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EFFECT OF THE LIPID-LOWERING DRUG LIFIBROL ON LIPID METABOLISM IN RAT MACROPHAGES AND IN ATHEROSCLEROTIC ARTERIES FROM SWINE AND WHHL RABBITS, *IN VITRO*

IMPLICATIONS IN ATHEROGENESIS

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Abstract—The effects of lifibrol on lipid metabolism in rat macrophages and swine and rabbit aortae were investigated. Resident peritoneal macrophages isolated from rats pretreated with lifibrol (50 mg/kg/7 days) showed a decreased capacity to synthesize cholesteryl esters from labeled precursors ([1-^{14}C]oleate and [4-^{14}C]cholesterol). Macrophages isolated similarly from non-treated rats demonstrated the ability to take up [^{14}C]lifibrol, *in vitro*. Modification of lipid metabolism in atherosclerotic aortae from swine and Watanabe heritable hyperlipidemic (WHHL) rabbits was also observed when the tissues were incubated *in vitro* in the presence of exogenous lifibrol. Concentrations of lifibrol of up to 50 μg/mL in the incubations selectively reduced the formation of cholesteryl esters from [1-^{14}C]acetate by 60–75%, whereas higher concentrations (100 μg/mL) resulted in a generalized inhibition of lipid biosynthesis of about 50% and of cholesteryl ester formation by up to 90%. The ability of lifibrol to directly affect these targets (i.e. macrophages and arterial tissue) has implications that extend beyond its confirmed plasma cholesterol-lowering activity since early stages of the atherogenic process involve an overall increase in arterial lipid synthesis and cholesteryl ester accumulation by monocyte-macrophages that infiltrate the vessel wall from blood.

The ability of lifibrol (K12.148; 4-[4′-*t*-butylphenyl]-1-[4′-carboxyphenoxy]-2-butanol) to reduce plasma lipid levels in humans, rats, swine, and other experimental animals has been amply demonstrated [1–3]. The therapeutic value in lowering plasma lipids, however, is the perceived benefit that will be derived at the arterial level through a reduction in atherosclerosis progression and perhaps through promotion of atherosclerosis regression. This being the case, it seemed relevant to investigate the possibility that lifibrol might even exert a direct effect on arterial metabolism that could augment or complement the benefits of plasma lipid reduction. Additionally, it seemed worthwhile to determine to what extent, if any, that lifibrol could affect macrophage metabolism. This latter question is important to consider in view of the fact that the cholesterol/cholesteryl ester-rich foam cells, which constitute an important component of atheromatous lesions, are in part derived from monocyte-macrophages that invade the vessel wall [4, 5]. The studies presented here, which were conducted *in vitro*, indicate that lifibrol has the potential to favorably affect lipid metabolism in atherosclerotic arterial tissue from swine and Watanabe heritable hyperlipidemic (WHHL)† rabbits and to favorably modify macrophage lipid metabolism.

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† Abbreviations: WHHL, Watanabe heritable hyperlipidemic; BSA, bovine serum albumin; FBS, fetal bovine serum; MEM, minimum essential medium; ACAT, acylCoA:cholesterol acyltransferase, EC 2.3.1.26; PL, phospholipids; DG, diglycerides; FFA, free fatty acids; TG, triglycerides; CE, cholesteryl esters; and LSC, liquid scintillation counting.

MATERIALS AND METHODS

Materials. Silica gel G-coated thin-layer plates (20 × 20 cm) were purchased from the Brinkmann Instruments Co., Westbury, NY. All radiolabeled substrates were obtained from Dupont NEN Research Products, Boston, MA ([1-^{14}C]acetate, sodium salt, 54.0 Ci/mol; [1-^{14}C]oleic acid, sodium salt, 56.0 Ci/mol; [4-^{14}C]cholesterol, 53.1 Ci/mol). Isotopically labeled lifibrol was synthesized at Upjohn Laboratories ([1-^{14}C]lifibrol, 6.8 Ci/mol). Roswell Park Memorial Institute (RPMI) 1640 culture medium, MEM (minimum essential medium) 199 and fetal bovine serum (FBS) were purchased from Gibco BRL, Grand Island, NY. Fatty acid-free bovine serum albumin (BSA) was purchased from the Sigma Chemical Co., St. Louis, MO.

Animals and diets. Homozygous WHHL female rabbits were purchased from The National Institutes of Health (NIH), Bethesda, MD. The rabbits were 1 year of age at the time of experimentation and had been maintained on pelletized chow (Purina Rabbit Chow Pellets). Spontaneously developed atheromatous lesions were present in their aortae and covered about 60% of the vascular surface [6, 7].

