BUTYRATE-INDUCED ALTERATION IN LIPID COMPOSITION OF HUMAN COLON CELL LINE HT-29

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SUMMARY. The effect of butyrate-induced differentiation on lipid composition of HT-29 cells were studied. It was observed that cell differentiation was associated with increased activity of alkaline phosphatase and changes in the lipid composition. Differentiated cells showed increased level of triacylglycerol, cholesteryl esters and decreased free cholesterol and phospholipids. Changes were also observed in individual phospholipid composition. It appears that butyrate induced differentiation is associated with increased esterification of neutral lipids.

INTRODUCTION

Butyrate has been shown to induce differentiation of several cell types (1). It has been shown that short chain fatty acids arrest proliferation of normal cells, while it is cytostatic to transformed cells (2). The morphological changes induced by butyrate are associated with changes in membrane lipid composition (3). In neonate cerebellum derived clonal oligodendrocyte cell line (CB-II), morphological transformation induced by butyrate was associated with changes in membrane phospholipids (4). Short chain fatty acids (butyrate, acetate, and propionate) are normal constituents of the colonic lumen and serve as respiratory fuel for the colonocytes. These short chain fatty acids are formed in the colon by the bacterial fermentation of dietary fibre (5).

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The HT-29 cell line is of human colon origin and the differentially HT-29 cells exhibit closest analogies to human fetal colon (6). This study looks at the effect of butyrate-induced differentiation on the lipid composition of HT-29 cells and compare with the undifferentiated cells.

**MATERIALS AND METHODS**

Butyric acid, lipid standards, bovine serum albumin, and p-nitrophenyl phosphate were obtained from Sigma Chemical Co. RPMI-1640 medium and fetal calf serum were obtained from Flow Laboratories. All other chemicals used were of analytical grade.

**Cell culture:** Human colon carcinoma cell line, HT-29 obtained from American Tissue Culture Collection (ATCC) was used in this study. Cell line was maintained in RPMI-1640 supplemented with 10% (v/v) fetal calf serum (FCS), 100 units/ml penicillin, 100 μg/ml streptomycin and 0.025 μg/ml fungizone. Cells were grown in 150 cm² flasks at 37° in 5% CO₂, fed at 2 days interval and passaged every four days interval. Stock cells were trypsinized, suspended at 10⁶ cells/ml in medium and 20 ml was seeded in tissue culture flask. Cells were allowed for 24h after seeding and then various filter-sterilised compounds were added from stock solution. Medium of experimental flasks was changed at 3 days interval. Confluent monolayers were used for this study.

**Determination of cell proliferation and differentiation:** Cell proliferation was followed by determining the total number of cells in each flask at specified intervals. At the end of each interval, the monolayer culture after removal of the culture medium, was washed with prewarmed phosphate buffered saline (PBS). Monolayer was harvested with 0.07% trypsin containing 0.03% EDTA and washed twice with PBS, finally suspended in PBS. The resulting cell suspension was appropriately diluted, stained with 0.1% trypan blue and counted in Coulter Hemocytometer. Cell differentiation was assessed by measuring the activity of alkaline phosphatase (7). Protein was measured using BSA as standard (8).

**Extraction and analysis of lipids:** Lipids from cell homogenates were extracted as described (9). The lower organic phase was evaporated to dryness, resuspended in a small volume of chloroform:methanol (2:1 v/v), and used for lipid analysis. Lipids were quantitated after separation by thin layer chromatography. Neutral lipids were separated on TLC with silica gel G plates using the solvent system hexane:diethylether:acetic acid (80:20:1 v/v). Separated lipids were visualized by exposure to iodine and individual spots scraped off, eluted with chloroform:methanol (2:1 v/v), and then dried using nitrogen gas. Cholesterol and cholesteryl ester were estimated as described (10). Triglycerides were quantitated colorimetrically (11). Individual phospholipids were separated on TLC with 1mM Na₂CO₃ impregnated silica gel H plates using the solvent system chloroform:methanol:acetic acid: H₂O (25:15:4:2 v/v) (12). Separated phospholipids were eluted after identification with iodine exposure and quantitated by phosphate estimation (13).
Statistical analysis: At least three separate experiments were carried out and results are presented giving the mean ± SEM. Mann-Whitney U test was done to compare the changes.

RESULTS AND DISCUSSION

Figure 1 shows the effect of 2 mM butyrate on cell number and alkaline phosphatase activity at different time intervals. As can be seen, the total number of cells decreased as the time of exposure to butyrate was increased (Fig. 1A). Concomitant with this, an increase in alkaline phosphatase activity was observed (Fig.1B). A similar increase in alkaline phosphatase activity was seen by others on butyrate treatment of HT-29 cells(14) and another colon adenocarcinoma cell line LS 174T (15). In the present study, the increase in alkaline phosphatase activity was 5 fold at 3 days and 7 fold at 6 days following butyrate treatment.

