The effect of lidocaine on cholesterol influx, esterification, and accumulation in cultured cells

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Lidocaine, a local anesthetic, inhibited cholesterol esterification in various cultured cells derived from tissues of the mouse, rat, hamster, monkey, and man. Esterification of exogenous [1-^{14}C]oleate, fatty acid synthesized *in situ* from [1-^{14}C]acetate, and exogenous [7-^{3}H]cholesterol was reduced 20–50% with 1.5 m*M* lidocaine in the culture medium. In Fu5AH rat hepatoma cells, incubated for 24 h with hyperlipemic serum lipoproteins and increasing levels of lidocaine up to 1.5 m*M*, unesterified (free) cholesterol mass of the cells increased about 25% whereas the cholesteryl ester mass fell about 40%. The net result was a reduction in total cellular sterol. When the cells were incubated with lidocaine and hyperlipemic serum lipoproteins labeled with [7-^{3}H(N)]cholesterol of known specific activity, incorporation of exogenous free cholesterol into cellular free cholesterol was constant, whereas there was a dose-dependent reduction in the amount of exogenous cholesterol appearing in cholesteryl esters. Additionally, a comparison of specific activities of cellular free cholesterol and cholesteryl esters at intervals over a 24-h period suggests that lidocaine also inhibits lysosomal cholesterol ester hydrolase (EC 3.1.1.13) in the Fu5AH cell line.

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La lidocaïne, un anesthésique local, inhibe l'estérification du cholestérol dans diverses cellules cultivées provenant des tissus de la souris, du rat, du hamster, du singe et de l'homme. En présence de lidocaïne 1,5 m*M* dans le milieu de culture, l'estérification du [1-^{14}C]oléate exogène, un acide gras synthétisé *in situ* à partir du [1-^{14}C]acétate et du [7-^{3}H]cholestérol exogène est réduite de 20 à 50%. Dans les cellules de l'hépatome de rat Fu5AH incubées durant 24 h avec des lipoprotéines d'un sérum hyperlipémique et des taux de lidocaïne croissant de 0 à 1,5 m*M*, la masse du cholestérol non estérifié (libre) des cellules augmente d'environ 25% tandis que la masse du cholestéryl ester diminue d'environ 40%. Il en résulte une réduction du stérol cellulaire total. Quand les cellules sont incubées avec la lidocaïne et des lipoprotéines d'un sérum hyperlipémique marquées avec le [7-^{3}H(N)]cholestérol d'activité spécifique connue, l'incorporation du cholestérol libre exogène dans le cholestérol libre cellulaire est constante alors qu'il y a réduction en fonction de la dose dans la quantité de cholestérol exogène apparaissant dans le cholestéryl ester. De plus, la comparaison des activités spécifiques de cholestérol libre cellulaire et des cholestéryl esters, à divers intervalles durant une période de 24 h, suggère que la lidocaïne inhibe aussi la cholestérol ester hydrolase (EC 3.1.1.13) lysosomique dans la lignée cellulaire Fu5AH.

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Introduction

Cholesteryl ester synthesis in tissues and isolated cell systems is principally the function of acylCoA:cholesterol acyltransferase (EC 2.3.1.26) which resides in the endoplasmic reticulum. Despite the ubiquity of the enzyme in animal tissues, cholesteryl esters usually represent a small proportion of total cellular cholesterol (1). Exceptions to this include (*a*) the normal adrenal gland in which 80–90% of the total cholesterol content may be esterified (1–4) and (*b*) arteries undergoing atherogenic change (5). In atheromatous arteries, cholesteryl ester is the lipid fraction showing the greatest relative increase and may attain levels 20-fold greater than normal (6). We have recently reported that acylCoA:cholesterol acyltransferase activity in liver, artery, and adrenal gland is inhibited by the local anesthetic lidocaine (7–9). In the present study, we have examined the effect of lidocaine on acylCoA:cholesterol acyltransferase activity in various cultured cell systems and, in addition, have examined the effect of an inhibition of the enzyme on influx, accumulation, and metabolism of exogenous cholesterol in Fu5AH rat hepatoma cells incubated with hyperlipemic plasma lipoproteins.

