Role of Multidrug Resistance P-glycoproteins in Cholesterol Esterification*

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Cholesterol esterification, catalyzed by acyl-CoA:cholesterol acyltransferase (ACAT), plays a central role in cellular cholesterol homeostasis and in physiologic processes that lead to coronary heart disease. Although ACAT resides in the endoplasmic reticulum (ER), the cholesterol substrate for esterification originates in the plasma membrane and must be transported to the ER for esterification. Progesterone inhibits esterification, possibly by blocking the transport of cholesterol to the ER. Recent studies suggest that progesterone acts by inhibiting the activity of one or more of the multidrug-resistant (MDR) P-glycoproteins. In the current manuscript, we demonstrate that progesterone's ability to inhibit esterification is not mediated through the progesterone receptor. We evaluate a series of steroid hormones and find a strong correlation between the steroid hormone's hydrophobicity and its ability to inhibit both cholesterol esterification and MDR-catalyzed drug efflux. We also find that cholesterol esterification is inhibited by nonsteroidal MDR inhibitors, and that this inhibition specifically affects the esterification of cholesterol derived from the plasma membrane. MDR inhibitors also inhibit cholesterol esterification in a wide range of cultured human cell lines. These observations suggest that MDR activity normally functions in a general process of intracellular cholesterol transport.

Cells maintain exquisite control over the level of free cholesterol through a complex set of homeostatic mechanisms that control the rates of cholesterol biosynthesis, cholesterol esterification, and receptor mediated endocytosis of cholesterol-rich low density lipoproteins (LDL); reviewed by Goldstein and Brown (1). Cholesterol esterification is catalyzed by acyl-CoA:cholesterol acyltransferase (ACAT) in the endoplasmic reticulum (ER) and involves the covalent attachment of fatty acids to the 3-position hydroxyl group of cholesterol to form cholesteryl esters. In a homeostatic process, excess free cholesterol stimulates esterification resulting in the storage of excess cholesterol in the form of cytosolic lipid droplets. While free cholesterol can be toxic to cells, cholesteryl esters can accumulate to relatively high levels. Under most physiologic conditions, the rate of cholesterol esterification is not limited by ACAT levels but rather by the availability of cholesterol substrate in the ER (2, 3). Very little cholesterol is found in the ER; 90% of all cholesterol resides in the plasma membrane (4), and most of the remainder is found in endocytic vesicles derived from the plasma membrane (5). Under conditions of excess cholesterol, cholesterol is transported from the plasma membrane to the ER where it is esterified by ACAT. The mechanism by which this transport occurs is poorly understood.

The delivery of plasma membrane cholesterol to the ER is also required for a number of other processes involved in cholesterol homeostasis. HMG-CoA reductase, the rate-limiting enzyme of cholesterol biosynthesis, resides in the ER even though both its substrate and product are cytosolic. Excess free cholesterol decreases HMG-CoA reductase activity by enhancing the rate of enzyme degradation. This enhanced rate of degradation requires the transmembrane portion of the protein that anchors it to the ER (6). In addition, DNA-binding proteins (SREBPs) that control transcription of HMG-CoA reductase, the LDL receptor, and a number of other enzymes involved in cholesterol metabolism are originally synthesized with a membrane spanning domain that anchors them to the ER (7). When sterol levels fall, specific proteolysis of SREBPs releases the DNA-binding domains which then enter the nucleus to promote transcription of sterol-regulated genes. Finally, enzymes involved in the final steps of cholesterol biosynthesis reside in the ER, and the delivery of sterol precursors to these ER-resident enzymes is required for the final steps of cholesterol biosynthesis (8, 9). Although the delivery of sterols to the ER clearly plays an important role in the regulation of cholesterol metabolism, very little is known about how sterols are transported to the ER and how this transport is regulated.

The distribution of sphingomyelin within the cell closely mimics that of cholesterol; greater than 90% of cellular sphingomyelin is found in the plasma membrane and very little is found in the ER (4). Cholesterol and sphingomyelin interact with high affinity in model membranes, and it has been suggested that this interaction is responsible for localizing cholesterol to the plasma membrane (10). In fact, reducing plasma membrane sphingomyelin levels by adding exogenous sphingomyelinase, releases cholesterol from the plasma membrane allowing it to enter intracellular pools which leads to increased cholesterol esterification (11) and HMG-CoA reductase degradation (11, 12).

