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

N S Dhanjal, I J Cox, S D Taylor-Robinson

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 processes, 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.4–6 Furthermore, methods of reducing artefacts from voluntary and involuntary motion are being addressed.6–8 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,9 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.10–13 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.14–16 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 intervention.15 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

Cancer

during development they might be de- 

like, enteric neurones, from the 

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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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heterozygosity (LOH) at the locus of the drome may arise in several organs, par
endocrine neoplasia type 1 (MEN-1). is provided by observations in multiple
liferation of neuroendocrine tumour cells
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certain. Clear examples of EEC hyperpla-
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hypergastrinaemia ECL cells have the
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proliferation of EEC hyperplasia in
intraepithelial neoplasia. EEC hyperplasia in
hypergastrinemic rats is differentially required for endocrine cell fate specification in the intestinal and gastric
neuroendocrine tumour cells.
neuroendocrine tumour) and in these
other cancers it is now clear
its action of TGFβ, and care should be
tumours. The relevant protein, menin,
the Smad signalling
Recent reports have suggested possible ways to block TGFβ signalling by deliv-
er of soluble TGFβ receptor protein constructs.1,2 In experimental models,
pro-oncogenic effects of TGFβ are
in myeloid leukaemia. However, because suppression of neuroendocrine tumour cell proliferation by TGFβ ap-
pears to be relatively well preserved, a primary objective in this case should be the maintenance and enhancement of
this action of TGFβ should be taken before considering whether inhi-
ition of TGFβ is worthwhile.

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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 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 synthesised. Each bile acid molecule is used multiple times before it is lost to the large intestine.3

Feedback inhibition of bile acid biosynthesis in the hepatocyte is well established experimentally.4 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.5 Binding of the bile acid molecule to FXR changes its confirmation. 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.6 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.7,8 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,9 hinting at downregulation of ileal transport in response to bile acid feeding. The first convincing experimental evidence for feedback inhibition of bile acid transport was reported by Lillienau and colleagues10 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 rats9 and humans.11 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,12 but using other experimental designs it was not confirmed in the rat (see Lanzini and colleagues13) or in the pig.14 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.15

Lillienau and colleagues16 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
colleagues in this issue of Gut [see
page 1371]. These workers used “Se-
SeHCAT, a selenium tagged homologue
to taurocholate, whose metabolism
was shown by Jazzari et al to be essentially
identical to that of taurocholate.” Because
SeHCAT is a gamma particle emit-
ting agent, it can be used to visualize the enter-
hepatic circulation and has been used for this
purpose to measure hepatic excre-
tory function non-invasively in patients
with cholestatic liver disease. SeHCAT
has also been used to measure the
persistence of bile acids in diarrhoeal conditions.

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
daily 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 t½ (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-
ples 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 diar-
hoeal 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 conser-
vation in cholestatic liver disease is
homeostasis gone awry.

Lanzini et al 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 bile acid transport, thus preventing the inappropria-
tive conservation of endogenous bile acids and restoring
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.

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 fra-
tion 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 re-
moves plasma bile acids and also de-
creases pruritus. A new bile acid se-
questrant, colesveal, 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 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 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 abt, 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 salt transporter (abt) which has been cloned and characterised
in the laboratory of Dawson. Development
of a potent inhibitor of abt 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 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 seque-
strant to a statin potentiates its
hypercholesterolaemic effect by still fur-
ther upregulating LDL receptor activity. Sequestrants are known to induce only mild bile acid malabsorption, suggesting
that a potent abt inhibitor (together with a statin) should be still more effec-
tive therapy for hypercholesterolaemia.

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