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Short communication

Induction of the differentiation of human HL-60 promyelocytic leukemia cell line by succinoyl trehalose lipids

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Abstract

Four analogs of succinoyl trehalose lipid-3 (STL-3) with saturated even-number or odd-number carbon chains, and unsaturated or halogenated fatty acids were examined for their ability to inhibit the growth and induce the differentiation of HL-60 human promyelocytic leukemia cells. The optimal concentration of STL-3 at which such activities were recognized was closed to the critical micelle concentration of STL-3. Analog of STL-3 with even-number or odd-number carbon chain and unsaturated fatty acids strongly inhibited growth and induced the differentiation of HL-60 cells, as evaluated in terms of nitroblue tetrazilium-reducing activity and the appearance of the CD36 antigen. An analog of STL-3 with halogenated fatty acids significantly inhibited proliferation but only induced the differentiation of HL-60 cells. Our results indicate that the effects of STL-3 and its analogs on HL-60 cells depend on the structure of the hydrophobic moiety of STL-3.

Abbreviations: STL, succinoyl trehalose lipid; FBS, fetal bovine serum; PBS, phosphate-buffered saline; CMC, critical micelle concentration; NBT, nitroblue tetrazolium.

Introduction

Many microorganisms produce surface-active compounds that are known collectively as biosurfactants. STL-3 (Figure 1) is a glycolipid biosurfactant that is produced by *Rhodococcus* sp. TB-42 when *n*-alkane is supplied as a source of carbon. Glycolipids produced by microorganisms are not identical to the components of mammalian cell membranes but their backbone structures are, in some case, similar to those of gangliosides, which are prominant components of cell membranes. Both glycolipids and gangliosides are amphiphilic compounds. Certain gangliosides induce the differentiation of leukemia cells when added to the culture medium (Saito et al., 1985; Nojiri et al., 1986, 1988). We reproted recently that STL-3 inhibits the growth and induces the monocytic differentiation of HL-60 cells (Isoda et al., 1997). The hydrophobic moiety of STL-3 is derived from components of the carbon source supplied to cultures of *Rhodococcus* sp. TB-42. In this study, we prepared analogs of STL-3 with different hydrophobic fatty acid moieties, namely, with even-number or odd-number carbon chains, and with unsaturated or halogenated fatty acids, using

Table 1. Relative leveles of fatty acids in analogs of STL-3 and their critical micelle concentrations (CMCs)

	Fatty acid composition (%)	CMC (μM)
Even-chain STL-3	Octanoic acid (27) Decanoic acid (67)	3.6
Odd-chain STL-3	Nonanoic acid (56) Undecanoic acid (39)	3.4
Unsaturated STL-3	Octanoic acid (15) Decanoic acid (51)	3.9
Halogenated STL-3	Chlorooctanoic acid (38) Chlorodecanoic acid (38)	4.6

n-hexadecane, *n*-pentadecane, 1-hexadecene and 1-chlorohexadecane as the carbon source, respectively. We report here the results of a comparative study of effects of the various analog of STL-3 on the growth and differentiation of HL-60 cells.

Materials and methods

Preparation of analog of STL-3

Analogs of STL-3 were extracted with ethyl acetate from the culture broth of *Rhodococcus* sp. TB-42 and purified by preparative thin-layer chromatography (Uchida et al., 1989). The fatty acid composition of these analog is shown in Table 1.

Culture of HL-60 cells and treatment with analogs of ATL-3

HL-60 cells were incubated in RPMI1640 medium (Nissui Pharmaceutical Co., Tokyo, Japan) that contained 10% fetal bovine serum in an atmosphere of 5% CO_2 in air at 37 °C. In some case, HL-60 cells were cultured in serum-free ERDF-ITES medium. ERDF medium was obtained from Kyokuto Pharmaceutical Industries Co. (Tokyo, Japan) and ITES consisted of insulin (5 μg ml^{-1}), iron-free human transferrin (10 μg ml^{-1}), ethanolamine (25 μM) and selenite (25 nM) (RD-1; Kyokuto Pharmaceutical Industries Co.) as described elsewhere (Isoda et al., 1997). Cells were counted in a hemocytometer and the viability of cells was estimated by the trypan blue-exclusion method. Analogs of STL-3 were sonicated in Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline (PBS^{-}) and sterilized by passage through a filter (0.45-μm pores) before addition to culture medium at the indicated concentration.

