RAPID COMMUNICATION

**Induction of glutathione-S-transferase-pi by short-chain fatty acids in the intestinal cell line caco-2**

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**Abstract.** Glutathione S-transferases (GSTs) are a multigene family of detoxification and metabolizing enzymes that have been linked with the susceptibility of tissues to environmental carcinogens. In addition to their role as the main energy source in the colonic mucosa, short-chain fatty acids (SCFAs) have been found to act as potent antiproliferative and differentiating agents in various cancer cell lines. The objective of this study was to evaluate the effects of SCFAs on the induction of GSTpi in the intestine as a possible new anticarcinogenic mechanism of SCFAs. Studies were performed in Caco-2 cells, a cell line resembling functionally normal enterocytes. Cells, cultured in DMEM supplemented with 10% fetal calf serum, were studied from day 0 dpc (days post confluence) until 21 dpc and culture. SCFAs (acetate, propionate, butyrate) were added to give a final concentration of 5 mmol L\(^{-1}\). At 0, 3, 6, 9, 15, and 21 dpc, protein, lactate dehydrogenase (LDH), alkaline phosphatase (AP) and GSTpi were measured. Butyrate supplementation significantly increased GSTpi levels compared with controls in a concentration-dependent manner. The effect was detectable within 3 dpc with a maximum at 15 dpc. In contrast to butyrate, the other SCFAs tested had no (acetate) or little effect (propionate). In conclusion, the data suggest that the anticancer effect of butyrate in part may be based on the induction of GSTpi activity, resulting in an enhanced detoxification capacity of the gut.

**Keywords.** Caco-2 cells, colon cancer, short-chain fatty acids.

**Introduction**

Glutathione S-transferases (GSTs) are a multigene family of proteins functioning as detoxification enzymes by catalysing the conjugation of potentially mutagenic electrophilic compounds with reduced glutathione [1]. In the intestinal tract the induction of the GST isoenzyme class has recently been discussed as a possible anticancer mechanism [2] of dietary compounds.

In addition, short-chain fatty acids (SCFAs) besides having a role as the main energy source in the colonic mucosa [3], have been found to act as potent antiproliferative and differentiating agents in various cancer cell lines. Thus, the fermentation of dietary fibres and non-absorbed starch to SCFAs by colonic bacteria appears to be a crucial component in colon cancer prevention [4,5]. In numerous studies butyrate has been shown to decrease growth and increase differentiation of various cell types *in vitro* [6–8]. Thus, SCFAs have the potential to modify growth of colonic neoplasms and perhaps even to protect against their genesis and modulation of cell differentiation by SCFAs may be of benefit in the management and prevention of colon carcinoma because the amounts of SCFAs in the colon can be modified by dietary means.

The present *in vitro* study was designed to investigate the effects of various SCFAs on the induction of GSTpi in the human colonic cell line Caco-2, which is able to undergo different patterns of intestinal differentiation depending on nutritional manipulations [9].

**Materials and methods**

All chemicals were purchased from Sigma (St. Louis, MO, USA). Cell culture medium and medium supplies were obtained from Gibco (Grand Island, NY, USA). Caco-2 cells were obtained from the German Centre of Cancer Research (Heidelberg, Germany) at passage 19, and experiments were performed using cells between passages 24 and 34. Cells were grown in 5% CO\(_2\)–95% air humidified incubator at 37°C in Dulbecco’s modified Eagle medium supplemented with 25 mmol L\(^{-1}\) NaHCO\(_3\), 25 mmol L\(^{-1}\) N-2-hydroxy-ethyl-piperazine-N\(^{\prime}\)-2-ethane sulphonic acid (Hepes), 50 IU mL\(^{-1}\) streptomycin, 50 \(\mu\)g mL\(^{-1}\) penicillin and 20% fetal calf serum. The culture medium was renewed on alternate days. Stock cultures were subcultured (1:3) weekly by trypsinization with 0.25% trypsin and 0.2%
EDTA in phosphate buffered saline (PBS). Caco-2 cells grown as described were found to exhibit a transepithelial electrical resistance of 300–400 Ω cm² (measured with Milli-ERS; Millipore, Bradford, MA, USA).

**Enzyme assays**

Cells (2 × 10⁶ mL⁻¹) were grown in flasks in media containing SCFAs. After incubation, the culture medium was discharged and the cell monolayer rinsed twice with 10 mmol L⁻¹ Tris-HCl buffer (pH 7.4). The cells were harvested by gentle scraping in 0.5 mol L⁻¹ of Caco-2 cells are shown in Table 1. The results of this long-term experiment confirm unambiguously the antiproliferative effect of butyrate and propionate in contrast to acetate. Since the lactate dehydrogenase activity in SCFA-treated cells was unaffected compared with control cells toxicity effects toward the cells could be excluded (data not shown).

Butyrate and, to a lesser extent, propionate supplementation significantly (P < 0.01) increased GSTpi levels compared with controls in a concentration-dependent manner. The effect was detectable within 3 dpc with a maximum at 15 dpc. Acetate showed no effect (Fig. 1).

