Trichostatin A and Herboxidiene Up-regulate the Gene Expression of Low Density Lipoprotein Receptor

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An abnormally elevated level of plasma cholesterol is one of the risk factors for the development of coronary heart disease. Low density lipoprotein (LDL) receptor binds plasma cholesterol-rich lipoproteins and transports them into cells to clear plasma cholesterol[1]. The synthesis of LDL receptor is regulated, to a large extent, by a sterol-mediated negative feedback mechanism at the level of gene transcription[2]. When cells are depleted of cholesterol, the LDL receptor gene is actively transcribed, and cholesterol is rapidly cleared from plasma. On the other hand, when cholesterol accumulates in cells, the expression of LDL receptor gene is repressed and plasma cholesterol level elevates. The inhibition of HMG-CoA reductase, a rate-limiting enzyme, in the cholesterol biosynthetic pathway depletes cellular cholesterol and activates LDL receptor gene transcription, thereby lowering plasma cholesterol[1]. The activation of LDL receptor gene transcription might be advantageous for ameliorating an elevated level of plasma cholesterol. In order to find compounds that activate the transcription of the LDL receptor, we screened buthanol extracts of microbial culture broths.

The transcription from LDL receptor promoter was assessed by a reporter assay using Chinese hamster ovary (CHO) cells transfected by a LDL receptor promoter-luciferase gene construct. The expression of luciferase activity in the transfectant was up- and down-regulated when cultured in the presence of lovastatin, an HMG-CoA reductase inhibitor, and LDL, respectively (data not shown). In the course of our screening, we isolated two active compounds which increased the luciferase activity from culture broths of unidentified actinomycete strains. NMR and MS data identified these compounds with trichostatain A and herboxidiene (Fig. 1), which had been reported as an antifungal antibiotic and a herbicidal compound, respectively[3,4]. As shown in Table 1, these compounds increased luciferase activity more potently than lovastatain.

By examining ^{125}I-LDL binding in an assay using human hepatoma HepG2 cells, we studied the effects of trichostatain A and herboxidiene on the amounts of LDL receptor synthesized on cell surface. Trichostatatin A and herboxidiene increased the specific binding of ^{125}I-LDL to LDL receptor by 48% and 31% at 10^{-7} M and 10^{-9} M, respectively, whereas lovastatin increased the specific binding by 17% at 10^{-6} M, taking the result for the cells cultured in the absence of compounds as 100%. The above result indicated that these compounds activated the synthesis of LDL receptor.

It has been reported that trichostatin A inhibits histone deacetylase and induces hyperacetylation of histone to stimulate the expression of several genes[5~7]. The activation of LDL receptor gene transcription by trichostatin A might be attributed to the histone hyperacetylation. The mechanism of activation by herboxidienen is not clear and merits further studies.

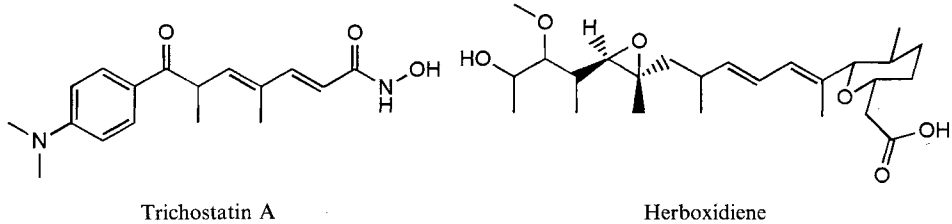
To our knowledge, this is the first report that trichostatain A and herboxidiene up-regulate the gene expression of LDL receptor.

Experimental

Reporter Assay

CHO cells were transfected with a plasmid construct containing human LDL receptor promoter region (−240

Fig. 1. Structure of trichostatin A and herboxidiene.



Trichostatin A Herboxidiene

Table 1. Effect of trichostatin A and herboxidiene on the expression of luciferase activity under the control of LDL recepter promotor in the transfected CHO cells.

Compound	Concentration (μM)	Luciferase activity (% of control[a])
Trichostatin A	0.1	268
	1	914
Herboxidiene	0.1	294
	1	4098
Lovastatin	5	634

[a] A control was the luciferase activity expressed in the cells cultured in the absence of test componds.

to -19 bp region, taking A of the start codon as $+1$[8]) linked to luciferase gene in the proper direction. The clone that expressed luciferase activity under the control of LDL receptor promoter was selected. The resultant recombinant cells (2×10^4 cells) were seeded into 96-well microplates and grown 24 hours at 37°C in 200 μl of complete F-12 medium (Gibco) supplemented with 10% fetal bovine serum (FBS). The cells were then washed with phosphate buffered saline (PBS) and incubated with or without samples to be tested for 48 hours at 37°C in complete F-12 medium supplemented with 10% lipoprotein-deficient serum (LPDS, Sigma). After the incubation, cells were washed two times with PBS, lysed in 50 μl of PicaGene TM Cell Culture Lysis Reagent Lucβ (Toyoinki Co.) and luciferase activity was measured by using PicaGene Luminescene kit (Toyoinki Co.).

LDL Binding Assay

Human hepatoma HepG2 cells (3×10^4 cells) were cultured for 48 hours at 37°C in complete MEM (Gibco) supplemented with 10% FBS by using 24-well microplates. The cells were washed with PBS and incubated with or without test samples for 24 hours at 37°C in complete MEM supplemented with 10% LPDS. ^{125}I-LDL (1 μg, 1.5×10^4 cpm) was then added to the culture and the incubation was continued for 3 hours. Thereafter, the cells were washed four times with 50 mM Tris-HCl (pH 7.5), 0.15 M NaCl, 2 mg of BSA per ml, and solubilized in 0.1 N NaOH. Total radioactivity was counted with liquid scintilator. Non-specific binding was measured by addition of 50-fold excess of non-radiolabeled LDL. The specific binding was obtained by subtracting the non-specific binding from total binding.

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