Effect of Cellular Fatty Acid Alteration on Hyperthermic Sensitivity in Cultured L1210 Murine Leukemia Cells

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ABSTRACT

We investigated the effect of fatty acid alteration on the thermosensitivity of a mammalian tumor cell, the L1210 lymphoblastic leukemia. Cells growing in Roswell Park Memorial Institute Medium 1640 with 5% fetal calf serum were modified with respect to their fatty acid composition by supplementing the media with either 40 μM docosahexaenoic acid (22:6) or oleic acid (18:1). A soft-agar clonogenic assay was used to assess viability following hyperthermic exposure. Cells grown in 22:6-supplemented media were more sensitive (D0 18.7 ± 0.4 min), and cells grown in 18:1-supplemented media were less sensitive (D0 46.2 ± 1.6 min) to a 42° exposure than were cells grown in unsupplemented media (D0 27.5 ± 1.5 min) (p < 0.001 for all comparisons). These contrasting effects on thermosensitivity increased as the concentration of supplemental fatty acid increased from 0 to 40 μM and were apparent at all temperatures from 41 to 44°C. When compared to cells grown in unsupplemented media, those grown in 22:6-supplemented media contained 300% more polyenoic and 70% less monoenic fatty acid in their cell phospholipids, while those grown in 18:1-supplemented media contained 37% less polyenoic and 17% more monoenic fatty acid. Similar changes were noted in neutral lipid fatty acid composition, while no changes were noted in cellular cholesterol or phospholipid content. Electron spin resonance studies suggested a significant increase in membrane fluidity when cells were grown in 22:6-supplemented media and a decrease when cells were grown in 18:1-supplemented media. These data demonstrate that modification of the cellular fatty acid composition of a mammalian tumor cell dramatically affects its thermosensitivity.

INTRODUCTION

There is a growing interest in hyperthermia which has stemmed from a number of observations which suggest that it may be a useful therapeutic modality in patients with cancer. Ample evidence in the literature documents the cytotoxic effect of mildly elevated temperatures both in vivo and in vitro (3). In addition, hyperthermia has been shown to interact synergistically with radiation (12, 29, 44), to potentiate the action of certain chemotherapeutic agents (18), and perhaps to preferentially kill neoplastic and transformed cells (7, 16, 17, 25). In vitro, these effects have sometimes been associated with a marked decrease in their maximal growth rate. Doubling times calculated during the logarithmic phase of growth were 12.5 ± 1.2 (S.E.), 12.5 ± 1.1, and 11.5 ± 1.5 hr for the cells grown in 22:6-supplemented, 18:1-supplemented, and unsupplemented media, respectively. Using the Escherichia coli mutant K1060, Yatvin (47) demonstrated that cells enriched with the polyunsaturated fatty acid linolenic acid (18:3; individual fatty acids are abbreviated as number of carbon atoms: number of double bonds) were more susceptible to thermal killing than were cells enriched with the monounsaturated acid, oleic acid (18:1). In addition, Overath et al. (37) found that the 18:3-supplemented cells were incapable of growth at temperatures greater than 40°C whereas the 18:1-supplemented cells could grow at temperatures up to 45°C. Similar observations have been made by McElhaney using Acholeplasma laidlawii B (33). Enrichment of these cells with fatty acids having a low melting point was associated with a marked decrease in their maximal growth temperature.

A full exploration of the usefulness of hyperthermia in the treatment of cancer would be greatly facilitated by the identification of those factors which govern its cytotoxic effect. It is therefore important to determine whether or not observations relating the thermosensitivity of bacterial cells to their fatty acid composition might extend to mammalian tumor cells. Recent observations by Hidvegi et al. (20) suggest this to be the case. Murine P388 cells grown in animals fed a diet high in polyunsaturated fatty acids were shown to be more thermosensitive than cells grown in animals fed a diet high in saturated fatty acids. We report here the results of studies which indicate that modifications of the cellular fatty acid composition of L1210 murine leukemia cells markedly influence their thermosensitivity.

