Nuclear gastroenterology: novel techniques in clinical and experimental gastrointestinal mobility, IBD and hepatology
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Citation for published version (APA):
Chapter 1

Introduction

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Chapter I

Introduction

Nuclear Gastroenterology

Most of the clinical manifestations of gastrointestinal (GI) disease reflect abnormal function. Dysphagia, dyspepsia, change in bowel habit, abdominal pain, GI bleeding, and jaundice are all manifestations of disturbed physiology. Whereas abnormal anatomy is usually best investigated by endoscopy, conventional radiology, ultrasound or computed tomography (CT), some functional abnormalities are better defined and explored by radionuclide techniques. Magnetic resonance imaging (MRI) has a large potential in the evaluation of GI physiology and motility disorders. However, the expensive technique, the limited expertise and availability for this kind of investigations and the lack of validation hamper wide implementation to date. Traditionally, investigating GI function requires intubation for sampling or prolonged intraluminal pressure monitoring. These techniques involve a degree of discomfort to the patient. Contrast studies and endoscopy, although excellent for anatomical detail, give only a very limited indication of functional disturbances. Scintigraphic studies show relatively poor anatomical detail but make their major contribution in the demonstration of abnormal function.

Major advances in positron emission tomography (PET) have resulted in a shift in nuclear medicine research with an exponential growth in submitted papers and with oncology as leading investigatory subject at the cost of conventional nuclear medicine. Except for GI oncology, PET has no major indications yet for GI motility disorders. However, PET can be used to localize a source of cerebral activity, which can be used in research assessing the brain's processing of visceral sensation and the role of abnormal visceral sensation in the pathophysiology of functional bowel disease.

Scintigraphy, however, has more potential than the routine clinical investigations currently available in most nuclear medicine facilities, frequently restricted to gastric emptying studies, limited hepatobiliary scintigraphy and localization of ectopic gastric mucosa in a Meckel's diverticulum. The current applicability of scintigraphic methods in gastroenterology is larger and includes for instance detection and quantification of gastroesophageal and enterogastric reflux, esophageal clearance, gastric emptying, small bowel and colonic transit, calculous and acalculous biliary disorders, localization of
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ectopic gastric mucosa in a Meckel's diverticulum, localization of sites of GI hemorrhage and identification of sites of inflammation in the GI tract. Research in GI motility, IBD and hepatology is making quantum leap progression increasing the demand for noninvasive validation of hypotheses and techniques. Therefore, the aim of this thesis was to further characterize existing nuclear medicine techniques in the evolving field of gastroenterology and to explore novel applications and techniques for clinical and experimental use in human and animal studies.

Gastrointestinal motility

Definition

GI dysmotility symptoms are a major problem in routine clinical practice. If after careful history-taking, thorough physical examination, appropriate biochemical and hematological screening and the conducting of examinations like radiology or endoscopy, with or without biopsy, no structural, infectious or biochemical cause can be found, the diagnosis of functional GI motility disorder can be made. The advantages of radionuclide imaging for studying GI tract function have remained the same since the oral administration of $^{51}$Cr porridge to measure gastric emptying was introduced 30 years ago. In contrast to manometry, scintigraphy is noninvasive, does not disturb normal physiology, and permits quantification of bulk transit of solids and liquids. Compared with radiographic methods, scintigraphy results in low radiation burden, is easily quantified, and uses commonly ingested foods rather than non-nutrient substrates.

