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ORIGINAL ARTICLE

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Delivery and cytotoxicity of RS-1541 in St-4 human gastric cancer cells in vitro by the low-density-lipoprotein pathway

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Abstract RS-1541 is a 13-*O*-palmitoyl derivative of rhizoxin, an inhibitor of tubulin polymerization. RS-1541 has been shown to bind preferentially to plasma lipoproteins and to exhibit selective and sustained uptake by tumors in mice. To elucidate a mechanism of RS-1541 cytotoxicity, the cellular uptake and the cytotoxicity of a complex of RS-1541 with human low-density lipoprotein (RS-1541/LDL complex) were investigated in cultured St-4 human gastric cancer cells. Both the cellular uptake and the cytotoxicity of the RS-1541/LDL complex were greater in cells with higher LDL-receptor activities than in control cells. Excess amounts of LDL or 1 μ*M* of monensin, a proton ionophore, significantly inhibited both the uptake and the cytotoxicity of the complex. Chloroquine, an inhibitor of lysosomal enzymes, decreased the intracellular level of rhizoxin liberated from RS-1541 and suppressed the cytotoxicity of the RS-1541/LDL complex. However, a detergent-aided solution of RS-1541 showed very low cellular uptake and cytotoxicity, irrespective of the LDL-receptor activities of these cells. These results demonstrate that the RS-1541/LDL complex is incorporated into the cells via the LDL receptor and that it manifests its cytotoxic activity after forming rhizoxin, the original antitumor agent, in the lysosomes.

Key words RS-1541 · Low-density lipoprotein · Targeting

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Introduction

Rhizoxin was discovered as an agent that induces rice seedling blight [16]. The toxicity of rhizoxin has been shown to result from the inhibition of tubulin polymerization [22], which thereby inhibits cell mitosis [26]. This mode of action suggested the application of rhizoxin as an antitumor agent [11]. Our laboratories have found in various in vivo tests that RS-1541, a 13-*O*-palmitoyl derivative of rhizoxin, exhibits much higher antitumor activity than rhizoxin [12]. RS-1541 inhibited the mitosis of P338 leukemia cells in vivo during the G_2-M phase in a manner similar to that of rhizoxin as determined by flow cytometry, indicating that RS-1541 and rhizoxin have the same mode of action [23]. In vitro, however, substitution of a hydroxyl group at the 13 position of rhizoxin is known to reduce markedly the inhibitory effect of rhizoxin on tubulin polymerization [21].

To elucidate the reason for the increased activity of RS-1541 relative to rhizoxin in vivo, we have previously compared the pharmacokinetics of RS-1541 with that of rhizoxin after intravenous administration to mice bearing M5076 sarcoma, a spontaneous murine reticulum-cell sarcoma [24]. RS-1541 bound preferentially to plasma lipoproteins and showed selective and sustained uptake by the tumor, whereas rhizoxin did not. A considerable amount of rhizoxin was detected in the tumor, and the rhizoxin formation in vivo was inhibited by chloroquine, an inhibitor of lysosomal enzymes.

One of the main problems in cancer chemotherapy is a lack of tumor selectivity. To circumvent this problem, several strategies have been explored by many investigators. Recently, low-density lipoprotein (LDL) has attracted attention for its use as an endogenous carrier of antitumor agents to malignant cells, with a view toward reducing undesired systemic effects [5, 15, 18, 20, 27]. The basis for this idea is the knowledge that as compared with normal cells, growing tumor cells vigorously incorporate LDL via LDL receptors [6, 13, 17, 28] and release low-molecular-

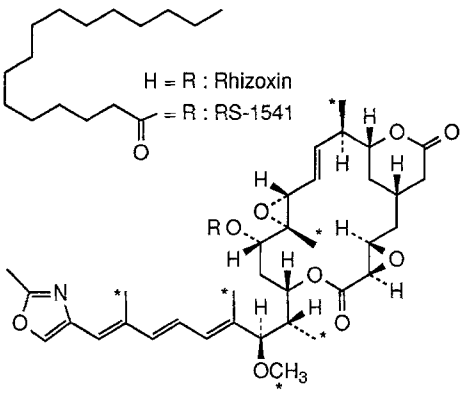

Fig. 1 Structures of [^{14}C]-RS-1541 and [^{14}C]-Rhizoxin and the labeled positions (★)

weight substances after degrading LDL particles by the action of lysosomal hydrolyzing enzymes [8, 19].