MATERIALS AND METHODS

Cell Culture. L1210 murine leukemia cells were grown at 37°C in media consisting of RPMI4 1640 (Grand Island Biological Co., Grand Island, N. Y.), 5% FCS (K. C. Biological Inc., Lenexa, Kans.), and gentamicin sulfate (40 μg/ml; Schering Corporation, Kenilworth, N. J.) under a humid atmosphere of 5% CO2:95% air. To produce modification in cellular and membrane fatty acid composition, cells were grown under identical conditions in media further supplemented with either docosahexaenoic acid (22:6) or 18:1. These fatty acids in >99% pure form were obtained from Nu Chek Prep, Inc. (Elysian, Minn.), and known quantities of their sodium salt were added dropwise to the FCS used to prepare the growth media. Unless otherwise stated, 22:6 and 18:1 were added to the FCS such that the concentration of added fatty acid in the final growth media was 40 μM. At this concentration of added fatty acid, cell viability as assessed by dye exclusion, and clonogenicity was not affected. In addition, no differences were apparent in cell growth rate. Doubling times calculated during the logarithmic phase of growth were 12.5 ± 1.2 (S.E.), 12.5 ± 1.1, and 11.5 ± 1.5 hr for the cells grown in 22:6-supplemented, 18:1-supplemented, and unsupplemented media. Reports have suggested a correlation between the thermosensitivity of bacterial cells and their cellular fatty acid composition. Using the Escherichia coli mutant K1060, Yatvin (47) demonstrated that cells enriched with the polyunsaturated fatty acid linolenic acid (18:3; individual fatty acids are abbreviated as number of carbon atoms: number of double bonds) were more susceptible to thermal killing than were cells enriched with the monounsaturated acid, oleic acid (18:1). In addition, Overath et al. (37) found that the 18:3-supplemented cells were incapable of growth at temperatures greater than 40°C whereas the 18:1-supplemented cells could grow at temperatures up to 45°C. Similar observations have been made by McElhaney using Acholeplasma laidlawii B (33). Enrichment of these cells with fatty acids having a low melting point was associated with a marked decrease in their maximal growth temperature.

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2 Supported by NIH Training Grant T32 HL 07344. To whom requests for reprints should be addressed, at the Department of Medicine, University Hospitals, Iowa City, Iowa 52242.
3 Recipient of USPHS Career Development Award CA 00324.

4 The abbreviations used are: RPMI, Roswell Park Memorial Institute medium; FCS, fetal calf serum; ESR, electron spin resonance.

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unsupplemented media, respectively. Cells were reseeded into fresh media every 3 days. To ensure substantial modification in the cellular fatty acid composition, cells were allowed to grow in the fatty acid-supplemented media for at least 10 days (3 passages) prior to their use in any experiments.

**Hyperthermia and Survival Determination.** In all cases, the cells were reseeded into fresh media complete with supplemental fatty acid 24 hr prior to being used to ensure that they were growing exponentially at the time of hyperthermic exposure. They were then resuspended at a concentration of \(4 \times 10^5\) cells/ml in flasks containing 10 ml of media which had been preheated to the temperature being studied. The caps of the flasks were tightened securely, and the flasks were then completely submerged in a water bath at the specified temperature for the duration of the heating period. The media in which cells were suspended during heating consisted of RPMI 1640 and 5% FCS with no additional fatty acids. The pH was adjusted to 7.4 and remained constant throughout the heating period. A circulating water bath (Neslab Instruments, Inc., Portsmouth, N.H.) which is capable of maintaining the desired temperature within ±0.02° was used in all experiments. Temperatures were measured using a thermometer calibrated in tenths of a degree against a National Bureau of Standards-certified thermometer (Fisher Scientific Co., Pittsburgh, Pa.). After various heating intervals, flasks were quickly transferred to a 37° water bath to terminate the hyperthermic exposure.