Materials and methods

Cells and media

The cells used in these studies were L929 (mouse connective tissue), ATCC CCL-25 WISH (human amnion), ATCC CCL-23 (HEp-2, human larynx epidermoid carcinoma), Vero

ABBREVIATIONS: MEM, minimal essential medium; sp.act., specific activity; dpm, disintegrations per minute; cpm, counts per minute.

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(African Green monkey kidney), diabetic hamster kidney (AC line) (10), and Fu5AH rat hepatoma cells (11). All culturing was done in a humidified CO_2 (5%) incubator at 37°C. Vero, WISH, HEp-2, and L929 cells were grown to confluency in 30-mL plastic culture flasks in MEM (Eagle's modified; Grand Island Biological Co., NY) containing 5% (v/v) calf serum, penicillin (50 U/mL), and streptomycin (50 μg/mL). The Fu5AH cells were cultured under the same conditions with the exception that 60-mm plastic tissue culture dishes were used rather than culture flasks. AC-line cells were grown to confluence in 100-mm plastic culture dishes in a 1:1 (v/v) mixture of Dulbecco's modified Eagle's medium and Ham's F-12 medium (Grand Island Biological Co., NY); calf serum was present at a final concentration of 2% (v/v) and levels of penicillin and streptomycin were as used above. All cells were permitted to reach confluence and were then rinsed twice with 3–5 mL of minimal essential medium without serum to remove nonadherent cells. The appropriate medium for the various incubation studies was then added to the cells. AC-line cells were incubated 3 h at 37°C in 5 mL of their original culture medium containing 0.4 μCi [1-^{14}C]oleate (New England Nuclear Corp., Boston, MA; sp.act. 56 Ci/mol, 1 Ci = 37 GBq) and lidocaine·HCl (Sigma Chemical Corp., St. Louis, MO) at a final concentration of 5.0 m*M*. The L929, WISH, Vero, and HEp-2 cells were incubated 3 h at 37°C in 20 mL of their original medium which contained 0.2 μCi [1-^{14}C]oleate and lidocaine·HCl at a level of 1.5 m*M*. The Fu5AH cells were incubated in the presence of minimal essential medium containing 2.5 mg delipidized calf serum protein (12), hyperlipemic rabbit serum lipoproteins (d < 1.063), [^{3}H]cholesterol, and lidocaine·HCl. The cholesterol-induced hyperlipemic rabbit serum was obtained from rabbits fed Purina rabbit chow supplemented with 2% cholesterol and 6% corn oil. The [7-^{3}H(N)]cholesterol (New England Nuclear Corp., Boston, MA; sp.act. 23.5 Ci/mmol) was added to the medium at a level of 0.3 μCi/mL by first solubilizing with ethanol prior to addition to the culture medium (13). Incubation times and levels of lidocaine used in the various experiments with Fu5AH cells are detailed in the tables and figure that follow. After the cells were incubated for the indicated time, the medium was aspirated and the cells were washed three times with cold phosphate-buffered saline. Cells grown in culture dishes (Fu5AH and AC-line cells) were harvested with a rubber policeman, whereas cells grown in culture flasks were removed with 0.25% trypsin in phosphate-buffered saline (Bacto-trypsin, catalogue No. 0153-59, Difco Laboratories, Detroit, MI). The cells were sonicated and analyzed for protein (or dry weight), lipids, and radioactivity as described in Analytical procedures. In studies with Fu5AH cells, the incubation medium was also analyzed for cholesterol content and radioactivity before addition to the cells as well as after incubation with the cells.

Assay of acylCoA:cholesterol acyltransferase (EC 2.3.1.26)

The microsomal fractions from normal rat liver and adrenal gland, and atheromatous rabbit aorta were isolated from homogenates prepared in 0.1 *M* phosphate buffer (pH 7.4) as previously described (14, 15). AcylCoA:cholesterol acyltransferase was assayed by following the incorporation of [1-^{14}C]oleoylCoA into [^{14}C]cholesteryl esters. The assay system consisted of 345 μL 0.1 *M* phosphate buffer (pH 7.4), containing 0.8–1.2 mg microsomal protein/mL, 2×10^5 dpm [1-^{14}C]oleoylCoA (sp.act. 49 Ci/mol, New England Nuclear Corp., Boston, MA) as described previously (7). Lidocaine was added to the incubations dissolved in 15 μL saline. The incubation time was 5 min at 37°C.