All of the results we previously obtained on RS-1541 in vivo using tumor-bearing mice [24] were consistent with the view that RS-1541 is taken up by the tumor via LDL receptors after binding to the lipoproteins, the uptake of which results in the formation of rhizoxin, the original antitumor agent, in the lysosome, although we need additional direct evidence for the participation of the LDL-receptor pathway in the manifestation of cytotoxicity of the lipoprotein-bound antitumor agent.

Recently, we successfully prepared a complex of RS-1541 with human LDL in vitro [25]. To obtain a rationale for the clinical use of RS-1541, we demonstrate in the present paper that the cellular uptake and the cytotoxicity of the RS-1541/LDL complex in St-4 human gastric cancer cells are dependent on the LDL-receptor activities. We also discuss the benefit in cancer chemotherapy of delivering an agent, chemically modified to bind preferentially to lipoproteins, to tumor cells via the LDL-receptor pathway.

Materials and methods

RS-1541, rhizoxin, and other reagents

Rhizoxin and [^{14}C]-rhizoxin (specific activity, 947.2 kBq/mg) was isolated from a culture broth of *Rhizopus chinensis* Rh-2 [10] in the Fermentation Research Laboratories of Sankyo Co. RS-1541 and [^{14}C]-RS-1541 (specific activity, 650.8 kBq/mg) were synthesized by palmitoylation of rhizoxin and [^{14}C]-rhizoxin, respectively, in the Bioscience Research Laboratories of Sankyo Co. The structures and labeled positions of [^{14}C]-RS-1541 and [^{14}C]-rhizoxin are shown in Fig. 1. [^{125}I]-Sodium iodide was purchased from NEN Research Products, Japan. Polyoxyethylene (60) hydrogenated castor oil (HCO60) was obtained from Nikkol, Tokyo. All other chemicals were commercially available and of reagent grade.

Cells

St-4, an established cell line of human poorly differentiated gastric cancer, was maintained in plastic dishes containing Roswell Park Memorial Institute medium 1640 supplemented with 5% calf fetal serum and kanamycin (100 μg/ml; hereafter referred to as growth medium) at 37 °C in a humidified atmosphere of 95% air and 5% CO_2. St-4 cells were seeded in 35-mm plastic dishes at a density of $3 \times 10^4 - 2 \times 10^5$ cells/dish. At 2 days after seeding, cells were cultured for the next 12 h in different combinations of media, i.e., medium containing 5% normal human serum (NS medium) and that containing 5% human lipoprotein-deficient serum (LPDS medium), as follows: (1) in NS medium for 12 h, (2) in NS medium for 9 h and in LPDS medium for another 3 h, (3) in NS medium for 6 h and in LPDS medium for another 6 h, and (4) in LPDS medium for 12 h. Thus, the cells were finally cultured in LPDS medium for 0, 3, 6, or 12 h to induce LDL-receptor activity of various degrees. Hereafter, the cells with high LDL-receptor activity, prepared by cultivation in LPDS medium for 12 h, are referred to as St-4[+] cells and those with low LDL-receptor activity, prepared by cultivation in NS medium for 12 h, are referred to as St-4[-] cells.

Lipoproteins

Human LDL (density, 1.019–1.063 g/ml) was prepared by differential ultracentrifugation [9] of serum from healthy volunteers and was dialyzed against buffer A [0.15 *M* NaCl, 0.24 m*M* ethylenediaminetetraacetic acid (EDTA)-2Na, penicillin (100 IU/ml), and streptomycin (100 μg/ml): pH 7.4]. Iodination of LDL was accomplished according to the modified iodine monochloride method of Bilheimer et al. [1]. The specific activity of [^{125}I]-LDL was 236 cpm/μg protein.