Evaluation of severe functional GI motility disorders requires an investigation of the entire GI tract. To determine the correct therapeutic option, it is important to differentiate between a diffuse GI motor disorder and the dysfunction of an isolated GI segment. Furthermore, it has been shown that whole gut transit scintigraphy frequently leads to a change in diagnosis and thus leads to improved patient management.
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Patient studies

Dual isotope scintigraphy is now widely used for clinical and investigative purposes. It is a noninvasive, physiologic, easy to perform, safe and quantitative technique with a low radiation burden.\textsuperscript{11} It has become the gold standard for the evaluation of gastric emptying for solids and liquids in all types of gastrointestinal disorders and for assessing the efficacy of gastrokinetic drugs and surgical procedures.\textsuperscript{11} Further investigation of the pathophysiology of functional dyspepsia focused on antral dysmotility, abnormal gastric pacemaker function and myoelectric activity.\textsuperscript{12-15} These investigations have led to the conclusion that delayed gastric emptying of a solid meal is related to antral hypomotility.\textsuperscript{16} It was demonstrated that dynamic gastric scintigraphy allows visualization and characterization of antral contractions.\textsuperscript{17} It can also be used simultaneously to evaluate the compartmentalization of food inside the stomach and to quantify the emptying of a radiolabeled test meal from each compartment.\textsuperscript{18}

The normal values for scintigraphic gastric emptying studies are based on male controls or a mixed control population. Historically it has been assumed that men and women have identical rates of gastric emptying.\textsuperscript{19,21} The effect of gender on gastric emptying remains controversial though there is more and more evidence that women have slower gastric emptying rates for solids\textsuperscript{22-24} and possibly also for liquids.\textsuperscript{25}

The aims of the study presented in chapter 2 were (1) to confirm the difference in gastric emptying for solid and liquid test meal between healthy men and women, and (2) to investigate the origin of this difference in absence of pathology by studying regional gastric emptying and antral motility.

In chapter 2, we confirmed a significant difference in gastric emptying of a solid test meal between healthy male and female volunteers. It has been demonstrated that gastric emptying is slower in healthy premenopausal women, where both half-emptying time (T\textsuperscript{1/2}) and lag-phase (Tlag) are significantly more prolonged than in men. When evaluating small-bowel transit as part of an entire bowel transit protocol, determination of the 10\% small bowel transit time (SBTT) for solid test meal could be different for male and female patients. Therefore, the purpose of the study presented in chapter 3 was to define normal values of small-bowel transit in men and women, and to assess if there is a gender difference, or a difference between solids and liquids.
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Accurate measurement of the oroceleal transit time (OCTT) is an important step in achieving better insight in detecting dysmotility of the upper GI tract. Moreover, intestinal transit influences the functions of the colon by the supply of substrates. Efficiency of colonic fermentation (i.e., the metabolism of unabsorbed dietary components) is greatly influenced by the motility of the upper intestinal tract. Hence, it is of major importance to be able to measure transit times, OCTT in particular, using a relatively simple, reproducible test. Methods for measuring intestinal transit should not interfere with normal GI functions and should cause minimal discomfort for the patient. Scintigraphy is usually considered the reference technique for measuring OCTT. Several drawbacks, however, limit its application in routine practice. Expensive equipment, time and specialized personnel are required, and the use of radioactive isotopes is associated with some irradiation (<3 mSv). It is not preferable to repeat the technique at short intervals in children, and in pregnant women the use of this technique should be avoided completely. Therefore, it was the aim of the study presented in chapter 4 to investigate the validity of the lactulose-[13C]ureide (LU) breath test (LUBT) by direct comparison with a well-established method, namely scintigraphy using 99mTc-sulfur colloid.

Animal studies

The use of mouse models has increased in GI research, particularly in GI oncology and GI immunology. In contrast, GI motility research would benefit from further development and application of mouse models. In the recent literature, a variety of techniques have been used to assess gastric emptying in small laboratory animals. In most techniques, the animals are sacrificed, the stomach and intestine are removed and weighed, and the content is analyzed, by measuring its radioactivity, counting the number of glass beads it comprises or assessing it with phenol red photospectrometry. Other techniques limit the number of animals needed for an experiment by measuring repetitively without sacrificing the animal, such as by using scintigraphy in a rat parabiosis model, MRI or 13C-octanoic acid breath testing in mice. The aims of the study presented in chapter 5 were: (1) to adapt a routine pinhole gamma camera system, suitable for murine gastric-emptying scintigraphy without the need of sedation or invasive parabiotic preparation, (2) to validate murine gastric emptying scintigraphy by
comparing it to an established technique such as phenol red recovery, and (3) to determine normal values for solid and liquid gastric emptying and evaluate effects of multiple handling, earlier sedation and/or analgesia on gastric emptying.

