In vivo imaging in drug development

Gamma scintigraphy is a non-invasive technique with applications in the development of drug products and the assessment of pharmacodynamic effects in humans. It enables the assessment of critical performance parameters that in vitro techniques attempt, but often fail, to predict. Quantification of pharmacodynamic effects (e.g., gastrointestinal transit; gall bladder emptying; lung mucociliary clearance) provides insights into the mode of action of drug candidates.

Methodology
Gamma scintigraphy is an imaging technique that enables the direct visualisation and quantification of events occurring in vivo, in real time.

Initially introduced as a diagnostic tool, the potential of this method was quickly realised within the pharmaceutical industry. Gamma scintigraphy was first reported for the measurement of transit times in 1966 (gastric emptying) followed swiftly by the assessment of drug product performance in 1976 (capsule disintegration).

Visualisation is achieved by the incorporation of short half-life gamma emitting radionuclides, e.g., technetium-99m (99mTc) and indium-111 (111In). The chosen radionuclide(s) is used to label the drug product or, for pharmacodynamic investigations, the component of interest (e.g., food or fluid for gastrointestinal transit; inhaled particles for mucociliary clearance). The radiation dose to the subject is minimal – often not exceeding that received from a single X-ray. A gamma camera is used to detect the gamma rays and record these as primary counts which are represented as an image (Figure 1).

Gamma scintigraphic investigations can be routinely incorporated into standard phase 1/2a studies.

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alongside safety, pharmacokinetic and other biomarker assessments.

**Applications in drug product development**

**Oral products**
The production of an oral product starts in the laboratory, where the pharmaceutical scientist is charged with developing a dosage form which meets a pre-determined specification for drug release. Release rate is measured by recognised methods, for example dissolution testing coupled with HPLC, to generate a profile of drug release versus time. The primary use of these data is for the comparison/differentiation of prototype formulations, and for quality control. However, the results are also often intended as a representation of formulation performance in simulated *in vivo* conditions and are used as a first stage tool for formulation selection. However, an *in vitro* method cannot take into account all of the physiological factors that influence formulation performance and even if an *in vitro-in vivo* correlation (IVIVC) can be established, this is only confirmed after completion of a clinical study.

Clinical studies designed to assess the performance of prototype formulations generate pharmacokinetic parameters. These data are at least one-step removed from formulation performance and so, when the pharmacokinetic profile is not as predicted, educated guesswork is needed to determine – and more importantly, fix – the cause.

Scintigraphic data provide the missing information, offering real-time visualisation and measurement of *in vivo* formulation performance. Key data are the rate of erosion of the dosage form – equating to release of drug (Case study 1). These data correspond to those obtained from *in vitro* dissolution, and assuming no other rate limiting factors may also parallel the appearance of drug in the systemic circulation (Figure 2).

A further level of detail is obtained by tracking the transit of the dosage form through the gastrointestinal tract. How long does a gastroretentive formulation remain in the stomach? To which regions does an extended release formulation deliver? How rapidly does an enteric coated formulation deliver drug after gastric emptying? Does a colon targeting formulation reproducibly deliver to the target site?

**Oral inhaled products**
The success of an orally inhaled product is a combination of the device, the formulation and the patient’s technique. As with oral formulations, development starts in the laboratory and the performance of prototypes is measured via particle size distribution (PSD) testing. While attempts continue to use PSD profiles as a predictor of *in vivo* deposition, the reality is that there is no direct correlation between individual or grouped

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**Figure 1:** Sample scintigraphic images. A: 99mTc-DTPA drink showing an outline of the stomach. B: Erosion of a tablet located in the colon. C: Nasal deposition, co-registered with MRI scan. D: Lung deposition following oral inhalation.

**Figure 2:** Correlation of plasma concentrations with *in vivo* tablet erosion (mean data). Quantitative assessment of the rate of loss of radiolabel from an oral dosage form, plotted as % radioactivity release versus time. Correlation with the plasma concentration profile reveals a strong relationship between erosion of the tablet and appearance of drug in the plasma.
Drug Development

Case study 1: Investigation of effect of food on rate of erosion from a HPMC MR formulation

Background
UK-294,315 is potent 1 adrenoreceptor antagonist and consistent with its mode of action, dose-limiting adverse effects related to Cmax (postural hypotension and fainting) were noted following dosing of an IR formulation. Consequently, modified release formulations were developed to minimise fluctuations in the plasma concentration profile.

Performance of the formulations in the fasted and fed states was assessed in a pharmacokinetic investigation. In the fed state, peak plasma concentration and exposure were increased significantly and absorption profiles were much more variable.