Male rats of the TUC-Sprague–Dawley strain

(Charles River Laboratories, Portage, MI) were used and weighed an average of 175 g. The rats were individually housed in wire-bottom cages with free access to a normal chow diet (Purina Rodent Chow No. 5002). Lifibrol, when administered, was provided as an admixture in the diet at a level calculated to provide 50 mg/kg/day based upon measured feed consumption.

The swine used were male Yorkshire (6 weeks old and 15–20 kg at outset) that were fed for 16 weeks with either normal hog chow diet (Purina Hog Chow) or a hyperlipidemic diet consisting of the normal chow supplemented with 1.5% cholesterol and 19.5% lard (w/w) [4]. The hyperlipidemic diet resulted in hypercholesterolemia development (but not hypertriglyceridemia) and the formation of fatty streak lesions (atherosclerosis) in the aortae [4].

Peritoneal macrophage isolation and incubation, in vitro. Resident peritoneal macrophages were harvested from rats via peritoneal lavage with 15 mL of phosphate-buffered saline following decapitation of the rats. The cells were sedimented at 275 *g* for 5 min at 10° and resuspended in RPMI 1640 that contained either 2% BSA or 5% FBS.

Lipid biosynthesis studies were conducted in the freshly isolated cells obtained from normal rats and lifibrol-treated rats (50 mg/kg/day for 7 days). The cells (2×10^6) were incubated in 13×100 mm glass tubes in 1.5 mL of RPMI 1640 + 2% BSA which contained either [1-^{14}C]acetate (3.3 μCi/mL), [1-^{14}C]oleate (1.0 μCi/mL), or [4-^{14}C]cholesterol (2.6 μCi/mL). All incubations were performed uncapped in a tissue culture oven (5% CO_2) for 3 hr at 37°. Following incubation, the cells were separated from the medium by centrifugation and extracted with chloroform–methanol mixtures [8]. The resulting extracts were fractionated by TLC on silica gel G-coated glass plates using a solvent system consisting of *n*-hexane:diethyl ether:glacial acetic acid (146:50:4, by vol.) [9]. The individual lipid classes were visualized on the chromatoplates with rhodamine 6G (0.5% in ethyl alcohol) and then scraped from the chromatoplates and assayed for radioactivity by liquid scintillation counting (LSC) (Beckman Instruments, Inc., Irvine, CA, model LS9800) [9]. Counting efficiency for ^{14}C averaged 94%, and quench corrections were made by internal standardization.

Studies of [^{14}C]lifibrol uptake by peritoneal macrophages were conducted with 2×10^6 cells in 2.0 mL RPMI 1640 + 5% FBS in 35-mm cluster wells (Costar, Cambridge, MA, Cat. No. 3406). The cells were permitted to attach to the plastic wells for 1 hr and then the medium was replaced with 2 mL of fresh RPMI 1640 + 5% FBS that contained 2 μCi of [^{14}C]lifibrol; the lifibrol was added via microsyringe in 10 μL ethanol. The cultures were then allowed to incubate for up to 4 hr. The experiments were terminated by removal of the medium followed by three washes with fresh medium. The cells were then killed and digested by addition of 100 μL of 0.1 N NaOH. The digests were then removed and the wells rinsed with five washes of hexane:ethanol (3:2, v/v). The combined extracts and digest were evaporated and assayed for radioactivity by LSC in Ready-Safe (Beckman Instruments, Inc., Fullerton, CA). The combined extract/digest from one 4-hr culture was neutralized with 0.1 N HCl, evaporated, and chromatographed by TLC [9] with authentic lifibrol; >90% of the radioactivity co-migrated with authentic lifibrol.

Swine and rabbit arterial tissue incubation. Swine were killed with an overdose of pentobarbital and the aortae were excised and rinsed in chilled phosphate-buffered saline. Aortic rings (about 2.5 cm long) were cut from the mid-thoracic region of a normal swine aorta and from the lower abdominal region of a hyperlipidemic swine aorta where fatty streak lesions had developed. The rings were stripped free of adventitia, opened longitudinally, and divided into 4–5 segments of approximately equal size (200–300 mg wet wt). The abdominal aortic tissue was divided so that each of the segments contained, to the visual extent possible, an equal amount of lesioned and non-lesioned tissue. The tissue segments were incubated in 25-mL Erlenmeyer flasks in 7.0 mL of normal swine serum:MEM 199 (1:1, v/v) [9] that contained 1.0 μCi [^{14}C]acetate/mL. Lifibrol, when present, was added to the incubation medium dissolved in 30 μL ethanol. The tissues were incubated at 37° for 90 min in a metabolic shaker while loosely covered with parafilm. After incubation, the tissues were rinsed in five changes of phosphate-buffered saline and frozen to await analysis. The tissues were subsequently thawed and the lipid was extracted by homogenization in chloroform:methanol (2:1, v/v) [8]. The lipid extracts were fractionated by TLC [9] and the various lipids assayed for radioactivity by LSC [9].