Progesterone has long been known to inhibit cholesterol esterification, and early studies suggested that this inhibition resulted from a direct interaction between progesterone and ACAT (13). However, more recent studies suggest that progesterone...
terone may indirectly block esterification by preventing delivery of cholesterol substrate to ACAT. In cultured cells, progesterone blocks transport of cholesterol from the plasma membrane to the ER (14) and causes sequestration of cholesterol within the lysosome (15). Progesterone interacts with two cellular proteins: the progesterone receptor (reviewed by Evans (16)) and members of the multiple-drug resistance (MDR) family of P-glycoproteins (17). Interaction of progesterone with the progesterone receptor alters the rate of transcription of progesterone-sensitive genes. Cellular events initiated by the progesterone receptor: 1) occur at nanomolar progesterone concentrations, 2) are inhibited by RU486, a specific antagonist of the progesterone receptor, and 3) demonstrate a characteristic lag due to the time required for altered transcriptional events to be manifested in altered protein and activity levels.

Progesterone also interacts with members of the MDR family of P-glycoproteins. MDR proteins act as energy-dependent drug efflux pumps that reduce drug cytotoxicity when overexpressed (reviewed by Ling (18)). Progesterone binds directly to the MDR pump to inhibit its activity (17, 19). In multidrug-resistant J774.2 cell lines, a number of other steroids, including deoxycorticosterone, testosterone, and corticosterone, also inhibit MDR activity, and although no specific structure explains their relative effectiveness, a correlation has been observed between steroid hydrophobicity and inhibition of MDR activity (17).

In the current studies, we exclude a role for the progesterone receptor in progesterone’s ability to inhibit cholesterol esterification. Rather, we show that progesterone’s ability to inhibit MDR activity is directly correlated with the inhibition of cholesterol esterification. We find that this inhibition results from a direct inability to esterify plasma membrane-derived cholesterol, and that these phenomena are observed in a wide range of cultured human cell lines. These findings, in conjunction with our recent observations that MDR activity is required for cholesterol biosynthesis (20, 21), suggest a general role for MDR in normal cellular cholesterol metabolism.

EXPERIMENTAL PROCEDURES

Materials—Newborn calf lipoprotein-deficient serum (d > 1.215 g/ml; cholesterol content of 33–61 μg/ml) and rabbit β-VLDL were prepared by ultracentrifugation as described (22). Progesterone, oleic acid, and human LDL were purchased from Sigma. Steroid hormones were purchased from Steraloids Inc. RU486 was the kind gift of Dr. Raymond Daynes (University of Utah). [9,10,13-3H]Oleic acid (5–8 Ci/mmol) and [3H]vinblastine sulfate (11 Ci/mmol), [1,2,6-3H]cholesterol (47 Ci/mmol), and [4-14C]cholesterol (52 mCi/mmol) were purchased from Amersham Life Sciences. Human cell lines were obtained from the American Type Culture Collection (ATCC). Other materials were obtained from previously reported sources (20, 21, 23, 24).

Cell Culture—All cells were grown in monolayer at 37°C in an atmosphere of 5% CO₂. CHO cells were maintained in medium A (1:1 mixture of Ham’s F12 medium and Dulbecco’s modified Eagle’s minimum essential medium containing 100 units/ml penicillin, 100 μg/ml streptomycin, and 5% (v/v) newborn calf lipoprotein-deficient serum). HeLa and Chang liver cells, initially grown in medium B (Dulbecco’s modified Eagle’s minimum essential medium with 100 units/ml penicillin, 100 μg/ml streptomycin, containing 10% (v/v) fetal calf serum) were gradually adapted to growth in medium A. CaCo2 and HepG2 cells did not grow well in medium A and consequently were maintained in medium B. At least 2 days prior to assay, all cell lines were switched to growth in medium A. Sterols and progesterone were added to the culture medium in ethanol; the final ethanol concentration did not exceed 0.5% (v/v).

Assays—The incorporation of [3H]oleate into cholesteryl [3H]oleate and [3H]triglyceride by cell monolayers was determined as described previously (22). Steroid hydrophobicity was estimated by measuring retention fraction (Rf) on Silica Gel G (Analtech) thin layer chromatography sheets developed in chloroform:methanol (12:1 v/v). Vinblastine accumulation was measured as described previously (21).

