The intracellular target of butyrate’s actions: HDAC or HDON’T?

Butyrate, the four-carbon short chain fatty acid, has special significance for clinicians and scientists interested in large bowel physiology. It is normally present in the colonic lumen at millimolar concentrations as a product of bacterial fermentation of luminal carbohydrates and is readily taken up by the colonic epithelium to be used as a major energy source via $\beta$-oxidation. Butyrate affects key functions of the colonic epithelium in vivo or at least in vitro in models of the colonic epithelium. These functions include promotion of sodium and water absorption, improvement of tight junction permeability, and acceleration of epithelial restitution. Thus, butyrate plays an important role in the maintenance of colonic mucosal health.

Butyrate has also been implicated in the pathogenesis of colonic diseases, especially colorectal cancer and ulcerative colitis. Butyrate’s role in the pathogenesis of ulcerative colitis has been a fascinating saga. In 1981, Roediger first reported that epithelial cells isolated from the rectum of patients with ulcerative colitis exhibited impaired $\beta$-oxidation of butyrate. $^1$ His “energy-deficiency” hypothesis created more attention when diversion colitis, which most likely to be found in the distal colon. Butyrate may affect cells via the supply of energy from its $\beta$-oxidation. This has been shown in vivo in the atrophic colon starved of short chain fatty acids $^3$ and in vitro in a cell line not able to meet its energy needs through other substrates. $^4$ However, apart from stimulation of proliferation under energy deficient conditions, the evidence is scanty for a role of $\beta$-oxidation in butyrate’s other cellular effects.

Most of butyrate’s effects seem to result from a direct action of butyrate itself on intracellular targets. A key target may be histone deacetylase (HDAC). Cells exposed to butyrate exhibit hyperacetylation of core histones, owing to the reversible inhibition of HDAC by butyrate. The importance of butyrate’s effect on HDAC has been highlighted by the demonstration that trichostatin A (TSA), which specifically inhibits HDAC, mimics many of the effects of butyrate on specific protein expression, such as interleukin 8 and urokinase receptor, cell proliferation and apoptosis, and epithelial functions, such as paracellular permeability and cell migration. However, Siavoshian and colleagues (see page 507) report that TSA does not mimic butyrate’s effect on markers of cell differentiation, specifically the activities of brush border hydrolases, in HT29 cells. This observation has also been recently reported in other colonic epithelial cell lines. $^5$ Does this mean that butyrate is acting on hydrolase activities via an intracellular target system that does not involve inhibition of HDAC?

Siavoshian et al examined whether the inhibition of cell proliferation induced by TSA in HT29 cells did truly mimic that of butyrate by comparing their effects on cell cycle events and on key intracellular molecules involved in those events. The effects of butyrate and TSA differed in the changes induced in cyclin dependent kinases and the stage in the cell cycle at which the cells were arrested (G1 for butyrate, G, and G, for TSA). Furthermore, the duration that histone H4 was hyperacetylated differed, with TSA having a short action (<15 hours) and butyrate still exerting its effect after 24 hours.

Interpretation of these findings is aided by an understanding of the emerging complexity of HDAC as a transcriptional regulatory system. Histone acetylation precedes transcription and alters nucleosome and chromatin structure. This enhances accessibility of transcription factors to nucleosomal DNA. It is a dynamic process involving two enzyme systems, histone acetyltransferase and HDAC, which catalyse rapid acetylation and deacetylation. HDACs comprise at least two families of proteins that are targeted to specific promoters through sequence specific DNA binding factors. $^6$ It seems likely, though not yet proved, that different HDAC proteins will target different promoters and, therefore, subserve different functions. In turn, different inhibitors may exert different spectra of inhibition of HDAC proteins. Siavoshian et al’s findings may reflect such an effect, in addition to the different kinetics of their actions. Inhibition of HDAC leads to increased transcription of HDAC mRNA. The recent report that exposure to butyrate or TSA induces differing patterns of mRNA for HDAC proteins $^7$ further supports the notion of heterogeneity in the patterns of inhibition of specific HDAC proteins.

