Use of the Entero-Test, a Novel Approach for the Noninvasive Capture of Biliary Metabolites in Dogs

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ABSTRACT:

Preclinical information on the biliary metabolites of a drug candidate is typically obtained through the collection of bile after surgical cannulation of the bile duct. In this study, we describe a novel approach using the Entero-Test, a simple device that facilitates the noninvasive sampling of duodenal bile. The Entero-Test was used to collect bile from six fasted dogs that had been dosed either orally with simvastatin (SV) or intravenously with simvastatin hydroxy acid (SVA), compounds that have been previously reported to undergo extensive metabolism and biliary secretion in the dog. The devices, consisting of a weighted gelatin capsule containing 90 cm of a highly absorbent nylon string, were swallowed by each dog with the proximal end of the string taped to the animal’s face. Once the weighted string had reached the duodenum, gallbladder contraction was stimulated to release bile. Each bile-stained string was then retrieved via the mouth and, after solvent extraction, samples were analyzed for drug-related material by ultraperformance liquid chromatography-mass spectrometry and NMR spectroscopy. Numerous metabolites of SV and SVA were observed, and, in general, the major metabolites have been reported previously from studies with bile duct-cannulated animals dosed with [14C]SV or [14C]SVA. The results from this study demonstrate the utility of deploying the Entero-Test in absorption, distribution, metabolism, and elimination studies to provide information on the nature of biliary metabolites, which, on occasion, may be sufficient to negate the need for more invasive sampling techniques. The benefits and limitations of the technique are discussed.

Biliary excretion is often a major route of elimination of drugs and their metabolites from the body, and elimination of compounds via the bile can affect the systemic exposure, toxicity, and pharmacological effects of drugs. It is important to characterize hepatobiliary elimination, because this is often the key site of drug-drug interactions, which may facilitate significant alterations in either systemic or hepatic exposure (Ghibellini et al., 2006).

Preclinical drug metabolism studies are typically conducted as part of the drug development process and are used to quantify and structurally identify drug-related material to help understand the absorption, distribution, metabolism, and elimination (ADME) characteristics of a drug. Coupled with having plasma, blood, fecal, and urine metabolite data available from such studies, any additional information obtained on the biliary disposition of drug-related material will be of benefit, because knowledge of the metabolic routes of elimination therein may help to contextualize not only the ADME profile but also the pharmacokinetic profile of the drug. Furthermore, information gathered preclinically on metabolites secreted in the bile is important, because it may help predict how the drug will behave in man. For example, certain conjugated metabolites (e.g., glucuronides) that are released into the bile are often subject to hydrolysis by the gut microflora; this phenomenon can lead to parent drug being made available for reabsorption, which in turn may lead to reexposure to the drug (enterohepatic cycling), thus prolonging the residence time of the drug in the body. Therefore, only by collecting bile before it is exposed to the gut microflora can these metabolites of interest be captured for analysis.

As part of ADME studies in dogs, bile can be obtained after surgical cannulation of the bile duct. This method is usually deployed as part of a mass balance investigation using radiolabeled drug. Nonetheless, the cannulation technique involves an invasive surgical procedure conducted under general anesthesia and is normally done only by a veterinary surgeon. Subsequently a long postsurgical recovery period is necessary before a dog is fit for study purposes, and there are often complications associated with such surgery, including peritonitis and infection.

A simple method for the collection of duodenal bile in human subjects has previously been reported. The method was based on administration to several human subjects of the Entero-Test, a commercially available device that is used to sample upper gastrointestinal fluid for diagnostic purposes. The aforementioned studies demonstrated that the device could be used reliably to collect bile to determine bile acid profiles (Vonk et al., 1986) and cholesterol saturation (Muraca et al., 1991) in human subjects. Despite these investigations, very little information exists on the use of the Entero-Test in dogs, with the exception that it has been shown to be a practical method for the diagnosis of Giardia infections (Hall et al., 2008). In addition, there are no reports in the literature on the use of the device specifically for bile sampling in dogs.