A scintigraphic study was performed to investigate the mechanism of this apparent failure in the fed state.

Methods
An open-label, randomised, single-dose three-way crossover investigation was performed in nine healthy subjects. Subjects received the IR tablet (fasted), the MR tablet (fasted) or the MR tablet (fed). The MR tablets were radiolabelled by the incorporation of a gamma emitting radionuclide (eg 99mTc) to the formulation. Scintigraphic images were acquired at 15min intervals until 12h post-dose, at 30-minute intervals until 15h post-dose and at 18h and 24h post-dose. Blood samples for PK analysis were collected at regular intervals until 48h post-dose. Scintigraphic data were analysed to determine the transit of the dosage form through the gastrointestinal tract, and the rate of erosion of the dosage form.

Results
The pharmacokinetic data confirmed the increased Cmax and variability following dosing in the fed state. Gastrointestinal transit of the MR tablet was as expected in the fasted state, with gastric emptying occurring on average at 1.15±1.19h, small intestinal transit of 2.88±1.24h and colon arrival at 3.90±0.67h. In the fasted state, tablet erosion was gradual, with drug being delivered throughout the gastrointestinal tract and complete tablet erosion occurred in the colon (9.38±1.95h). In the fed state, limited data on transit were obtained since seven of the nine dosage forms were still located in the stomach at the time of complete erosion (5.90±2.18h). This, plus an increased rate of erosion resulted in drug delivery to the upper small intestine.

Conclusions
The scintigraphic data showed a clear difference in the tablet erosion profiles following fed and fasted administration, providing an explanation for the unexpected pharmacokinetic results in the fed state.

Drug Development
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Oral inhaled route, the use of in vitro assessments and the quantification of lung deposition via imaging are already recognised as supporting data – although pharmacokinetic data are still deemed to be advantageous. The regulators now also recognise that the use of comparative clinical trials is inefficient and prohibitively expensive for locally acting molecules delivered to the gastrointestinal tract. As part of the FDA Critical Path Initiative, in vivo imaging has been suggested as a method to directly assess the rate of drug release at the target site. Scintigraphic data provides a measure of both the location and rate of drug release, and comparative assessments of innovator versus test product can be performed.

Applications in clinical pharmacology
Gastrointestinal transit
Drug molecules often have an impact on gastrointestinal transit. This may be an unwanted side effect, such as opioid-induced constipation, or a key element of the molecule’s pharmacology, e.g., 5-HT4 agonists. This impact can be measured in a variety of ways – from changes in acetaminophen kinetics, to the 13C octanoic acid or hydrogen breath tests, the use of radio-opaque markers and the measurement of pH changes. However, each of these approaches provides limited insight into the extent of the effect exerted by the drug molecule.

Scintigraphic imaging is a convenient method for

Figure 3: Calculation of colonic transit using geometric centre analysis. The proportion of the radionuclide distributed within each region of the colon is multiplied by the region’s associated weighting factor. The sum of each of these values generates a single ‘GC value’ for each selected time point.

Calculation of GC value:
80% in region 1 = 80/100 = 0.8
20% in region 2 = 20/100 = 0.4
0.8 + 0.4 = GC value of 1.2 at Xh post-dose
Drug Development

Case study 2: Assessment of the impact of oxycodone/naloxone combination on colon transit compared with oxycodone alone

Background
Opioid-induced bowel dysfunction is reported in the majority of patients treated with opioids. Oxycodone exhibits this effect as a result of the presence of μ-opioid receptors in the gastrointestinal wall. Naloxone is an opioid receptor antagonist which acts locally at the receptors and is able to counteract the opioid-induced constipation. An oxycodone/naloxone combination product has been developed to provide analgesia locally at the receptors and is able to counteract the opioid-induced constipation. An oxycodone/naloxone combination product has been developed to provide analgesia

Methods
An open-label, randomised, single-dose, placebo controlled five-way crossover study was performed in 15 healthy subjects. Subjects received: 10mg oxycodone PR tablet, 20mg oxycodone PR tablet, 10/5mg oxycodone/naloxone PR tablet, 20/10mg oxycodone/naloxone PR tablet and placebo tablet. To determine the impact on gastrointestinal transit, radiolabelled ion-exchange resin (111In) was also administered. The test formulations were also radiolabelled to permit correlation of the intestinal transit, radiolabelled ion-exchange resin (111In) was also administered. The test formulations were also radiolabelled to permit correlation of the intestinal transit, radiolabelled ion-exchange resin (111In) was also administered. The test formulations were also radiolabelled to permit correlation of the intestinal transit, radiolabelled ion-exchange resin (111In) was also administered. The test formulations were also radiolabelled to permit correlation of the intestinal transit,

Results
The scintigraphic data confirmed that colon transit was significantly longer for the 10mg and 20mg oxycodone compared with placebo. The presence of naloxone at the 10mg oxycodone dose had no effect on colon transit time. The presence of naloxone at the 20mg oxycodone dose resulted in a significant reduction in colon transit time. Only one incidence of constipation was reported throughout the study, confirming that the effect on colon transit time was sub-clinical.