The fraction of cells surviving the heating interval was then determined using a soft-agar clonogenic assay. In this assay, cells were suspended in media consisting of RPMI 1640, 20% horse serum (Grand Island Biological Co.), gentamicin sulfate (40 μg/ml), and Bacto agar (0.3 g/dl; Difco Laboratories, Detroit, Mich.) and plated onto 10- x 35-mm tissue culture dishes (Biolabs, Inc., Northbrook, Ill.). After the agar was allowed to solidify, dishes were incubated at 37° for 1 week at which time colonies consisting of at least 50 cells were counted using an inverted microscope. In this assay, the cloning efficiency of unheated cells was 75%, and a linear relationship existed between the number of cells plated and the number of colonies formed.

**Lipid Analysis.** Cells growing logarithmically were washed 3 times in phosphate-buffered saline prior to removal of an aliquot for protein determination (31). The remainder of the cells were extracted using CHCl₃:CH₃OH (2:1, v/v) (14). Aliquots of the cell lipid extract were then used to determine phospholipid (38), cholesterol (45), and acylglycerol (45) content.

An additional aliquot of cell lipid extract was used to determine fatty acid composition. Neutral lipids and phospholipids were separated using silicic acid chromatography (8). Each fraction was then saponified for 60 min at 56° in 1.2 N KOH and 8% ethanol (1). Fatty acids in the saponifiable fraction were methylated for 10 min at 95° with 14% BF₃:CH₃OH (36), and the methyl esters were separated by gas chromatography using a 1.8-m column packed with 10% SP 2340 on 100/200 mesh (Chromosorb (Supelco, Inc., Bellefonte, Pa.). Peak areas were quantitated and identified by comparison of retention times to those of standards obtained from Supelco, Inc. Statistical analysis was done using the t test.

**ESR Analysis.** The doxyl-substituted analog of stearic acid, 2-(3-carboxypropyl)-4,4-dimethyl-2-tridecyl-3-oxazolidinylxoyl, was purchased from Syva (Palo Alto, Calif.), and a stock solution at a concentration of 0.1 μM was prepared using 100% ethanol. Washed L1210 cells were incubated for 15 min at 37° in 15 ml of Hankos’ buffer (Grand Island Biological Co.) containing 5 μl of the stock solution. Cells were then washed with Hankos’ buffer, and ESR spectra were obtained using a Varian E-104 spectrometer equipped with a variable-temperature accessory. Temperatures were monitored with a Hewlett-Packard 346 FB Digital multimeter using a Teflon:coppper:constantan thermocouple placed directly above the sample in the ESR cavity. First-derivative absorption spectra were recorded with a 100-G field sweep, a scan time of 4 min, and a peak-to-peak modulation amplitude of 1 G. Microwave power was kept at 10 milliwatts to avoid signal saturation or sample heating. Recorder scan width was calibrated with Freny's salt and was operating in the X-band that was linear and accurate to ±0.1 G.

Order parameters (S) were calculated from the inner and outer extrema of the hyperfine splittings of the ESR spectra using the following relationship (15):

\[
S = \left( \frac{T_1 - T_2 - C}{T_1 + 2T_2 + 2C} \right) \times 1.723
\]

where 2\(T_1\) and 2\(T_2\) are the distances between outer and inner extrema, respectively, and \(C = 1.4 G - 0.053 (T_1 - T_2)\).

In preliminary ESR studies, a supplementary technique was used which has been applied previously to mouse L-cells and human lymphocytes and platelets (24, 39). Kaplan et al. (24) have reported that the decay of spin labels that occurs with time can be reversed with potassium ferricyanide. Since intact cells are relatively impermeable to this compound, it has been argued that a ferricyanide-regenerated spectrum arises from spin label probe incorporated into the plasma membrane (24). We therefore compared initial and ferricyanide-regenerated spectra in an attempt to determine if the order parameters taken from intact cells might be used as approximations of plasma membrane fluidity. After ESR spectra were recorded, cells were incubated at 37° for 30 min, and the spectra were allowed to decay completely. Spectra were then regenerated by the addition of 1 mM ferricyanide and immediately recorded. Order parameters calculated from the initial and regenerated spectra did not differ. In addition, a comparison of the height of the central band amplitudes of the initial and regenerated spectra showed that greater than 95% of the initial spectral intensity was immediately recoverable by the addition of ferricyanide.