Determination of the critical micelle concentration (CMC)

The CMC was calculated from a graph of surface tension vs. oncentration as described by Kwan and Rosen (1980). Surface tension was determined with a Whilhelm-type automatic tensiometer (CBVP-A3; Kyowa Kaimenkagaku, Tokyo, Japan) at 25 °C.

Indices of cell differentiation

The reduction of nitroblue tetrazolium (NBT) was monitored by a modified version of the method reported by Takeda et al. (1988). The percentage of cells containing blue-black formazan deposits was caluculated after examination of at least 200 cells under a light microscope. The expression of the surface membrane antigen CD36 was assessed by flow cytometry after labeling with antibodies against CD36 (Serotec Co., Oxford, UK).

Results

Effects of analogs of STL-3 on the viability of HL-60 cells

Cells were seeded at an initial density of 2×10^5 cells per ml and cultured with or without an analog of STL-3. Viable cells were counted two days later. The various analogs had a dose-dependent inhibitory effect on the growth of HL-60 cells (Figure 2). The anti-proliferative effects were dramatically enhanced when the concentration of each analog was greater than its critical micelle concentration (CMC). However, no further obvious increases in inhibitory effects were noted above the respective CMCs. Therefore, the optimal concentration of each analogs for inhibition of growth appeared to correspond to the respective CMC (Table 1).

NBT-reducing activity

The ability to reduce NBT is often used as a marker of the differentiation of neutrophiles, monocytes, macrophages and lymphocytes. Therefore, we examined

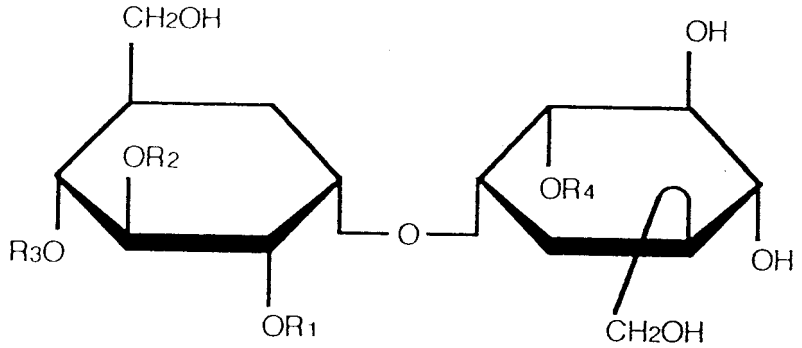

Figure 1. Structure of analogs of succinoyl trehalose lipid-3 (STL-3). R_1 thorough R_4 = 1 × succinic acid, 3 × fatty acid.