WHHL rabbits were killed by exsanguination while under pentobarbital anesthesia, and the aortae were excised and rinsed in chilled phosphate-buffered saline. The descending thoracic aorta from each rabbit was opened longitudinally and bisected lengthwise and then divided into four segments to yield two sets of horizontally paired tissue segments. The tissues were incubated as above for 3 hr at 37° in 25-mL Erlenmeyer flasks in 7.0 mL FBS:MEM 199 (1:1, v/v) that contained 2.6 μCi [1-^{14}C]acetate/mL or 1.3 μCi [1-^{14}C]oleate/mL. Lifibrol, dissolved in 28 μL ethanol, was added to one member of each tissue pair to a concentration of 100 μg/mL; the paired control segment received 28 μL ethanol vehicle alone. The experiments were terminated by rinsing the tissues in five changes of phosphate-buffered saline; subsequent handling and analysis were precisely as described above for swine aortae.

Plasma cholesterol. Plasma total cholesterol was measured with an Ecktachem DT60 analyzer (Eastman Kodak Co., Rochester, NY).

Data presentation and statistical analyses. Data are presented as individual observations or the mean of multiple observations ± SD. When appropriate, statistical analyses were performed using one-way analysis of variance. Differences were considered to be statistically significant when the P value was equal to or less than 0.05.

RESULTS

Swine arterial studies. The effect of lifibrol on

Table 1. Effect of lifibrol on lipid synthesis from [^{14}C]-acetate in normal swine aorta, *in vitro*

Lifibrol concn (μg/mL)	[^{14}C]Acetate incorporation (dpm/g wet wt)					
	PL	DG	FFA	TG	CE	Total
0	2780	580	1160	160	120	4800
10	2210	560	1140	170	100	4180
25	3270	630	1410	270	150	5730
50	2510	470	1250	280	160	4670

Thoracic aorta from a normocholesterolemic swine (total plasma cholesterol, 71 mg/dL) was divided into four segments of 200–300 mg each. The segments were incubated separately with various concentrations of lifibrol at 37° for 90 min in 7.0 mL normal swine serum : MEM 199 (1 : 1, v/v) that contained 1.0 μCi [1-^{14}C]acetate/mL as the substrate for lipid synthesis. Lifibrol was added to the incubations, at the outset, dissolved in 30 μL of ethanol vehicle; the control incubation received vehicle alone. Values shown are data obtained from each thoracic aortic segment.

arterial tissue was examined in normal and atherosclerotic swine aortae, *in vitro* (Tables 1 and 2). In the normal swine aorta (Table 1), the presence of exogenous lifibrol at concentrations up to 50 μg/mL had essentially no effect on lipid biosynthesis from [^{14}C]acetate [i.e. neither the total incorporation of [^{14}C]acetate into lipid (dpm/g wet wt of tissue) nor the percentage distribution of the label amongst the individual lipid classes showed any major change].

The studies with atherosclerotic aorta are presented in Table 2. The total lipid synthesis capacity (dpm/g wet wt) of the atherosclerotic tissue was characteristically greater than in the normal tissue (Table 1 vs Table 2) by a factor of approximately two with the greatest proportionate increase being in the cholesteryl ester (CE) fraction which was increased about 10-fold above normal. At concentrations of lifibrol up to 50 μg/mL, the drug reduced the incorporation of [^{14}C]acetate into CE by 60–75% while having essentially no effect on incorporation into the free fatty acid (FFA) fraction or the glycerolipid fractions [diglycerides (DG), triglycerides (TG) and phospholipids (PL)]. However, at 100 μg/mL lifibrol reduced the incorporation of [^{14}C]acetate into all lipid fractions. Incorporation into CE was reduced to the greatest extent (90%), whereas incorporation into all other fractions was about 50%. These modifications at 100 μg/mL made the lipid synthesis profile of the atheromatous tissue indistinguishable from that of normal aorta (Table 2 vs Table 1).