Cell differentiation is known to alter the lipid composition. Table 1 shows the lipid composition of the control and butyrate differentiated HT-29 cells both at 3 days and 6 days. Differentiated cells showed increased triacylglycerol which was prominent at 6 days. Similarly, cholesteryl ester level was also increased in differentiated cells as compared to control cells. Simultaneously a decrease in free cholesterol level was observed in the differentiated cells. Total phospholipids decreased following differentiation. Diacylglycerol, monoacylglycerol and free fatty acid were not present both in the control and differentiated cells. Among the individual phospholipids, phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine and phosphatidylinositol were decreased in differentiated cells, whereas other phospholipids were unaltered. In an earlier study, differentiation of HT-29 cells by glucose deprivation showed changes in phospholipids (16). It was also shown that these differentiated cells have increased activity of acylcholesterol acyltransferase (ACAT) which resulted in increased cholesteryl ester formation (17). Fatty acid esterification to triacylglycerol by increased utilization of diacylglycerol have also been demonstrated during differentiation of the human intestinal cell line Caco-2 (18). An increase in triacylglycerol level was also
Fig. 1. Effect of 2 mM butyrate on cell number and alkaline phosphatase activity at different days of culture. Each value represents mean ± SEM of three separate estimations. A, Number of cells significantly decreased in butyrate treated cells when compared to control (p < 0.05). B, Alkaline phosphatase significantly increased in butyrate treated cells when compared to control (p < 0.05). ■ Control ■ Butyrate treated.

seen in this study. A similar increase in cholesteryl ester and triacylglycerol formation was seen in the present butyrate induced differentiation. It may be due to increased esterification or decreased hydrolysis of these lipids in differentiated cells. Since earlier study has suggested an increase in ACAT activity in differentiated cells, it is likely that butyrate induced
Table 1. Lipid composition of control and butyrate treated HT-29 cells

<table>
<thead>
<tr>
<th>Lipids</th>
<th>3 days Control</th>
<th>+ Butyrate</th>
<th>6 days Control</th>
<th>+ Butyrate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n mole /mg protein)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>74.4 ± 3.3</td>
<td>60.3 ± 1.6</td>
<td>76.9 ± 2.4</td>
<td>40.7 ± 1.8</td>
</tr>
<tr>
<td>Cholesteryl esters</td>
<td>21.6 ± 1.7</td>
<td>29.3 ± 1.2</td>
<td>19.0 ± 1.7</td>
<td>37.7 ± 1.9</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>102.0 ± 4.9</td>
<td>126.5 ± 4.5</td>
<td>100.9 ± 7.5</td>
<td>184.7 ± 3.1</td>
</tr>
<tr>
<td>Total phospholipids</td>
<td>135.3 ± 8.0</td>
<td>112.4 ± 9.5</td>
<td>125.1 ± 7.3</td>
<td>84.9 ± 5.6</td>
</tr>
<tr>
<td>Individual phospholipids</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphatidyl choline</td>
<td>48.9 ± 2.8</td>
<td>39.9 ± 3.7</td>
<td>50.3 ± 2.5</td>
<td>35.1 ± 1.5</td>
</tr>
<tr>
<td>Phosphatidyl ethanolamine</td>
<td>33.2 ± 1.0</td>
<td>26.7 ± 2.1</td>
<td>31.8 ± 1.7</td>
<td>16.9 ± 1.0</td>
</tr>
<tr>
<td>Phosphatidyl serine +</td>
<td>21.2 ± 1.9</td>
<td>15.0 ± 1.0</td>
<td>19.7 ± 1.1</td>
<td>9.2 ± 1.2</td>
</tr>
<tr>
<td>Phosphatidyl inositol</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Spingomyelin</td>
<td>10.3 ± 0.8</td>
<td>8.9 ± 1.3</td>
<td>8.4 ± 0.9</td>
<td>9.5 ± 1.2</td>
</tr>
<tr>
<td>Cardiolipin</td>
<td>12.1 ± 0.6</td>
<td>12.6 ± 1.1</td>
<td>8.1 ± 0.7</td>
<td>7.5 ± 0.8</td>
</tr>
<tr>
<td>Lysophosphatidyl choline</td>
<td>9.5 ± 0.9</td>
<td>9.3 ± 1.8</td>
<td>6.7 ± 0.4</td>
<td>6.8 ± 0.5</td>
</tr>
</tbody>
</table>

Each value represents mean ± SEM of three separate estimations.
* Indicates a significant difference (p < 0.05) between control and treated cells.

differentiation also increases esterification especially of neutral lipids.

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REFERENCES