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The Utilization of Short and Medium Chain Length Fatty Acids by L1210 Murine Leukemia Cells*

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Abstract—*Many neoplasms are reported to be unable to utilize acetate for oxidation and synthesis of cell lipids. On this basis, it has been postulated that a metabolic deletion involving the utilization of short and medium chain fatty acids might be a general defect of neoplastic cells. In this study, we have demonstrated that the L1210 murine leukemia cell is able to effectively utilize six short and medium chain fatty acids. The rate of oxidation was greater for those acids with shorter chain lengths. Glucose had a sparing effect on the oxidation of all fatty acids except acetate. The total uptake tended to increase with carbon chain length and this increase was due in part to greater incorporation into triglycerides. These data indicate that the absence of short and medium chain fatty acid activating enzymes is not a general metabolic deletion in neoplastic cells.*

INTRODUCTION

MANY investigators working with a variety of tumors have reported impaired utilization of short and medium chain length fatty acids [1–6]. It has been suggested that this may be a metabolic defect characteristic of the neoplastic cell [7, 8]. However, Gellhorn *et al.* demonstrated some incorporation of tracer amounts of radioactive acetate into L1210 cell lipids [9]. Because of this apparent discrepancy, we examined the ability of the L1210 cell to oxidize and incorporate into cell lipids six short and medium chain length fatty acids.

MATERIALS AND METHODS

L1210 cells were harvested from male DBA/2j mice 7 days after intraperitoneal injection of 1×10^5 cells [10]. Cells obtained from 6–8 mice were pooled, and separated from the ascites plasma by centrifugation at 480 ***g*** for 5 min at 0°C. Erythrocytes were removed by hypotonic hemolysis [11] and the washed cells were resuspended in either Eagle's minimum essential medium with glutamine (Grand Island Biological Company, Grand Island, NY) or in phosphate buffered saline, pH 7.25. Cell counts were determined in duplicate after Turk's staining using a hemocytometer. Viability by staining with Erythrosin B averaged greater than 91% just prior to incubation. Short and medium chain fatty acids were added to 0.6 mM bovine serum albumin as the sodium soap. These solutions contained phosphate buffered saline and were adjusted to pH 7.4. Fatty acid concentrations were determined by titration [12], and protein content was measured by the method of Lowry [13].

Incubations were carried out in rubber-stoppered 50 ml glass flasks at 37°C with a gas phase of 95% air–5% CO_2. Each flask contained 5×10^7 L1210 cells, 3.6 μmole of a single fatty acid and 1.2 μmole of albumin in a total vol of 5 ml. Previous studies with palmitate had failed to detect any appreciable differences in the rate of utilization at various incubation times [10]. Therefore, for the present study of multiple fatty acids, we used one appropriate time point. A substrate concentration was chosen which resembles that of L1210 ascites fluid (free fatty acids 0.24–1.11 μg/ml) and to obtain a physiologic molar ratio of fatty acid to albumin [10]. A kinetic

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study of the utilization of decanoate was carried out to eliminate the possibility of toxicity of the shorter chain fatty acids during incubation with the L1210 cells. After incubation and three washes, the cells were extracted with 20 ml $CHCl_3$:CH_3OH (2:1, v/v). The $CHCl_3$ phase was evaporated to dryness under N_2 and redissolved in 5 ml $CHCl_3$. One milliliter of the $CHCl_3$ solution was dried and analyzed for total lipid radioactivity using a dioxane-water (5:1, v/v) scintillation solution containing 5.83 g 2,5-diphenyloxazole, 0.25 g 1,4-bis-5(5-phenyloxazolyl-2)benzene and 83 g napthalene per liter. The lipids contained in another aliquot of the $CHCl_3$ were separated by one-dimensional thin-layer chromatography on silica gel G [11]. Two-dimensional thin layer chromatography was used to separate individual phospholipids [14]. The segments of silica gel containing lipids were scraped into the dioxane scintillation solution for measurement of radioactivity. Incubations for radioactive CO_2 determination were terminated by adding 1 ml of 35% $HClO_4$, and the CO_2 was collected in 3N NaOH added to the center well [10].