The plasma membrane cholesterol pools of cell monolayers were labeled with [3H]cholesterol at 15°C for 20 min as described previously (14). Equilibrium labeling of cellular cholesterol pools was accomplished by continuously growing cells in [3H]cholesterol for 14–20 h. To measure the amount of [3H]cholesterol incorporated into [3H]cholesteryl esters, labeled monolayers were washed twice with 2 ml of ice-cold 5 mM Tris HCl (pH 7.4), 150 mM NaCl, 0.2% bovine serum albumin, and once with 2 ml of ice-cold 5 mM Tris HCl (pH 7.4), 150 mM NaCl. One milliliter of ice-cold hexane:isopropanol alcohol (3:2 v/v) containing 10 μl of a recovery solution (200 cpm of [14C]cholesterol, 4 μg/ml cholesterol, 2 μg/ml cholesteryl oleate in chloroform:methanol (2:1 v/v)) was added to each well. After 30 min, the steroids were transferred to 13 x 100 mm tubes, dried, resuspended in hexane:isopropanol alcohol, and resolved on Silica Gel thin layer chromatography (TLC) plates in heptane:ether:acetic acid (20:5:1 v/v/v). Cholesteryl ester and cholesterol bands were scraped and counted using a liquid scintillation counter. The amount of cholesterol converted to cholesteryl ester is reported as a percentage of the total cell-associated [3H]sterol after subtracting the background counts observed in parallel incubations that lacked cells.

RESULTS

The Progesterone Receptor Does Not Mediate the Effects of Progesterone on Cholesterol Esterification—To determine if the progesterone receptor is involved progesterone’s ability to inhibit cholesterol esterification, we tested for three characteristics of progesterone receptor-mediated events. Progesterone receptor-mediated events typically: 1) occur at nanomolar concentrations, 2) are prevented by RU486, a specific antagonist of the progesterone receptor, and 3) demonstrate a characteristic lag due to the time required for altered transcriptional events to be manifested in altered protein and activity levels. We first measured the concentration of progesterone required to inhibit esterification in intact cells by monitoring the incorporation of [14C]oleate into cholesteryl [14C]oleate. Pretreating CHO cells with progesterone for 2 h had little effect on esterification at concentrations below 1 μM; 50% inhibition occurred at approximately 10 μM progesterone (Fig. 1). This concentration is many orders of magnitude higher than typically required for progesterone receptor-mediated events, suggesting that the progesterone receptor is not involved. Furthermore, addition of RU486 failed to prevent inhibition by progesterone (Fig. 1) as would be expected if the progesterone receptor was involved. Finally, to test for the characteristic time lag associated with progesterone receptor-mediated events, we preincubated cells...
with progesterone for various lengths of time prior to measuring the rate of cholesterol esterification (Table I). In the absence of β-VLDL, CHO cells esterified cholesterol at a modest rate that increased nearly 7-fold upon addition of β-VLDL. Preincubation with 40 μM progesterone for 6 h reduced the rate of cholesterol ester synthesis more than 10-fold, both in the absence and presence of β-VLDL. This inhibition was observed even when progesterone was added simultaneously with \([^{3}H]\)oleate, demonstrating that there is no detectable lag between the time of progesterone addition and its effects on cholesterol esterification. Taken together, these findings demonstrate that the progesterone receptor is not involved in progesterone’s ability to inhibit cholesterol esterification.

**Correlations between Steroid Hydrophobicity, MDR Activity, and Cholesterol Esterification**—We next compared progesterone to other steroids in their ability to inhibit the incorporation of \([^{3}H]\)oleate into cholesteryl \([^{3}H]\)oleate and \([^{3}H]\)triglyceride (Fig. 2). Many of the steroids inhibited esterification (panel A). Progesterone was the most potent, followed by androsterone, pregnenolone, dehydroepiandrosterone (DHEA), and deoxycorticosterone. In contrast, cortisol, DHEA-sulfate, and pregnenolone-sulfate had little or no effect. As a control, none of the steroids significantly affected the incorporation of \([^{3}H]\)oleate into \([^{3}H]\)triglycerides (panel B). Although there was no obvious correlation between the potency of the steroids and specific chemical structures, we did note a direct relationship between potency and the steroid’s general hydrophobicity as estimated by the retention fraction \((R_f)\) in thin layer chromatography (Fig. 3). More hydrophobic steroids migrated further up the TLC sheet, had higher \(R_f\) values, and were more potent inhibitors of cholesterol esterification. This relationship was evident at steroid hormone concentrations of 10 μM (panel A) and 40 μM (panel B).

