released, leading to a prolonged and larger oxidation of exogenous glucose.

COLONIC MICROFLORA AND METABOLIC FUNCTIONS

Molecular mechanisms of butyrate action on HT-29 intestinal epithelial cell proliferation. S. Siavoshian, J.P. Segain, C. Cherbut, J.P. Galmiche, H.M. Blottière (Human Nutrition Research Center, CRI Inserm 95-08, CHU Hôtel-Dieu, Inra, 44035 Nantes cedex 01, France).

Sodium butyrate, a product of colonic bacterial fermentation, is able to inhibit cell proliferation and to induce the differentiation of colonic epithelial cells in culture. In a variety of cell systems, butyrate has been found to block cells in the G1 phase. D type cyclins appear early in the G1 phase and complex with cdk4 or cdk6. Then, they can bind to the unphosphorylated form of pRb, thus allowing E2F transcription factor to activate gene transcription. The p21 protein inhibits the action of G1/S cdk-cyclin complexes. The aim of our study was to investigate the mechanisms by which butyrate inhibits cell cycle progression toward S phase.

HT-29 cells were cultured in the presence or absence of increasing concentration of sodium butyrate (from 2 to 8 mM) for 24 h. Proteins were extracted, and cyclin D1, D3, E2F-1 and p21 expression were studied by western blotting. mRNA were extracted, and cyclin D1, D2 and p21 expression were studied by RT-PCR.

Butyrate inhibited cyclin D1 mRNA expression, without affecting its protein level. In contrast, butyrate stimulated cyclin D3 protein expression. We failed to detect any mRNA for cyclin D2 in HT-29 cells. Moreover, a dose-dependent decrease in E2F-1 expression was observed in HT-29 exposed to butyrate. In addition, after 6 h of incubation with butyrate, p21 mRNA was detected and mRNA expression reached a plateau in between 12 and 24 h. At the protein level, no p21 was detected at 6 h. At 12 h, p21 was detected and the optimal detection was observed at 24 h.

Our results suggest that the inhibition of cell cycle progression by sodium butyrate may be explained by a modulation of cell cycle regulatory proteins such as cyclin D3 and p21.

Measurement of short chain fatty acids produced from resistant starch fermentation in the portal blood: A study in a pig model. L. Martin[a], H. Dumon[a], G. Lecannu[b], M. Champ[b,c] ([a]Ecole nationale vétérinaire, Unité de nutrition et alimentation, BP 40706, 44307 Nantes cedex 03; [b]Inra, Laboratoire de technologie appliquée à la nutrition, BP 71627, 44316 Nantes cedex 03; [c]CRNH groupe métabolisme, Hôtel Dieu, Place A. Ricordeau, 44093 Nantes cedex 01, France).

Colonic fermentation of resistant starch (RS) produces short chain fatty acids (SCFA) and an especially high proportion of butyric acid (*n*C4). This SCFA plays a specific role in the wholeness of colonic mucosa. In this study, we tested the hypothesis that the rate of starch fermentation could influence the production of nC4 and its utilization by the colonocytes in vivo.

Material and methods: Three experimental diets were formulated with three different resistant starches selected on the basis of their in vitro fermentation kinetic: raw potato starch (PoS), high amylose corn starch (HS) and retrograded high amylose corn starch (RHS). The animals ate 15 g of RS per experimental meal. Four pigs were fitted with two indwelling catheters (portal vein and carotid artery) and a flow probe (Transonic™) was placed around the portal vein. Results were analysed by ANOVA.

Results: The mean total quantities of SCFA in the portal blood were, respectively, for PoS, HS and RHS: 131.4 ± 25.8, 85.0 ± 13.3 and 163.0 ± 35.5 mmol with HS statistically different from PoS and RHS. The mean total quantities of *n*C4 measured in the portal vein varied significantly between starches: PoS (15.2 ± 2.8 mmol) versus HS (0.2 ± 0.2 mmol) and RHS (3.1 ± 1.1 mmol).

Conclusion: The mean total quantities of SCFA measured in the portal vein agree with previous studies. The *n*C4 produced from HS and RHS fermentation seems to be completely used up by the colonic mucosa. On the other hand, after consumption of the PoS diet, a part of the *n*C4 appears in the portal blood. This result could indicate a saturation of its utilization by the colonocytes when a large amount of nC4 is produced, as previously observed in vitro.

Measurement of the colonic acetate production after lactulose ingestion in humans – using isotopic dilution. E. Pouteau[a,c], K. Vahedi[b], B. Messing[b], B. Flourié[b], P. Nguyen[c], M. Krempf[a] ([a]Groupe métabolisme, CRNH, Nantes; [b]Inserm 290, Hôpital St Lazare, Paris; [c]Unité de nutrition et alimentation, ENV, Nantes, France).

Lactulose is a disaccharide which is not digested or entirely degraded by colonic fermentation. This results in an exogenous acetate supply. Our aim was to evaluate quantitatively this colonic acetate production following lactulose ingestion. Six healthy volunteers (24–38 years, 21.7 ± 1.5 $kg \cdot m^{-2}$) were given a low fiber diet for 3 days (< 5 $g \cdot d^{-1}$). They then received a prime of [1-^{13}C] acetate (33 ± 10 $\mu mol \cdot kg^{-1}$) intravenously and were infused with the same tracer (1.0 ± 0.1 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) for 7 h. At t = 60 min, the subjects received an oral dose of 20 g of lactulose. Breath samples and arterial blood samples were collected every 15 min throughout the study. The isotopic enrichment levels and the concentrations were measured by gas chromatography/mass spectrometry. The acetate turnover was calculated, after being validated, with the steady state equation at each sample point. During the initial period, the hydrogen and methane levels were 7 ± 2 and 10 ± 4 ppm, respectively. The concentration and the acetate turnover were 141 ± 14 $\mu mol.L^{-1}$ and 6.0 ± 0.7 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, respectively. At t = 195 min, the hydrogen and acetate concentrations reached 63 ± 15 ppm and 313 ± 25 $\mu mol \cdot L^{-1}$, the methane level remained unchanged. Simultaneously, the whole body acetate turnover increased to 9.8 ± 1.5 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ and then decreased, to close to the initial value, at the end of the study. The area under the curve of the whole body acetate turnover variations minus the constant initial turnover, supposed to be endogenous, represents the colonic exogenous acetate supply. It was 140 ± 12 mmol, which was 86 % of the expected stoichiometric amount. This approach is unique. It made it possible to quantitatively estimate the colonic acetate production and thus the colonic fermentation, in vivo, in humans.

In vitro fermentation of Acacia gum by human fecal flora: effects on the bacterial populations and on the production of short-chain fatty acids. C. Michel, T. Kravtchenko, C. Cherbut (CRNH de Nantes, Inra, BP 71627, 44316 Nantes cedex 03, France).

Several carbohydrates have been identifed as prebiotics. They are not digested in the small intestine but are highly fermented in the colon where they specifically promote the growth of lactic acid bacteria. Although prebiotics are mainly oligosaccharides, high molecular weight polymers are likely to exert similar effects. This study aimed to determine the effects