Preparation of the [^{14}C]-RS-1541/LDL complex

[^{14}C]-RS-1541 (1.0 mg) was dissolved in 0.1 ml of dimethylacetamide containing 2.0 mg of HCO60, a detergent. The solution was further diluted in 9.9 ml of 0.15 *M* NaCl with sonication. The detergent-aided solution of [^{14}C]-RS-1541 (20 μ*M*) was then incubated with LDL (800 μg/ml) for 6 h at 37 °C. The incubation mixture was dialyzed against buffer A, sterilized by filtration through a 0.22-μm Millex GV Millipore filter, and stored at 4 °C as the [^{14}C]-RS-1541/LDL complex, which was used within 1 week. The purity of the complex was analyzed by both gel-filtration chromatography through a Superose 6HR 10/30 column (Pharmacia, Uppsala) and 1% agarose-gel electrophoresis. Precipitation of LDL was performed by the dextran sulfate/$MnCl_2$ method [4].

Metabolism of [^{125}I]-LDL by St-4 cells

St-4 cells were incubated with [^{125}I]-LDL in 1 ml of the medium containing 5% LPDS at 37 °C. After incubation for 0, 1, 3, and 6 h, the medium was removed and each dish, washed. The cells were solubilized in 1 ml of 1 *N* NaOH and measured for radioactivity and protein concentration [14]. The degradation of [^{125}I]-LDL was determined from the noniodide trichloroacetic acid-soluble radioactivity in the incubation medium [7]. The radioactivity incorporated into the cells and that of the degraded LDL in the medium were combined and regarded as the total uptake.

Cellular uptake of [^{14}C]-RS-1541/LDL

St-4 cells were incubated with either the [^{14}C]-RS-1541/LDL complex or the detergent-aided solution of [^{14}C]-RS-1541 in 2 ml of the medium containing 5% LPDS at 37 °C. After incubation for 0, 1, 3, and 6 h, the medium was removed and the cells were washed. The cells were solubilized and measured for radioactivity and protein concentration.

Calculation of uptake clearance

Uptake clearance of [^{125}I]-LDL and the [^{14}C]-RS-1541/LDL complex was determined according to the following equation. For calculation of

the uptake clearance of [^{125}I]-LDL, total uptake (uptake plus degradation) was used:

$$\text{Uptake clearance } (\mu l\ h^{-1}\ mg\ protein^{-1}) = \frac{\text{Initial rate of uptake } (ng\ h^{-1}\ mg\ protein^{-1})}{\text{Medium concentration } (\mu g/ml)} =$$

Effect of chloroquine on the rhizoxin content of cells

Cells were incubated with the [^{14}C]-RS-1541/LDL complex for 6 h in the medium containing 5% LPDS and were then washed and incubated for a further 18 h in the growth medium in either the presence or the absence of chloroquine (100 μM), which is an inhibitor of lysosomal enzymes [3]. The cells were extracted with 0.5 ml of acetonitrile, and the resultant extracts were separated by thin-layer chromatography using a silica-gel thin-layer plate [F_{254}, 0.25 mm, Merck Co., Darmstadt; benzene/isopropanol (5/1)]. Radioactive spots of RS-1541 and rhizoxin were detected by a Bioimage Analyzer (Fuji Photo Film Co., Tokyo) and from these, the percentages of rhizoxin and RS-1541 were determined.

Evaluation of cytotoxicity

Cells were treated with the RS-1541/LDL complex or RS-1541 solution for 6 h in the medium containing 5% LPDS and were then washed and incubated for a further 42 or 66 h in the growth medium. The cells were next washed with Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline (buffer B) and then treated with a solution of 0.05% trypsin (Boehringer Manheim, Germany) containing 0.02% EDTA-2Na. Buffer B containing 1% fetal calf serum was added to neutralize the trypsin. The cells were suspended by pipetting and then counted by a Model ZM Coulter Counter.

Results

Formation of the [^{14}C]-RS-1541/LDL complex

The detergent-aided solution of [^{14}C]-RS-1541 (20 μM) was incubated with LDL (800 µg/ml) for 6 h at 37 °C. The resultant mixture was analyzed by analytical gel-filtration chromatography. Radioactivity was found to be associated mostly with LDL as shown in Fig. 2. On agarose-gel electrophoresis, a single protein band was observed at the same electrophoretic migration as native LDL. More than 80% of the radioactivity detected in the incubation mixture was precipitated with dextran sulfate/$MnCl_2$. No aggregation or precipitation was noted after storage of the [^{14}C]-RS-1541/LDL complex for 1 week at 4 °C, demonstrating the good stability of this preparation.