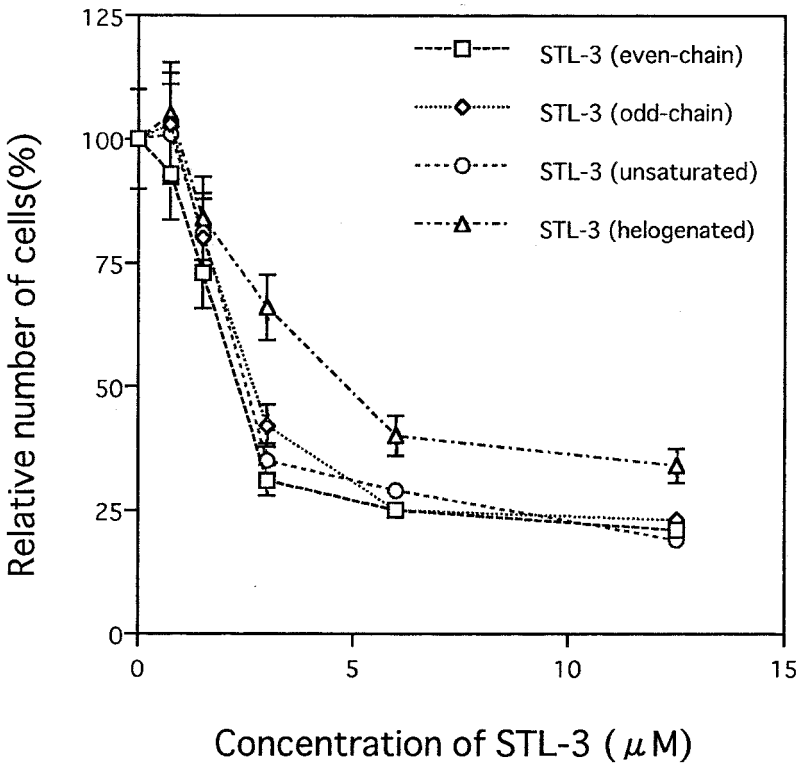

Figure 2. Effects of analogs of STL-3 on growth of HL-60 cells. HL-60 cells were cultured with analogs of STL-3 for two days. Vaiable cells are cultured as described in Materials and Methods. The number of control cells was taken as 100%. All values are means of results of three experiments and the standard deviation for each value is indicated.

the effects of the analogs of STL-3 on the NBT-reducing activity of HL-60 cells (Figure 3). In the absence of analogs of STL-3, only 8% of HL-60 cells was positive for reduction of NBT. By contrast, cells incubated with an analog of STL-3 of 3 μM had significantly increased NBT-reducing activity. In particular, treatment of HL-60 cells with STL-3 that contained saturated fatty acids (even-chain STL-3 and odd-chain STL-3) resulted in a 4.0- to 4.8-fold increase in NBT-reducing activity compared with that of control HL-60 cells (Figure 3). Treatment of HL-60 cells with halogenated STL-3 resulted only a 1.6-fold increase in NBT-reducing activity.

Expression of CD36

CD36 is a membrane glycoprotein that is used as a marker of the differentiation of monocytes and macrophages (Prieto et al., 1994). We examined the expression of CD36 48 h after the start of incubation

