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, 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. The average endoscopist, faced with the clinical burden of disease and an ever growing case load, requires an emerging clinical technique to robustly deliver reproducible clinically relevant data without obfuscation by artefact. The questions therefore arise of how feasible will it be for ESR spectroscopy to be implemented in the clinical arena and what additional information can be given to the average busy gastroenterologist?

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 is 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.1-3 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 microsecond and not millisecond as in NMR.4 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.5-10 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 colleagues11 have used ESR spectroscopy to investigate changes in mucosal sulphhydril 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-tetramethylpyrrolidine-1-oxyl (carbamoyl-PROXYL) as a “spin probe” for measuring oxidative stress in the murine liver.12 The same technique has been extended to experimental colitis, where it was argued that adequate levels of mucosal sulphhydril 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.13 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.14 Furthermore, methods of reducing artefacts from voluntary and involuntary motion are being addressed.15 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,11 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.16-17 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.18-20 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 studies.21,22 Therefore, having an endoscope with inbuilt 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...
Keeping neuroendocrine cells in check: roles for TGFβ, Smads, and menin?

G J Dockray

Neuroendocrine tumour cells of the gastroenteropancreatic tract are subject to paracrine and autocrine growth inhibition by transforming growth factor β which may account for the low cell proliferation of this tumour

The endocrine cells of the gastrointestinal epithelium sense the luminal contents and through secretions at their basolateral side signal both to other epithelial cells and to subepithelial cells, including smooth muscle, neurones, and inflammatory cells.1 Some of the features of these cells are clearly neurone-like and for a time it was thought that during development they might be derived, like enteric neurones, from the neural crest. This now seems unlikely, and instead it is thought that normally they arise from the pluripotent stem cells that also give rise to the other epithelial cell lineages.2 However, in some circumstances at least, these cells appear to have the capacity for proliferation, and in extreme cases this gives rise to tumours that are called “neuroendocrine” as they exhibit some of the features of neurones and endocrine cells. There are many similarities between neuroendocrine tumours of the gastrointestinal tract and pancreas. In general, these tumours grow slowly and the reasons for this are unknown. Wimmelm and colleagues now present evidence that transforming growth factor β (TGFβ) is produced by neuroendocrine tumours and through autocrine and paracrine mechanisms restrains tumour cell proliferation [see page 1308].

There are over a dozen major enterodocrine cell (EEC) types, most with a restricted distribution along the gut.3 The cellular mechanisms that normally determine the differentiation of these cells and, their numbers relative to other epithelial cells in each region of the gut, are only now beginning to clear. For example, the basic helix-loop-helix (bHLH) transcription factor neurogenin 3 is required for the development of intestinal and pancreatic endocrine cells and for the main pyloric antral endocrine cells (G and D cells), but not for endocrine cells of the gastric corpus such as enterochromaffin-like (ECL) and X

Cancer

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cells. 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 hyperplasia also play a part in the EEC hyperplasias found in different clinical settings remains uncertain. Clear examples of EEC hyperplasia in patients include ECL cell hyperplasia in hypergastrinaemia,2 G cell hyperplasia in achlorhydria,3 and rectal EEC hyperplasia in Campylobacter enteritis.4 Of these, ECL cell hyperplasia in patients with duodenal ulcer disease is the best understood. Thus hypergastrinaemia in several different clinical settings, including gastrinoma, pernicious anaemia, and peptic ulcer disease that in both patients and experimental animals, hypergastrinaemia is also associated with ECL cell dysplasia and with a tendency to develop ECL cell carcinoid tumours. Moreover, there is evidence that in the setting of pernicious anaemia these tumours may regress after antrectomy compatible with the view that gastrin provides a primary drive to proliferation.14 At the cellular level, a clue to the mechanisms that might regulate the proliferation of neuroendocrine tumour cells is provided by observations in multiple endocrine neoplasia type 1 (MEN-1). Even in this relatively simple condition, neuroendocrine tumours may arise in several organs, particularly the pancreas, parathyroid, and pituitary glands. In addition, loss of heterozygosity (LOH) at the locus of the menin gene occurs in about 75% of ECL cell carcinoid tumours in patients with gastrinoma on a background of MEN-1, compared with <15% of patients with ECL cell carcinoids on a background of hypergastrinaemia due to chronic atrophic gastritis.15 Interestingly, LOH at this locus was not observed in mid and hindgut carcinoid tumours.15 These observations implicate the product of the menin gene in the inhibition of proliferation of both pancreatic and gastric endocrine tumours. The relevant protein, menin, binds several signalling proteins, including the transcription factors Jun-D and Smad3.16,17 Smad3 is a downstream mediator of TGFB signalling, and as loss of menin appears to downregulate Smad3 function, it seems reasonable to suppose that TGFB might be a negative regulator of proliferation in at least some neuroendocrine tumours. The idea is attractive not least because TGFB is known to inhibit the proliferation of other cell types.

