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FR901512, a Novel HMG-CoA Reductase Inhibitor Produced by an Agonomycetous Fungus No. 14919

II. Biological Profiles

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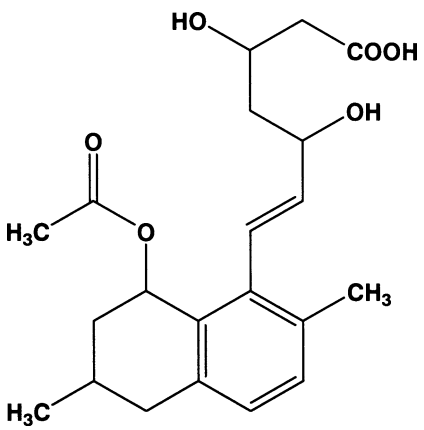
FR901512, a new specific inhibitor of HMG-CoA reductase, was isolated from the culture of an agonomycetous fungus No. 14919. FR901512 inhibited cholesterol synthesis from [^{14}C] acetate in Hep G2 cells with an IC_{50} of 1.0 nM. An increase of cell surface LDL receptors observed on the FR901512 treated human hepatoma cell line Hep G2 cells. Single oral administration of FR901512 strongly inhibited sterol synthesis in rats. Daily oral administration of FR901512 to beagle dogs decreased plasma cholesterol levels.

In the previous paper, we reported production, isolation, physico-chemical properties and *in vitro* biological activities of novel and potent 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (EC 1.1.1.34) inhibitor FR901512 (Fig. 1) and its lactone form FR901516 produced by an agonomycetous fungus No. 14919[1]. FR910512 strongly inhibited HMG-CoA reductase activity in Hep G2 cell lysate and potently inhibited cholesterol synthesis from acetic acid (Table 1), but not from mevalonic acid, which is product of HMG-CoA reductase. HMG-CoA reductase is the major rate limiting enzyme in the cholesterol synthetic pathway[2], and is considered to be a prime target for pharmacological intervention. It is well known that inhibitors of HMG-CoA reductase inhibitors are effective in lowering the level of blood plasma cholesterol, especially low density lipoprotein cholesterol (LDL-C)[3,4].

Fig. 1. Structure of FR901512.



To investigate the hypolipidemic effects of the newly isolated HMG-CoA reductase inhibitor FR901512, we tested its up-regulating effects on LDL receptors on Hep G2 cells, its inhibitory effect on hepatic sterol synthesis in rats and lipid lowering effects in a dog model.

Materials and Methods

^{125}I-LDL Binding Assay

Hep G2 cells were cultured as described previously[5].

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Table 1. Inhibitory activities of FR901512 on HMG-CoA reductase and cholesterol synthesis *in vitro*.

Assay system	IC_{50} (nM)
HMG-CoA reductase	0.95
Cholesterol synthesis	1.0

Human LDL (purchased from Sigma, USA) was used for ^{125}I iodination by the iodine monochloride method as described by FIELDING *et al.*[6]. Hep G2 cells in 24-well culture plates were incubated with or without test samples for 48 hours at 37°C in Eagle's modified minimum essential medium (MEM) supplemented with 10% human lipoprotein deficient serum. After incubation, the cells were washed three times with MEM containing 10 mM HEPES (pH 7.4), 10 mg/ml BSA (binding medium) at 4°C. Binding medium containing ^{125}I-LDL (2 μg) was added to the culture and incubated at 4°C for 2 hours. The cells were washed three times with 20 mM Tris-HCl (pH 7.4), 0.15 M NaCl, 1 mg/ml BSA, and solubilized in 0.5 N NaOH. Total radioactivity was counted with gamma counter. 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.

Assay of *In Vivo* Sterol Synthesis in Rats

FR901512 was suspended in 0.5% methylcellulose and administrated orally to male Sprague-Dawley rats (two rats per group). Dosage levels used in this experiment are 0.01, 0.1, 1 and 10 mg/kg. The control group received the suspending vehicle only. Fifty minutes after compound administration, [1-^{14}C] sodium acetate was injected intraperitoneally. Fifty minutes after receiving the [^{14}C] acetate, animals were anesthetized with ether and blood (3 ml) was collected by heart puncture. Plasma was obtained by centrifugation at 1100×g for 10 minutes. One ml of plasma samples were saponified in 15% KOH in 95% EtOH at 80°C for 2 hours. Non-saponifiable substances were extracted with *n*-hexane, and digitonin precipitable [^{14}C] sterols were measured through liquid scintillation counting.

