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Antitumoral activity of low density lipoprotein-aclacinomycin complex in mice bearing H_{22} tumor

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INTRODUCTION

Cancer cells, which proliferate rapidly need large amounts of cholesterol for new membrane synthesis, and high LDL receptor (LDLR) activity. LDL has been proposed as a useful discriminatory vehicle for the delivery of cytotoxic drugs to tumor cells[1,2].

LDL presents many advantages as drug carriers that may circumvent many problems encountered with synthetic carriers[3,4]: ① LDL may deliver highly lipophilic compounds with promising cytotoxic effect. On the other hand, the seques trated drug is protected from enzymatic action. ② Furthermore, LDL is not clear ed by the monocytic macrophagic system and may prolong the serum half-life of antineoplastic drugs. ③ Tumor cells internalise and degrade LDL by the LDL rece ptor pathway. This potentially high efficient process may lead to different phar macological effects.

It is possible to incorporate aclacinomycin (ACM) into LDL to form low-density lipoprotein-aclacinomycin (LDL-ACM) complex[5,6]. In our recent study, the therapeutic activity of LDL-ACM complex and that of ACM were compared in experimental mice tumor with the potential effect of LDL-ACM complex.

MATERIALS AND METHODS

Materials

Male or female KM mice, 6-8 weeks old, were purchased from Experimental Animal C enter of Shandong Medical University. Murine Hepatoma 22 (H_{22}) cells were bought from Pharmaceutical Institute, Shandong Academy of Medical Sciences, and ACM from Shenzhen Wanle Pharmaceutial Co., Ltd.

Lipoproteins

Human LDL (density, 1.019 kg/L - 1.063 kg/L) was isolated by ultra centrifugation of serum from healthy blood donors[7]. The isolated LDL was dialyzed against LDL buffer (Na_2HPO_4 50 mmol/L, NaCl 100 mmol/L, Na_2EDTA 0.1 g/L, pH7.4) for 48 h, filtered through a me mbrane filter (Millipore, 0.22 μm pore size) and stored at 4 ℃. Lipoprotein purity was assessed by agarose gel electrophoresis. All concentrations of LDL given refer to protein. The protein concentration was determined according to the method of Lowry *et al*.

Incorporation of ACM into LDL[5]

ACM was incorporated into LDL by incubating 36 mg LDL with 40 mg ACM in LDL buffer for 3 h at 40 ℃. The mixture was then subjected to extensive dialysis for 48 h aganist LDL buffer to remove free drug and thereafter filtered and stored at 4 ℃. The LDL-ACM complex obtained had a drug: LDL protein weight ratio 0.35. The concentrations of LDL-ACM complex given in the text refer to ACM.

Assay for ACM

ACM was assayed fluorometrically in a HITACHI spectrophotofluorometer model F-3000 using 430 nm and 580 nm as excitation and emission wavelengths, respectively[5]. ACM was extracted with chloroform: methanol (4:1 V/V). When LDL-ACM complex was assayed, the standards also contained LDL.

Therapeutic activity of LDL-ACM complex

On day 0, H_{22} cells (2×10^6) were injected subcutaneously in the right upper armpit of KM mice. Free ACM (3.5 mg/kg) or LDL-ACM complex (3.5 mg/kg) was given intravenously in the tail vein q.d. on days 1-7. After 9 days, the animals were killed. Tumor and body weights were

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recorded.

On day 0, H_{22} cells (2×10^5) were injected intraperitoneally in KM mice. Free ACM (3.5 mg/kg) or LDL-ACM complex (3.5 mg/kg) was given intraperitoneally q.d. on days 1-7. Then survival times and body weights of mice were recorded.

In both types of experiments, eight mice were used for each schedule of administration and controls.

Statistical analysis

Results are expressed as $\bar{x}\pm s$. Difference between mean values was compared by the Student's *t* test.