Pharmacokinetic data was compared with in vivo tablet performance and revealed that the majority of absorption was occurring in the small intestine, before the tablets reached the colon.

Conclusions
Scintigraphic data confirmed that a 20mg dose of oxycodone resulted in a significant slowing of colon transit, and that the effect was reduced by the simultaneous administration of naloxone.

Mucociliary clearance
Mucociliary clearance is the primary physiologic defence mechanism, protecting the lungs from damage caused by inhaled particles and micro-organisms. Impairment of mucociliary clearance function plus airway inflammation in conditions such as chronic obstructive pulmonary disease (COPD) and cystic fibrosis (CF) may contribute to the increased morbidity from airway infections that are part of these conditions. Consequently, the quantification of the impact of drug molecules on the rate of mucociliary clearance is a key biomarker for products in development for the treatment of respiratory disease.

Scintigraphic assessment of mucociliary clearances enabled by administration to the lungs of radiolabelled non-absorbable particles, including providing direct, detailed, quantitative assessment of gastrointestinal transit. The radiolabel is administered in food and/or fluid, or as a multi-particle formulation, which provides a marker for gastrointestinal contents without concomitant administration of calories and induction of the fed state.

The method permits a detailed assessment of the effect of a drug molecule on gastric emptying. Solid and liquid phase gastric emptying, which are regulated by different mechanisms and hence can be affected differentially, can be measured concurrently by the simultaneous use of two radionuclides. Detailed analysis of intra-gastric distribution can also be performed.

Similarly, the impact of a drug molecule on colon transit can be a desired pharmacological effect, ie the normalisation of transit times to treat diarrhoea or constipation, or it can be an adverse effect resulting in the generation of these symptoms.

The rate of colon transit is measured by a technique termed geometric centre (GC) analysis. The method was first presented in 1986, and has developed since then to offer a non-invasive measure of transit through the colon versus time (Figure 3). The data generated are both stable and clinically relevant.

A healthy volunteer model for the assessment of the sub-clinical effect of drug molecules on colon transit is available (Case study 2). Effects on colon transit are observed, following single doses, prior to the onset of any signs or symptoms of disruption of the normal pattern of motility. This provides the opportunity to assess the effect of new drug products in simple, efficient investigations that can be performed early in the development process.
Following deposition, the rate of removal of these particles is primarily governed by mucociliary clearance. Sequential scintigraphic images are acquired and the rate of clearance quantified. Results for the test regimen can be compared with baseline, placebo or positive control (e.g. hypertonic saline). Dose ranging assessments can also be performed.

Gallbladder emptying

The utilisation of gamma scintigraphic techniques within the pharmaceutical industry is continually progressing, and the latest transition from diagnosis (hepatobiliary disease) into clinical research is the impact of drug molecules on the rate of gallbladder emptying. The drive for this transition was the concern voiced by the FDA of the potential for GLP-1 analogues and agonists to induce acute pancreatitis following post-marketing reports for exenatide. This led to the requirement for the assessment of gallbladder emptying for new molecules within this class.

A number of variations of the method are routinely used within a diagnostic setting. In order to ensure consistent and comparable data, the method was adapted and standardised. Recommended parameters have been published. For drug product development, it is the only direct method of assessment of critical-to-performance parameters which in vitro methods attempt but often fail to predict. It also permits direct quantification of pharmacodynamic effects, providing insight into mode of action of drug candidates and essential biomarker data. The data generated are accurate, detailed, comprehensive and clinically relevant, resulting in greater confidence in decision making leading to more effective drug product development.

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Dr Alyson Connor gained her PhD in 1998 and has 15 years’ experience in the pharmaceutical industry. For the past 12 years Alyson has fulfilled the role of Senior Research Fellow at Quotient Clinical, a provider of early drug development services. As lead scientist she is responsible for all oral scintigraphic studies performed and is an acknowledged expert in the use of scintigraphy to assess product performance and pharmacodynamic effect. She regularly publishes on this subject.
Drug Development

References