In Vivo Serum Cholesterol Lowering Effect in Beagle Dog

Pure-bred beagle dogs weighing 8～10 kg were housed individually and fed a commercial dog food (three dogs per group). FR901512 was orally administrated in gelatin capsules once daily for 15 days. FR901512 was administrated at a dose of 0.32 mg/kg body weight. On day 0, 5, 8, 12 and 15, blood samples (3 ml) were collected from jugular or saphenous vein. Plasma samples were obtained by centrifugation at 1100×g for 10 minutes and plasma cholesterol levels were determined by commercial kit (Wako pure chemical, Co.). Data from this experiment were expressed as percent decrease of pretreatment (values were expressed as mean±S.E.).

Results

Up-regulation of Cell Surface LDL Receptors

Since Hep G2 cells have been shown to possess specific LDL receptors and HMG-CoA reductase inhibitors up-regulates cell surface LDL receptors[7,8], we first tested the LDL-receptor up-regulating effect of FR901512. The binding activity of ^{125}I-LDL to HepG2 cells was determined after incubation of the cells for 48 hours with FR901512 at the indicated concentrations (Table 2). FR901512 up-regulated the receptor mediated binding of ^{125}I-LDL in Hep G2 cells in a dose-dependent manner.

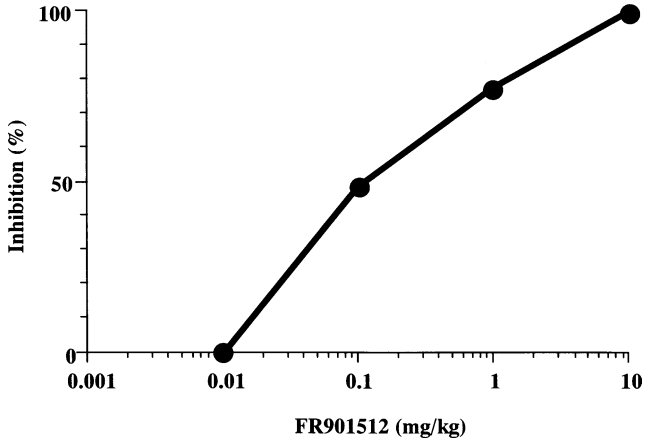
Inhibition of Sterol Synthesis in Rats by FR901512

Single oral administration of FR901512 inhibited rat hepatic sterol synthesis from [^{14}C]-acetic acid in a dose dependent manner with an ED_{50} value at 0.18 mg/kg (Fig. 2). This demonstrates that FR901512 can serve as an effective inhibitor of HMG-CoA reductase *in vivo*.

In Vivo Serum Cholesterol Lowering Effect of FR901512 in Beagle Dog

As shown in Table 3, daily oral administration of 0.32 mg/kg of FR901512 to dogs gradually decreased plasma cholesterol, and reached 34% decrease after 15 days of dosing.

Fig. 2. Inhibition of rat hepatic sterol synthesis by FR901512.



Discussion

In this paper we evaluated the *in vivo* effects of a novel and potent HMG CoA reductase inhibitor, FR901512. This compound was discovered during a screening program for cholesterol synthesis inhibitors targeting earlier stages of cholesterol synthetic pathway than mevalonic acid formation. FR901512 strongly inhibited HMG-CoA reductase activity with an IC_{50} value of 0.95 nM, and inhibited cholesterol synthesis in Hep G2 cells from ^{14}C-acetic acid with an IC_{50} value of 1.0 nM (Table 1). This compound did not inhibit the cholesterol biosynthesis from ^{14}C-mevalonate (data not shown). The mechanism of lipid lowering effects of HMG-CoA reductase inhibitors has been well established. The depletion of a critical intrahepatic pool of cholesterol stimulates transcription of LDL receptors in the liver, which enhances the removal of LDL particles from the circulation and contributes significantly to lowering of the cholesterol[9,10]. Lowered LDL levels are maintained in steady state by compensatory mechanisms of increased cholesterol synthesis by induction

Table 2. Effects of FR901512 on the binding of ^{125}I-LDL to HepG2 cells.

FR901512 (μM)	^{125}I-LDL binding (% of control)
0.001	115
0.01	125
0.1	162
1	186

Table 3. Effect of oral administration of FR901512 on plasma cholesterol levels in dog.

Day	Plasma cholesterol* (% decrease of pretreatment)
5	19.9 ± 7.6
8	27.8 ± 6.8
12	36.7 ± 4.7
15	34.3 ± 4.2

*mean ± S.E.

of HMG-CoA reductase levels[11].