Steroid hormones have previously been shown to bind to MDR P-glycoproteins and inhibit their activity (17, 19). From a series of four steroid hormones, Yang et al. (17) concluded that the ability of a steroid to inhibit MDR activity is correlated with the general hydrophobicity of the steroid. To confirm and extend these observations, we measured the ability of 12 steroid hormones to inhibit MDR-mediated drug efflux (Fig. 4) using a \([^{3}H]\)vinblastine accumulation assay. Vinblastine passively enters cells and is pumped out by MDR. The rate of drug efflux dictates the steady-state intracellular level of vinblastine; inhibiting MDR decreases drug efflux and consequently increases the intracellular accumulation of \([^{3}H]\)vinblastine. Using this assay, we measured the accumulation of \([^{3}H]\)vinblastine in the presence of either 10 μM (panel A) or 40 μM (panel B) of each of the steroids listed in Fig. 2. We observed a strong correlation between the hydrophobicity of a steroid and its ability to inhibit MDR pump activity; steroids with large \(R_f\) values were more potent inhibitors of MDR activity. This relationship was evident at steroid hormone concentrations of 10 μM (panel A) and 40 μM (panel B).

To more directly demonstrate a correlation between cholesterol esterification and MDR activity, we simultaneously assayed cholesterol esterification and vinblastine accumulation in cells treated with various steroid hormones at either 10 μM (Fig. 4, panel C) or 40 μM (panel D). A good correlation is observed at both steroid concentrations; steroids that were potent inhibitors of esterification were also potent inhibitors of vinblastine efflux. These correlations suggest that one or more members of the MDR family of proteins may be required for cholesterol esterification. To test this hypothesis, we measured cholesterol esterification in cells treated with nonsteroidal in-

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**Table I**

| Preincubation in progesterone | Cholesteryl \([^{3}H]\)oleate formed | \(\text{nmol h}^{-1}\text{mg protein}^{-1}\) 
|-----------------------------|---------------------------------|----------------------|
|                             | \(-\beta\)-VLDL                  | \(+\beta\)-VLDL       |
| No Progesterone             | 2.4                             | 16.5                 |
| 0 h                         | 0.3                             | 0.5                  |
| 1 h                         | 0.2                             | 0.4                  |
| 3 h                         | 0.2                             | 0.5                  |
| 6 h                         | 0.2                             | 0.6                  |

**Fig. 2. Effect of other steroid hormones on cholesterol esterification.** Cells were plated for experiments on day 0 and grown through day 2 as described in the legend to Fig. 1. On day 3, cells were refed 0.5 ml of the medium A containing 30 μg/ml β-VLDL and the indicated additions of steroid hormones. Cells were then labeled for 2 h with 0.4 mCi \([^{3}H]\)oleate and extracted for cholesteryl \([^{3}H]\)oleate and \([^{3}H]\)triglyceride as described in the legend to Fig. 1.
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FIG. 3. Correlations between steroid hydrophobicity and cholesterol esterification. Cells were plated and grown through day 2 as described in the legend to Fig. 2. On day 3, cells were refed 0.5 ml of the medium A containing 30 μg/ml β-VLDL and either 10 μM (panel A) or 40 μM (panel B) of the same steroid hormones listed in Fig. 2. Steroid hydrophobicity was estimated by measuring retention fraction (Rf) on Silica Gel G (Analtech) thin-layer chromatography sheets developed in chloroform:methanol (12:1). Each value represents the average of triplicate incubations. Trendlines and correlation coefficients result from fitting the data using a second order polynomial least-squares method.

FIG. 4. Correlations between steroid hydrophobicity and [3H]vinblastine accumulation. Cells were plated and grown through day 2 as described in the legend to Fig. 1. On day 3, the medium was replaced with medium A containing 20 mM HEPES (pH 7.2). One hour later, cells were refed 0.5 ml of identical medium containing 50 nM [3H]vinblastine as described in the legend to Fig. 3. Parallel cultures were assayed for [3H]vinblastine accumulation as described in the legend to Fig. 4. Each value represents the average of triplicate incubations. Trendlines and correlation coefficients result from fitting the data using a second order polynomial least-squares method.