RESULTS

Initial kinetic studies were carried out on a representative saturated, medium chain length fatty acid, decanoate. There was a constant rate of uptake of the fatty acid radioactivity of the cells for at least 60 min. The incorporation of radioactivity into phospholipids and glycerides increased linearly during the study period. This is analogous to results obtained with the long chain fatty acid palmitate [10] and indicates maintenance of cell metabolic processes during the study interval chosen. All of the fatty acids studied were utilized (oxidation plus incorporation into cell lipids) at a rapid rate (Fig. 1). The total amounts taken up were dependent on the molecular structure of the fatty acid. Dodecanoate and acetate were utilized at the greatest rates. Except for acetate, the fatty acids were utilized at a rate that tended to increase with increasing chain length.

All six fatty acids were oxidized at a brisk rate (Table 1). The rate in the presence of glucose was highest for acetate and lowest for dodecanoate. The rate of oxidation tended to deline as the chain length increased. With acetate, the oxidation was 47% of the total radioactivity taken up. The percentage of the uptake that was oxidized declined as the carbon chain lengthened, and for dodecanoate it was only 10%. When the rates were expressed on a carbon atom basis, the differences among the fatty acids were less marked. In the absence of glucose, there was a considerably higher rate of oxidation except in the case of acetate. Those fatty acids with the highest rates of oxidation were not those with the most rapid rates of total utilization. This suggests that differences in the rate of oxidation of the various fatty acids is not due entirely to a variation in rate of transport across the plasma membrane. The incorporation of each of the six fatty acids into the separated lipid fractions is shown in Table 2. The major labelled fraction when the cells were incubated with radioactive acetate, butyrate or hexanoate was phospholipids. In contrast, a majority of the radioactivity from decanoate and dodecanoate was recovered in triglycerides. In all cases, only small amounts of fatty acid radioactivity were incorporated into mono- and diglycerides, cholesterol esters and cellular fatty acids. The incorporation into phospholipid increased approximately 2-fold as the fatty acid chain was lengthened from butyrate to dodecanoate. An even more marked increase was noted with triglycerides, where incorporation increased 12-fold over this range of chain length. Therefore, the

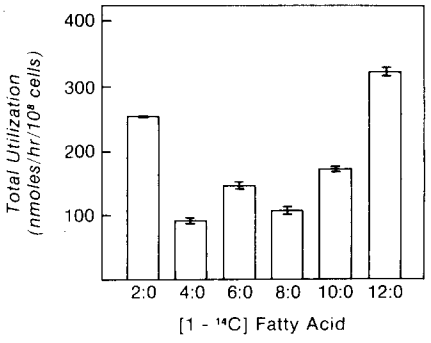

Fig. 1. Relationship of total utilization of fatty acids to their structure. 5×10^7 L1210 *cells obtained* 7 days *after transplantation were washed and incubated with* [1-^{14}C]*acetate (specific activity* 0.280 mCi/mmole), [1-^{14}C]*butyrate (specific activity* 0.211 mCi/mmole), [1-^{14}C] *hexanoate (specific activity,* 0.272 mCi/mmole), [1-^{14}C] *octanoate (specific acticity,* 0.314 mCi/mmole), [1-^{14}C] *decanoate (specific activity,* 0.577 mCi/mmole), *or* [1-^{14}C] *dodecanoate (specific activity,* 0.221 mCi/mmole) *in minimal essential medium for* 60 min. *Each flask contained* 3.6 μmole *of fatty acid and* 1.2 μmole *of albumin. The incubations were terminated by transferring the contents of each flask into ice-cold phosphate buffered saline followed by centrifugation. Lipids were extracted with chloroform: methanol* (2:1) *and the lipid fractions were separated by thin-layer chromatography. Separate incubations were performed for the determination of* CO_2 *production. Values shown include incorporation into cellular lipids and oxidation to* CO_2, *and are the mean and* S.E. *of* 3–4 *determinations.*

tendency toward increasing total uptake with increasing carbon chain length could be accounted for to a large extent by the increase of incorporation into the lipid storage form, triglyceride.