**RESULTS**

**Thermosensitivity of L1210.** Chart 1 illustrates the survival curves for L1210 cells heated at 42°. As has been noted with many cell lines (3), including the L1210, survival curves are characterized by an initial shoulder followed by an exponential decline in surviving fraction. Cells grown in 22:6-supplemented media were remarkably more sensitive to the cytotoxic effects of heat than were cells grown in 18:1-supplemented media. Statistically significant differences in surviving fraction were noted at all time points exceeding 30 min, and with a 3-hr exposure to hyperthermia there was nearly a 2-log difference in the surviving fractions of cells grown in the 22:6-versus the 18:1-supplemented media. When compared to cells grown in unsupplemented media, the addition of 22:6 and 18:1 to the growth media led to contrasting effects on the thermosensitivity of the cells. Thus, supplementation of the media with 22:6 resulted in an increase in thermosensitivity, while supplementation with 18:1 resulted in a decrease in thermosensitivity. \(D_0\) values were 18.7 ± 0.4 min for cells grown in 22:6-supplemented media, 46.2 ± 1.6 min for cells grown in 18:1-supplemented media, and 27.5 ± 1.5 min for cells grown in unsupplemented media. These values are all significantly different at \(p < 0.001\).

We next determined whether these differences in thermosensitivity were a function of the concentration of supplemental fatty acid and whether they would persist at lesser concentrations. Cells grown in 10 to 40 μM fatty acid were heated for 2 hr at 42°, and the surviving fractions were determined (Chart 2). With increasing concentrations of 22:6, there was an increase in thermosensitivity. Even at a supplemental 22:6 concentration of only 10 μM, thermosensitivity was greater than that seen with cells grown in the unsupplemented media (\(p < 0.001\)). Increasing concentrations of supplemental 18:1 re-
The percentage of surviving cells was determined using a clonogenic assay.

**Chart 1.** Effect of type of fatty acid added to culture media on thermosensitivity at 42°. L1210 cells were grown in media supplemented with either 22:6 or 18:1 at a concentration of 40 μM and then heated at 42° for the designated times. The percentage of surviving cells was determined using a clonogenic assay. Points, mean of at least 5 determinations; bars, S.E.

**Chart 2.** Effect of concentration of supplemental fatty acid on thermosensitivity. L1210 cells were grown in media supplemented with either 22:6 or 18:1 at concentrations ranging from 0 to 40 μM and then heated at 42° for 2 hr. The percentage of surviving cells was determined using a clonogenic assay. Points, mean of at least 5 determinations; bars, S.E.

**Table 1.** Effect of type of fatty acid added to culture media on thermosensitivity at 41-44°

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Time (min)</th>
<th>22:6 supplemented</th>
<th>18:1 supplemented</th>
</tr>
</thead>
<tbody>
<tr>
<td>41.0°</td>
<td>90</td>
<td>90.4 ± 2.9</td>
<td>97.8 ± 1.6</td>
</tr>
<tr>
<td>41.5</td>
<td>90</td>
<td>67.2 ± 3.5</td>
<td>88.6 ± 2.6</td>
</tr>
<tr>
<td>42.0</td>
<td>90</td>
<td>24.6 ± 1.4</td>
<td>52.3 ± 2.4</td>
</tr>
<tr>
<td>42.5</td>
<td>90</td>
<td>1.1 ± 0.3</td>
<td>4.7 ± 0.9</td>
</tr>
<tr>
<td>43.0</td>
<td>30</td>
<td>16.2 ± 0.4</td>
<td>58.3 ± 5.8</td>
</tr>
<tr>
<td>44.0</td>
<td>30</td>
<td>0.5 ± 0.1</td>
<td>7.9 ± 2.0</td>
</tr>
</tbody>
</table>

- Differences between cells grown in the 22:6- and 18:1-supplemented media were significant at each temperature (p < 0.05).
- Mean ± S.E. of determinations made on 4 individual samples of cells.
- With a 90-min heating interval at 43.0° and 44.0°, cell survival was not detected. Therefore, a 30-min heating interval was used.

