In vivo electron spin resonance spectroscopy: what use is it to gastroenterologists?

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Electron spin resonance (ESR) spectroscopy may have a role in the future in assessing the mucosal integrity of the colon non-invasively in the otherwise normal looking colon of patients with quiescent colitis. Like all techniques that strive to bridge the gap between laboratory science and clinical medicine, electron spin resonance (ESR) spectroscopy builds on established applications in biochemistry and chemistry and, following on from its discovery by Professor EK Zavoisky and colleagues in 1944 at Kazan State University, situated deep within the Tatarstan Republic of the Russian Federation, formerly the Soviet Union. However, it is only now that developments in technology may perhaps allow the endoscopist of the future to acquire information on gut mucosal integrity in vivo during a procedure. This is an intriguing prospect, although there are a number of practical problems to be solved before the in vivo clinical potential of this sensitive and specific technology is realised.

To delve into the basic physics of the technique for a moment, ESR, also known as electron paramagnetic resonance (EPR) spectroscopy, describes the resonant absorption of microwave radiation by paramagnetic materials—that is to say, materials with an unpaired electron such as free radicals and transition metal ions—in the presence of a static magnetic field. Specifically, with respect to in vitro ESR spectroscopy, which is a well-used biochemical tool, the sample, placed in a resonant chamber in a magnetic field and microwave frequency is then applied. The resulting ESR spectrum illustrates net absorption of microwaves at a specific frequency, which is dependent on the atomic and molecular structure of the sample under analysis. While an individual electron spin contributes to the magnetic moment of an atom, the majority of materials are not amenable to study by ESR spectroscopy as their electrons are paired and there is therefore no net bulk magnetism. This means that the region under scrutiny must contain a paramagnetic substance and so, for clinical applications, either a free radical must be administered or a so-called “spin trap” must be utilised to provide a mechanism for detection of reactive naturally occurring free radicals, present only in very low concentrations. By way of comparison, nuclear magnetic resonance (NMR) spectroscopy is based on the property of nuclear spin and there are a number of similarities between these two non-invasive techniques. Owing to the fact that electrons have a greater magnetic moment than nuclei, ESR spectroscopy is more sensitive than NMR spectroscopy. ESR spectroscopy also has the advantage of being highly specific, although it clearly can be a disadvantage that most chemical and biological materials are not paramagnetic. ESR spectroscopy has the scope for studying faster dynamics than NMR spectroscopy as the ESR timescale in the time domain is nanoseconds and not milliseconds as in NMR. The ESR technique has more recently been harnessed to study the presence and generation of free radicals in intact cells, perfused organs, and in small animals in vivo. For practical purposes, ESR spectroscopy allows some insight into tissue inflammation through measurement of free radicals. Taken to its logical conclusions in the clinical context, an endoscope with ESR spectroscopy capabilities could, for example, be of use for surveillance when the mucosal surface may otherwise appear normal.

In this issue of Gut, Togashi and colleagues have used ESR spectroscopy to investigate changes in mucosal sulphydryl compounds in an animal model of colitis (see page 1291). These authors have previously evaluated the ESR active compound 3-carbamoyl-2,2,5,5- tetramethylypyrroline-1-oxyl (carbamoyl-PROXYL) as a “spin probe” for measuring oxidative stress in the murine liver. The same technique has been extended to experimental colitis, in which it was argued that adequate levels of mucosal sulphydryl compounds, such as reduced glutathione, are critical in the prevention of tissue damage from the generation of reactive oxygen species in inflammatory conditions, such as ulcerative colitis. This technique provides a non-destructive method of assessing oxidative stress in small animals and these authors have produced a very elegant study using their in house, low frequency, 700 MHz microwave ESR spectroscopy apparatus. The authors are developing new ESR spectroscopy equipment with a surface coil-type resonator, which may be applicable to clinical colonoscopy.

The development of low frequency ESR spectroscopy, combined with the introduction of surface coil-type resonators, has opened up a wide range of applications for ESR as the depth sensitivity of the technique has improved and the required sample size is less restricted by the dimensions of the resonator. Furthermore, methods of reducing artefacts from voluntary and involuntary motion are being addressed. As with all new techniques, safety issues must be considered as magnetic fields and microwave power are integral to the ESR spectrometer, albeit at low levels, and because paramagnetic materials may be administered. The current generation of ESR spectrometers have quite limited physical space, as illustrated in the equipment used in the study of Togashi and colleagues, and therefore larger magnets are required for interventional clinical applications. With regard to the development time to clinical usage, there are some parallels with NMR spectroscopy. The NMR phenomenon itself was discovered shortly after World War II, but it was not until the mid-1980s that human NMR spectroscopy studies started on liver and in muscle using whole body magnets. In that sense, NMR spectroscopy was ahead of the game compared with ESR spectroscopy but there were still many years of proving the value of NMR spectroscopy before clinical studies were undertaken in earnest. In fact, for gastroenterologists, the liver remains the main focus of interest for NMR spectroscopy as in vivo studies on the gut are fraught with technical difficulties whereas the liver as a solid organ is a much easier focus for NMR study. Therefore, having an endoscope with in built NMR spectroscopy capabilities is still on the drawing board, rather than being a practical reality.

