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Notes & tips

Lipid-based transfection reagents can interfere with cholesterol biosynthesis

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Lipid-based transfection reagents are widely used for delivery of small interfering RNA into cells. We examined whether the commonly used commercial transfection reagents DharmaFECT-4 and Lipofectamine 2000 can interfere with lipid metabolism by studying cholesterogenesis. Cholesterol de novo synthesis from [^{14}C]acetate was assessed in human hepatocyte-derived Huh-7 cells. The results revealed that DharmaFECT, but not Lipofectamine, markedly inhibited cholesterol biosynthesis by approximately 70%. Cell viability was not significantly altered. These findings suggest that caution is required in the choice of certain lipid-based transfection reagents for gene silencing experiments, particularly when assessing cholesterol metabolism.

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Studies in culture cells make use of lipid-based transfection reagents to transfect small interfering RNA (siRNA).[1] In this study, we examined whether the commonly used commercial transfection reagents Lipofectamine and DharmaFECT can affect the cellular synthesis of cholesterol.

The human hepatocyte-derived cell line Huh-7 (American Type Culture Collection) was maintained at 37 °C under a 5% CO_2 atmosphere in Dulbecco's modified Eagle's medium (DMEM, Gibco) supplemented with 100 U/ml penicillin and 100 mg/ml streptomycin and 10% fetal bovine serum (FBS). The medium was changed every other day, and cells were trypsinized after reaching confluency [1]. For the experiments, the cells were seeded in 6-well tissue culture plates and grown for 24 h until they reached approximately 70% confluence (i.e., ~300,000 cells/well). Cells were treated with 8 μl of Lipofectamine 2000 (Thermo Fisher Scientific) or 5 μl of DharmaFECT-4 (Dharmacon) alone following the corresponding manufacturer's protocol for siRNA transfection. Controls received no treatment. After 48 h, cells were incubated with 1 μCi [^{14}C]acetate for 4 h to assess cholesterol de novo synthesis [2]. After lipid extraction [3], the resulting [^{14}C]cholesterol was separated by silica thin-layer chromatography with hexane, diethyl ether, and acetic acid (70:30:1) as solvent system. After visualization with iodine vapor and disappearance of the color, the spots were scraped from the plates and ^{14}C quantitated by liquid scintillation counting [2].

Before assessing cholesterol synthesis, some treated and untreated cells were incubated for 6 h with DMEM containing 5% fetal calf lipoprotein-deficient serum (LPDS, Sigma) to stimulate cholesterol synthesis. Lactate dehydrogenase (LDH) activity was used as a measure of cell viability by measuring the enzyme leakage with an LDH assay kit (Wiener Lab, Rosario, Argentina) [1]. Proteins were determined according to Lowry and coworkers using bovine serum albumin as a standard [4].

As shown in Fig. 1, cholesterol de novo synthesis in Huh-7 cells incubated with 10% FBS or 5% LPDS was slightly reduced by Lipofectamine 2000 (~15%), whereas DharmaFECT-4 induced a major inhibition of approximately 70%. According to the LDH leakage, delivery reagents caused very low toxicity in Huh-7 cells (Fig. 1).

Differences in the lipid formulation between Lipofectamine 2000 and DharmaFECT-4 are expected to explain the dissimilar effects of these two reagents on cholesterol synthesis. Most of the lipid-based transfection reagents contain cationic and neutral lipids. Nevertheless, to our knowledge, formulations for Lipofectamine 2000 and DharmaFECT-4 have not yet been disclosed. Interestingly, our findings are in line with recently published data indicating that other commercial lipid-based transfection reagents can directly affect hepatocyte lipid metabolism by altering gene expression [5].

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[1] *Abbreviations used:* siRNA, small interfering RNA; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; LPDS, lipoprotein-deficient serum; LDH, lactate dehydrogenase.

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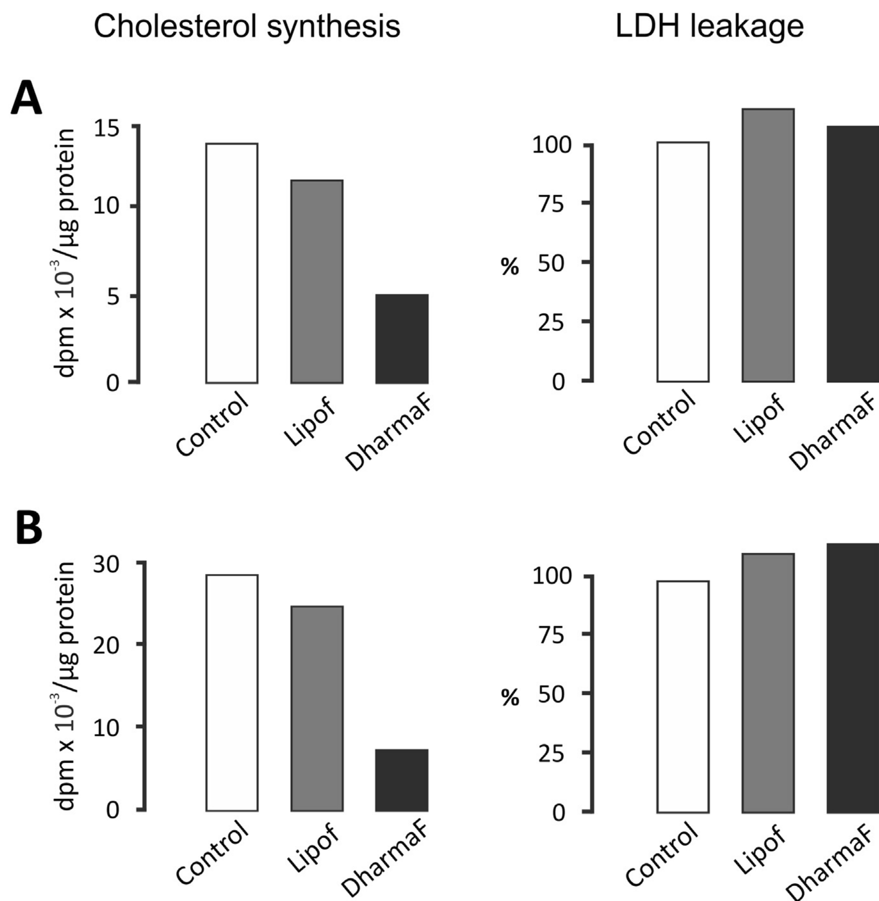

Fig. 1. Human hepatocyte-derived Huh-7 cells were treated with Lipofectamine 2000 (Lipof) or DharmaFECT-4 (DharmaF) reagents following the corresponding manufacturer's protocol for siRNA transfection. Controls were untreated. After 48 h, cells in DMEM containing 10% FBS (A) or 5% LPDS (B) were incubated with [^{14}C]acetate for 4 h to assess cholesterol de novo synthesis. After 48 h of treatment with transfection reagents, cell viability was assessed by LDH leakage assay.

In conclusion, our data indicate that the commonly used commercial transfection reagent DharmaFECT-4, but not Lipofectamine 2000, markedly inhibited cholesterol synthesis in human hepatocyte-derived cells. These findings suggest that caution is required in the choice of certain lipid-based transfection reagents for gene silencing experiments, particularly when studying cholesterol metabolism.

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