Analytical procedures

Lipids of all cells, media, and microsomal assay mixtures were extracted by the method of Bligh and Dyer (16) and protein was assayed according to Lowry *et al.* (17). Lipid-free dry weight of cells was determined after overnight drying at 60°C. The cholesterol and cholesteryl ester fraction of the lipid extracts were obtained by thin-layer chromatography on silica gel G using petroleum ether – diethyl ether – glacial acetic acid (70:29:1, v/v/v) as a developing solvent. The cholesterol and cholesteryl ester bands were scraped from the chromatoplates and either assayed directly by liquid scintillation counting or extracted with $CHCl_3$ to recover the cholesterol which was assayed for both radioactivity and cholesterol content. Quantitation of cellular and medium cholesterol was done by gas–liquid chromatography using coprostanol as an internal standard (13). Values given for the incorporation of exogenous unesterified cholesterol into cellular sterols were obtained by using the specific activity of the labeled free (unesterified) cholesterol in the medium at the start of the experiment.

Results

Incorporation of [^{14}C]oleate and [^{14}C]acetate into cellular cholesteryl esters

The effects of lidocaine on the esterification of cholesterol with [1-^{14}C]oleate in various cultured cells is shown in Table 1. In all cells examined, lidocaine inhibited the esterification of cholesterol to [^{14}C]oleate. At a level of 5 m*M*, inhibition of esterification in AC-line cells was virtually complete (i.e., 98%). Sterol esterification in Vero and WISH cells was 85–90% inhibited at 1.5 m*M* lidocaine. This same concentration inhibited cholesterol esterification in HEp-2 and L929 cells by 50–55%.

The effect of lidocaine on the esterification of cholesterol with fatty acid synthesized endogenously from [1-^{14}C]acetate was also investigated in AC-line,

TABLE 1. Effect of lidocaine on the incorporation of [^{14}C]oleate into steryl esters by cells in culture (dpm per milligram dry weight)

	Control	Lidocaine	% inhibition
AC	25820	555	98
Vero	18480	1935	90
WISH	2375	345	86
HEp-2	26380	11705	56
L929	28750	14135	51

NOTE: Cells were incubated for 3 h at 37°C. AC-line cells were incubated in 5 mL of medium containing 0.4 μCi [1-^{14}C]oleate (sp.act. 56 Ci/mol) with and without (control) lidocaine at a level of 5.0 m*M*. All other cells were incubated in 20 mL of medium containing 0.2 μCi [1-^{14}C]oleate with and without (control) lidocaine at a level of 1.5 m*M*. Values are means of duplicate cultures.

TABLE 2. Effect of lidocaine on cholesterol content of rat hepatoma cells exposed to hyperlipemic rabbit serum lipoproteins ($d < 1.063$)

Lidocaine (m*M*)	Cholesterol content (μg/mg protein)		
	Free	Ester	Total
0	24	69	93
0.5	27	55	82
1.0	28	55	83
1.5	29	41	70

NOTE: Cells were incubated 24 h in medium supplemented with hyperlipemic rabbit serum lipoproteins equivalent to 5% serum. All values are average of duplicate cultures.

HEp-2, and WISH cells under identical conditions to those given in Table 1. The incorporation of labeled fatty acid into cholesteryl esters was inhibited 83% in the AC-line, 43% in HEp-2, and 30% in WISH cells.