Thus, whether HDAC is the major intracellular target for butyrate’s actions remains unresolved, but it cannot be assumed that, if TSA does not mimic butyrate’s action, then HDAC is not involved. Nevertheless, it seems more likely that butyrate has other intracellular targets, particu-
larly as, unlike TSA, butyrate probably does not directly bind to HDAC and requires phosphatase activity to exert its inhibitory effect. Resolution of these issues is eagerly awaited as definition of the molecular pathways by which butyrate acts will greatly improve our understanding of multiple cellular processes in general and may be used specifically in the design of new therapeutic agents with—for example, antitumorigenic properties.

University of Melbourne Department of Medicine, The Royal Melbourne Hospital, Victoria, 3050 Australia


4 Singh B, Haleslap AP, Paraskeva C. Butyrate can act as a stimulator of growth or inducer of apoptosis in human colon epithelial cell lines depending on the presence of alternative energy sources. Carcinogenesis 1997;18:1265–70.


See article on page 540
family based AALD data sets would be useful to exclude population mismatching but their collection would be problematic. Replication of the data of Grove et al in a further independent data set is however vital, even if this is in the form of a second population based case control study. While confirmatory findings in an ethnically distinct population as opposed to similar ethnicity would be desirable, this is not essential. Replication of these data would strengthen the argument for subsequent functional molecular biological studies to help “nail” the primary aetiological disease causing mutation.

As with the study of all complex diseases, establishment of large multiple independent data sets is vital in attempts at identifying susceptibility loci which are likely to be exerting small but clinically significant effects. It is unlikely that any one group will have sufficient numbers of patients to establish more than one data set and, therefore, working collaborations between groups should be encouraged. With the ongoing emergence of more detailed genetic maps, including the availability of in silico candidate SNP markers and the development of technology capable of performing large scale genotyping, those groups who have established large data sets will be the first to identify susceptibility genes.

SC Gough
Department of Medicine, University of Birmingham, Birmingham Heartlands Hospital, Bordesley Green East, Birmingham B9 5SS, UK
Email: s.c.gough@bham.ac.uk

LKM antibody: getting in some target practice

In the current issue of the journal (see page 553), Muratori et al provide convincing evidence that cytochrome P4502D6 (CYP2D6) is present on the liver cell plasma membrane. This finding has important implications because CYP2D6 is the main target of liver kidney microsomal antibody type 1 (LKM1). Not only is LKM1 the serological hallmark of autoimmune hepatitis type 2 but it is also found in up to 10% of patients with chronic hepatitis C virus (HCV) infection where it appears to single out the form of a second population based case control study. While confirmatory findings in an ethnically distinct population as opposed to similar ethnicity would be desirable, this is not essential. Replication of these data would strengthen the argument for subsequent functional molecular biological studies to help “nail” the primary aetiological disease causing mutation.

As with the study of all complex diseases, establishment of large multiple independent data sets is vital in attempts at identifying susceptibility loci which are likely to be exerting small but clinically significant effects. It is unlikely that any one group will have sufficient numbers of patients to establish more than one data set and, therefore, working collaborations between groups should be encouraged. With the ongoing emergence of more detailed genetic maps, including the availability of in silico candidate SNP markers and the development of technology capable of performing large scale genotyping, those groups who have established large data sets will be the first to identify susceptibility genes.

SC Gough
Department of Medicine, University of Birmingham, Birmingham Heartlands Hospital, Bordesley Green East, Birmingham B9 5SS, UK
Email: s.c.gough@bham.ac.uk

LKM antibody: getting in some target practice

In the current issue of the journal (see page 553), Muratori et al provide convincing evidence that cytochrome P4502D6 (CYP2D6) is present on the liver cell plasma membrane. This finding has important implications because CYP2D6 is the main target of liver kidney microsomal antibody type 1 (LKM1). Not only is LKM1 the serological hallmark of autoimmune hepatitis type 2 but it is also found in up to 10% of patients with chronic hepatitis C virus (HCV) infection where it appears to single out the form of a second population based case control study. While confirmatory findings in an ethnically distinct population as opposed to similar ethnicity would be desirable, this is not essential. Replication of these data would strengthen the argument for subsequent functional molecular biological studies to help “nail” the primary aetiological disease causing mutation.