The distribution of radioactivity into specific types of phospholipid is shown in Table 3. Of the radioactivity incorporated into phospholipids, 74–80% was recovered in choline and ethanolamine phosphoglycerides. These are the phospholipids found in the highest quantities in the L1210 cell [10].

of a compensatory metabolic process. However, our studies indicate that the L1210 cells utilize acetate at a rapid rate for synthesis of cell lipids and oxidation to CO_2. In addition, this leukemic cell briskly utilizes other short and medium chain fatty acids. In order for the fatty acids to be utilized, they had to be activated. Since a deficiency of the activating enzyme for these fatty acids does not occur in the L1210 cell, such a deletion cannot be a uniform characteristic of all malignant cells.

Table 1. *Comparison of the rates of oxidation of various fatty acids*

	Oxidation to CO_2			
Fatty acid	(+) Glucose		(−) Glucose	
	nmole/hr per 10^8 cells	n atoms/hr per 10^8 cells*	nmole/hr per 10^8 cells	n atoms/hr per 10^8 cells
Acetate	119.4 ± 10.6†	238.8‡	120.1 ± 5.0	240.2
Butyrate	38.9 ± 0.9	155.6	86.5 ± 2.3	346.0
Hexanoate	69.1 ± 1.8	414.6	160.1 ± 2.3	960.6
Octanoate	43.6 ± 1.3	348.8	83.1 ± 6.9	664.8
Decanoate	40.7 ± 1.8	407.0	—	—
Dodecanoate	30.6 ± 0.4	367.2	90.4 ± 2.1	1084.8

*Obtained by multiplying rate of incorporation expressed as nanomoles by the number of carbon atoms in the molecule. We assumed complete catabolism of the molecule.
†Mean and S.E. of 3–4 determinations.
‡Mean of 3–4 determinations.

L1210 cells were incubated with labelled fatty acids for 60 min at a molar ratio of fatty acid to albumin of 3 in the presence or absence of 5.5 mM glucose. Incubations were terminated by the addition of 1 ml of 35% $HClO_4$, and radioactive CO_2 was collected in 3N NaOH added to removable center wells in the sealed incubation flasks.

DISCUSSION

Early studies by Weinhouse, Allen and Millington on several animal tumors suggested a limited ability to oxidize short chain fatty acids as compared to long chain fatty acids [3]. More recently, it has been shown that neither acetate nor octanoate are oxidized or incorporated into cell lipids appreciably by Ehrlich ascites cells [6]. Hepp *et al.* have presented evidence suggesting that the low utilization of acetate is due to a lack of a short chain fatty acid activating enzyme [7, 8]. Based on these results, it has been suggested that the inability to utilize the shorter chain fatty acids might be a general metabolic deletion in neoplastic cells. Such a defect could result in vulnerability to chemotherapy directly at the deletion or indirectly at the site

The rate of oxidation of each short and medium length fatty acid except acetate in the presence of glucose was only about 50% of the values of the incubations carried out in the absence of glucose. This is similar to studies on the Ehrlich ascites tumor cell [15]. It is likely that the glucose dilutes the intercellular acetyl CoA pool, thereby decreasing the radioactivity recovered in CO_2. Except for acetate and disregarding the loss of labelled lipids from the cell, the uptake of fatty acid increased with lengthening carbon chain. Just as is the case with the longer chain fatty acids, the penetration of these short and medium chain acids appears to be a function of chain length and lipid solubility. The increased rate of total uptake with longer chain length could also be due in part to increased rates of esterification.