ABBREVIATIONS: ADME, absorption, distribution, metabolism and elimination; SV, simvastatin; SVA, simvastatin hydroxy acid; HPLC, high-performance liquid chromatography; UPLC, ultraperformance liquid chromatography; MS, mass spectrometry; CCK, cholecystokinin.
The objectives of the work described here were 1) to evaluate the safety and tolerability of the device in dogs and 2) to determine whether it could be used to noninvasively capture small bile samples from dogs, which would facilitate the qualitative characterization of the biliary disposition of a drug using spectrometric and spectroscopic techniques. Nonradiolabeled simvastatin (SV) and simvastatin hydroxy acid (SVA) were selected as being appropriate tool compounds for the evaluation of the device, because in dogs these compounds are known to undergo extensive phase I and II metabolism (including the formation of glucuronide conjugates) before being secreted into the bile (Prueksaritanont et al., 2002). In this work, we attempted to compare the biliary metabolites of SVA and SV captured noninvasively using the device with those that had previously been described from bile samples collected from bile duct-cannulated dogs dosed with [14C]SVA or [14C]SV (Vickers et al. 1990a; Prueksaritanont et al., 2002; Subramanian et al., 2002).

Materials and Methods

Materials. Entero-Test devices [pediatric version (90 cm) part 101–01] were purchased from HDC Corporation (Milpitas, CA). SV and SVA were purchased from Toronto Research Chemicals Inc. (North York, ON, Canada). Ceruletide (caerulein, sulfated, synthetic) was purchased from Sigma-Aldrich Company Ltd. (Poole, UK). HPLC-grade acetonitrile and methanol were obtained from Thermo Fisher Scientific (Loughborough, UK). Analytical-grade ammonium acetate was purchased from BDH (Poole, UK). Deionized water was generated in the laboratory using a Milli-Q water filter unit (Milton, MA). Grade ammonium acetate was purchased from BDH (Poole, UK). Deionized water was generated in the laboratory using a Milli-Q water filter unit (Milton, MA). Deuterium oxide was purchased from GOSS Scientific Ltd. (Molsheim, France). Deuterium oxide was purchased from GOSS Scientific Ltd. (Molsheim, France). Deuterium oxide was purchased from GOSS Scientific Ltd. (Molsheim, France).

During withdrawal, the small steel weight that is attached to the distal end of the capsule dissolves in the stomach and the string, which is weighted at its distal end, passes into the duodenum. After a period of approximately 4 h, the string with any adsorbed gastrointestinal fluid is withdrawn through the mouth. During withdrawal, the small steel weight that is attached to the distal end of the string detaches and is eliminated in the stool. The collection of the string for microbiological analysis.

In Vivo Metabolism Studies. All procedures were performed under the authority of a Project License granted by the United Kingdom Home Office issued under the Animals (Scientific Procedures) Act 1986. Six health-checked, male beagle dogs, each weighing between 9 and 14 kg, were used in this study.

Oral Leg. Three dogs were fasted overnight. Each dog was dosed orally with SV formulated as a suspension in 0.5% hydroxypropylmethylcellulose at a dose level of 30 mg/kg and at a dose volume of 5 ml/kg. Approximately 1 h after dosing, each dog was given a single food pellet to stimulate the release of bile from the gallbladder after overnight fasting. At 2 h after oral dosing, each dog swallowed an Entero-Test capsule with the proximal string taped to the face. The dogs were placed into restraining slings for the remainder of the procedure. Two and one-half hours after capsule swallowing, each dog was fed a single food pellet to stimulate gallbladder emptying. Forty-five minutes later the strings were withdrawn from each dog, and the bile-soaked sections were immediately eluted using the method described below (see Elution of Bile).

Intravenous Leg. Three dogs were fasted overnight before being placed into restraining slings. Each dog then swallowed an Entero-Test capsule, with the proximal string taped to the face. Approximately 1 h after capsule swallowing, each dog was given a single food pellet to stimulate the release of bile from the gallbladder after overnight fasting. One hour later (by which time the device had been adjudged to have reached the duodenum), each dog was dosed intravenously with SVA formulated as a saline solution containing 20% (w/v) polyethylene glycol 200 at a dose level of 2 mg/kg and at a dose volume of 1 ml/kg. Fifteen minutes after intravenous dosing, each dog was fed a single food pellet to stimulate gallbladder emptying and bile release. Forty-five minutes later, the strings were withdrawn from each dog, and the bile-soaked sections were immediately eluted using the method described below (see Elution of Bile).