RESULTS

Antitumoral activity of LDL-ACM complex in mice bearing subcutaneous H_{22} tumor (Table 1)

When given intravenously, the complex had a marked therapeutic effect on mice with subcutaneous H_{22} tumor ($P<0.05$), whereas ACM had not. The average weight for mice in the group of LDL-ACM complex or ACM lost less during the treatment period.

Table 1 Antitumoral activity of LDL-ACM complex and free ACM agains t murine subcutaneous H_{22} tumor

Drugs	Mean weight change (g)	Tumor weight ($\bar{x}\pm s$, g)	Inhibition rate (%)
Control	+5.4	1.74±0.60	
Free ACM	+3.2	1.30±0.57	25.3
LDL-ACM	+3.5	0.86±0.44	50.2

Antitumoral activity of LDL-ACM complex in mice bearing intraperitoneal H_{22} tumor (Table 2)

The survival times of mice bearing intraperitoneal H_{22} tumor were all significantly prolonged in the groups of ACM ($P<0.05$) and LDL-ACM complex ($P<0.01$) when compared with that in the group of control. Administrat ion of LDL-incorporated ACM resulted in a significant increase in antitumoral a ctivity at the same dose as compared with the free ACM ($P<0.05$). Abnor mality in body weight has been noted in any lot of mice.

Table 2 Antitumoral activity of LDL-ACM complex and free ACM agains t murine intraperitoneal H_{22} tumor

Drugs	Survival time ($\bar{x}\pm s$, d)	Life-prolonging rate (%)
Control	17.1±3.44	
Free ACM	24.6±7.50	43.9
LDL-ACM	33.4±7.67	95.3

DISCUSSION

An LDL-ACM complex containing 212 drug molecules per LDL particle could be obtained by incubating LDL with a large excess of ACM at 40 ℃. High-performance liquid chromatography studies of the mixture before and after the incubation showed that ACM was stable under these conditions[5].

We investigated antitumoral activity of LDL-ACM complex in mice bearing H_{22} tumor. Results showed that entrapment of ACM into LDL particles increased the antitumoral activity as compared with the free ACM in H_{22} tumor model. The improved antitumoral activity could be the fact that the catabolism of the complex was related to the LDLR pathway. By LDLR pathway, tumor tissue took up more ACM by the LDL-ACM complex than normal tissue. Such targeting could lead to in creased exposure of ACM to the tumor and consequently, increase the antitumoral efficacy.

LDL-drug complex might result in lessions in the organs like the liver and adrenals known to be the normal tissues with the highest LDL uptake. This problem might be circumvented since animal studies indicate that it is possible to down-r egulate the LDL-uptake in these organs by pretreatment with bile acids and steroids without affecting the uptake by the tumor[8]. Acetyl LDL (Ac-LDL) and Methyl LDL (Me-LDL) are rapidly cleared from the plasma. The endothelial cells of the liver have scavenger receptors and can remove these modified lipoproteins from the circulation. The endothelial cells of the spleen, bone marrow, adrenal, and ovary also participate in this rapid clearance. Internalization of modified LDL leads to the accumulation of large amounts of cholesteryl esters in the cells, which decreases the LDLR activity of these cells. Modified LDL does not affect the tumor LDLR activity. So giving Ac-LDL or Me-LDL in advance may protect the normal tissue having rich LDL receptors from LDL-drug complex during t he treatment of tumors[9,10].

This study has been performed with human LDL. Even if human LDL is recognised by the animal's receptor[11], the competition from endogenous LDL is very different from the one in the human situation since there are pronounced species differences in the plasma lipoprotein pattern and metabolism. Therefore, the results must be interpreted with caution. It would be suitable to use an anim al model close to the human one for the lipoprotein metabolism such as hamster or guinea pigs.

We used a technique which allowed a high entrapment level of lipophilic ACM into LDL. The

cytotoxic LDL-ACM complex so formed exhibited an increasing antitumoral activity in a murine tumor model. Further human clinical trials will depend o n a better knowledge of the expression and the regulation of the LDL receptors by the tumor cells. Studies in this optic are now in progress in our department.

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