**Table 2.** Fatty acid composition of L1210 cell phospholipids

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>22:6 supplemented</th>
<th>18:1 supplemented</th>
<th>Unsupplemented</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0</td>
<td>17.3 ± 0.3</td>
<td>12.3 ± &lt;0.1</td>
<td>11.4 ± 0.1</td>
</tr>
<tr>
<td>16:1</td>
<td>2.8 ± 0.1</td>
<td>2.8 ± &lt;0.1</td>
<td>3.7 ± &lt;0.1</td>
</tr>
<tr>
<td>18:0</td>
<td>18.5 ± 0.2</td>
<td>11.4 ± 0.1</td>
<td>18.3 ± 0.2</td>
</tr>
<tr>
<td>18:1</td>
<td>14.8 ± 0.1</td>
<td>60.7 ± 0.6</td>
<td>51.2 ± 0.2</td>
</tr>
<tr>
<td>18:2</td>
<td>1.2 ± &lt;0.1</td>
<td>1.8 ± &lt;0.1</td>
<td>2.3 ± 0.1</td>
</tr>
<tr>
<td>20:4</td>
<td>2.9 ± 0.2</td>
<td>2.6 ± 0.2</td>
<td>4.2 ± &lt;0.1</td>
</tr>
<tr>
<td>22:6</td>
<td>36.1 ± 0.3</td>
<td>0.5 ± 0.1</td>
<td>0.8 ± &lt;0.1</td>
</tr>
<tr>
<td>Other</td>
<td>8.0 ± 0.5</td>
<td>8.0 ± 0.6</td>
<td>7.7 ± 0.6</td>
</tr>
</tbody>
</table>

- Differences between the 22:6- and 18:1-supplemented cells are highly significant (p < 0.001) for all the fatty acids except 20:4.
- Mean ± S.E. of determinations made on 4 individual samples of cells.

The overall changes in the fatty acid composition of the phospholipids and neutral lipids are summarized in Table 4. Within the phospholipid fraction, growth in 22:6-supplemented media resulted in less impressive changes in thermosensitivity compared to cells grown in unmodified media, with statistical significance being obtained only at 40 μM supplemental 18:1 (p < 0.05). However, a trend for decreasing thermosensitivity with increasing concentration of supplemental 18:1 was apparent. Most importantly, at all concentrations studied, there was a large difference between cells grown in 22:6- and 18:1-supplemented media.

It has been suggested that the mechanism by which hyperthermia kills cells may be different at different temperatures. It was therefore important to determine whether these striking differences in thermosensitivity persisted at temperatures other than 42°. As shown in Table 1, cells grown in the 22:6-supplemented media were more thermosensitive than were cells grown in the 18:1-supplemented media at all temperatures from 41 to 44°. While differences were significant at all temperatures (p < 0.05), they were most impressive at 44° where more than a 1-log difference in surviving fraction was apparent with only a 30-min heating interval.

**Hypermefic Sensitivity and Fatty Acids**
media resulted in a greater than 300% increase in polyenoics, a 70% decrease in monoenicis, and a 20% increase in saturates when compared to cells grown in unsupplemented media. Growth of cells in 18:1-supplemented media resulted in changes in the opposite direction with a 37% decrease in polyenoics, a 17% increase in monoenicis, and an 18% decrease in saturates contained in phospholipids. These changes resulted in a nearly 3-fold increase in the average number of double bonds per fatty acid molecule in cells grown in 22:6-supplemented media. A slight reduction in the number of double bonds per fatty acid molecule was apparent in the cells grown in the 18:1-supplemented media. While growth in the 22:6-supplemented media resulted in an increase in the average carbon atom chain length (when compared to unsupplemented, p < 0.001), growth in the 18:1-supplemented media was associated with a slight but significant decrease (when compared to unsupplemented, p < 0.005). The modifications in neutral lipids were similar.