Elution of Bile. Immediately after removal from each dog, the proximal end of each bile-stained string was taped to a retort stand such that it hung vertically with the distal end placed into a glass vial. The bile sample (obvious by the yellow color) was eluted from the string by gently pipetting 2 ml of 0.2 M ammonium acetate buffer (pH 4 with acetic acid)-methanol (3:1, v/v) onto the string, which was then collected into the glass vial. The string was eluted for a second time using 3 ml of methanol, which was collected into the same vial. Any residual material was scraped from the string into the methanol sample using a gloved thumb and forefinger, slide down the length of the string. Samples were immediately stored at −20°C, pending further processing.

Sample Processing. On the day of processing, the samples were allowed to warm to room temperature. The samples were then concentrated to a volume of approximately 1000 μl under nitrogen gas using a DDA-X solvent evaporator (Genevac, Ipswich, Suffolk, UK) at 37°C. Approximately 200 μl of each bile sample concentrate was taken for ultraformance liquid chromatography (UPLC)-mass spectrometry (MS) analysis, and the remainder from each sample (approximately 800 μl/sample) was pooled for additional NMR spectroscopy analysis. In addition, a control bile sample collected from an undosed dog was also processed and analyzed by UPLC-MS to discriminate drug-related from endogenous components in the bile.

UPLC-MS Analysis. UPLC separation was performed on an Acquity liquid chromatography system (Waters, Milford, MA) using an Acquity BEH C18 column (100 × 2.1 mm, 3 μm) (Waters). The column temperature was maintained at 50°C. The mobile phase consisted of 0.1% aqueous formic acid (v/v, solvent A) and acetonitrile (solvent B) and was delivered at a constant flow rate of 0.3 ml/min with an initial gradient of 5% B held for 0.2 min, increasing linearly to 95% B at 10 min. Fifteen-microliter injections of individual and pooled bile samples together with an appropriate control sample were made.

The ultraformance liquid chromatograph was coupled to an Acquity photodiode array detector (Waters) and a Q-ToF Premier Mass Spectrometer (Waters) controlled with MassLynx software (version 4.1; Waters, Manchester, UK) with electrospray ionization in the negative ionization mode. A capillary voltage of 2.7 kV, cone voltage of 35 V, collision energies of 5 and 20 eV, and source and desolvation temperatures of 150 and 400°C, respectively, were used. Leucine enkephalin (200 ng/ml) was introduced via the lock spray inlet at 10 μl/min using a Waters Reagent Manager pump to act as a lock mass. Mass resolution was set at 8000 full-width at half-maximum at 500 Da. MetaboLynx processing software (Waters) was used to assist molecular ion detection, and a combination of accurate mass and tandem mass spectrometry data on the molecular ions was used for structural identification of individual metabolites. Peak areas for metabolites of interest were automatically generated on the relevant ion traces using MassLynx integration tools to compare concentrations across the samples.

Preparative HPLC. An aliquot (approximately 3.5 ml) of pooled bile extracts was separated by preparative HPLC using a Series 1100 Preparative LC System (Agilent Technologies, Waldbronn, Germany). Separations were performed on an Xbridge Prep-Phenyl HPLC column (250 × 10 mm i.d.; Waters) at ambient temperature with a mobile phase of 0.1% aqueous formic acid (v/v, solvent A) and acetonitrile (solvent B) at a constant flow rate of 4 ml/min with an initial gradient of 5% B held for 1 min, increasing linearly to 95% B at 42 min and held at 95% B for a further 3 min. The HPLC eluent was collected into fractions, in a time-slice mode, into two 96-drop-deep plates using a frequency of 15 s/fraction. This resulted in 180 fractions, each containing 1 ml of column eluent. The flow was split 100:1 into a Micromass ZQ mass spectrometer (Waters) fitted with an electrospray source operated in both positive and negative ionization modes. System control was mediated through MassLynx and FractionLynx (Waters). The fractions were taken to dryness under nitrogen at 37°C within the 96-drop-deep plates using a Micro DS96 dry down station (Porvair Scientific Ltd., Shepperton, UK) and then reconstituted in approximately 0.6 ml of deuterium oxide-acetonitrile (1:1) before being transferred to 5-mm NMR tubes.