Table 2. Incorporation of labelled fatty acids into cellular lipid fractions

Fatty acid	Distribution				
	Phospholipids	Mono- and diglycerides	Free fatty acids	Triglycerides	Cholesterol esters
Acetate	62.6±1.0 (46.7)*	9.3±0.7 (6.9)	2.1±0.2 (1.6)	48.2±0.9 (35.9)	12.0±0.6 (8.9)
Butyrate	23.7±0.5 (44.3)	4.5±0.3 (8.5)	1.0±0.1 (1.9)	19.5±0.6 (36.5)	4.7±0.2 (8.8)
Hexanoate	36.4±2.0 (47.3)	5.0±0.4 (6.5)	1.2±0.1 (1.5)	27.8±1.3 (36.1)	6.7±0.4 (8.6)
Octanoate	25.7±1.6 (40.5)	3.8±0.2 (6.1)	0.9±0.1 (1.5)	28.7±1.9 (45.5)	4.0±0.4 (6.3)
Decanoate	40.1±0.6 (30.8)	8.6±0.3 (6.7)	1.4±0.1 (1.1)	76.6±1.1 (58.8)	3.6±0.2 (2.7)
Dodecanoate	48.5±1.5 (16.6)	7.8±0.6 (2.7)	1.8±0.4 (0.6)	231.2±4.3 (79.0)	3.2±0.7 (1.1)

*Parentheses enclose the percentage of radioactivity in each fraction.

L1210 leukemia cells obtained 7 days after transplantation were harvested, washed, and incubated at 37°C with [1-^{14}C] fatty acid for 60 min at a molar ratio of fatty acid to albumin of 3. The incubations were terminated by transferring the contents of each flask into ice-cold PBS followed by centrifugation. Lipids were extracted with chloroform:methanol (2:1) and the lipid fractions were separated by thin-layer chromatography. Radioactivity was determined with a liquid scintillation spectrometer. Values are in nmole/hr/10^8 cells and are expressed as mean±S.E. of 5–6 determinations.

Table 3. Distribution of radioactivity in phospholipids

Fatty acid	Distribution					
	Ethanolamine phosphoglyceride	Choline phosphoglyceride	Serine and inositol phosphoglyceride	Sphingomyelin	Choline lyso-phosphoglyceride	Other phospholipids
Acetate	12.3±0.3 (20.2)*	33.0±1.1 (53.8)	7.3±0.1 (11.9)	1.5± <0.1 (2.4)	3.8±0.2 (6.2)	3.0±0.3 (4.3)
Butyrate	4.8±0.2 (19.8)	13.1±0.3 (54.0)	3.3±0.1 (13.6)	0.7±0.3 (2.7)	1.3± <0.1 (5.4)	1.1±0.2 (4.4)
Hexanoate	7.5±0.8 (20.1)	20.0±1.5 (54.0)	5.0±0.5 (13.5)	1.0±0.1 (2.7)	2.0±0.2 (5.4)	1.5±0.4 (4.3)
Octanoate	4.7±0.4 (18.2)	15.2±1.1 (58.8)	3.2±0.4 (12.2)	0.5± <0.1 (2.1)	1.3± <0.1 (5.0)	1.0±0.2 (3.8)
Decanoate	5.4±0.5 (13.7)	24.9±0.2 (63.3)	4.3±0.2 (11.0)	1.6±0.1 (4.1)	1.7± <0.1 (4.2)	1.5±0.5 (3.7)
Dodecanoate	5.0±0.2 (10.2)	35.5±4.3 (69.5)	4.7±0.4 (4.5)	1.9±0.7 (3.9)	2.4±0.2 (4.9)	3.3±1.0 (4.7)

*Parentheses enclose the percentage incorporation of each phospholipid.

L1210 leukemia cells were incubated with [1-^{14}C] fatty acids in minimal essential medium for 60 min at a molar ratio of fatty acid to albumin of 3. The phospholipids in the cell lipid extract were separated using 2-dimensional thin-layer chromatography. The regions of the gel that contained lipid material were scraped, and the radioactivity was determined by liquid scintillation spectrometry. Values are the absolute rate of incorporation of each phospholipid expressed as nmole/hr/10^8 cells and are the mean and S.E. of 4 determinations.

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