The data reported by Wimmel et al support the idea that TGFB inhibits neuroendocrine tumour cell proliferation. The authors showed by immunohistochemistry that TGFB1 was expressed in 50–80% of fore, mid, and hindgut neuroendocrine tumour cells as well as by mesenchymal cells, and that the two relevant receptors, TGFBRI and TGFBRII, were also highly expressed by these tumours. There was similar expression in two neuroendocrine cell lines (BON cells, from a functional human panceatic islet cell carcinoma, and LGC-18 cells from a non-functional colorectal neuroendocrine tumour) and in these cells TGFB was shown to increase p15 and decrease c-myc, causing arrest in the G1 phase of the cell cycle. Moreover, neutralising antibodies to TGFB, or treatment with a dominant-negative receptor, increased proliferation of responsive neuroendocrine cell lines. Taken as a whole, these findings provide direct evidence for the importance of TGFB as a paracrine/autocrine inhibitor of neuroendocrine tumour cell proliferation. Further experimental findings are generally compatible with data in other systems that indicate inhibition of proliferation by TGFB mediated by the Smad pathway and directed at decreased expression of c-myc and induction of p15 and p16. The role of TGFB in tumorigenesis is however more complicated. In particular, in other cancers it is now clear that TGFB can act both as an enhancer of tumour progression as well as a suppressor. The picture emerging over the last few years indicates that TGFB also stimulates tumour cell migration, promotes epithelial to mesenchymal transition (EMT), as well as increasing the production of matrix metalloproteinases (MMPs); together these effects lead to tumour cell invasion and metastasis.21 Interestingly, while the Smad signalling pathway appears to be required for inhibition of proliferation, other signalling systems including the MAPkinase, PI-3kinase, and protein phosphatase2A/p70s6k pathways are implicated in the pro-oncogenic effects of TGFB. The mechanisms responsible for the shift in TGFB signalling from a tumour suppressor mode to a tumour enhancer are still unclear. Wimmel et al did not specifically address the question of whether TGFB stimulates invasion, EMT, or expression of MMPs in neuroendocrine tumour cells. However, as these cells appear to retain the inhibitory effects of TGFB on proliferation, they may provide a useful model for further studies of the relative importance of the tumour suppressor and pro-oncogenic actions of TGFB.

Recent reports have suggested possible ways to block TGFB signalling by delivery of soluble TGFB receptor protein constructs.22 In experimental models, this approach appears to inhibit carcinoid cell invasion, and so may be valuable in preventing cancer progression. However, because suppression of neuroendocrine tumour cell proliferation by TGFB appears to be relatively well preserved, a primary objective in this case should be the maintenance and enhancement of this action of TGFB in cancer care should be taken before considering whether inhibition of TGFB is worthwhile.
Liver

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

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 transport in the biliary tract and small intestine.

Development of the enterohepatic circulation and gall bladder storage resulted in far more bile acids being available for digestion than those recently synthesised. Each bile acid molecule is used multiple times before it is lost to the large intestine.1

Feedback inhibition of bile acid biosynthesis in the hepatocyte is well established experimentally.2 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.3 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.4 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.4,5

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,6 hinting at downregulation of bile acid transport in response to bile acid feeding. The first convincing experimental evidence for feedback inhibition of bile acid transport was reported by Lillienau and colleagues7 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_sp" for ileal transport in rats and humans.8 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,9 but using other experimental designs it was not confirmed in the rat (see Lanzini and colleagues10) or in the pig.11 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.12

Lillienau and colleagues13 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 specula-
tion has now been confirmed in an
important clinical study by Lanzini and
colleagues6 in this issue of Gut [see
page 1371]. These workers used “Se-
SeHCAT, a selenium tagged homologue
of taurocholate, whose metabolism
was shown by Jazrawi et al to be essentially
identical to that of taurocholate.6 Because
SeHCAT is a gamma particle emit-
ter, it can be used to visualise the entero-
hepatic circulation and has been used for
this purpose to measure hepatic excret-
tory function non-invasively in patients
with cholestatic liver disease.17 SeHCAT
has also been used to measure the
efficiency of ileal conservation of bile
acids in diarrhoeal conditions.18