FIG. 5. Effect of nonsteroidal MDR inhibitors on cholesterol esterification and [3H]vinblastine accumulation. Cholesterol esterification rates were measured as described in the legend to Fig. 3 in cells grown in the presence of the indicated additions of 40 μM progesterone, 40 μM verapamil, and 10 nM Triton X-100. Parallel cultures were assayed for [3H]vinblastine accumulation as described in the legend to Fig. 4. Each value represents the average of duplicate incubations. Although progesterone could act directly on ACAT activity, the fact that these agents are known to interact with MDR and the direct correlations observed between MDR activity and cholesterol esterification make it likely that the effects of these agents are mediated through their effects on MDR activity.

MDR Inhibitors Block Esterification of Plasma Membrane-derived Cholesterol—In order to determine if MDR activity is specifically required for esterification of plasma membrane cholesterol, we used a combination of plasma membrane [3H]cholesterol labeling and sphingomyelinase treatment. Cells were labeled to equilibrium for 16 h with [3H]cholesterol. Under these conditions, the labeled cholesterol assumes a distribution similar to that reported for the endogenous cholesterol pools with greater than 93% of the [3H]cholesterol residing in cellular fractions consistent with plasma membrane localization (data not shown). Six hours prior to harvest, labeled cholesterol was removed and the cells were incubated in either the absence or presence of sphingomyelinase. Exogenously added sphingomyelinase catalyzes the conversion of sphingomyelin to ceramide and phosphocholine in the plasma membrane. This conversion reduces sphingomyelin levels in the membrane and releases cholesterol to enter intracellular pools (25). Sphingomyelinase treatment increased cholesterol esterification in CHO cells over 4-fold (Fig. 6, panel A). A considerable portion of the cholesterol substrate used in this esterification must have derived from the plasma membrane; over 20% of the entire cholesterol pool was esterified while less than 7% of the pool exists outside of the plasma membrane. Progesterone blocked esterification >2-fold in the absence of sphingomyelinase treatment and by >10-fold following sphingomyelinase treatment. The ability of MDR inhibitors to block the effects of sphingomyelinase is not due to inhibition of sphingomyelinase digestion of plasma membrane sphingomyelin. Koval and Pagano (26) have shown that the hydrolysis of fluorescent sphingomyelin to ceramide is associated with the movement of the fluorescent label from the plasma membrane to the Golgi. In control experiments, we have found that progesterone and other MDR inhibitors had no effect on the movement of fluorescent label from the plasma membrane to the Golgi following sphingomyelinase treatment (data not shown).

In order to more specifically demonstrate that MDR inhibitors block the esterification of plasma membrane-derived cho-
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1.6 plates containing 2 ml of medium A. On day 2, oleic acid was added to ice-cold phosphate-buffered saline and fed into [3H]cholesteryl esters as described under “Experimental Procedures.” In panel B, 1 × 10^4 cells were plated on day 0 in 24-well Linbro plates containing 2 ml of medium A. On day 2, oleic acid was added to 1.6 μM, and the cells were incubated an additional 2 h at 37°C. Cells were then incubated in ice-cold phosphate-buffered saline containing [3H]cholesterol at 15°C for 20 min. The cells were then washed with ice-cold phosphate-buffered saline and fed 250 μl of medium A containing the indicated additions of 40 μM progesterone, 40 μM DHEA, 40 μM verapamil, and 50 μl of sphingomyelinase. The cells were then incubated at 37°C for an additional 30 min. Cells were harvested and assayed for the incorporation of [3H]cholesterol into [3H]cholesteryl esters as described under “Experimental Procedures.”

We next tested whether MDR inhibitors also blocked esterification of cholesterol specifically derived from the plasma membrane. In CHO cells, approximately 1% of the cell-associated cholesterol was esterified in this short incubation time. Sphingomyelinase treatment increased esterification 2.5-fold. Progesterone, DHEA, verapamil, or β-estradiol completely blocked the esterification of [3H]cholesterol by these agents. These results demonstrate that steroidal and nonsteroidal MDR inhibitors block esterification of cholesterol specifically derived from the plasma membrane.