An application of the model described and validated in chapter 5 is illustrated in chapter 6. In this study, our aim was to show in a murine model for postoperative ileus that leukocyte infiltrates recruited in the intestinal muscularis by selective small-intestinal manipulation affect the motility of parts of the gastrointestinal tract, distant from the site of manipulation, by triggering an inhibitory neural pathway. Gastrointestinal motility was assessed with pinhole gastric emptying scintigraphy in mice.

**Gastric volume scintigraphy**

Dyspepsia refers to pain or discomfort centered in the upper abdomen in the absence of structural or biochemical abnormalities. The pathophysiology of functional dyspepsia is largely unknown. Proximal stomach dysfunction has been demonstrated in patients with functional dyspepsia, with impaired gastric accommodation to meal ingestion and hypersensitivity to fundic distention. However, controversy remains and therapeutic effects of drugs targeting these mechanisms are often disappointing.

The gastric accommodation response enables relaxation of the proximal stomach, providing a reservoir for food ingestion without a rise in pressure. Impaired relaxation of the proximal stomach may contribute to the development of meal-induced symptoms in conditions such as functional dyspepsia, diabetes mellitus or postfundoplication syndrome. To facilitate research on impaired gastric accommodation, a noninvasive and easy-to-perform test is needed to select patients for pharmacological testing and evaluate effects of therapeutic strategies.

Measurement of the gastric accommodation response to meal intake is technically difficult and available techniques have methodological drawbacks. The current gold standard for the measurement of accommodation is the gastric barostat, involving the introduction of a balloon into the gastric fundus. In addition to the discomfort associated with this invasive, and time-consuming procedure, the presence of a balloon in the stomach has been shown to cause dilatation of the antrum as a result of meal displacement and induction of exaggerated proximal gastric relaxation. Nonradiation
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techniques, such as ultrasound or MRI have technical limitations or, in the case of MRI, potential advantages but limited availability for this type of studies.\textsuperscript{51, 64}

A scintigraphic technique based on pertechnetate uptake in gastric mucosa for measurement of gastric accommodation has been described.\textsuperscript{65-70} However, this technique requires relative high exposure to ionizing radiation and imaging can only be performed within a relatively short time interval after isotope injection.\textsuperscript{63} Despite these limitations, the technique could be applicable in clinical practice to select patients with impaired accommodation that might benefit from fundic relaxant agents.\textsuperscript{71}

The aim of the study presented in \textit{chapter 7} was to refine the scintigraphic method, limiting the radiation dose applied and increasing possible postinjection imaging time span without losing image quality, so that sequential (multiple measurements within 1 test) and repetitive (measurements before and after treatment) measurements within 1 subject become possible without increasing radiation burden.

The technique of gastric volume scintigraphy has been shown to record changes in postprandial volume to a similar extent as the gastric barostat.\textsuperscript{66} It should be emphasized that in this study the barostat balloon was positioned in the proximal stomach during SPECT scanning, providing a positive pressure to the gastric wall and thereby actively distending the stomach. Under more physiological conditions, however, the stomach will remain collapsed. Consequently, the volumes measured by SPECT scanning will reflect intragastric content such as intragastric secretions, swallowed air and ingested foods or liquids. Based on these assumptions, we hypothesized in \textit{chapter 8} that in the absence of a barostat balloon, SPECT scanning will largely detect the volume effect of meal ingestion and will be a rather insensitive tool to detect fundic relaxation. To test this hypothesis, the ability to detect fundic relaxation of comparable magnitude evoked by meal ingestion or glucagon was compared between the two techniques performed at separate days. In addition, symptoms reported during the study were evaluated as indirect measure of procedure related discomfort.
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Inflammatory bowel disease

Patient studies

Ulcerative colitis (UC) is a chronic inflammatory bowel disease accompanied by frequent acute exacerbations. It is usually diagnosed by rectosigmoidoscopy, colonoscopy or double-contrast barium enema.