NMR Spectroscopy. NMR experiments were performed on all 180 fractions using a Bruker AVII+ spectrometer equipped with an inverse 5-mm TXI
CryoProbe (1H/13C/15N) operating at 600.4 MHz under the control of TopSpin (version 2.1; Bruker, Rheinstetten, Germany). 1H NMR spectra were acquired using a standard nuclear Overhauser effect spectroscopy presaturation pulse sequence for solvent suppression with time-shared double presaturation of the water and acetonitrile frequencies. In these experiments, 128 transients were acquired into 48K data points over a spectral width of 12,019 Hz (20 ppm) with an interscan delay of 3 s, giving a pulse repetition time of 5 s. Fractions observed to contain drug-related components were subsequently reacquired with 1200 transients to improve the signal/noise ratio. Routinely, the optimum receiver gain is determined solely by residual solvent signals because of the small amounts of material present in the isolated fractions; therefore, to improve intersample reproducibility an identical receiver gain was used for all data acquisitions. Before Fourier transformation, an exponential line-broadening function of 0.3 Hz was applied to each spectrum to improve the signal/noise ratio. Appropriate peaks were quantified using the proton integration feature of TopSpin 2.1 software.

Results

Safety and Tolerability of the Entero-Test in Dogs. Before this work, initial investigations were conducted to elucidate the safety and tolerability of the Entero-Test device in beagle dogs. During these investigations, which involved administering the device to 20 dogs on more than 50 occasions, no adverse clinical signs were noted. It was also determined that the 90-cm (pediatric) version of the device was more successful in capturing bile than the 140-cm (adult) version, which would often be contaminated by what appeared to be fecal material at the distal end. It was noted that the device needed to be in place for at least 2 h to ensure that it had reached the duodenum; removal of the device before this time often led to incomplete capsule digestion and/or nondetachment of the steel weight. On two occasions, the whole string was ingested owing to poor attachment of the string to the face using adhesive tape; however, in both instances these were retrieved whole in the feces within 24 h of swallowing. In addition, fecal samples collected from dogs for up to 24 h after they had swallowed the device were subject to X-ray to confirm passage of the 1-g steel weight. The dogs were held in slings after Entero-Test swallowing, primarily so that a single animal technician could readily watch over six dogs on a study at any one time. Subsequent studies have also used unrestrained dogs housed in steel metabolism cages to facilitate the collection of urine and feces (as well as bile) as part of a mass balance study using radiolabeled drug without issue. In later studies, it was also established that the levels of bile-associated, drug-related material could be increased by fitting the dogs with two Entero-Test devices simultaneously.

Identification of Metabolites. UPLC-MS data obtained after dosing by either route were qualitatively similar. A total of 31 metabolites were detected comprising multiple oxidations, hydrations, and glucuronide conjugations of SV or SVA. Only the major metabolites will be discussed in this article because the objective of our investigations was to focus primarily on those components for which NMR data were obtainable, thereby allowing comparison of definitive structures with metabolites previously described in the literature.

Unequivocal structural assignments were made for SV, for SVA, and for three metabolites, namely, 3′,5′β,6′-dihydrotriol-SVA (M1), 6′-hydroxy-SVA (M2), and 3′-hydroxy-SVA (M3) (Fig. 1), all of which have been identified previously as notable metabolites in dog bile (Vickers et al., 1990a,b; Subramanian et al., 2002). An O-glucuronidated SVA metabolite (M4) was also identified, but

![Fig. 1. Structures of SV, SVA, and major metabolites identified in dog bile captured using the Entero-Test.](image-url)
the $^1$H NMR chemical shifts indicated an ether glucuronide (chemical shift range typically 4.0–4.8 ppm) rather than the acyl glucuronide (typically 5.5–6.0 ppm) reported previously (Prueksaritanont et al., 2002). The anomeric proton of SVA $O$-glucuronide (M4) exhibited an $^1$H chemical shift of 4.50 ppm, which appears as a doublet with a scalar coupling constant of 7.9 Hz, consistent with the $\beta$-anomer (Fig. 2). Total correlation NMR spectroscopy showed couplings to other glucuronide resonances at 3.42 and 3.17 ppm. Slight downfield shifting of proton 3 supported glucuronidation at this position; however, insufficient material for nuclear Overhauser enhancement experiments meant glucuronidation at the 5-position could not be totally discounted. Nonetheless, the data were consistent with an ether $O$-glucuronide conjugate and not an acyl $O$-glucuronide.