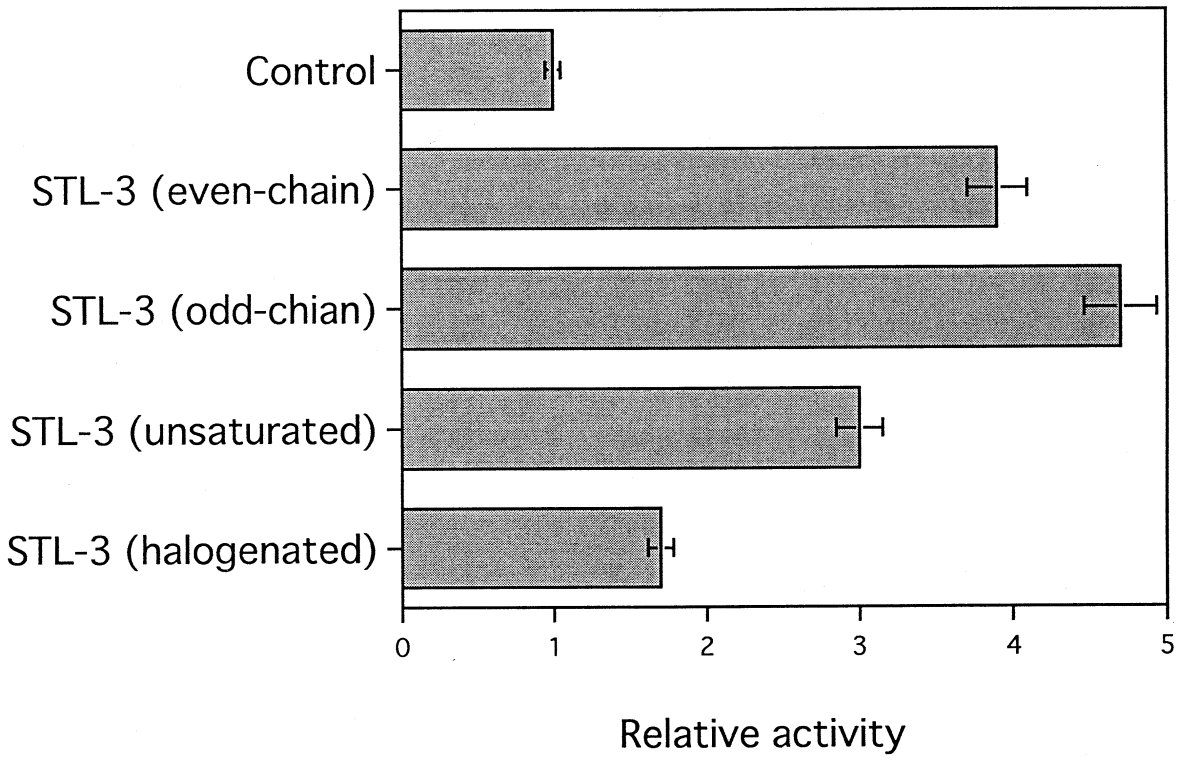

Figure 3. NBT-reducing activity of HL-60 cells treated with analogs of STL-3 at 3 μM for two days. NBT-reducing activity of HL-60 cells without treatment with an analog was taken as 1.0. All values are the means of results of three experiments and the standard deviation for each value is indicated.

of HL-60 cells with analogs of STL-3 using a fluorescence activated cell sorter (FACS). Treatment with STL-3 analogs of 3 μM increased the expression of detectable CD36 antigen. Significantly increased expression of CD36 was recognized when HL-60 cells had been cultured with analogs with even- or odd-chain saturated fatty acids (Figure 4). Analogs of STL-3 with unsaturated fatty acids also stimulated the expression of CD36 but to a lesser extent. By contrast, treatment of HL-60 cells with analogs with halogenated fatty acids did not significantly increase numbers of CD36-positive cells. These data reflected the NBT-reducing activity of the various samples.

Discussion

We have described here the ability of analogs of succinoyl trehalose lipid-3 (STL-3) to induce the differentiation of HL-60 human leukemia cells. Each of the tested analogs inhibited the growth of HL-60 cells in a dose-dependent manner (Figure 2) and those with saturated even-number or odd-number carbon chain fatty acids clearly increased the cellular NBT-reducing activity, which is a marker of differentiation to monocytes. Furthermore, enhancement of the expression of CD36 was also significant. Thus, it appears that STL-3 analogs with saturated even-number or odd-number carbon chain fatty acids are potent inducers of the monocytic differentiation of HL-60 cells. Analogs with halogenated fatty acids did not induce the monocytic differentiation of HL-60 cells. The mechanism(s) responsible for the distinct effects of the analogs of STL-3 on the induction of the differentiation of HL-60 cells remains to be clarified.

The optimal concentration of the analogs of STL-3 for induction of differentiation and inhibition of proliferation was around the CMC of each, suggesting that micelle formation might be essential for biological activity. It was reported recently that the glycolipid biosurfactant has a lower CMC and greater ability to reduce surface tension and interfacial tension than might be expected from its bulky structure (Ishigami, 1996). These features of STL-3 may be attributable to the molecular orientation at the interfaces of hydrophilic and hydrophobic domains. However, the nature