Rabbit arterial studies. Additional arterial studies were conducted *in vitro* employing atherosclerotic aortae from WHHL rabbits (Table 3). The studies were conducted in the presence or absence of 100 μg/mL lifibrol in the tissue incubation medium. The effect of lifibrol on [^{14}C]acetate incorporation into arterial lipids resembled that observed with the 100 μg/mL concentration in the atherosclerotic swine aorta (Table 2) in that synthesis of all lipid classes was reduced in the presence of drug. The effect of lifibrol on cholesterol esterification capacity of WHHL aorta was also evaluated specifically by incubation of the tissue with [^{14}C]oleate and measuring its incorporation into the CE fraction of the tissues (Table 3). At a concentration of 100 μg/mL, lifibrol decreased the incorporation of [^{14}C]-oleate into cholesteryl esters by an average of 40% in paired aortic segments.

Rat peritoneal macrophages. Lipid biosynthesis was also investigated in resident peritoneal macrophages that were harvested from normal rats treated with lifibrol for 7 days at a dose of 50 mg/kg (Tables 4 and 5). Cells from both the untreated (control) and

Table 2. Effect of lifibrol on lipid synthesis from [^{14}C]acetate in atherosclerotic swine aorta, *in vitro*

Lifibrol concn (μg/mL)	[^{14}C]Acetate incorporation (dpm/g wet wt)					
	PL	DG	FFA	TG	CE	Total
0	7,410	1,130	2,760	530	1,990 (14.4)*	13,820
10	5,130	900	2,280	390	450 (4.9)	9,150
25	6,420	1,160	1,980	390	780 (7.2)	10,730
50	6,630	1,000	2,850	470	530 (4.6)	11,480
100	2,550	430	1,250	260	150 (3.3)	4,640

Atherosclerotic abdominal aorta from a hypercholesterolemic swine (total plasma cholesterol, 576 mg/dL) was divided into five segments of 200–300 mg each. The segments were incubated separately *in vitro* with various concentrations of lifibrol under the same conditions as described in the legend of Table 1. Values shown are data obtained from each abdominal aortic segment.

* Values in parentheses are the percentage distribution of [^{14}C]acetate incorporated into CE.

Table 3. Effect of lifibrol on lipid synthesis from [^{14}C]acetate and [^{14}C]oleate in atherosclerotic aortae from WHHL rabbits, *in vitro*

Paired aortic segments	[^{14}C]Acetate incorporation (dpm/mg dry wt)						[^{14}C]Oleate incorporation (dpm/mg dry wt)
	PL	DG	FFA	TG	CE	Total	CE
Control-1	80	45	15	10	30	180	510
Lifibrol-1, 100 μg/mL	45	25	15	5	20	110	350
Control-2	600	140	60	60	150	1010	350
Lifibrol-2, 100 μg/mL	245	40	30	15	45	375	180
Control-3	250	30	15	5	20	320	240
Lifibrol-3, 100 μg/mL	30	10	5	1	5	51	150

Atherosclerotic aortae from three WHHL rabbits (average plasma total cholesterol, 530 mg/dL) were each divided into four segments for paired comparisons of lipid synthesis in the presence and absence of lifibrol (100 μg/mL). The tissues were incubated separately *in vitro* at 37° for 90 min in 7.0 mL FBS:MEM 199 (1:1, v/v) that contained either 2.6 μCi [1-^{14}C]acetate/mL or 1.3 μCi [1-^{14}C]-oleate/mL, as detailed in Materials and Methods. Lifibrol was added to the incubations at the outset, dissolved in 28 μL of ethanol vehicle; paired control segments received vehicle alone. Values shown are data obtained from each individual segment.

Table 4. Metabolism of [^{14}C]oleate and [^{14}C]cholesterol by peritoneal macrophages isolated from rats pretreated with lifibrol (50 mg/kg/7 days)

	[^{14}C]Oleate incorporation (dpm/10^6 cells)					[^{14}C]Cholesterol incorporation (dpm/10^6 cells)	
	PL	DG	TG	CE	Total	CE	Total
Control	20,390 ± 3,590	830 ± 90	1,330 ± 260	1,020 ± 310	23,570 ± 3,710	1,070	355,000
Lifibrol	22,740 ± 5,390	920 ± 310	1,480 ± 400	360 ± 60*	25,500 ± 5,670	740	380,000

Resident peritoneal macrophages (2 × 10^6 cells) isolated from lifibrol-treated and untreated (control) rats were incubated *in vitro* for 3 hr at 37° in 1.5 mL RPMI 1640/2% BSA that contained [1-^{14}C]oleic acid (1 μCi/mL) or [4-^{14}C]cholesterol (2.6 μCi/mL) as substrates for esterified lipid synthesis. Values are means ± SD of N = 4 incubations with [^{14}C]oleate and means of N = 2 incubations with [^{14}C]cholesterol.