Induction of LDL-receptor activity in St-4 cells

St-4 cells were cultured in lipoprotein-deficient medium for 0, 3, 6, or 12 h, and the J_{max} (the maximal uptake rate) and K_m (the concentration at $J_{max}/2$) values for [^{125}I]-LDL in these cells were calculated. K_m was not altered (ca. 20 µg/ml) by treatment for induction, whereas J_{max} increased with increasing induction periods. The J_{max} value in St-4[+] cells (1.94 µg/mg protein per 6 h) was about 3 times greater than that of St-4[-] cells (0.59 µ g/mg protein per 6 h). The doubling time of the cells (ca. 24 h) was not changed by this treatment.

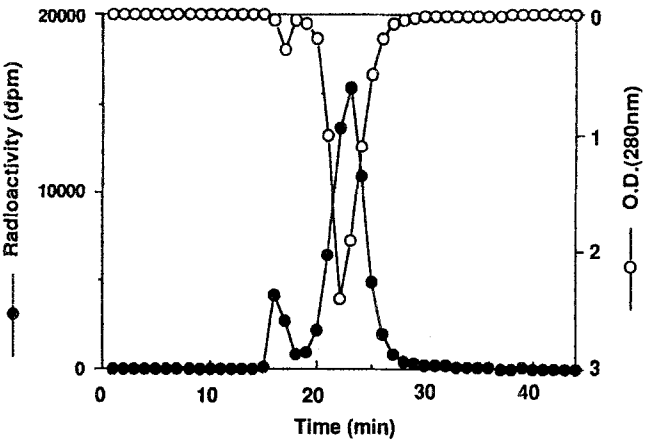

Fig. 2 Column elution profiles for the [^{14}C]-RS-1541/LDL complex. A 40 µl aliquot of the solution was applied to a Superose 6HR 10/30 column and eluted with 0.15 M NaCl, 0.01% EDTA 2Na and 0.02% NaN_3 (pH 7.4) at 0.5 ml/min. Radioactivity and optical density were monitored as indicated

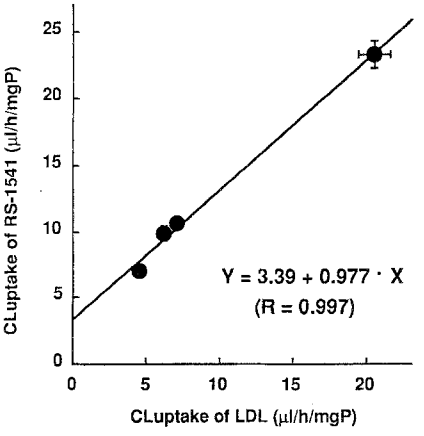

Fig. 3 Correlation between the uptake clearance of [^{14}C]-RS-1541/LDL complex and [^{125}I]-LDL by St-4 cells, which were preincubated for 0, 3, 6, and 12 h in the lipoprotein-deficient medium

Comparison of cellular uptake of [^{125}I]-LDL and [^{14}C]-RS-1541/LDL

The uptake of both [^{125}I]-LDL and the [^{14}C]-RS-1541/LDL complex at 40 µg-Equiv. of LDL/ml was measured in the cells, which were preincubated for 0, 3, 6, and 12 h in the lipoprotein-deficient medium. The uptake clearances of the [^{14}C]-RS-1541/LDL complex correlated well with those of [^{125}I]-LDL, and the correlation coefficient was 0.997 with a slope of 0.977 (Fig. 3), indicating that the [^{14}C]-RS-1541/LDL complex was recognized as native LDL and was incorporated according to the density of LDL receptors on the cell surface.

Uptake of [^{14}C]-RS-1541/LDL by St-4[+]and St-4[-] cells

When the cells were incubated with the [^{14}C]-RS-1541/LDL complex, both St-4[-] and St-4[+] cells showed uptake