The quantities of phospholipid, cholesterol, and acylglycerol found in the L1210 cells grown in the supplemented media are shown in Table 5. The addition of fatty acid to the growth media had no significant effect on cellular phospholipid and cholesterol content. In contrast, media supplementation with either 22:6 or 18:1 did result in highly significant increases in cellular acylglycerol content (p < 0.001), indicating the accumulation of storage lipids within the cells grown in both supplemented media.

ESR Data. First-derivative ESR spectra from the spin-labeled cells were recorded. The order parameter, S, was calculated for the unsupplemented cells (p < 0.025 at each temperature). In contrast, growth of cells in 18:1-supplemented media was associated with increases in the order parameters (indicating a lesser fluidity) which were statistically significant at 37°C (p < 0.025). Differences between the cells grown in the 22:6- and 18:1-supplemented media were highly significant at all temperatures (p < 0.001).

**DISCUSSION**

In this study, we have demonstrated that the addition of fatty acid to the media in which a mammalian tumor cell line is grown has a pronounced effect on its thermosensitivity. Furthermore, the type of effect noted is dependent upon the specific fatty acid added to the media. Thus, an increase in thermosensitivity was observed when cells were grown in media supplemented with either 22:6 or 18:1 at a concentration of 40 μM. After the cells were spin labeled with 5-nitroxyosrenate, ESR spectra were recorded, and order parameters were calculated as defined under "Materials and Methods."

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**Table 3**

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>22:6 supplemented</th>
<th>18:1 supplemented</th>
<th>Unsupplemented</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0</td>
<td>15.4 ± 0.5</td>
<td>18.0 ± 0.9</td>
<td>21.9 ± 1.2</td>
</tr>
<tr>
<td>16:1</td>
<td>3.9 ± 0.2</td>
<td>6.0 ± 1.2</td>
<td>7.0 ± 0.3</td>
</tr>
<tr>
<td>18:0</td>
<td>11.6 ± 0.6</td>
<td>12.9 ± 1.2</td>
<td>13.1 ± 1.4</td>
</tr>
<tr>
<td>18:1</td>
<td>19.3 ± 1.2</td>
<td>40.4 ± 2.1</td>
<td>25.1 ± 0.7</td>
</tr>
<tr>
<td>18:2</td>
<td>10.2 ± 2.6</td>
<td>6.4 ± 0.8</td>
<td>13.9 ± 2.1</td>
</tr>
<tr>
<td>20:4</td>
<td>0.6 ± 0.1</td>
<td>1.1 ± 0.1</td>
<td>0.9 ± 0.2</td>
</tr>
<tr>
<td>22:6</td>
<td>28.7 ± 2.5</td>
<td>0.1 ± 0.1</td>
<td>ND</td>
</tr>
<tr>
<td>Other</td>
<td>10.3 ± 1.1</td>
<td>15.0 ± 0.9</td>
<td>18.1 ± 0.8</td>
</tr>
</tbody>
</table>

* Differences between the 22:6 and 18:1 supplemented cells were significant (p < 0.05) for all fatty acids except 18:0 and 18:2.
* Mean ± S.E. of determinations made on 4 individual samples of cells.
* ND, not detected.