* Statistically significantly different from the corresponding control mean ($P < 0.007$).

Table 5. Effect of lifibrol on lipid synthesis from [^{14}C]acetate in peritoneal macrophages isolated from rats pretreated with lifibrol (50 mg/kg/7 days)

	[^{14}C]Acetate incorporation (dpm/10^6 cells)					
	PL	DG	FFA	TG	CE	Total
Control	1120 ± 150	1060 ± 630	220 ± 160	40 ± 20	20 ± 10 (0.84 ± 0.21)*	2460 ± 730
Lifibrol	1170 ± 230	1680 ± 1120	300 ± 250	50 ± 10	15 ± 5 (0.56 ± 0.20)	3215 ± 1600
	NS†	NS	NS	NS	NS, $P < 0.06$	NS

Resident peritoneal macrophages (2 × 10^6 cells) isolated from lifibrol-treated and untreated (control) rats were incubated as described in the legend to Table 4 with the exception that the medium contained [1-^{14}C]acetate (3.3 μCi/mL) as the substrate for lipid synthesis. Values are means ± SD of N = 5 incubations.

* Values in parentheses are the percentage distribution of [^{14}C]acetate incorporated into CE.

† NS = not significant.

drug-treated animals actively incorporated [^{14}C]-oleate into complex lipids. The total incorporation of [^{14}C]oleate (dpm/10^6 cells) did not differ significantly between the groups. However, lifibrol treatment resulted in a significant ($P < 0.007$) reduction of incorporation of labeled oleate into CE. This effect of treatment was selective in that incorporation into other esterified lipids (DG, TG, and PL) was not

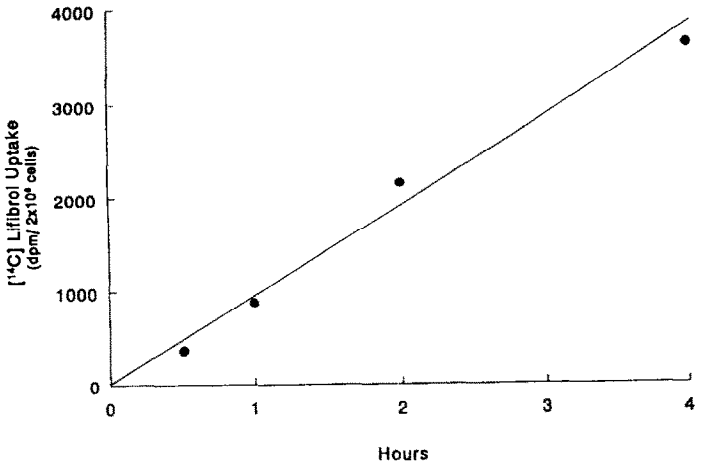

Fig. 1. Typical time–course for uptake of [1-^{14}C]lifibrol by rat resident peritoneal macrophages. Resident macrophages were isolated from the peritoneal cavity of a normal rat by lavage with 15 mL of phosphate-buffered saline. The cells were harvested by centrifugation, resuspended in RPMI 1640/5% FBS, and allowed to adhere in plastic culture plates for 1 hr before replacement with fresh medium containing [1-^{14}C]lifibrol, as detailed under Materials and Methods.

affected significantly (Table 4). Confirming evidence for decreased cholesterol esterification was also obtained in an experiment where [^{14}C]cholesterol (rather than a fatty acid) was evaluated as the esterification substrate in the macrophages. Total [^{14}C]cholesterol uptake by control and treated cells was similar (355×10^3 vs 380×10^3 dpm/10^6 cells, respectively) but the formation of CE was reduced 31% in the treated cells (i.e. 1070 vs 740 dpm/10^6 cells). Similar experiments were performed with the macrophages from untreated and drug-treated rats using [^{14}C]acetate as a lipid precursor (Table 5). The cells clearly demonstrated the capability to synthesize the full complement of lipids from [^{14}C]-acetate; however, the total incorporation of [^{14}C]-acetate into lipid products was relatively low when compared to the [^{14}C]oleate studies. This lower incorporation is typical and reflects the relatively low rate of *de novo* fatty acid synthesis in these normal rat cells. Although there were no statistically significant differences between the groups, it is of interest to note that the mean incorporation (and percent distribution) of [^{14}C]acetate (dpm/10^6 cells) into CE was somewhat lower in the treated group, whereas its mean incorporation into all other lipid classes was somewhat higher.

