyranosyl)-*sn*-glycerol (**3**), characterized by the hexanoyl chain at position 6 of the sugar moiety.



1 2 3

2. Experimental procedures

2.1. Test products

1-*O*-Hexanoyl-2-*O*-β-D-glucopyranosyl-*sn*-glycerol (**1**) ($M_w = 352.4$), 1-*O*-hexanoyl-2-*O*-β-D-galactopyranosyl-*sn*-glycerol (**2**) ($M_w = 352.4$), and 2-*O*-(6-*O*-hexanoyl-β-D-galactopyranosyl)-*sn*-glycerol (**3**) ($M_w = 352.4$) were prepared by *Pseudomonas cepacia* lipase (lipase PS, Amano Pharmaceutical Co.) catalysed transesterification of 2-*O*-β-D-glucopyranosyl-*sn*-glycerol [4] or 2-*O*-β-D-galactopyranosyl-*sn*-glycerol [5] respectively, as previously described. Their physicochemical properties were consistent with the literature data [4,5].

2.2. Mice

Male Swiss mice, 6–8 weeks old, obtained from Nossan (Italy), were housed in temperature-controlled rooms (22 ± 1 °C) and received food and water ad libitum.

2.3. T-cell proliferation assay

Single lymph node cell suspension was obtained from popliteus lymph nodes removed from mice killed with CO_2. Cells were suspended (2.5×10^6 cells/ml) in the RPMI-1640 culture medium containing 10% foetal calf serum, L-glutamine (2 mM), penicillin (100 U/ml), streptomycin (100 μg/ml) and 2-mercaptoethanol (50 μM). The cell suspension was dispensed at 100 μl/well in 96-well flat-bottomed plates (Nunk, Roskilde, Denmark), stimulated with Concanavalin A (Con A; 0.5 μg/ml) in the presence or in the absence of the test compounds and incubated for 24 h at 37 °C in an atmosphere of 5% CO_2 and 95% O_2. Cultures, in triplicates, were pulsed with 1 μCi/well ^{3}H-thymidine (47 Ci/mmol, Amersham Int., Amersham, UK) for the final 6 h of incubation, then harvested and counted in a β-scintillation counter.

2.4. Statistics

Each experiment was repeated three times. All results are expressed as mean ± SD. Statistical analysis of the data was performed using a PHARM/PCS computer program. Means were compared by Student's test for unpaired data.

3. Results

When tested [8] on murine immune-system T-cells stimulated with Con A, 1-*O*-hexanoyl-2-*O*-β-D-glucopyranosyl-*sn*-glycerol (**1**) and 1-*O*-hexanoyl-2-*O*-β-d-galactopyranosyl-*sn*-glycerol (**2**) showed a significant and dose-dependent inhibitory activity on their proliferation. In fact, compound **1** caused a significant ($P < 0.001$) inhibition of proliferation by 64% at a concentration as low as 0.1 μg/ml, which raised to 76% at 10 μg/ml (Fig. 1), whereas compound **2** significantly ($P < 0.05$) inhibited T-cell proliferation by 22% at 0.01 μg/ml and by 77% at 10 μg/ml (Fig. 1).

On the contrary 2-*O*-(6-*O*-hexanoyl-β-D-galactopyranosyl)-*sn*-glycerol (**3**) turned out to be completely ineffective in inhibiting ^{3}H-thymidine incorporation even at 10 μg/ml (Fig. 1).

Also, in order to verify a possible cytotoxicity of test compounds, we assessed the viability of cells by Trypan blue exclusion after 72 h of incubation. We found that 85 ± 3.5% of the cells treated with the highest dose of test compounds were still viable. This proportion was not significantly different from the viability of control cells (87 ± 3.3%). Respiratory metabolism was also examined by using MTT assay [9]. Under this respect there was no significant difference among compounds treated cells and control cells (data not shown).

4. Discussion

Proliferation inhibitions caused by compounds **1** and **2** were similar to those displayed by the natural marine glycolipids simplexides [2], which were proved to be capable of inhibiting proliferation of T-cell without killing them.

Although the above synthetic glycolipids analogues and the natural ones are remarkably different, their

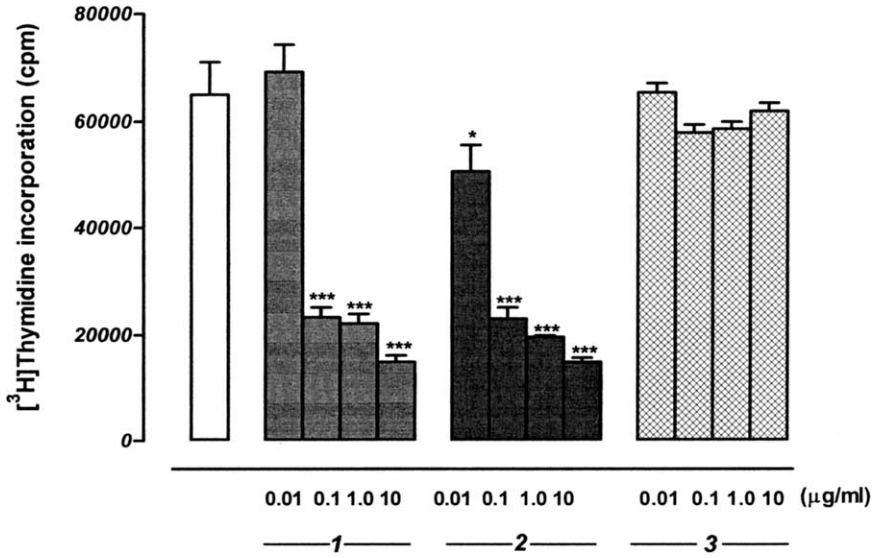


Fig. 1. Effect of compounds **1**, **2** and **3** on T-cell proliferation. Cells were stimulated with Con A at 0.5 μg/ml in the absence (empty column) or presence of test compounds. Data are expressed as means ± SD of T-cell proliferation evaluated as ^{3}H-thymidine incorporation (cpm ± SD, $n = 3$). $^*P < 0.05$; $^{***}P < 0.001$ vs. control cells (Con A stimulated cells in the absence of test compound).

basic structural features, i.e. the hydrophilic head due to one or two carbohydrate residues, the alkyl chains (or chain) located on the aglycone moiety and the β-anomeric configuration, confer to all the compounds potent immunosuppressive properties, as shown by the inhibition of the Con A induced T-cell proliferation in the absence of cytotoxicity.

Also, the location of the lipophilic residue appears strictly related to the immunomodulating properties of such glycolipids. In fact, when compound **3**, in which the acyl chain is located at position 6 of the sugar (and not on the aglycone moiety), was tested on murine immune-system T-cells stimulated with Con A, no immunomodulating activity was observed suggesting that for a significant activity at least one lipophilic chain, even if only six carbon long, should be present on the aglycone.

In conclusion, although the mechanism by which compounds **1** and **2** exert the immunosuppressive activity deserves further investigation, our data indicate that the structural features of these glycolipids analogues permit to maintain the proliferation inhibiting properties associated to the natural glycolipids simplexides.

Acknowledgements

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