Colonoscopic and contrast radiographic procedures have inherent disadvantages and can be cause of severe complications. In severe cases of the disease, colonoscopy should not be performed to assess the severity and the extent of the disease. Caution must be exercised in performing barium studies in acutely ill patients with severe colitis, because bowel preparation may worsen the disease or precipitate toxic dilatation of the colon. However, knowing the severity of disease is important for estimating the intensity of anti-inflammatory therapy and the risk of complications. Some recommendations for therapy are based on extent and severity of disease activity.\textsuperscript{72,73}

Despite convincing evidence in the recent literature about the potential benefits of \textsuperscript{99m}Tc-hexamethylpropylene amine oxime (HMPAO)-labeled leukocyte scintigraphy in adults,\textsuperscript{74-78} and children,\textsuperscript{79,80} the procedure is not performed routinely for UC in many hospitals.

The aim of the study presented in chapter 9 was to determine if \textsuperscript{99m}Tc-HMPAO labeled WBC scintigraphy could be an alternative to endoscopy with biopsy to determine the extent and the severity of the disease in these critically ill patients.

Treatment of ulcerative colitis is mainly based on corticosteroids, administered orally, parenterally or rectally.\textsuperscript{72} A rapid and sustained response is usually seen within a few days following initiation of treatment. However, the overall rate of steroid treatment failure in acute exacerbations of UC remains high, and 20-30\% of patients ultimately require surgery.\textsuperscript{81,82} The introduction of cyclosporine as a potent immunosuppressive drug for treatment of corticosteroid-refractory UC benefits the short-term management in 60-80\% of patients.\textsuperscript{83,84} However, several questions remain regarding the long-term maintenance strategies, since long-term prognosis is not overall impressive and cyclosporine treatment is known for its possible important side effects.\textsuperscript{83,85}
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Prediction of the long-term prognosis of UC at the time of diagnosis and early identification of which patients with a severe exacerbation will respond to therapy remains difficult. However, it is of great clinical importance to be able to predict a need for step-up medical treatment or colectomy at an early stage of the acute attack.\(^{85,86}\)

Therapeutic action is based on reduction of inappropriate gut inflammation, by means of glucocorticoids, immunomodulators like cyclosporine or biological therapeutic intervention in mucosal homing.\(^ {87,88}\) Since scintigraphy and histology correlate very well in this particular field, WBC scintigraphy could become a reliable, noninvasive prognostic tool in prediction of therapeutic response and the follow-up of treatment for CU.

The aim of the study presented in chapter 10 was to determine if WBC scintigraphy can predict early treatment failure in patients with acute attacks of UC.

Gantry validation

During the development of new radiopharmaceuticals, the biological behavior and dosimetry are frequently studied by ex vivo measurements in animals.\(^ {89,90}\) However, the benefits of an in vivo technique, such as single photon emission computed tomography (SPECT), are numerous. The number of animals needed within an in vivo approach is far less compared to ex vivo measurements, where for a single measurement the animal has to be sacrificed. The reduced number of animals required within an in vivo approach is beneficial from an ethical point of view, but may also save research time and may therefore be more cost-effective.