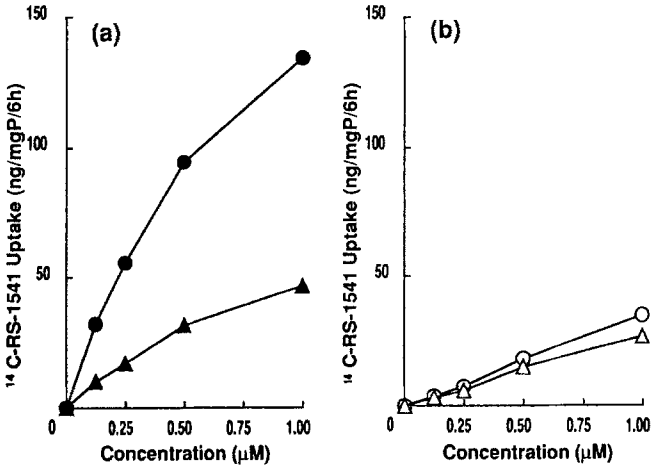

Fig. 4 Cellular uptake of the radioactivity after incubation of the [^{14}C]-RS-1541/LDL complex (**a**) and the detergent-aided solution of [^{14}C]-RS-1541 (**b**) with St-4[+] cells (●, ○) or St-4[-] cells (▲, △). Each value represents the mean ±SE (n = 3)

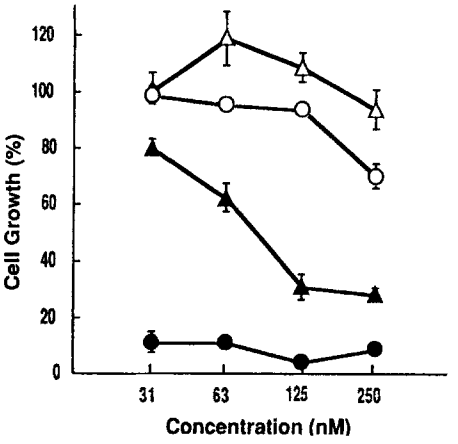

Fig. 5 Cytotoxicity of the RS-1541/LDL complex (closed) and detergent-aided solution of RS-1541 (open) against St-4[+] cells (●, ○) and St-4[-] cells (▲, △). These cells were treated for 6 h, and then washed and incubated for a further 42 h in the growth medium. Each value represents the mean ±SE (n = 3)

saturation with increasing concentrations of [^{14}C]-RS-1541 (Fig. 4a). The uptake by St-4[+] cells was always higher than that by St-4[-] cells (ratio, ~3). On the other hand, when the cells were incubated with the detergent-aided solution of [^{14}C]-RS-1541, both the uptake by St-4[+] cells and that by St-4[-] cells were low and increased linearly with the concentration of [^{14}C]-RS-1541 (Fig. 4b).

Effects of inhibitors on the uptake of [^{14}C]-RS-1541/LDL by St-4[+] cells

Unlabeled LDL inhibited the uptake of the [^{14}C]-RS-1541/LDL complex (40 μg/ml as LDL) by St-4[+] cells in a concentration-dependent manner (Table 1). The unlabeled LDL at 320 μg/ml decreased the cellular uptake of [^{14}C]-RS-1541/LDL to 20.1%. Also, 1 μ*M* of monensin, a proton ionophore [2], significantly inhibited the cellular uptake (to 24.3%) of the complex. Incubation at 4 °C resulted in an uptake of only 4.8% of that observed at 37 °C.

Effect of chloroquine on intracellular metabolism of [^{14}C]-RS-1541

As shown in Table 2, after incubation of St-4[+] cells with [^{14}C]-RS-1541/LDL for 6 h, the amounts of RS-1541 and rhizoxin detected in the cells were 71.2% and 4.3%, respectively. After 18 h of further incubation of the cells in the medium lacking the [^{14}C]-RS-1541/LDL complex, the percentage of RS-1541 decreased to 55.6%, whereas that of rhizoxin increased to 17.4%, indicating that the formation of rhizoxin from RS-1541 occurs in the cells. On the other hand, in the cells incubated in the presence of chloroquine (100 μ*M*), the portion of rhizoxin remained at a low level (3.8%) after the second incubation, suggesting that the metabolic activation of the prodrug occurs in the lysosomes.