Uptake, accumulation, and esterification of [7-^{3}H(N)]-cholesterol by Fu5AH cells

The results presented in Tables 2 and 3 illustrate the effect of lidocaine on the accumulation of cholesterol by Fu5AH rat hepatoma cells elicited by exposure of these cells to lipoproteins obtained from cholesterol-fed rabbits. At the time of exposure to the rabbit lipoproteins, the cells contained 22 μg of total cholesterol/mg protein. Approximately 90% of the cellular cholesterol was unesterified. After 24 h of exposure to the hyperlipemic lipoproteins there was a fourfold accumulation of cholesterol, with 75% present in cells as cholesterol ester (Table 2). Increasing concentrations of lidocaine, up to 1.5 m*M*, reduced total cholesterol by 25%, with the major reduction (40%) in the cholesterol ester pool. Table 3 shows the fate of the radiolabeled free cholesterol which was incorporated by the cells from the hyperlipemic lipoproteins. Total cholesterol incorporation was reduced, although the amount of incorporated cholesterol recovered in cells as free cholesterol remained constant. There was, however, a 73% reduction in the utilization of exogenous cholesterol for cellular cholesterol ester synthesis. The cellular esterification of exogenous cholesterol was reduced in a dose-dependent manner from 60% in controls to only 30% when 1.5 m*M* lidocaine was present. Table 3 also shows the specific activity of cellular cholesterol. The presence of lidocaine resulted in a small reduction in cellular-free cholesterol specific activity and a large decrease (54%) in the specific activity of the accumulated cholesterol ester. A time course of the changing cellular cholesterol specific activity in control and lidocaine-treated cells is shown in Fig. 1. A small, but consistent, reduction in free cholesterol specific activity was observed at all time points. In contrast, the cholesterol ester specific activity

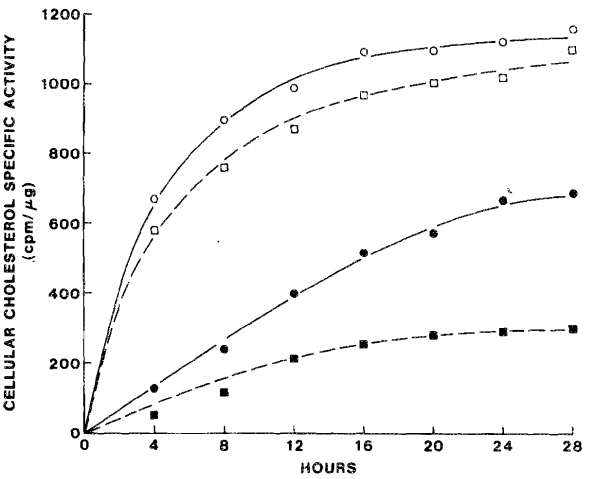

FIG. 1. Fu5AH rat hepatoma cells were cultured in 60-mm plates and allowed to reach confluence. When almost confluent, the plates were washed, and 5 mL of either the control medium alone or the control medium containing lidocaine (1.0 m*M*) was added to the plates; both media contained [7-^{3}H(N)]cholesterol. Delipidized serum protein was at a level of 2.5 mg/mL and hyperlipemic rabbit serum was at a level of 5% (v/v). Duplicate plates from each group (i.e., control and lidocaine) were taken every 4 h for 28 h. The medium was aspirated and each plate was washed three times with cold phosphate-buffered saline. The cells were harvested and analyzed as described in Materials and methods. Free (unesterified) cholesterol specific activity for both control and lidocaine media was 2076 cpm/μg. Free cholesterol, control, ○; esterified cholesterol, control, ●; free cholesterol, lidocaine, □; esterified cholesterol, lidocaine, ■.

showed a marked reduction that was accentuated as the exposure to the lidocaine was prolonged.

Assay of AcylCoA:cholesterol acyltransferase (EC 2.3.1.26) in isolated microsomes

The effect of lidocaine on cholesterol esterification (principally a function of microsomal acylCoA:cholesterol acyltransferase) was examined directly by assaying the enzyme in isolated microsomes from rat adrenal, rat liver, and atheromatous rabbit aorta (Table 4). Inhibition of acylCoA:cholesterol acyltransferase as evidenced by a reduction in the incorporation of [1-^{14}C]-oleoylCoA into labeled cholesteryl esters was similar in microsomes from each of the three tissues and ranged from 41 to 65% at 1.0 m*M* lidocaine.

