Proceedings of the Nutrition Society (2008), **67 (OCE)**, E87 doi:10.1017/S0029665108007192

Diet and Chronic Disease, Nutrition Society Summer Meeting, 16–19 July 2007

Role of DNA-mismatch repair in anti-neoplastic effects of butyrate

S. S. Dronamraju[1], J. M. Coxhead[1], S. B. Kelly[2] and J. C. Mathers[1]
[1]*Human Nutrition Research Centre, Newcastle University, Newcastle Upon Tyne NE2 4HH, UK and* [2]*North Tyneside General Hospital, North Shields NE29 8NH, UK*

Colo-rectal cancer (CRC) is the second-most-common cause of cancer-related death in the Western world[(1)]. DNA-mismatch repair (MMR) genes regulate key cellular processes, including correction of DNA replication errors[(2)]. Impaired functioning of MMR has been implicated in the aetiology of hereditary non-polyposis colon cancer and in ≤15% of sporadic CRC[(2)]. The C_4 fatty acid, butyric acid, which is produced by bacterial fermentation of resistant starches in the large bowel, has potent anti-neoplastic effects on colon cancer cells[(3)]. Recent *in vitro* studies have indicated that MMR status may modulate the anti-neoplastic effects of butyrate[3]. The present study aimed to investigate the mechanisms underlying these differential effects of butyrate on colon cancer cells.

SW48 colon cancer cells, in which the MMR gene *MLH1* is silenced by promoter hypermethylation, were treated with the demethylating agent 5-aza 2′deoxycytidine to partially demethylate and reactivate the *MLH1* gene. The native SW48 cells and their demethylated counterparts were treated with butyrate (0–5 mM) for 8 d and the effects on cell proliferation, *MLH1* gene promoter methylation (combined bisulfite restriction analysis assay) and expression of two butyrate-responsive genes, i.e. *CDK4* and *GADD45A*, were assessed (real-time RT–PCR).

Butyrate (0.5–5 mM) suppressed proliferation ($P<0.001$) and reduced *MLH1* promoter methylation ($P<0.05$) in SW48 cells. However, in demethylated SW48 cells butyrate caused a small but significant increase in cell proliferation ($P<0.05$; Fig. 1) and promoter methylation ($P<0.05$). *CDK4* expression was higher ($P=0.02$) in demethylated SW48 cells compared with native SW48 cells. There was little effect of butyrate on *CDK4* expression in SW48 cells, but this was reduced markedly in the demethylated cells ($P=0.025$ for cell line × butyrate interaction; Fig: 2). Further there was more than two fold up regulation of *GADD45A* expression following butyrate (1 mM) treatment in native SW48 cells as compared with demethylated SW48 cells in which *GADD45A* expression was down regulated ($P=0.045$ for cell line × butyrate interaction; Fig: 2).

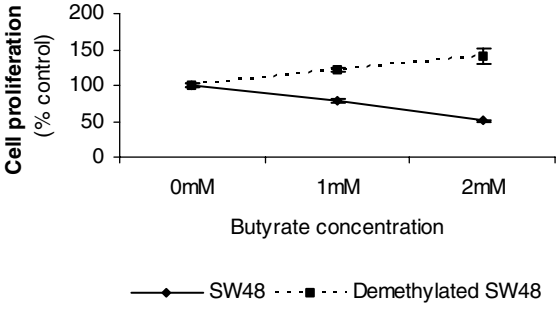

Fig. 1

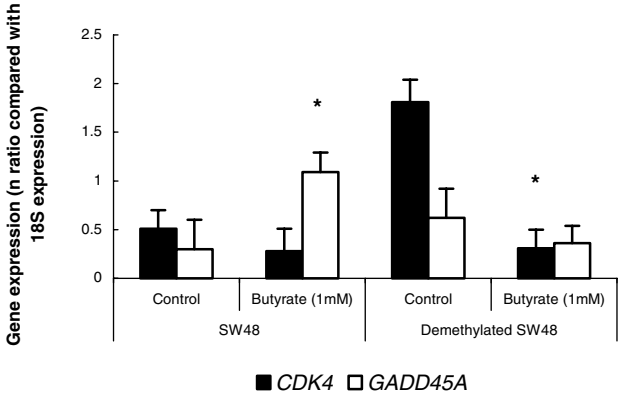

Fig. 2

The present study suggests that butyrate may have more potent anti-neoplastic effects on colon cancer cells, with epigenetic silencing of *MLH1* function. Although butyrate showed differential expression of *CDK4* and *GADD45A* genes, it does not explain its effects on cell proliferation. It is essential to investigate the effects of butyrate on more cell-cycle regulatory genes to understand the molecular mechanisms underlying these differential effects.

This study was supported by the Biotechnology and Biological Sciences Research Council (grant no.13D/20173).

1. Cancer Research UK (2007) http://info.cancerresearchuk.org/cancerstats
2. Wheeler JM (2005) *Ann R Coll Surg Engl* **87**, 15–20.
3. Coxhead JM, Williams EA & Mathers JC (2005) *Biochem Soc Trans* **33**, 728–729.