Returning to the problem in hand, the study by Togashi et al illustrates that it...
could be very desirable to have ESR spectroscopy capabilities for a new generation of future endoscopes in order to assess the mucosal integrity of the colon non-invasively in the otherwise normal looking colon of patients with quiescent colitis. However, so that this goal can become a reality, a range of safety and practical issues need to be overcome, obviously initially in the domain of research institutes where clinician scientists can conduct small scale research studies on selected patients with specialist equipment. While there are some potential pitfalls, we do suggest that you follow the development of clinical ESR spectroscopy enthusiastically. Nevertheless, it remains to say that time will tell whether the technique becomes sufficiently robust to join the diagnostic armamentarium of the busy clinical gastroenterologist.

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cell. Another bHLH transcription factor, BETA2/NeuroD, is required for the development of intestinal secretin and cholecystokinin cells. In contrast, the bHLH transcriptional repressor Hes1 is a negative regulator of endocrine cell numbers and in mice with deletion of the Hes1 gene there is hyperplasia of pyloric antral and intestinal endocrine cell populations.

The extent to which the mechanisms determining EEC differentiation also play a part in the EEC hyperplasias found in different clinical conditions remains uncertain. Clear examples of EEC hyperplasia in patients include ECL cell hyperplasia in Campylobacter enteritis. Of these, ECL cell hyperplasia in patients with chronic hypergastrinaemia due to chronic atrophic gastritis. Hypergastrinaemia ECL cells have the potential to form functional neuroendocrine tumours. The idea is attractive not least because TGF-β is known to inhibit the proliferation of other cell types. Other causes of hypergastrinaemia include para- and autocrine inhibition of neuroendocrine tumour cell proliferation. The finding is generally compatible with data in other systems that indicate inhibition of proliferation by TGF-β mediated by the Smad pathway and directed at decreased expression of c-myc and induction of p21(WAF1) and p15(INK4B). The role of TGF-β in tumorigenesis is however more complicated. In particular, in other cancers it is now clear that TGF-β can act both as an enhancer of tumour progression as well as a suppressor. The picture emerging over the last few years indicates that TGF-β also stimulates tumour cell migration, promotes epithelial to mesenchymal transition (EMT), and increases the production of matrix metalloproteinases (MMPs); together these effects lead to tumour cell invasion and metastasis.

Interestingly, while the Smad signalling pathway appears to be required for inhibition of proliferation, other signalling systems including the MAP kinase, PI-3 kinase, and protein phosphatase2A/p70s6k pathways are implicated in the pro-oncogenic effects of TGF-β. The mechanisms responsible for the shift in TGF-β signalling from a tumour suppressor mode to a tumour enhancer are still unclear. Wimmel et al did not specifically address the question of whether TGF-β stimulates invasion, EMT, or expression of MMPs in neuroendocrine tumour cells. However, as these cells appear to retain the inhibitory effects of TGF-β on proliferation, they may provide a useful model for further studies of the relative importance of the tumour suppressor and pro-oncogenic actions of TGF-β. Recent reports have suggested possible ways to block TGF-β signalling by delivery of soluble TGF-β receptor protein constructs. In experimental models, this approach appears to inhibit tumour cell invasion, and so may be valuable in preventing cancer progression. However, because suppression of neuroendocrine tumour cell proliferation by TGF-β appears to be relatively well preserved, a primary objective in this case should be the maintenance and enhancement of this action of TGF-β hormone, should be taken before considering whether inhibition of TGF-β is worthwhile.

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Liver

Inappropriate ileal conservation of bile acids in cholestatic liver disease: homeostasis gone awry

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Patients with cholestatic liver disease are likely to inappropriately conserve bile acids. Ursodiol corrects the defect, but is this enough?

Conjugated bile acids are water soluble amphipathic end products of cholesterol metabolism that promote lipid transport in the biliary tract and small intestine by forming mixed micelles.1 Bile acids are formed in pericentral hepatocytes by a complex multienzyme process whose details have at last been largely elucidated.2 After formation, their acidic group is linked (“conjugated”) with the amino group of glycine or taurine in an amide bond that is resistant to the proteolytic enzymes present in pancreatic secretion and on the surface of the enterocyte brush border. Conjugated bile acids differ from unconjugated bile acids in being membrane impermeable and water soluble at the pH conditions prevailing in the biliary tract and small intestine.