Discussion

The present studies clearly demonstrate that lidocaine is an inhibitor of cholesterol esterification in cultured cell systems. This inhibition is apparent using exogenously added fatty acid (i.e., [^{14}C]oleate), endogenously generated fatty acid (i.e., from [^{14}C]acetate), or exogenously added [^{3}H]cholesterol. The ability of lidocaine

TABLE 3. Effect of lidocaine on the incorporation and esterification of free cholesterol by rat hepatoma cells

Lidocaine (m*M*)	Exogenous free cholesterol incorporated (μg/mg protein)				Cellular cholesterol specific activity (cpm/μg)	
	Total	Free	Esterified	Cellular esterification (%)	Free	Esterified
0	37	15	22	60	1219	618
0.5	30	15	15	50	1129	532
1.0	25	15	10	40	1058	363
1.5	20	15	6	30	1021	284

NOTE: Cells were incubated 24 h in medium supplemented with hyperlipemic rabbit serum lipoproteins (5%) containing [7-^{3}H(N)]cholesterol (sp.act. 2083 cpm/μg). All values are average of duplicate cultures.

TABLE 4. Effect of lidocaine on acylCoA:cholesterol acyltransferase activity in isolated microsomes from various tissues

Tissue	Lidocaine (m*M*)	[^{14}C]OleoylCoA incorporation into cholesteryl esters (dpm/mg protein)		% inhibition
		Control	Lidocaine	
Aorta	1.0	7620	2670	65
Liver	1.0	5430	3210	41
Adrenal	1.0	1920	810	58

NOTE: The enzyme was assayed by measuring the incorporation of [1-^{14}C]oleoylCoA into cholesteryl esters during a 5-min incubation at 37°C. The incubation mixtures consisted of 345 μL 0.1 *M* phosphate buffer (pH 7.4) containing 0.8–1.2 mg microsomal protein and 2×10^5 dpm [^{14}C]oleoylCoA (sp.act. 49 Ci/mol). Lidocaine was added dissolved in 15 μL saline. All values are the mean of duplicate incubations.

to inhibit cholesterol esterification in the cells can be explained by its ability to inhibit microsomal acylCoA:cholesterol acyltransferase as previously demonstrated (7–9) and confirmed in Table 4. Our initial observation of the effect of lidocaine in cultured cells prompted us to examine its effect in the Fu5AH rat hepatoma cell line which has been used extensively for studies on cellular cholesterol metabolism (11, 18–20). It has been demonstrated that exposure of these cells to lipoproteins from cholesterol-fed animals results in the accumulation of large intracellular pools of cholesterol ester which are derived primarily through the action of acylCoA:cholesterol acyltransferase (13, 19). Both exogenous free cholesterol and cholesterol liberated via the action of lysosomal cholesterol ester hydrolase (EC 3.1.1.13) serve as substrates for intracellular esterification (19, 20). Previous investigations have demonstrated that quantitating the incorporation and esterification of exogenous free cholesterol serves as a reliable indicator of cholesterol ester deposition (13). The data presented in Fig. 1 and Table 2 demonstrate that lidocaine effectively reduces both the mass of cholesterol ester accumulated in these cells and the conversion of free cholesterol to cholesterol esters. Although these data clearly indicate that acylCoA:cholesterol acyltransferase activity is reduced, the results presented in Fig. 1 and Table 3 on cholesterol specific activity suggest that lidocaine may also have a second mode of action in this cell system. In this study acylCoA:cholesterol acyltransferase activity was reduced more effectively than actual cholesterol ester mass, as reflected by the reduction in cholesterol ester specific activity observed with increasing lidocaine concentration (Table 3) and increasing incubation times (Fig. 1). The reduced specific activity results from a disproportionate increase in an unlabeled cholesterol ester pool. Inhibition of lysosomal cholesterol ester hydrolysis could produce an accumulation of exogenous unlabeled cholesterol esters which would result in the decrease in cholesterol ester specific activity observed in these experiments. Thus, the results obtained from the hepatoma studies suggest that lidocaine may have an action both at the level of acylCoA:cholesterol acyltransferase and lysosomal cholesterol ester hydrolysis; such a combination of effects seems probable in view of the fact that lidocaine has previously been shown to inhibit cholesterol esterase (EC 3.1.1.13) in the cytosolic fraction of normal rat liver (21). Lidocaine may be useful as a tool for studying the

metabolic fate of sterol entering cells and the factors controlling its distribution between esterified and unesterified forms.

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