Evidence that peritoneal macrophages are able to take up lifibrol was suggested from studies in which peritoneal macrophages from untreated rats were incubated with [^{14}C]lifibrol for up to 4 hr *in vitro* (Fig. 1). Although the radiolabeled material accumulated by the cells was not chemically characterized, it co-chromatographed with authentic lifibrol (data not shown), thus suggesting that changes induced in the macrophages from lifibrol-treated rats described above may be in response to *in vivo* uptake of lifibrol by the cells.

DISCUSSION

The development of atherosclerosis in humans and experimental animals involves a complex interplay between plasma lipid/lipoproteins, the formed elements of the blood, and cellular and biochemical responses of the vessel wall [10]. To date, most drug development activity aimed at atherosclerosis therapy has focused on pharmacologic agents that reduce plasma lipids, particularly cholesterol [11, 12]. However, drugs that can act directly upon the arterial wall to alter its metabolism or that modify monocyte-macrophage recruitment, infiltration, and metabolism are also likely to be of importance in the treatment of atherosclerotic vessel disease [10, 13, 14]. In this regard, the results presented here seem pertinent. Previous studies have reported an effect of lifibrol in liver of treated rats [2, 3], while the present work demonstrates that peritoneal macrophages are also affected by host exposure to the drug and atherosclerotic arterial tissue undergoes modification of lipid metabolism when exposed to lifibrol *in vitro* (Tables 2–4). The ability of lifibrol to affect these particular targets offers the possibility that the benefits of lifibrol therapy could reach beyond its confirmed action of reducing plasma cholesterol [1–3]. The ability of lifibrol to inhibit the formation of CE was a common observation in both the macrophage and in atherosclerotic (but not normal) arteries (Tables 2–4). This has significance in view of the fact that CE constitute a significant proportion of the total amount of cholesterol that deposits within the lipid-filled foam cells of atherosclerotic lesions [15]. The accumulated esters are largely the product of ACAT (acylCoA : cholesterol acyltransferase) activity expressed within the vessel wall. Under atherogenic conditions (e.g. hypercholesterolemia) where ACAT activity is augmented [9, 15], ACAT functions as a biochemical trapping mechanism for cholesterol influxing from plasma. Although some of the esters may undergo hydrolysis by cholesterol ester hydrolase, the bulk of the esters accumulate

in intracellular lipid droplets, which give the characteristic histological appearance to the monocyte/macrophage-derived foam cells and, later, to the smooth muscle cell-derived foam cells of the arterial lesions [4, 5, 16]. Inhibition of arterial ACAT has been postulated as a therapeutic approach to limiting the vascular response to atherogenic stimuli and thereby reducing the rate of the atherogenic process [15].

The highest concentration of lifibrol employed here (100 μg/mL) decreased the overall enhanced lipid synthesis of the atherosclerotic swine artery to the level of normal swine artery, but not beyond; this suggests that there is a basal level of lipid synthesis which can be maintained in the presence of lifibrol and may explain the apparent neutral impact of lifibrol on normal swine artery (Table 1).

The impact of lifibrol on macrophages from drug-treated rats was most clearly illustrated in the studies with [^{14}C]oleate (Table 4) and [^{14}C]cholesterol (see Results) where its ability to reduce the formation of CE from these directly-employable substrates was evident. Parallel studies using [^{14}C]acetate, while reflecting the same trend (Table 5), channelled little of the ubiquitous precursor into CE. This altered metabolism displayed by the peritoneal macrophages from lifibrol-treated rats presumably indicates that the cells acquire the drug from the host and that the effects are direct effects of the drug on the cells. This presumption is strengthened by the demonstration that peritoneal macrophages take up [^{14}C]lifibrol from their culture medium, *in vitro* (Fig. 1).

In conclusion, the studies presented here suggest that lifibrol has the potential to directly favorably modify lipid metabolism in atherosclerotic arteries and in monocyte-macrophage progenitors of the lipid-filled foam cells which contribute to the development of atherosclerotic lesions [7]. Such effects, if operable in humans, should operate synergistically with the clinically demonstrable hypocholesterolemic action of the drug [1] to reduce the risk of development of cardiovascular disease.

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