In the experiments reported by Lan-
zini et al, SeHCAT was used as a surrogate
to taurocholate, and its turnover rate
quantified by measuring gall bladder
radioactivity daily for several days. The
depth 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.6

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 tv (equal to 0.69 divided by
the fractional turnover rate) was corre-
spondingly increased. Thus in these
patients with all stages of PBC, bile acids
were inappropriately retained. The sim-
plest 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
processes because of increased bile acid
loads thereby protecting the liver. When
the bile acid pool is lost, as in acute diar-
rhoeal 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
hepatoctye because of biliary ductule
obstruction. Inappropriate ileal conserved
in cholestatic liver disease is homeostasis gone awry.

Lanzini et al4 made a second important
observation. Inappropriate ileal conser-
vation 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 cir-
culation has an additional input (prob-
ably 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 conserva-
tion of endogenous bile acids and restor-
ing the fractional turnover rate to nor-
mal. 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 hepatic excretory function.21

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.6 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.17
Emerick and Whittington have treated intractable pruritus in children by partial
biliary diversion which prevents a fra-
tion of secreted bile acids from reaching
the ileum.20 Another surgical approach
reported to be successful is ileal bypass
which should have the same effect as
partial biliary diversion.21 The technique
of extracorporeal albumin dialysis re-
moves plasma bile acids and also de-
creases pruritus.22 A new bile acid se-
questrant, colesevelam, 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.23 The
majority of the 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 cytoxic bile acids so
that the input of bile acids to the liver
will be enriched in the recently injected
ursodiol.

The last approach to be considered is
inhibition of asbt, the apical transporter
of the ileal enterocyte. Ileal absorption of
bile acids begins with transport into the
enterocyte mediated by the apical so-
dium dependent bile acid transporter (asbt)
which has been cloned and characterised in
the laboratory of Dawson.19 Development
of a potent inhibitor of asbt has been the
goal of several pharmaceutical
companies.20 The target disease for such
an inhibitor of bile acid transport was
not cholestatic liver disease, but hyper-
cholesterolaemia, a far more prevalent
problem. The rationale for the develop-
ment of such inhibitors was the observa-
tion that addition of a bile acid sequest-
trant to a statin potentiates its
cytoxic effect. Hypercholesterolaemic effect by still fur-
ther upregulating LDL receptor activity.21
Sequestrants are known to induce only
mild bile acid malabsorption, suggesting
that a potent asbt inhibitor (together
with a statin) should be still more effec-
tive therapy for hypercholesterolaemia.
Although bile acids have not been proven
in animal studies, it is not clear that
they will reach the market. Newer more
potent statins are quite effective without
adjunct therapy and older statins will
soon become available as generic drugs.
In addition, bile acid malabsorption
caused by ileal blockade appears to
induce diarrhoea in humans because of
the cathartic effect of malabsorbed bile
acids. This adverse effect has dampened
the enthusiasm of the drug development
groups. From a commercial standpoint,
cholestatic liver disease is unlikely to
ever be a target of drug development
by ‘big pharma’ because the market is too
small. Let us hope, none the less, that
these new potent ileal uptake blockers will be
made available to hepatologists so that
their value, if any, in treating cholestatic
liver disease can be assessed rigorously.
The side effect of diarrhoea observed in
hypercholesterolaemic patients might be
less of a problem in cholestatic patients
as the compensatory increase in bile acid
synthesis might be dampened because of
liver disease.

The paper of Lanzini et al is an import-
ance advance in our understanding of the
pathophysiology of cholestatic liver
disease. The enterohepatic circulation of
bile acids arose in vertebrate evolution
to promote nutrition, not to deal with the
problem of cholestatic liver disease.
Ursodiol therapy corrects the defect in
inappropriate conservation. Whether
this is enough or whether we should fur-
ther reduce ileal transport can be tested
if the newly developed asbt inhibitors
become available to the liver community.
Still, all of the approaches discussed
above are palliative and we must con-
tinue to seek therapeutic approaches
that deal with the fundamental aetiology
of these conditions, which is likely to be
infected and/or autoimmune.

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