Efficient ileal conservation of bile acids results in the accumulation of a mass of bile acids termed the bile acid “pool”. Between meals, most of the pool is stored in the gall bladder; with meals, the gall bladder discharges bile into the small intestine where bile acids promote lipid absorption. Both bile acid synthesis and ileal conservation continue after a meal but the gall bladder does not increase in volume in proportion to the amount of bile acids it contains because of its continuous concentration of bile. The gall bladder appears early in vertebrate evolution and genes for gall bladder development appear to have evolved at the same time as genes for bile acid synthesis and intestinal conservation.

Development of the enterohepatic circulation and gall bladder storage resulted in far more bile acids being available for digestion than those recently synthesized. Each bile acid molecule is used multiple times before it is lost to the large intestine.

Feedback inhibition of bile acid biosynthesis in the hepatocyte is well established experimentally.7 Interruption of the enterohepatic circulation causes increased bile acid synthesis. This may be modest, for example, increases of 3–4 times are seen in patients taking bile acid sequestrants for hypercholesterolaemia; or it may be marked, for example, increases of 10–15 times are seen with an ileal resection causing severe bile acid malabsorption. Bile acid feeding of any of the natural bile acids occurring in human bile (cholic acid (CA), chenodeoxycholic acid (CDCA), deoxycholic acid (DCA)) suppresses bile acid synthesis, but the effect is relatively small (about a 50% decrease).

The mechanism by which the concentration of bile acids in the hepatocyte regulates bile acid synthesis has been elucidated only recently. Bile acids enter the nucleus and bind to a heterodimeric protein composed of two nuclear receptors, FXR and RXR.9 Binding of the bile acid molecule to FXR changes its configuration. This in turn leads to a complex sequence of events resulting ultimately in increased synthesis of one or more inhibitory proteins. The inhibitory protein(s) repress(es) the activity of the gene for cholesterol 7 alpha hydroxylase, the rate limiting enzyme in bile acid biosynthesis.10,11 FXR, the bile acid nuclear receptor, has now been crystallised, its structure determined by x ray crystallography, and the shape of the cavity that holds the conjugated bile acid elucidated in the last few months.12,13

Transport of bile acids by the ileal enterocyte is also modulated in a homeostatic manner analogous to feedback inhibition of bile acid biosynthesis in the hepatocyte. Early studies of bile acid secretion at the Mayo Clinic reported that bile acid secretion increased only modestly or not at all when bile acids were fed,14 hinting at downregulation of bile acid transport in response to bile feeding. The first convincing experimental evidence for feedback inhibition of bile acid transport was reported by Lillienau and colleagues15 who performed experiments in the guinea pig. These workers measured total ileal absorptive capacity for conjugated bile acids by perfusing bile acids at such a high rate that the intraluminal concentration remained constant. This technique had been used previously in studies that defined the T_{max} for ileal transport in rats and humans.16 Lillienau et al found that the ileal transport capacity for bile acids decreased after bile acid feeding and was increased by addition of cholestyramine to the diet. This finding was confirmed for the mouse,17 but using other experimental designs it was not confirmed in the rat (see Lanzini and colleagues)18 or in the pig.19 Thus in this area of physiology there are marked species differences, a problem that continues to bedevil those who try to understand the intricacies of bile acid metabolism. The mechanism by which the concentration of bile acids in the ileal enterocyte modulates enterocyte transport is under active investigation at the moment. As in the hepatocyte, regulation is likely to involve interaction of bile acids with nuclear receptors such as FXR.20

Lillienau and colleagues19 speculated that “patients with cholestatic liver disease are likely to inappropriately conserve endogenous dihydroxy bile acids such as CDCA and DCA, which are
known to be hepatotoxic”. This speculation has now been confirmed in an important clinical study by Lanzini and colleagues in this issue of Gut [see page 1371]. These workers used “SeHCAT, a selenium tagged homologue of taurocholate, whose metabolism was shown by Jazrawi et al to be essentially identical to that of taurocholate.” Because SeHCAT is a gamma particle emitter, it can be used to visualise the enterohepatic circulation and has been used for this purpose to measure hepatocellular excretory function non-invasively in patients with cholestatic liver disease. SeHCAT has also been used to measure the efficiency of ileal conservation of bile acids in diarrhoeal conditions.