To investigate the effect of FR901512 on the cell surface LDL receptors, we carried out the receptor binding assay using Hep G2 cells and ^{125}I-LDL as a ligand. When the cells were cultured in the presence of several concentrations of FR901512 at 48 hours, the specific binding of ^{125}I-LDL was increased in a dose dependent manner. Preincubation of 1 μM FR901512 increases cell surface LDL receptors at 186% compared to control well. This result indicated that FR901512 activated the expression of cell surface LDL receptors.

When administrated orally in rat, FR901512 inhibited hepatic sterol synthesis with an ED_{50} value at 0.18 mg/kg (Table 2). This demonstrates that FR901512 can serve as an effective inhibitor of cholesterol biosynthesis *in vivo*.

Finally we investigated the hypolipidemic effect of FR915152 using normolipidemic beagle dogs. Daily oral administration of FR901512 gradually reduces the serum cholesterol levels in dogs, and at day 12 the cholesterol levels are lowered 36.7% compared to pretreatment levels. FR901512 is expected to have hypolipidemic effect in humans.

Reference

1) HATORI, H.; B. SATO, I. SATO, T. SHIBATA, Y. TSURUMI, K. SAKAMOTO, S. TAKASE, H. UEDA, M. HINO & T. FUJII: FR901512, a novel HMG-CoA reductase inhibitor produced by an agonomycetous fungus No.14919. I. Taxonomy of the producing organism, fermentation, isolation and physico-chemical properties. J. Antibiotics 57: 264～270, 2004
2) RODWELL, V. W.; J. NORDSTORM & J. J. MITSCHELEN: Regulation of HMG-CoA reductase. Adv. Lipid Res. 1～74, 1976
3) ENDO, A.: The discovery and development of HMG-CoA reductase inhibitors. J. Lipid Res. 33: 1569～1582, 1992
4) TSUJITA, Y.; M. KURODA, Y. SHIMADA, K. TANZAWA, M. ARAI, I. KANEKO, M. TANAKA, H. MASUDA, C. TARUMI & Y. WATANABE: CS-514, a competitive inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase: tissue-selective inhibition of sterol synthesis and hypolipidemic effect on various animal species. Biochim. Biophys. Acta 877: 50～60, 1986
5) HATORI, H.; T. SHIBATA, Y. TSURUMI, T. NAKANISHI, M. KATSUOKA, Y. OHTSU, K. SAKAMOTO, S. TAKASE, H. UEDA, M. HINO & T. FUJII: FR171456, A novel cholesterol synthesis inhibitor produced by *Sporormiella minima* No. 15604. I. Taxonomy, fermentation, isolation, physico-chemical properties. J. Antibiotics 57: 253～259, 2004
6) FIELDING, C. J.; I. VLODAVSKI, P. E. FIEDING & D. GOSPODAROWICZ: Characteristics of chylomicron binding and lipid uptake by endothelial cells in culture. J. Biol. Chem. 254: 8861～8868, 1979
7) HAVEKES, L.; V. V. HINSBERGH, H. J. KENPEN & J. EMEIS: The metabolism *in vitro* of human low-density lipoprotein by the human hepatoma cell line Hep G2. Biochem. J. 214: 951～958, 1983
8) COHEN, L. H.; M. GRIFFIOEN, L. HAVEKES, D. SCHOUTEN, V. VAN HINSBERGH & H. J. KEMPEN: Effects of compactin, mevalonate and low-density lipoprotein on 3-hydroxy-3-methylglutaryl-coenzyme A reductase activity and low-density-lipoprotein-receptor activity in the human hepatoma cell line Hep G2. Biochem. J. 222: 35～39, 1984
9) BROWN, M. S. & J. L. GOLDSTEIN: A receptor-mediated pathway for cholesterol homeostasis. Science 232: 34～47, 1986
10) DAWSON, P. A.; S. L. HOFMANN, D. R. VAN DER WESTHUYZEN, T. C. SUDHOF, M. S. BROWN & J. L. GOLDSTEIN: Sterol-dependent repression of low density lipoprotein receptor promoter mediated by 16-base pair sequence adjacent to binding site for transcription factor Sp1. J. Biol. Chem. 263: 3372～3379, 1988
11) GRUNDY, S. M.: HMG-CoA reductase inhibitors for treatment of hypercholesterolemia. N. Engl. J. Med. 319: 24～33, 1988