Fig. 2. Comparison of $^1$H NMR resonances to assign glucuronide regiochemistry in M4 and M5.
In addition, limited NMR data were obtained on another glucuronidated metabolite (M5) exhibiting a deprotonated molecular ion at 607.2764 Da, which is consistent with glucuronidated hydroxydehydrogenated SV (theoretical mass 607.2755 Da). Relative to SVA, the proton chemical shifts of the dimethylbutyric acid side chain, 2'- and 6'-methyls, and 3'-, 4'-, and 5'-protons were barely altered, indicating no metabolic changes to the decalin ring nor to the dimethylbutyric acid side chain. No other 1H NMR signals were identified and a Markush structure for M5 is shown in Fig. 2. It was noted that to date, this structure had not been reported in the literature.

**Quantitative Evaluation of Major Metabolites.** Relative concentrations of SV, SVA, and the major metabolites in the pooled bile sample were estimated from the 1H NMR data by integration of a common proton using methodology similar to that described by Dear et al. (2008). Absolute levels of observed drug-related material in bile were estimated by comparison of the 1H NMR integrals with a 1 mg/ml standard solution of SVA. The total amount of observed drug-related material in the pooled bile sample was estimated to be 30 μg, with individual components representing between 1 and 10 μg.

Subsequent UPLC-MS analyses of both pooled and individual samples were conducted. Comparison of MS responses for each metabolite in the pooled sample (where levels had been quantified by NMR) with those in individual bile samples allowed an assessment of relative levels of each metabolite to be made after oral and intravenous dosing. After oral dosing with SV, the major biliary metabolites included 3',5',6'-dihydrotriol-SVA (M1) and 3'-hydroxylated SVA (M3). After intravenous dosing with SVA, the major biliary metabolites included M1 and M3 together with SVA O-glucuronide (M4). In general, low levels of SV and SVA were observed after both oral and intravenous dosing.

The UPLC-UV chromatograms at 240 nm (Fig. 3) are an indicator of the relative levels of drug and metabolites in the samples. However, a notable difference was observed in the UV response for M1 because of the loss of the diene chromophore.

**Discussion**

This work describes the first time use of the Entero-Test to harvest bile from dogs with a view to structurally identifying drug-related material. Before the experimental work conducted with SV and SVA, pilot experiments were conducted, which confirmed that dogs would tolerate the device and that by optimizing the experimental procedure, consistent bile sampling was achievable. In addition, experiments were used to evaluate whether a food stimulus or a chemical stimulus was required to initiate gallbladder contraction to increase the likelihood of bile being collected onto the string. During fasting, bile is stored in the gallbladder until the ingestion of food causes the release of cholecystokinin (CCK) from endocrine cells into the circulation, which subsequently induces gallbladder contraction (Bridger et al., 2008). More successful sampling occasions (i.e., bile-soaked string retrievals) were generally achieved when the dogs were given a small food morsel to eat (i.e., a single dog treat) in preference to allowing them to simply smell or lick the same food source, with the optimal time of applying the food stimulus being 30 to 45 min before string withdrawal. Other studies have successfully used a chemical stimulus with the CCK analog ceruletide to induce gallbladder contraction in both dogs and human subjects (Krishnamurthy et al., 1985; Bridger et al., 2008). The utility of administering ceruletide to dogs was evaluated during our pilot investigations, with some dogs receiving an intramuscular injection of ceruletide (aqueous) at a dose level of 0.3 μg/kg b.wt. instead of a food stimulus. Replacing the food stimulus with ceruletide administration produced a satisfactory bile sample in terms of volume on every occasion (typically producing 30 cm of bile-soaked string, equivalent to approximately 500 μl of bile), whereas on a few occasions use of the food stimulus resulted in poor sample collection if the dog exhibited signs of nervousness or when the food pellet was refused. A food morsel was used in preference to the chemical stimulus in the study described here, because we were developing the technique to be as noninvasive as possible from an...
animal welfare perspective. Nonetheless, should the chemical stimulant be used in future studies, this result is unlikely to have any unwanted effects on the ADME or pharmacokinetic properties of the drug under investigation, because of the extremely low dose of ceruletide, which only differs in structure by a single amino acid from CCK that is released naturally in response to eating (Krishnamurthy et al., 1985). Another potential benefit of a chemical stimulus is that there could be no food contamination of the string, which may cause interference with spectrometric analysis of drug-related material.