Table 1 Effects of unlabeled LDL, monensin, and low temperature on uptake of the [^{14}C]-RS-1541/LDL complex by St-4[+] cells

		Uptake for 6 h (% control)
[^{14}C]-RS-1541 (1 μM)/LDL (40 μg/ml) at 37 °C		100
+ unlabeled LDL	40 μg/ml	65.9±2.7
	80 μg/ml	51.2±2.1
	160 μg/ml	32.0±0.4
	320 μg/ml	20.1±0.4
+ monensin	1 μM	24.3±0.9
[^{14}C]-RS-1541 (1 μM)/LDL (40 μg/ml) at 4 °C		4.8±0.1

Each value represents the mean ±SE of three experiments

Table 2 Effect of chloroquine on intracellular metabolism of the [^{14}C]-RS-1541/LDL complex in St-4[+] cells

Incubation	Control		Chloroquine (100 μM)	
	RS-1541	Rhizoxin	RS-1541	Rhizoxin
	(% total radioactivity)			
With complex (6 h)	71.2±1.9	4.3±0.1	70.2±1.5	5.1±0.3
Without complex (+18 h)	55.6±1.9	17.4±0.1	71.2±0.7	3.8±0.1

Each value represents the mean ±SE of 3 experiments

Cytotoxicity of the RS-1541/LDL complex

As shown in Fig. 5, the RS-1541/LDL complex caused a strong growth inhibition in St-4[+] cells (12% of control growth), even at the lowest concentration tested (31.3 n*M*). The growth inhibition induced by the RS-1541/LDL complex in St-4[-] cells was very weak (80%) at 31.3 n*M*, but increased with increasing concentration, reaching 28% at 250 n*M*. On the other hand, the detergent-aided solution of RS-1541 showed only weak cytotoxicity (70%–94%) on both types of cell, even at the highest concentration tested. Rhizoxin, which did not bind to lipoproteins, was equally

Table 3 Effect of unlabeled LDL, monensin, and chloroquine on cytotoxicity of the RS-1541/LDL complex against St-4[+] cells

		Cell growth (% control)
Control growth		100
RS-1541 (500 nM)/LDL (20 μg/ml)		1.0 ± 1.4
+ native LDL	320 μg/ml	75.3 ± 2.4
+ monensin	1 μM	38.1 ± 7.2
+ chloroquine	4 μM	7.7 ± 1.4
+ chloroquine	10 μM	18.6 ± 1.0

Cells were treated with the RS-1541/LDL complex for 6 h, and then washed and incubated for a further 66 h in the growth medium. Each value represents the mean ± SE of three experiments

cytotoxic to both St-4[+] and St-4[-] cells at 0.3 n*M*, irrespective of the presence of LDL (data not shown), indicating that the cytotoxicity of rhizoxin against the St-4 cells was not affected either by LDL-receptor induction or by the presence of LDL in the medium

Inhibitory effects of agents modifying the LDL-receptor pathway on the cytotoxicity of RS-1541/LDL

The growth of St-4[+] cells was strongly inhibited by treatment with the RS-1541/LDL complex (500 n*M* as RS-1541) to 1.0% as shown in Table 3. Excess amounts of LDL (320 μg/ml) and monensin (1 μ*M*) effectively weakened the cytotoxicity of the complex, increasing cell growth to 75.3% and 38.1%, respectively. Chloroquine had a weak but significant effect against the cytotoxicity of the complex (recovery to 18.6% at 10 μ*M*). In the absence of the complex, addition of these agents did not alter the control growth at the concentrations used.

Discussion

Considerable evidence that the RS-1541/LDL complex is incorporated into St-4 human gastric cancer cells via the LDL receptor was obtained in the present study. The uptake of the [^{14}C]-RS-1541/LDL complex correlated well with that of [^{125}I]-LDL, with a correlation coefficient of 0.997. The uptake of the RS-1541/LDL complex was dependent on the LDL-receptor density on the St-4 cells as well as on the incubation temperature and was inhibited by excess amounts of LDL. Monensin, an agent that prevents internalization processes of endocytosis, significantly inhibited the uptake of the complex. The detergent-aided solution of RS-1541, however, showed very low cellular uptake, regardless of the LDL-receptor activities of the cells.

It is well-known that LDL is degraded in lysosomes and that it supplies free cholesterol to the cells from cholesterol esters by the action of hydrolyzing enzymes [8]. In a similar way, after chase-labeling with the RS-1541/LDL complex, a significant amount of rhizoxin, the de-esterified form of RS-1541, was produced in the tumor cells after further incubation. Chloroquine, an inhibitor of lysosomal enzymes, markedly inhibited rhizoxin formation. Thus, RS-1541 was demonstrated to produce rhizoxin, an active metabolite, in the lysosomes after internalization.