In the experiments reported by Lanzini et al, SeHCAT was used as a surrogate for taurocholate, and its turnover rate quantified by measuring gall bladder radiolabelled bile on several days. The rate of decline in radioactivity with time gives the fractional turnover rate of the endogenous bile acid pool. The method used by Lanzini et al does not provide information on bile acid synthesis, which is the product of pool size and turnover rate.

Lanzini et al found that the fractional turnover rate of 14 women with primary biliary cirrhosis (PBC) was, on average, one half that of 14 age matched healthy women. The t1/2 (equal to 0.69 divided by the fractional turnover rate) was correspondingly increased. Thus in these patients with all stages of PBC, bile acids were inappropriately retained. The simplest interpretation of this novel finding is that the ileum has sensed a lowered intraluminal bile acid concentration and reacted by increasing its efficiency of bile acid conservation. However, a sensing of the elevated plasma level of bile acids might also contribute. In health, the ileum efficiently downregulates transport in response to increased bile acid loads thereby protecting the liver. When the bile acid pool is lost, as in acute diarrhoeal disease, the ileum upregulates to regenerate the bile acid pool as quickly as possible. In cholestatic liver disease, the signal of decreased intraluminal bile acid concentration acts to mislead the ileal transport system, which cannot know that bile acids are being retained in the hepatocyte because of biliary ductule obstruction. Inappropriate ileal conservation in cholestatic liver disease is hence arrest of the disease.

Lanzini et al made a second important observation. Inappropriate ileal conservation of bile acids was abolished by administration of ursodiol at the usual dose of 15 mg/kg/day. Although ursodiol is fairly well absorbed, it does not suppress endogenous bile acid synthesis because it does not interact with the nuclear receptor FXR. Thus in patients receiving ursodiol, the enterohepatic circulation has an additional input (probably 10–12 mg/kg/day) of exogenous bile acids, far exceeding endogenous bile acid synthesis (3–5 mg/kg/day). Presumably, ursodiol conjugates secreted by the liver compete for active ileal transport, thus preventing the inappropriate conservation of endogenous bile acids and restoring the fractional turnover rate to normal. Ursodiol is non-cytotoxic and has multiple effects on the hepatocyte that appear to decrease the injurious effects of retained endogenous bile acids and to promote hepatocellular excretory function.

A major question remaining for the hepatologist is whether downregulation of ileal bile acid transport to its normal level by ursodiol therapy is optimal therapy in cholestatic liver disease, or whether it is desirable to decrease the efficiency of ileal conservation to a still greater degree, thereby reducing the return of bile acids to the hepatocyte that is already impacted with bile acids.

Historically, bile acid drainage was used to treat the pruritus of cholestatic liver disease. When cholestyramine was introduced, it was also shown to decrease pruritus that, then and still now, is considered by many to arise from increased plasma levels of bile acids. Emerick and Whittington have treated intractable pruritus in children by partial biliary diversion because this prevents a fraction of secreted bile acids from reaching the ileum. Another surgical approach reported to be successful is ileal bypass which should have the same effect as partial biliary diversion. The technique of extracorporeal albumin dialysis removes plasma bile acids and also decreases pruritus. A new bile acid sequestrant, colesuvelam, has binding properties for bile acids that are superior to those of cholestyramine and has been reported to be more effective than cholestyramine in treating cholestatic pruritus in open label studies. The majority of these cholestatic patients were already receiving ursodiol so that these adjuvant therapeutic approaches appear to add efficacy to that achievable by ursodiol therapy alone. All of these approaches will result in less absorption of endogenous cytotoxic bile acids so that the input of bile acids to the liver will be enriched in the recently ingested ursodiol.

The last approach to be considered is inhibition of asbt, the apical transporter of the ileal enteroctye. Ileal absorption of bile acids begins with transport into the enteroctye mediated by the apical sodium dependent bile salt transporter (asbt) that has been cloned and characterised in the laboratory of Dawson. Development of a potent inhibitor of asbt has been the goal of several pharmaceutical companies. The target disease for such an inhibitor of bile acid transport was not cholestatic liver disease, but hypercholesterolaemia, a far more prevalent problem. The rationale for the development of such inhibitors was the observation that addition of a bile acid sequestrant to a statin potentiates its hypercholesterolaemic effect by still further upregulating LDL receptor activity. Sequestrants are known to induce only mild bile acid malabsorption, suggesting that a potent asbt inhibitor (together with a statin) should be still more effective therapy for hypercholesterolaemia. Although bile acid transport inhibitors might also contribute. In health, the signal of decreased intraluminal bile acid concentration and bile acid malabsorption of endogenous bile acids so that the input of bile acids to the liver will be enriched in the recently ingested ursodiol.
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