MDR Inhibitors Block Cholesterol Esterification in Other Cell Types—We next tested whether MDR inhibitors also blocked esterification in human cell lines (Fig. 7). LDL stimulated esterification by >4-fold in CHO cells. Progesterone inhibited esterification in the presence of LDL >10-fold, while verapamil inhibited over 4-fold. Similar results were observed in HeLa cells which derive from a human cervical carcinoma. Although LDL failed to stimulate esterification to the same degree in two cell lines derived from human liver (HepG2 and Chang liver cells), both progesterone and verapamil were potent inhibitors of esterification in these cells. LDL completely failed to stimulate esterification in CaCo2 cells, a human cell line derived from intestinal epithelia. There was no apparent change in the rate of esterification following treatment with either progesterone or verapamil in CaCo2 cells. These results demonstrate that in human cells that responded to LDL, esterification can be inhibited by both steroidal (progesterone) and nonsteroidal (verapamil) inhibitors of MDR. These results further suggest that MDR plays a central role in a general process required for cholesterol esterification.

DISCUSSION

MDR P-glycoproteins were originally identified for their ability to confer drug resistance when overexpressed; however, their normal physiologic function is not well understood. The ability of P-glycoproteins to catalyze the efflux of unnatural drugs led to the hypothesis that MDR normally functions in detoxification. Support for this hypothesis comes from studies of mice with a homozgyous disruption of the mdr3 gene which is primarily expressed at the blood-brain barrier. These mice are phenotypically normal unless challenged with drugs; the mice demonstrate increased sensitivity to amphiphilic drugs, especially in the brain (27). Other studies suggest that the normal physiologic role of MDR is to catalyze the transport of normal cellular lipids across membranes. Mice with a homozygous disruption of mdr2, which is expressed predominantly in bile canicula (28), develop severe liver disease due to a lack of phosphatidylcholine and cholesterol production into bile (29). In addition, mdr2 catalyzes the ATP-dependent translocation of phosphatidylcholine across secretory vesicles when expressed in yeast (30). Targeted disruption of the mdr1 (mdr1b) gene in a mouse adrenal cell line decreased the stimulated secretion of steroid hormones from the cell (31). These experiments strongly suggest that MDR proteins catalyze the transport of normal cellular lipids across membranes.

A number of recent lines of evidence suggest that MDR activity is required for cholesterol esterification. A series of amphiphilic agents, known to modulate MDR activity, blocked transport of cholesterol substrate from the plasma membrane to ER-resident ACAT in a cultured rat hepatoma cell line (32). However, the cell line used has never been shown to have MDR activity. Known inhibitors of MDR also inhibited the movement of cholesterol from the plasma membrane to the ER in CaCo2 cells (33), which are known to express MDR activity. Although
these studies suggest a role for MDR in cholesterol esterification, no direct correlation between MDR activity and cholesterol esterification has yet been provided.

In the current studies, we exclude a role for the progesterone receptor in cholesterol esterification and demonstrate a direct correlation between measured MDR activity and cholesterol receptor in cholesterol esterification and demonstrate a direct correlation between measured MDR activity and cholesterol receptor in cholesterol esterification and demonstrate a direct correlation between measured MDR activity and cholesterol receptor in cholesterol esterification and demonstrate a direct correlation between measured MDR activity and cholesterol receptor. This correlation is observed with both steroidal and nonsteroidal MDR inhibitors. Since progesterone, verapamil, and β-estradiol are all known to physically interact with MDR to inhibit its activity (19, 34), the most likely explanation for the observed correlation is that changes in cholesterol esterification are a consequence of the effect of these inhibitors on MDR activity. However, our studies do not formally exclude the possibility that cholesterol esterification is required for MDR activity or that both processes are sensitive to common change in membrane composition introduced by amphiphilic compounds.

We also demonstrate a direct correlation between MDR activity and cholesterol esterification in a number of cultured human cell lines. These results extend previous observations by implicating MDR as being involved in a general cellular process that is required for esterification. Recently we have demonstrated that MDR activity is also required for the late steps of cholesterol biosynthesis (20, 21), a process that requires the transport of sterol precursors from the plasma membrane to the ER (8, 9). MDR inhibitors also block cholesterol biosynthesis in a wide range of cultured human cell lines, supporting the observation that MDR activity is required for a general cellular process involved in sterol transport. Although these studies demonstrate that MDR is required for cholesterol esterification, further studies are required to determine the mechanism by which it participates in this process.

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