As with the study of all complex diseases, establishment of large multiple independent data sets is vital in attempts at identifying susceptibility loci which are likely to be exerting small but clinically significant effects. It is unlikely that any one group will have sufficient numbers of patients to establish more than one data set and, therefore, working collaborations between groups should be encouraged. With the ongoing emergence of more detailed genetic maps, including the availability of in silico candidate SNP markers and the development of technology capable of performing large scale genotyping, those groups who have established large data sets will be the first to identify susceptibility genes.

Combination therapy of hepatitis B

There are now two licensed therapies for chronic hepatitis B: interferon α and lamivudine. Interferon α was first shown to have activity against hepatitis B in 1976, but was not formally approved for use in chronic hepatitis B until 1992. The currently recommended regimen for interferon is 5 million units (mu) given daily or 10 mu given three times a week by subcutaneous (sc) injection for four to six months. This regimen results in long term beneficial responses in roughly 33% of patients. Lamivudine was first shown to have activity against hepatitis B virus (HBV) in 1992 and was approved for use in chronic hepatitis B in 1998. The currently recommended regimen for lamivudine is 100 mg given daily by mouth for one year. This regimen results in beneficial responses in 16–20% of patients with typical chronic hepatitis B.

Thus, there are now two choices for therapy. Which should be used? Which should be used first? Should the second be tried if the first fails? What about combining the two? These are simple questions, but they do not have simple answers.

In this issue (see page 562), Schalm and collaborators report results of a large, multinational, randomised, double blind, placebo controlled trial of lamivudine, interferon α and the combination of both in 230 patients with typical chronic hepatitis B. This trial was one of the largest studies ever conducted in hepatitis B and was large enough to have answered the questions posed above, at least in part. In the study, 18% of patients receiving lamivudine alone, 19% receiving interferon alone and 29% receiving the combination had a beneficial response, the higher response rate with combination therapy being of borderline statistical significance. Side effects from the combination were no greater than those with interferon alone. These results suggest that the combination of lamivudine and interferon α is the optimal initial therapy of this disease.

Before such recommendations are embraced, however, a closer look at this study is needed. Unfortunately, the study suffered in several regards: in design, conduct and analysis.

The design of this three arm study was complex. The first two arms were standard. One group received the recommended regimen of lamivudine and the second, the recommended regimen of interferon. The combination group, however, received an unusual and irregular regimen of both: lamivudine was given for six months only and interferon was started late, two months after initiating lamivudine therapy. The “lead in” phase of lamivudine before combination therapy was done because of previous studies suggesting that interferon is more effective in patients with lower levels of viral DNA. The difficulty is that interferon is also more effective in patients with higher serum aminotransferase activities, and lamivudine therapy often lowers serum enzyme activities. Furthermore, the complex regimen made the blinding of placebo treatment difficult.

Another major problem with the design was the differences in the timing of the end point evaluation in relation to therapy. Thus, the end points were measured in patients receiving lamivudine while they were still on treatment, but in those receiving interferon or the combination at a point six months after stopping therapy. This variability complicated the comparison of end points among groups. Thus, at the one year point, HBV DNA levels, aminotransferases and liver histology may well have been affected by the continuation of lamivudine therapy. Furthermore, scant information was given about follow up of treated patients after stopping lamivudine. Of course, it is difficult to compare therapies if the durations are different between groups (and this problem has plagued other studies of therapy of viral hepatitis). This discrepancy can only be overcome by adequate follow up, which would be at least 12 months after stopping lamivudine and, therefore, 18 months after stopping interferon with or without lamivudine.

The conduct of the trial was also problematic. The number of protocol violations was high, occurring in 50 of the 230 randomised patients. These violations consisted of dropouts, errors in randomisation, errors in assignment of inclusion and exclusion criteria, use of other antiviral agents during the study, and other problems. The number of protocol violations may have been because of the complexity of the design, lack of acceptance of a placebo control, the diversity of the therapies, and the large number of geographically dispersed centres involved.