A prerequisite for the replacement of ex vivo experiments by SPECT imaging is that it enables an accurate quantification of tracer uptake and kinetics. For in vivo experiments with smaller animals, such as mice, hamsters, or rats, a SPECT system encounters its physical limits for resolution and sensitivity. The pinhole collimator has been used extensively to obtain greater detail in planar imaging. The application of pinhole SPECT, however, is difficult since it requires a heavy collimated detector to rotate around a small object with a precisely constant radius of rotation. To circumvent this problem, we have developed an animal pinhole SPECT system in which the mechanical misalignment is minimized.
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Chapter 11 describes a dedicated animal pinhole SPECT device, in which the gantry and collimator are fixed and the animal rotates. To ensure that the rotation of the object does not introduce artifacts, calibration experiments were performed. Phantom experiments were done to investigate the extent to which accurate quantification of tracer uptake may be possible with this new system. In vivo experiments were performed using 2 animals of different size, a rat and a hamster, to test the clinical feasibility of the system.

Animal studies

Although the initiating events of IBD are still unknown, increasing evidence indicates that mucosal CD4+ T cells activated by enteric bacteria initiate and perpetuate the inflammation in genetically susceptible individuals. Molecular understanding of the mechanisms that guide lymphocyte trafficking into the gut is rapidly increasing. Molecular control of lymphocyte trafficking in mucosal tissues under physiological and pathological conditions is a well-orchestrated interaction between lymphocyte receptors and corresponding endothelial ligands. In an inflammatory state endothelial adhesion molecules are upregulated and lymphocytes home to their target tissue. Imbalance of the immunomodulating mechanisms can result into a chronic nonspecific inflammation in IBD where a down-regulated response would be appropriate. Aberrant (excessive or inappropriate) homing of these cells to mucosal sites is believed to be a central element in the pathogenesis of IBD. This knowledge has fueled enthusiasm for the development of novel anti-inflammatory strategies, by blunting the multistep lymphocyte adhesion cascade. To this end, designer drugs for IBD are created and subsequently tested in animal models for clinical potential. However, these animals are expensive and large numbers can be required.

A noninvasive technique, able to assess inflammatory activity in bowel segments or lymphocyte recirculation and kinetics in the follow-up of experimental treatment for IBD, would not only benefit longitudinal studies, but also reduce the number of animals required for testing. Radioactive labeled purified lymphocytes have been shown to allow study of inflammatory activity, short-term lymphocyte recirculation kinetics and homing. In-oxine is the most appropriate reagent for radiolabeling lymphocytes for in
vivo distribution studies. The 2,4,6-trinitrobenzene sulfonic acid (TNBS) model of colitis is characterized by a T helper 1 (TH1) cell mediated transmural colitis and resembles human Crohn's disease in regard to many features, both on a histological and immunological level. Dedicated pinhole single photon emission computed tomography (SPECT) cameras have been shown to be able to image radioactivity distribution in vivo in small animals with a spatial resolution on the order of a few mm. Therefore, the aim of the study presented in chapter 12 was to construct and validate an animal model for IBD, in which intestinal lymphocyte homing can be monitored non-invasively in vivo by means of pinhole SPECT.

Under normal conditions, naïve T lymphocytes migrate randomly from blood to secondary lymphoid tissues for immune surveillance. When naïve T lymphocytes encounter an antigen in the draining lymph nodes of the gut, they differentiate into memory/effector cells. If such an encounter takes place in the mesenterial lymph nodes, migration is subsequently directed back to the intestinal mucosa. This process is controlled by the expression of different sets of adhesion molecules and chemokines. The integrin alpha(4) beta(7) is the principal gut-homing receptor and functions at several steps in the adhesion cascade by interacting with the mucosal addressin MAdCAM-1 present on endothelial cells. Naïve lymphocytes express low levels of alpha4beta7 but upon activation a significant amount of functionally active alpha4beta7 appears on the surface. During intestinal inflammation MAdCAM-1 is upregulated resulting in an increased influx of lymphocytes.