**Results**

The effects of SCFAs on cell growth and differentiation of Caco-2 cells are shown in Table 1. The results of this

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**Table 1.** Protein content (mg per dish) and enzyme activities (mU mg⁻¹ protein) of Caco-2 cells from day 0 dpc (days post confluence) until day 21 dpc. SCFAs are added at final concentration of 5 mmol L⁻¹ (n = 6–8)

<table>
<thead>
<tr>
<th>Protein</th>
<th>Control</th>
<th>Acetate</th>
<th>Propionate</th>
<th>Butyrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 3 dpc</td>
<td>1.73 ± 0.34</td>
<td>1.47 ± 0.13</td>
<td>1.67 ± 0.32</td>
<td>1.2 ± 0.68</td>
</tr>
<tr>
<td>Day 6 dpc</td>
<td>3.39 ± 0.21</td>
<td>2.99 ± 0.40</td>
<td>1.84 ± 0.30</td>
<td>1.64 ± 0.98*</td>
</tr>
<tr>
<td>Day 9 dpc</td>
<td>4.44 ± 0.26</td>
<td>4.11 ± 0.35</td>
<td>1.95 ± 0.19*</td>
<td>1.8 ± 0.16*</td>
</tr>
<tr>
<td>Day 15 dpc</td>
<td>4.96 ± 0.46</td>
<td>4.93 ± 0.14</td>
<td>2.10 ± 0.21*</td>
<td>1.4 ± 0.40*</td>
</tr>
<tr>
<td>Day 21 dpc</td>
<td>6.10 ± 0.86</td>
<td>5.49 ± 0.30</td>
<td>1.61 ± 0.14*</td>
<td>1.1 ± 0.32*</td>
</tr>
</tbody>
</table>

Alkaline phosphatase

| Day 3 dpc | 25.3 ± 3.64 | 24.4 ± 5.0 | 43.9 ± 6.1 | 98.2 ± 12.2* |
| Day 6 dpc | 34.3 ± 6.14 | 57.9 ± 14.4 | 167.0 ± 33.6* | 320.0 ± 48.8* |
| Day 9 dpc | 49.4 ± 2.27 | 61.2 ± 16.19 | 196.0 ± 29.6* | 420.0 ± 46.8* |
| Day 15 dpc | 87.8 ± 5.63 | 77.5 ± 4.92 | 204.2 ± 46.9* | 1240.0 ± 128.8* |
| Day 21 dpc | 97.7 ± 11.27 | 98.5 ± 4.96 | 285.7 ± 38.5* | 940 ± 124.4* |

*P < 0.05 or better compared with control.

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**Discussion**

The protective effect of dietary fibres against colorectal cancer is now widely accepted. Nevertheless, the exact mechanism by which such dietary compounds prevent carcinogenesis of the large bowel is poorly understood. Various antiproliferative effects of sodium butyrate on

![Figure 1](image-url). Evolution pattern of GSTpi in Caco-2 cells cultured in the presence of FCS alone (control) or in the presence of different SCFAs (mmol L⁻¹). Each curve is the mean ± SEM of 4–6 different experiments. * Significant different (P < 0.05 or better) compared with control (●). Error bars are not shown if they are smaller than the symbols. □, Acetate; ○, propionate; ■, butyrate.
different types of cells in vivo and in vitro have been described [13–16]. It has also been shown that butyrate can inhibit the development of grafted tumours in mice [17]. Given the difficulty of studying in vivo the anti-proliferative influence of SCFAs on colonic carcinogenesis, we have chosen to study their effect on the established intestinal cell line Caco-2.

Our study provides evidence that SCFAs (butyrate > propionate) under normal culture conditions enhance differentiation of the human colon cancer cell line Caco-2. These observations are consistent with previous findings in other tumour cell lines [8]. The concentrations of SCFAs used in our study are lower than those found in the lumen of the large bowel [18], suggesting that the observed effect may be of physiological relevance. In all experiments, acetate fails to affect cell growth and differentiation, which could be explained by a rapid metabolism of this SCFA by Caco-2 cells. On the other hand, butyrate, which has been shown in our study to be the most potent anti-proliferative acid, is also known to be highly metabolized in colonic cells [3]. Further work will be necessary to clarify the molecular mechanisms whereby butyrate and propionate induce cell differentiation of intestinal mucosal cells. Recently Gamet et al. [19] could demonstrate that the anti-proliferative effect of SCFAs is associated with the inhibition of ornithine decarboxylase (ODC), the key enzyme of polyamine synthesis.

The second obvious effect of SCFAs on the Caco-2 cell line presented in this investigation was the induction of glutathione S-transferase-pi. GSTs represent a family of dimeric isoenzymes in which the subunits belong to a supergene family of four classes, alpha, mu, pi and tetra [20,21], functioning as highly potent detoxification enzymes. In addition, GSTs have been shown to protect against the damaging of DNA by metabolites of polycyclic hydrocarbons or aflatoxin B, both by catalysing the conjugation of reactive metabolites with glutathione and by covalent binding of reactive species [22,23]. Similar effects for sodium butyrate have recently been shown in the SW620 tumour cell line. In this study the glycoprotein P-170 level increased 25-fold after sodium butyrate treatment. It is likely that GST as a membrane-associated phosphoglycoprotein has an important role in detoxification in tissues such as colon, kidney and liver [24].

Based on these data one can assume that the concentration reactive metabolites of drugs, plant toxins and environmental pollutants will decrease more rapidly when GST and/or P170 levels are higher.

In conclusion, our study suggests both butyrate and propionate may play an important role in vivo in inhibiting growth of neoplastic colonic cells. These compounds could account for the protective effect of dietary fibres in colon carcinogenesis. Therefore it is possible to speculate that both butyrate and propionate could play a dual regulatory role in colonic function and structure, activating both normal mucosal differentiation and detoxifying enzymes such as glutathione S-transferases.

References