The analysis and presentation of results of this trial were also complex and difficult. Results were presented using five different populations of patients enrolled: the total “as treated” population of 230 patients, the “intention to treat” population of 226 patients, the “per protocol” population of 180 patients (minus the violations), the population of 212 patients who reached the 12 month point for analysis, and the population of 165 patients reaching the 15 month point. The shifting denominator of number of patients makes it difficult to state what the response rates were in terms that are understandable clinically. It is also difficult to say which group should have been used. For instance, in the “per protocol” analysis, the response rate to combination therapy was 36%, quite a bit better and supportive of combination therapy than the 22% for interferon alone and 19% for lamivudine alone.
A final critical issue in analysis was the end point used to define benefit. A “response” was defined as seroconversion from HBeAg to anti-HBe (and absence of HBV DNA by hybridisation testing) by the 12 month point. This end point differs from most trials of antiviral therapy of hepatitis B which used the loss of HBcAg (with or without development of anti-HBe) as an end point. Using loss of HBeAg as a definition of response (from table 2 and the population of 214 patients), 29% of lamivudine treated, 36% of interferon treated, and 51% of combination treated patients responded. Loss of HBeAg is a surrogate marker that has been found to be reasonably reliable in predicting a long term remission after interferon therapy of chronic hepatitis B as shown in several long term follow up studies. Whether loss of HBeAg or seroconversion to anti-HBe is a reliable surrogate marker for a sustained remission after nucleoside analogue therapy is unknown. In this study, nine of 11 patients who lost HBeAg during lamivudine therapy remained HBeAg negative three months later (data from panel A of figure 2 are, however, incompatible with this statement from the text). This number represents a 18% relapse rate within three months of stopping therapy, quite high when one considers that this is a chronic, often life-long disease. Despite these shortcomings, this trial provides a large amount of important information about therapy of hepatitis B. Additional information would have been helpful, especially with regard to the effect of the lead in phase on HBV DNA and alanine aminotransferase activities and whether responses were sustained long term. Additionally, use of a more sensitive assay for HBV DNA levels (such as PCR) might have helped to resolve differences in the three groups.

What can be recommended from this study? Another study, certainly. But if a clinician plans to treat patients with hepatitis B, a combination approach is reasonable and should probably employ a conventional regimen of both agents, starting both interferon and lamivudine at the same time, continuing interferon for four months and lamivudine for 12 months. In clinical practice, as in clinical research studies, long term follow up is needed of all treated patients to document absence of reactivation and continued resolution of disease activity.

Antiviral therapy of hepatitis B has entered adolescence, the awkward age—restive and resistant to management. The availability of two agents to treat this disease has provided more opportunities, but with it goes more responsibilities and more difficult choices. A safe and secure maturity will probably require a third or fourth agent active against this disease.

E DOO

Liver Diseases Section,
Digestive Diseases Branch,
National Institute of Diabetes and Digestive and Kidney Diseases,
Building 10, Room 9B06,
NIH, Bethesda, MD 20892, USA
Email: Dooe@intramid.niddk.nih.gov

JH HOOFNAGLE

Liver Diseases Section,
Digestive Diseases Branch,
National Institute of Diabetes and Digestive and Kidney Diseases,
Building 31, Room 9A23,
NIH, Bethesda, MD 20892, USA
Email: HoofnagleJ@extranidk.nih.gov

Conflict of Interest Statement: The authors are associate investigators in a clinical trial of lamivudine therapy at the National Institutes of Health supported in part by Glaxo-Wellcome, but neither has received direct funding, salary or honoraria from Glaxo-Wellcome.

LKM antibody: getting in some target practice

D VERGANI

Gut 2000 46: 449-450
doi: 10.1136/gut.46.4.449

Updated information and services can be found at:
http://gut.bmj.com/content/46/4/449

These include:

References
This article cites 8 articles, 1 of which you can access for free at:
http://gut.bmj.com/content/46/4/449#BIBL

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/