The same observations were applied to the cytotoxicity of RS-1541. The cytotoxicity of the RS-1541/LDL complex against St-4 cells was also dependent on the expression of surface receptors for LDL on the cells. It was markedly reduced by excess amounts of LDL, monensin, and chloroquine. Furthermore, the detergent-aided solution of RS-1541 showed very low cytotoxicity, irrespective of the LDL receptor activities of the cells. These results led to the conclusion that the RS-1541/LDL complex is incorporated into St-4 cells via the LDL receptors and that it displays cytotoxic activity after forming rhizoxin in the lysosomes.

It should be noted that our complex was obtained simply by incubating human LDL with the detergent-aided RS-1541 solution. We recently demonstrated that 99% of RS-1541 binds to human lipoproteins after incubation with human serum and that 10% of the total drug distributes to the LDL fraction [25]. Therefore, it is likely that a complex will be produced in plasma after administration of the detergent-aided solution of RS-1541 to patients, raising the possibility for the use of LDL as an endogenous targeting carrier of RS-1541 into tumor cells, which have higher LDL-receptor activities. Considering that RS-1541 is stable and less toxic in plasma [24], it would be expected to produce fewer adverse effects in peripheral tissues than those caused by rhizoxin. Recently, it was reported that the neurotoxic side effects commonly seen during conventional vincristine therapy appeared to be reduced in cancer patients treated with a complex of vincristine with LDL [5], suggesting the possible low toxicity of an agent delivered by the LDL-receptor pathway.

Our results indicate that the chemical modification of a chemotherapeutic agent to give it high lipophilicity will enable it to bind to lipoproteins after intravenous administration. These modifications have advantages for clinical use, as there is no need for elaborate preparation of an LDL complex of the agent.

References

1. Bilheimer DW, Eisenberg S, Levy RI (1972) The metabolism of very low density lipoproteins. I. Preliminary in vitro and in vivo observations. Biochim Biophys Acta 260: 212
2. Blomhoff R, Drevon CA, Eskild W, Helgerud P, Norum KR, Berg T (1984) Clearance of acetyl low density lipoprotein by rat liver endothelial cells. J Biol Chem 259: 8898
3. Brown MS, Dana SE, Goldstein JL (1975) Receptor-dependent hydrolysis of cholesterol esters contained in plasma low density lipoprotein. Proc Natl Acad Sci USA 72: 2925
4. Burstein M, Scholnick HR (1973) Lipoprotein-polyanion-metal interaction. Adv Lipid Res 11: 67
5. Filipowska D, Filipowski T, Morelowska B, Kazanowska W, Laudanski T, Lapinjoki S, Åkerlund M, Breeze A (1992) Treatment of cancer patients with a low-density-lipoprotein delivery vehicle containing a cytotoxic drug. Cancer Chemother Pharmacol 29: 396