In inflammatory bowel disease, increased numbers of activated CD4+ TH1 lymphocytes, that locally produce proinflammatory cytokines, such as interferon gamma (IFN-γ) and tumor necrosis factor alpha (TNF-α), mediate mucosal inflammation and tissue damage. Blockade of lymphocyte recruitment to the intestinal mucosa is considered a useful therapy for inflammatory bowel disease. Indeed, administration of antibodies that bind either the alpha4 integrin alone or in combination with beta7 has resulted in therapeutic benefit in experimental colitis and in patients with active Crohn's disease. Although these results are promising, the number of in vivo studies is limited. More insight into the complex migratory pathways of lymphocytes to the intestine and the role chemokines and their receptors play herein, is necessary.
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In the study presented in chapter 13, we used dedicated animal SPECT for assessment of radioactive labeled $^{111}$In-oxinate purified T helper lymphocyte homing to the gut in TNBS-induced experimental colitis in mice. Further, we applied this technique to study blockade of intestinal lymphocyte influx in TNBS colitis with an anti-α4 integrin antibody.

Liver disease

Assessment of hepatocellular function

Hepatic resection is the therapy of choice for malignant and symptomatic benign, hepatobiliary tumors. Recent years have shown a marked decrease in morbidity and mortality rates after major liver resections.\textsuperscript{108, 109} Refinements in operative techniques, better selection of patients and advances in perioperative care are thought to be responsible for this improvement.\textsuperscript{110} Nevertheless, perioperative blood loss and postoperative liver failure have remained the most significant complications after liver resections, particularly in patients with suboptimal liver function due to parenchymal liver disease such as cirrhosis or steatosis.\textsuperscript{111} The major cause of mortality after liver resection consequently is liver failure.\textsuperscript{108} For this reason it is important to estimate total and regional liver function before planning partial resection of the liver in order to predict function of the remnant liver.

To date, the most frequently used test for evaluating preoperative liver function is the indocyanine green (ICG) clearance test.\textsuperscript{112} Alternatively, $^{99m}$Tc-labeled iminodiacetic acid (IDA) analogues, transported in blood by binding to albumin in the same manner as ICG, can be used for hepatobiliary scintigraphy (HBS) in the assessment of liver function.\textsuperscript{113} HBS, requiring a single intravenous injection, provides visual and quantitative information of global and regional liver function as well as excretory function (intrahepatic and extrahepatic bile transport). Both ICG and $^{99m}$Tc-mebrofenin are excreted in bile by the hepatocytes\textsuperscript{114} by the ATP-dependent export pump multidrug-resistance associated protein 2, without undergoing biotransformation during their transit through the hepatocyte.\textsuperscript{115, 116} Therefore, these agents are well suited for the study of
hepatic transport. The aim of the study presented in chapter 14 was to examine correlation of the ICG clearance test with the uptake of $^{99m}$Tc-mebrofenin as determined from the blood as well as from scintigraphical assessment.

The maximum extent of resection compatible with a safe postoperative outcome remains unknown, but it is generally believed that the risk for perioperative complications increases when the remnant liver volume is too small.\textsuperscript{117} Therefore, preoperative assessment of hepatic function and remnant liver volume (RLV) is advocated.\textsuperscript{118} Measurement of preoperative and remnant liver volumes can be accurately estimated by CT.\textsuperscript{119,120} However, most strategies evaluating preoperative hepatic function reserve and estimating the RLV rely on a homogeneous liver function.\textsuperscript{121} Unlike patients undergoing liver resection for metastatic cancer or benign liver conditions, patients with hepatocellular carcinoma (HCC) or obstructing tumors like cholangiocarcinoma may have underlying chronic liver disease or cholestasis with primary or secondary impaired total or segmental liver function.\textsuperscript{122-124}

There is a good correlation between the preoperative $^{99m}$Tc-mebrofenin liver uptake rate and the ICG clearance test in patients scheduled for major liver surgery.\textsuperscript{125} Therefore, the aim of the study presented in chapter 15 was to assess total and regional liver function before and after major liver surgery and to compare scintigraphic results with volumetric data and ICG clearance test results. Furthermore, the correlation between the immediate postoperative remnant liver function predicted on preoperative scintigraphy and measured 24 h after surgery with scintigraphy was assessed. Finally, the relation between liver function regeneration determined with HBS and volumetry was assessed.

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