**Table 4**

<table>
<thead>
<tr>
<th>Lipid</th>
<th>% saturated</th>
<th>% monoenoic</th>
<th>% polyenoic</th>
<th>No. of double bonds</th>
<th>Chain length</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phospholipids</td>
<td>44.1 ± 0.3</td>
<td>6.5 ± 0.5</td>
<td>2.7 ± &lt;0.1</td>
<td>19.2 ± &lt;0.1</td>
<td>18.0 ± 0.2</td>
</tr>
<tr>
<td>22:6 supplemented</td>
<td>36.6 ± 0.2</td>
<td>66.2 ± 0.6</td>
<td>16.6 ± 0.2</td>
<td>44.1 ± 0.3</td>
<td>2.7 ± &lt;0.1</td>
</tr>
<tr>
<td>18:1 supplemented</td>
<td>24.9 ± 0.1</td>
<td>65.5 ± 0.3</td>
<td>10.3 ± 0.2</td>
<td>17.8 ± &lt;0.1</td>
<td>17.9 ± &lt;0.1</td>
</tr>
<tr>
<td>Unsupplemented</td>
<td>30.4 ± 0.3</td>
<td>56.5 ± 0.3</td>
<td>10.3 ± 0.2</td>
<td>17.8 ± &lt;0.1</td>
<td>17.9 ± &lt;0.1</td>
</tr>
<tr>
<td>Neutral lipids</td>
<td>42.7 ± 1.1</td>
<td>2.4 ± 0.1</td>
<td>18.7 ± 0.1</td>
<td>18.7 ± 0.1</td>
<td>18.7 ± 0.1</td>
</tr>
<tr>
<td>22:6 supplemented</td>
<td>28.9 ± 0.9</td>
<td>42.7 ± 1.3</td>
<td>2.4 ± 0.1</td>
<td>18.7 ± 0.1</td>
<td>18.7 ± 0.1</td>
</tr>
<tr>
<td>18:1 supplemented</td>
<td>33.9 ± 1.0</td>
<td>8.9 ± 1.2</td>
<td>0.8 ± &lt;0.1</td>
<td>17.3 ± &lt;0.1</td>
<td>17.3 ± &lt;0.1</td>
</tr>
<tr>
<td>Unsupplemented</td>
<td>38.9 ± 1.4</td>
<td>32.9 ± 0.6</td>
<td>16.5 ± 2.0</td>
<td>0.8 ± &lt;0.1</td>
<td>17.5 ± &lt;0.1</td>
</tr>
</tbody>
</table>

* Mean ± S.E. of determinations on 4 individual samples of cells.
with the highly polyunsaturated fatty acid 22:6, and a decrease in thermosensitivity was noted when cells were grown in media supplemented with the monounsaturated fatty acid 18:1. These differences, which were significant at all heating intervals from 30 to 180 min, increased as the concentration of fatty acid added to the media increased and were apparent over the range of temperatures studied from 41 to 44°C.

In all studies, cells were handled in an identical fashion with the only variable being the addition of either 22:6 or 18:1 to the growth media. In an attempt to explain our observations, we therefore searched for differences in cellular lipid composition which might correlate with the differences observed in thermosensitivity. The addition of 22:6 to the growth media resulted in changes in the phospholipid fatty acid composition which were in general the opposite of those observed when 18:1 was added to the media. Of particular interest, however, were the differences in the percentage of polyenoic fatty acids. When cells were grown in 18:1-supplemented media, there was a reduction in the percentage of polyenoic fatty acids such that they comprised less than 10% of the total phospholipid fatty acids. This contrasts with the dramatic increase in the percentage of polyenoic fatty acids (44%) seen when cells were grown in 22:6-supplemented media. While previous reports have shown that it is possible to modify the phospholipid fatty acid composition by altering the fatty acids supplied to a cell in its media (2, 28, 30, 43, 46), our studies are noteworthy for the extent of modification brought about when 22:6 was used to supplement the media. This unusual highly polyunsaturated fatty acid normally comprises only a small fraction of the cellular fatty acids. To our knowledge, enrichment of phospholipids and neutral lipids with this fatty acid to the extent which we have reported here has not been described previously. Indeed, the ability of the L1210 cell to readily incorporate such large amounts of 22:6 into its lipids suggests that it may be an ideal model in which to study further the effects of this fatty acid in a variety of cellular processes.

Since phospholipids are important components of cell membranes, such changes in their fatty acid composition might be expected to have an important influence on various membrane properties. The determinants of membrane fluidity have recently been reviewed (22), and previous reports have noted a relationship between the fatty acid composition of phospholipids and membrane fluidity (5, 27, 28). In particular, it is known that an increase in the percentage of polyenoic fatty acids with a resultant increase in the mean number of double bonds per fatty acid molecule exerts a membrane-fluidizing effect. We have used an ESR technique to make fluidity measurements, and the values obtained suggest that the growth of L1210 cells in 22:6-supplemented media resulted in an increase in membrane fluidity and that growth in 18:1-supplemented media lead to a decrease in fluidity. It is necessary to emphasize that these measurements have been made using intact cells and not isolated cell membranes. The results may therefore be criticized because of the possibility that the spectra recorded arose from probe which was distributed throughout all cell lipids and not just within membrane structures. We have shown previously, however, that order parameters obtained from fatty acid-altered intact L1210 cells closely approximate those obtained from their isolated plasma membranes (5, 41). In addition, we have shown that changes in order parameters brought about by the modification of the fatty acid composition of L1210 cells cannot be explained by the influence of probe located within cytoplasmic lipid droplets (41). Thus, although our measurements of membrane fluidity have been made using intact cells and must be interpreted cautiously, in light of our previous studies involving fatty acid modified L1210 cells and particularly considering the dramatic differences observed in phospholipid fatty acid composition, it is likely that significant differences in membrane fluidity were indeed present.