6. Gal D, Ohashi M, MacDonald PC, Buchsbaum HJ, Simpson ER (1981) Low-density lipoprotein as a potential vehicle for chemotherapeutic agents and radionucleotides in the management of gynecologic neoplasms. Am J Obstet Gynecol 139: 877
7. Goldstein JL, Brown MS (1974) Binding and degradation of low density lipoproteins by cultured human fibroblasts. J Biol Chem 249: 5153
8. Goldstein JL, Brown SB (1977) The low-density lipoprotein pathway and its relation to atherosclerosis. Annu Rev Biochem 46: 897
9. Havel RJ, Eder MA, Bradgon SM (1955) The distribution and chemical composition of ultracentrifugally separated lipoproteins. J Clin Invest 34: 1345
10. Iwasaki S, Kobayashi H, Furukawa J, Namikoshi M, Okuda S, Sato Z, Matsuda I, Noda T (1984) Studies on macrocyclic lactone antibiotics. VII. Structure of a phytotoxin rhizoxin produced by *Rhizopus chinensis*. J Antibiot 37: 354
11. Kerr DJ, Bisset D, Graham.M, Setanoians A, Chadwick G, Cassidy J, Henrar R, Kaye SB (1992) A phase I and pharmacokinetic study of rhizoxin (abstract). Proceedings, 7th NCI-EORTC Symposium on New Drugs in Cancer Therapy, Amsterdam, March 17–20, p 120
12. Kobayashi T, Sasagawa K, Hirai K, Nishimura T, Tsuruo T, Iwasaki S, Tsukagoshi S, Okuda S (1989) Antitumor activity of new antitumor antibiotic derivatives, acylated rhizoxins. Proceedings, 16th International Congress of Chemotherapy, Jerusalem, June 11–16, p 739
13. Lombardi P, Norata G, Maggi FM, Canti G, Franco P, Nicolin A, Catapano AL (1989) Assimilation of LDL by experimental tumours in mice. Biochim Biophys Acta 1003: 301
14. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ (1951) Protein measurment with the Folin phenol regent. J Biol Chem 93: 265
15. Lundberg B (1992) Assembly of prednimustine low-density-lipoprotein complexes and their cytotoxic activity in tissue culture. Cancer Chemother Pharmacol 29: 241
16. Noda T, Hashiba T, Sato Z (1980) The structural changes in young swollen roots of rice seedlings infected with *Rhizopus chinensis* Saito. Ann Phytopathol Soc Jpn 46: 40
17. Norata G, Canti G, Ricci L, Nicolin A, Trezzi E, Catapano AL (1984) In vivo assimilation of low density lipoproteins by a fibrosarcoma tumour line in mice. Cancer Lett 25: 203
18. Peterson CO, Masquelier M, Rudling M, Söderberg-Reid K, Vitols S (1991) Lipoproteins, malignancy, and anticancer agents. In: Shaw JM (ed) Lipoproteins as carriers of pharmacological agents. Marcel Dekker, New York, p 175
19. Samadi-Baboli M, Favre G, Bernadou J, Berg D, Soula G (1990) Comparative study of the incorporation of ellipticine-esters into low density lipoprotein (LDL) and selective cell uptake of drug-LDL complex via the LDL receptor pathway in vitro. Biochem Pharmacol 40: 203
20. Samadi-Baboli M, Favre G, Canal P, Soula G (1993) Low density lipoprotein for cytotoxic drug targeting: improved activity of an elliptinium derivative against B-16 melanoma in mice. Br J Cancer 68: 319
21. Takahashi M, Iwasaki S, Kobayashi H, Okuda S (1987) Studies on macrocyclic lactone antibiotics. XI. Anti-mitotic and anti-tubulin activity of new antitumor antibiotics, rhizoxin and its homologues. J Antibiotics 40: 66
22. Takahashi M, Iwasaki S, Kobayashi H, Okuda S, Murai T, Sato Y (1987) Rhizoxin binding to tubulin at the maytansine-binding site. Biochim Biophys Acta 926: 215
23. Takatori T, Koizumi T, Tokui T, Shiraishi A, Tsuruo T (1994) Intracellular activation and cytotoxic action of RS-1541 (palmitoyl-rhizoxin) against cultured human tumor cells. Cancer Chemother Pharmacol 35: 283
24. Tokui T, Kuroiwa C, Tokui Y, Sasagawa K, Kawai K, Kobayashi T, Ikeda T, Komai T (1994) Targeting of an antitumor agent, RS-1541 (palmitoyl-rhizoxin) via low-density lipoprotein receptor. Biopharm Drug Dispos 15: 93
25. Tokui T, Tokui Y, Ishigami M, Tanzawa K, Ikeda T, Komai T (1994) Contribution of serum lipoproteins as carriers of antitumor agent: RS-1541 (palmitoyl-rhizoxin) in mice. Int J Pharm 110: 277
26. Tsuruo T, Oh-hara T, Iida H, Tsukagoshi S, Sato Z, Matsuda I, Iwasaki S, Okuda S, Shimizu F, Sasagawa K, Fukami M, Fukuda K, Arakawa M (1986) Rhizoxin, a macrocyclic lactone antibiotic, as a new agent against human and murine tumor cells and their vincristine-resistant sublines. Cancer Res 46: 381
27. Vitols S, Söderberg-Reid K, Masquelier M, Sjöström B, Peterson CO (1990) Low density lipoprotein for delivery of a water-insoluble alkylating agent to malignant cells. In vitro and in vivo studies of a drug-lipoprotein complex. Br J Cancer 62: 724
28. Vitols S, Peterson C, Larsson O, Holm P, Åberg B (1992) Elevated uptake of low density lipoproteins by human lung cancer tissue in vivo. Cancer Res 52: 6244