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Original article

An evaluation of the non-invasive faecal pellet assessment method as an early drug discovery screen for gastrointestinal liability

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ABSTRACT

Introduction: Gastrointestinal adverse effects contribute significantly to drug attrition as well as reduced patient compliance. Determination of gastrointestinal liability early in a compound's preclinical development would be a valuable tool. We evaluated the non-invasive faecal pellet method in the rat, assessed the feasibility of adding the endpoint to other study types and investigated correlation with the charcoal meal method. **Methods:** Han Wistar rats, pair housed in metabolism cages, received a single dose of vehicle, atropine, bethanechol, loperamide or metoclopramide. The number, weight and appearance of pellets produced were assessed over 10 h and at 24 h post-dose. The endpoint was also added to a modified Irwin screen (testing atropine, theophylline, clonidine, amphetamine, baclofen or quinine) and a whole body plethysmography study (testing theophylline or bethanechol). Pellets were collected from home cages out to 4 h post-dose (Irwin) or following a 45 minutes plethysmography session. To assess correlation with stomach emptying and intestinal transit charcoal meal data was generated where published data was not available. **Results:** Atropine decreased, while bethanechol and metoclopramide increased the number and weight of faecal pellets produced. Atropine produced darker, harder pellets and bethanechol lighter, softer pellets. Loperamide reduced pellet production at later time points only. Theophylline increased (Irwin and plethysmography) and atropine (Irwin) decreased pellet number and weight. Effects were maximal at the T_{max} and detected in all study environments. Primary data generation was not affected by pellet collection. Pellet findings were generally comparable to charcoal meal transit data, with compounds showing an inhibition (atropine, loperamide, amphetamine, baclofen, clonidine, quinine) or stimulation (bethanechol) in both models. **Discussion:** We have demonstrated that the faecal pellet method can detect expected reference compound induced changes in pellet transit. The technique is a useful non-invasive 'add-on' to other study types allowing gastrointestinal effects to be flagged earlier in preclinical development.

1. Introduction

Gastrointestinal (GI) adverse events (AEs; undesirable secondary events [or effects] occurring in addition to the desired therapeutic effects) contribute significantly to the high incidence of drug attrition and reduced patient compliance (Keating et al., 2010; Leong & Chan, 2006; Lewis, 1986; Valentin & Hammond, 2008). Furthermore GI AEs have been reported to account for almost 20% of total adverse drug reactions (ADRs; harmful, unintended reactions occurring at therapeutic doses) and around 20 to 40% of ADRs in hospitalised patients (Lewis, 1986).

The presence of GI AEs is not restricted to a particular drug class; antipsychotics, cytotoxics, antidepressants, antibacterials and anti-inflammatories have all been shown to result in GI AEs in the clinic (Chassany, Michaux, & Bergmann, 2000) and it has previously been reported that there are over 700 drugs implicated causing diarrhoea (Chassany et al., 2000). The majority of GI AEs observed in the clinic are functional in nature and include altered GI transit, which can manifest itself as diarrhoea, and/or constipation (Ghahremani, 1999; Gore, Levine, & Gharemani, 1999; Lat, Foster, & Erstad, 2010). Indeed diarrhoea has been reported to account for around 7% of all drug adverse events (Chassany et al., 2000). The presence of recurring GI AEs and ADRs can greatly reduce the quality of life for patients such that compliance may be reduced and already fragile physical and mental states may be compromised further.

In addition to the detrimental impact GI AEs and ADRs can have in patients, altered GI function can also result in dose-limiting toxicity and cause altered pharmacokinetic profiles in preclinical studies.

Abbreviations: ADRs, adverse drug reactions; AEs, adverse effects; BPM, breaths per minute; DRF, dose range finding; GI, gastrointestinal; ICH, International Conference on Harmonisation; MTD, maximum tolerated dose; MV, minute volume; NSAID, non-steroidal anti-inflammatory drugs; RR, respiratory rate; SC, subcutaneous; TV, tidal volume; WBP, whole body plethysmography.

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The ability of a candidate drug compound to induce marked GI effects can be assessed pre-clinically and in general there would appear to be a good correlation between GI data generated in animals and GI-related AEs in humans (Fletcher, 1978; Greaves, Willliams, & Eve, 2004; Olson et al., 2000; Valentin, Keisu, & Hammond, 2009). While larger species such as the dog may be more physiologically similar to that of the human (Greaves et al., 2004), the rat is one of the preferred rodent species in the GI safety evaluation of candidate compounds and is accepted by regulatory authorities as suitable for pharmacodynamic studies (Anon, 2001 [ICH S7A]).

In Safety Pharmacology studies, the charcoal meal method (Harrison, Erlwanger, Elbrond, Andersen, & Unmack, 2004) is commonly used to examine the effects of candidate compounds on GI function in rodents (gastric emptying and small intestinal transit) and it may help to predict the prevalence of GI effects experienced in the clinic (Redfern et al., 2010). Although this is a simple method, it is a terminal procedure, which can require a period of fasting on the morning of dosing (Prior, Ewart, Bright, & Valentin, 2012). Due to the terminal nature of the method a stand-alone study is normally required and as a result the endpoint cannot be easily added to other study types. The compound usage and resource associated with the charcoal meal method may also be limiting for use in early drug discovery.