These studies therefore suggest a correlation between the thermosensitivity of a mammalian cell and the fatty acid composition and fluidity of its cellular membranes. While it is possible that these are not causally related, several additional observations in the literature would indicate that this is unlikely. (a) In response to changes in environmental temperature, many organisms have been shown to alter their phospholipid fatty acid composition (13, 35, 47). This process, termed "homeoviscous adaptation," is believed to represent an attempt to minimize the influence of temperature on the fluidity of membrane lipids (42). It suggests that membrane fluidity as influenced by the phospholipid fatty acid composition may be an important determinant of the ability of an organism to survive a temperature shift. (b) Yativ (47) using the E. coli mutant K1060 and Yau (48) using 2 mammalian tumor cell lines have shown that procaine, which is known to have a membrane-fluidizing effect, increases thermosensitivity. (c) Cress and Gerner (9) have demonstrated that the cellular cholesterol content of 5 mammalian cell lines correlates inversely with their thermosensitivity. While we noted no changes in cholesterol content in our studies, their data suggest that changes in thermosensitivity might be brought about by modifying other membrane lipids which are likely to affect the physical properties of the membrane. (d) Hahn et al. (19) have shown that amphotericin B, a drug known to interact with membrane sterols and to increase membrane permeability, heightens the thermosensitivity of Chinese hamster ovary cells. Our observations, taken together with these, strongly suggest that the chemical and physical properties of membrane lipids are important determinants of the thermosensitivity of mammalian cells.

The mechanism whereby changes in membrane fatty acid composition and fluidity might alter thermosensitivity is speculative. (a) It is possible that the incorporation of highly unsaturated fatty acids into the lipid bilayer may lead to a membrane which is structurally unstable at elevated temperatures. (b) The fatty acid composition and fluidity of membranes have been shown to have an effect on several membrane properties including permeability (34), endocytosis (32), and the activity of membrane-associated enzymes (8, 10, 26) and transport systems (5, 23). Optimal function of these and similar membrane-related processes might be of importance if a cell is to survive a potentially lethal insult such as hyperthermic exposure (4). (c) It is possible that the fatty acid composition and fluidity of the lipids surrounding critical membrane proteins might affect their heat denaturability. Although data directly pertaining to this hypothesis are limited, studies correlating the thermosensitivity of E. coli K1060 with its membrane fatty acid composition suggest that this is probably not the case (11).

The alterations in thermosensitivity such as we have brought about may have therapeutic uses. Techniques for the perfusion of the liver or an isolated extremity for the delivery of chemotherapeutic agents.
therapy have been developed. These could be adapted to allow delivery of perfusion fluid containing a high concentration of albumin-bound polysaturated fatty acid to the region containing the tumor followed by localized delivery of hyperthermia. Alternately, dietary alteration of cellular fatty acids could be attempted prior to the local delivery of heat directly to a tumor. We are now investigating an animal model of the latter possibility.

In summary, we interpret these data to indicate that experimental modification of the fatty acid composition of the L1210 cell lipids results in a marked alteration of hyperthermic sensitivity. This effect is related to the type of fatty acid in which the cell is enriched. Although we have suggested that the changes in thermosensitivity may be ascribed to the changes in fluidity, it is possible that these are unrelated. We believe that these observations may have long-range therapeutic implications.

REFERENCES


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