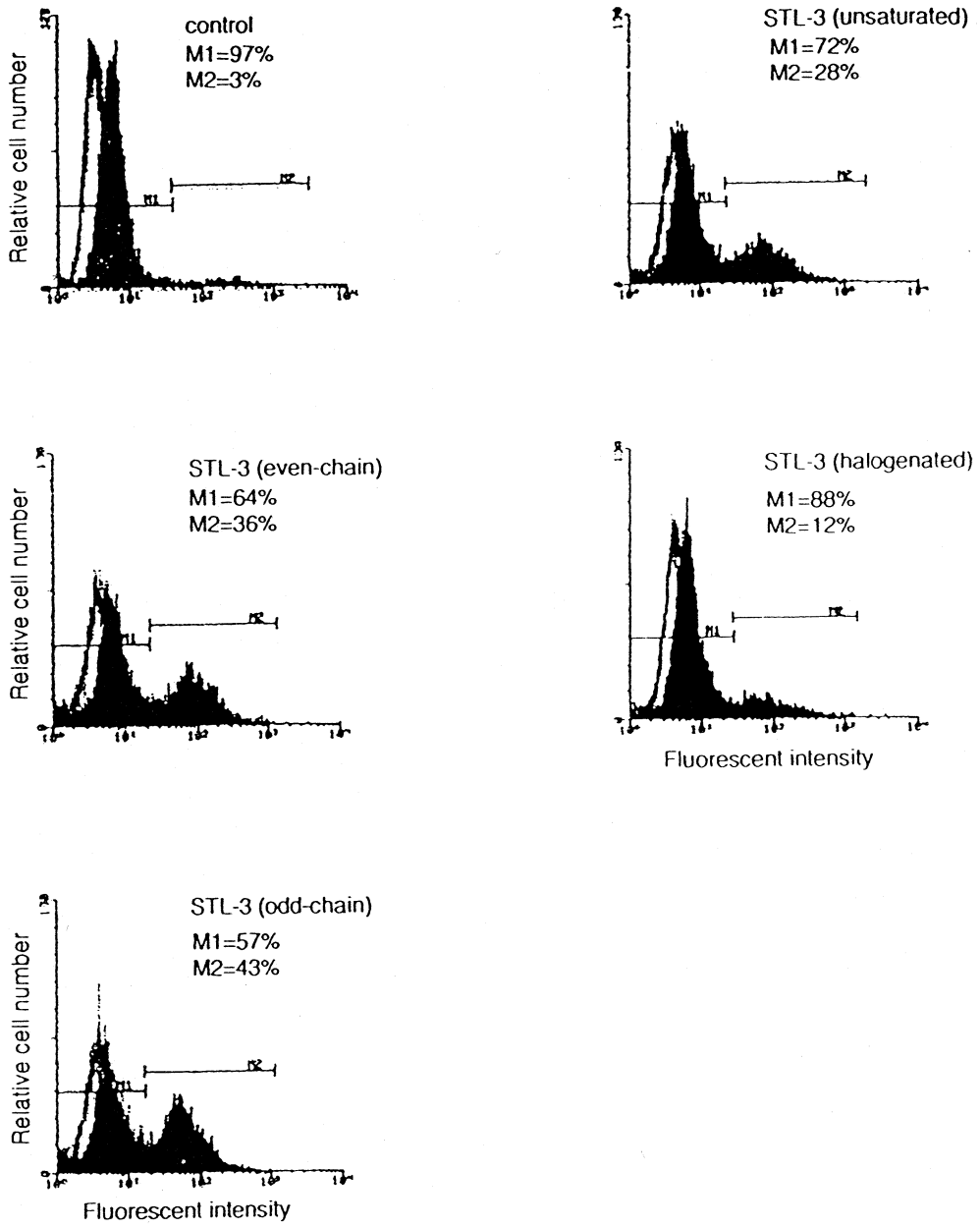

Figure 4. Flow-cytometric analysis of the expression of CD36 by HL-60 cells that had been exposed to analogs of STL-3 for two days. Open peaks, control HL-60 cells stained with irrelevant monoclonal antibodies; solid peaks, staining with fluorescein isothiocyanate-labeled antibodies specific for CD36. M1 and M2 indicate the populations of CD36-negative and CD36-positive cells, respectively.

of the molecular association of micelles of this biosurfactant with components of the cell membrane remains unclear. Further insight into the mechanism of micelle formation and the actions of analogs of STL-3 might ultimately lead to an understanding of the way that the signal triggered by an external glycolipid is transmitted and acts in the nucleus of host cells.

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