A primary concern surrounding the use of the Entero-Test was the possibility that the device may become contaminated by the oral dose, which could lead to difficulty in differentiating unab sorbed drug-related material from that which had been absorbed and then secreted into the bile, together with any dose that may have undergone metabolism in the gut. Low levels of SV were observed after oral dosing (Fig. 3), providing evidence that the string had not been excessively contaminated with the oral dose. It was estimated, based on fasted dog stomach emptying times (Karali 1995), that the device should not be swallowed by the dogs for at least 2 h after oral compound administration to minimize the risk of contamination.

The metabolism of SVA in preclinical species is well documented. In a study with bile duct-cannulated dogs, within 24 h after intravenous administration of [14C]SVA dosed at 1.2 mg/kg, approximately 65% of total radioactivity was detected in the bile, with unchanged parent drug accounting for less than 5% of the total recovered, suggesting that SVA underwent extensive metabolism. These studies also reported the presence of an acyl glucuronide of SVA (approximately 20% of the dose) and its lactone, SV (Prueksaritanont et al., 2002). Another study reported biliary secretion to be a major route of elimination after oral administration of [14C]SVA (60 mg/kg) to dogs, with several metabolites secreted in both lactone and hydroxy acid forms, of which 3′- and 6′-hydroxylated metabolites of SV were the major biliary components. In addition, the study reported that the 6′-isomer readily undergoes rearrangement to the 3′-isomer under acidic conditions and that SV, a lactone prodrug, undergoes spontaneous, reversible metabolism to its hydroxy acid form in vivo (Vickers 1990b). A further study reported 3′,5′,6′,6′-dihydrotriol-SVA in dog bile after intravenous administration of [14C]SVA (Subramanian et al., 2002).

Each of these reported metabolites was captured using the Entero-Test at sufficient levels to produce interpretable 1H NMR spectra. However, our 1H NMR data support the presence of an ether O-glucuronide (M4) in preference to an acyl glucuronide, contradicting reported data. Prueksaritanont et al. (2002) described the 1H NMR of an acyl glucuronide obtained using a metabolite isolated from dog liver microsomes, which was subsequently compared using chromatographic retention time and MS data with the O-glucuronide metabolite observed in dog bile. Therefore, it is feasible that these two metabolites gave comparable chromatographic and MS data but, in fact, were different and would only have been distinguishable had NMR been performed on the latter bile sample. It should be noted that the detection of the O-glucuronide in bile after oral administration of SV represents the first time that glucuronidation of SV has been confirmed via this dosing route.

In addition, 26 components were detected by UPLC-MS only; however, insufficient data were obtained to enable unequivocal structural identification. These metabolites were formed via multiple oxidations, hydrations, and glucuronide conjugations.

A methyl ester of M3 was also detected by UPLC-MS and NMR; however, it was concluded that this was an artifact and was probably formed during the processing of the strings. It is therefore recommended that methanol be replaced with acetonitrile during the extraction procedure.

In conclusion, the results from this work demonstrate the utility of the Entero-Test for the assessment of compounds that undergo hepatic metabolism and/or biliary secretion in the dog. Using the device to establish, for example, that glucuronidation occurs, when this route cannot be detected in plasma or excreta, demonstrates that the Entero-Test may occasionally offer a viable alternative to bile duct cannulation. If radio-labeled drug is dosed, metabolite standards are available for UPLC-MS quantification or alternatively, sufficient bile volumes are collected that facilitate NMR quantification (as described here), then it may be feasible to provide a quantitative “snapshot” of the levels of metabolites present in bile. However, the Entero-Test does not facilitate quantitative evaluation of the percentage of the dose eliminated via the bile. Consequently, this limits the interpretation of the biliary data compared with that typically generated using radio-labeled drug administered to bile duct-cannulated animals. It is also anticipated that the device may not be suitable or indeed successful in providing biliary data for all compounds under investigation, for example, because of inappropriate ADME or pharmacokinetic properties of the compound or the use of very low doses that may preclude the characterization of metabolites notwithstanding, given the simplicity and noninvasive nature of the technique, we believe the device is worthy of investigation ahead of using surgical techniques, particularly when quantitative information is not essential during the early drug development cycle. In addition, from an ethical standpoint and within the spirit of the 3Rs in animal experimentation (replacement, refinement, and reduction) we believe that the technique may, on occasion, provide sufficient metabolism data to negate further investigations using surgically prepared dogs. Finally, given the encouraging results achieved in dogs and the fact that the Entero-Test is routinely used in the clinic, we have now initiated studies to explore the utility of the device in capturing human biliary information for drug candidates.

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References


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