Rather than initiating complex standalone studies early in drug discovery, a basic non-invasive method that could be incorporated into existing discovery study types would be an attractive option for project teams hoping to optimise information on drug candidates without over stretching resource and delaying progression. The use of a non-invasive method for the determination of compound-induced GI effects may be of value for incorporation into maximum tolerated dose (MTD)/dose range finding (DRF) toxicity studies where it could be used to flag marked GI effects earlier in a compound's development. The incorporation of a GI endpoint into an MTD/DRF study would optimise the data generated from a single set of animals while the availability of a non-invasive method may also promote the refinement of other toxicity or Safety Pharmacology study types; both 3R considerations.

A number of published rodent studies have demonstrated that an assessment of faecal pellet number, weight and appearance can be used to determine compound-induced effects on GI function (Bass, Kennedy, & Wiley, 1972; Charoenthongtrakul et al., 2009; Enck & Holtman, 1992; Raehal, Walker, & Bohn, 2005). These studies have assessed reference compound induced changes in the number and weight of faecal pellets produced in the mouse and rat and marked changes in pellet transit have been detected. Unlike the charcoal meal method, the faecal pellet method does not allow an assessment of upper versus lower GI function but could still be used as an initial flag for a GI effect.

We set out to perform an in-house evaluation of the faecal pellet method to determine whether it was sensitive enough to detect reference compound-induced effects on pellet transit in Han Wistar rats. A number of reference compounds were chosen which would be expected to result in the stimulation or inhibition of GI function. In order to determine how well the pellet data correlated with the routinely used (gold standard) preclinical charcoal meal model pellet data was compared to charcoal meal data for the reference compounds assessed. In addition the optimal experimental setting (or study type) for the faecal pellet assessment was investigated including the addition of the endpoint to whole body plethysmography (WBP) and modified Irwin study types.

2. Methods

All protocols and experiments were performed under the authority of a valid Home Office Project Licence and conformed to UK Governmental regulations regarding laboratory animal use and care (National Archives, UK Animals (Scientific Procedures) Act, 1986).

232 Han Wistar rats (207 males and 25 females) (285 to 342 g; Harlan, Bicester, UK Ltd. or Charles River, UK) were housed in same sex groups of 3 or 4 with aspen chip bedding, sizzle nest (Datesand Ltd., Manchester, UK) and fun tunnels. From arrival and throughout the study period each rat was offered Rat and Mouse 1 (E) SGC food Diet 2041 (Special Diets Services, Essex, UK) and drinking water ad libitum. Chew sticks were also provided (Tapvei, Estonia). Room temperature and relative humidity were maintained at 16 °C to 23 °C and 40% to 70%, respectively. The animal room was illuminated by artificial light from fluorescent tubes on a 12 hour light/dark cycle. On arrival, animals were allowed to acclimatise for a minimum of 4 days prior to the first day of dosing.

2.1. Faecal pellet assessment phase

Animals were dosed orally (by gavage) with vehicle, 0.5, 1 or 5 mg/kg atropine sulphate, 1 or 10 mg/kg loperamide, 5 or 15 mg/kg metoclopramide, or subcutaneously (sc) with vehicle, 0.5 or 2 mg/kg bethanechol (all Sigma-Aldrich, Gillingham, UK). Each vehicle and treatment group consisted of 6–8 animals.

To facilitate the removal of pellets and to prevent coprophagia, rats were placed in pairs into grid floor metabolism cages (type 2154, Tecniplast UK). The removable trays below the cages were lined with absorbent paper, to soak up urine and to further aid pellet collection. A measured weight of food was provided in each cage to allow food consumption (in grams [g]) to be monitored and the number of pellets produced per g of food consumed to be calculated. Two chew sticks were provided per cage; all other environmental enrichment was removed. Clinical observations were recorded.

The number and wet weight of pellets produced in each cage by each pair of animals were recorded at 30 min, and then at 1 hourly intervals out to 10 h and at 24 h following administration of vehicle, atropine or bethanechol or at 30 min, and then at 1 hourly intervals out to 4 h and at 24 h following administration of loperamide or metoclopramide. Animals were removed from the metabolism cages after a maximum of 10 h, and placed back into their home cages. They were then placed into the metabolism cages for a further hour the following morning (23 to 24 h post-dose) to allow a measurement of any recovery at 24 h post-dose. The pellets collected were 'scored' according to their shape and appearance using a scoring system adapted (for rats) from published paper containing faecal scales (Pedersen & Toft, 2011) (Table 1).

As the pellet collection points were close together, the drying out of pellets, which may affect the ability to class appearance, was not considered to be likely to be an issue.

Total pellet number and weight over the collection period were calculated for the animals in each group and these totals are presented in addition to the number and weight of pellets present at the estimated T_{max} of the test compounds.

Table 1
Rat faecal pellet scoring classification. A normal pellet was classed as '3C'. (adapted from Pedersen & Toft, 2011).

Number	Shape
1	Loose, watery, runny
2	Mushy, soft, flattened surface
3	Smooth cylindrical
4	Cylindrical but with cracks or dry looking
Letter	**Colour**
A	Yellow/brown
B	Pale brown
C	Medium brown
D	Dark brown/black