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Original article

Effects of fasting on evaluation of gastrointestinal transit with charcoal meal

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Abstract

Introduction: At the present time, most studies investigating gastrointestinal transit time with charcoal are conducted in fasted rats. It seems reasonable to hypothesize that the fasting state of rats could influence the effect a compound had on gastrointestinal transit time. The purpose of this study was to investigate the effects of food on the pharmacological effects on gastrointestinal transit. **Methods:** For each drug investigated, two sets of 32 male Sprague–Dawley rats were used. One set was studied after being fasted for approximately 6 h, the second set was studied after free access to food. Each set had 4 groups of animals ($n=8$/group) that were administered different doses, allowing the assessment of the drug effect after fasting and after free access to food. Animals were administered 0, 10, 25, and 75 mg/kg of morphine; 0, 10, 20, and 40 mg/kg loperamide, or 0, 0.05, 0.5, and 3.0 mg/kg clonidine. At predetermined times, an activated charcoal suspension was administered by oral gavage. Thirty minutes after receiving the charcoal meal, rats were euthanized and the small intestine was removed. The length of the small intestine and the distance traveled by the charcoal were recorded. For each animal, gastrointestinal transit was calculated as the percentage of the distance traveled relative to the total length of the small intestine. **Results:** Baseline (vehicle dosed animals) gastrointestinal transit was significantly greater in fasted versus fed rats. In fasted rats, morphine did not have a significant effect on transit. In fed rats, 25 and 75 mg/kg morphine caused a significant decrease in transit. In fed and fasted rats, 0.5 and 3 mg/kg clonidine caused a significant decrease in transit. Loperamide did not affect gastrointestinal transit in fed or fasted rats at doses up to 40 mg/kg. **Discussion:** These data demonstrate that food does not reduce the sensitivity of the gastrointestinal transit time.

Keywords: Clonidine; Gastrointestinal transit; Loperamide; Morphine; Methods; Rats

1. Introduction

Historically, the effects of drugs on gastrointestinal function have commonly been investigated by safety pharmacologists (Erlwanger, Elbrond, Anderson, & Unmack, 2004; Mortin, Horvath, & Wyand, 1997). Gastrointestinal transit time is probably the most common assay utilized and has most often been investigated by dosing the rodents with an aqueous suspension of charcoal. The compound is administered to the animal and the distance traveled by the charcoal is measured at a predetermined time. The distance traveled by the charcoal is a measurement of gastrointestinal motility.

These studies are most commonly conducted in animals that have been fasted for 18–24 h (Marona & Lucchesi, 2004). Recent studies have demonstrated that the fasting period can be reduced (Marona & Lucchesi, 2004; Vermeulen, De Vries, Schlingmann, & Remie, 1997). However, to our knowledge, no studies have investigated the potential to eliminate fasting from gastrointestinal studies.

It seems reasonable to hypothesize that the fasting state of rats could influence the effect a compound has on gastrointestinal transit time. This is due to the different baseline gastrointestinal motility found during fed and fasted conditions (Kihara et al., 2001; Ruckebusch & Fioramonti, 1975; Weisbrodt, 1981; Zenilman, Parodi, & Becker, 1992). In the fasted state, cyclic changes of gastrointestinal pressure waves are detected in the duode-

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num, including periods of relatively low amplitude contractions (Phase I) followed by a grouping of strong contractions (Phase-III). After food intake, irregular contractions of high frequency with no clear Phase III contractions are observed.

A recent study (Baldrick, Bamford, & Tattersall, 1998) did investigate the effects of a number of compounds (diazepam, metoclopramide, bethanechol, domperidone, CCK-8) known to affect gastrointestinal motility. Metoclopramide, bethanechol, and domperidone did not affect gastrointestinal transit time, leading the authors to conclude that the results were not in agreement with previous literature. The authors hypothesized that one potential reason for the equivocal results was the fact that the mice were not fasted. However, no studies have actually looked at the effect of fed versus fasted state on pharmacological activity in the gastrointestinal transit assay. The purpose of the present study was to investigate the effects of fasting on gastrointestinal transit time and to assess whether it is appropriate to conduct general screening studies for gastrointestinal effects under fed conditions. The advantages of being able to conduct these studies under fed conditions would be the elimination of the additional step of food removal and it would reduce potential stress on the animals caused by fasting (Marona & Lucchesi, 2004; Vermeulen et al., 1997).

2. Methods

2.1. Approvals

The study was approved by the Institutional Animal Care and Use Committee and conformed with the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institute of Health (NIH Publication No. 85-23, revised 1996).

2.2. Drugs

Morphine sulfate solution (15 mg/ml) was ordered from Butler Co. and further diluted in deionized water. Loperamide hydrochloride and clonidine hydrochloride were all purchased from Sigma Chemical Co. Clonidine was dissolved in deionized water and loperamide was dissolved in 10% ethanol. The stock drug concentrations prepared for loperamide and clonidine were 40 mg/ml and 50 mg/ml, respectively. All formulations were prepared within 4 days of dosing and stored refrigerated and in an amber jar.

2.3. Experimental protocol

Male Sprague–Dawley rats were obtained form Charles River (Kingston, NY). The rats weighed between 200 and 250 g at the time of dosing. For each drug investigated, two sets of 32 rats were used. One set was studied after being fasted for approximately 6 h, the second set was studied after free access to food. A 6 h fast was chosen because research has shown that this is an adequate time period to assure that the stomach will be empty (Vermeulen et al., 1997). Each set had 4 groups of animals ($n=8$/group) that were administered different doses, allowing the dose response effect of each drug to be assessed after fasting and after free access to food.

One set of animals was administered 0, 10, 25, and 75 mg/kg of morphine sulfate by oral gavage followed by administration of an activated charcoal suspension 2 h later. The second set of animals was administered 0, 10, 20, and 40 mg/kg of loperamide by oral gavage followed by administration of an activated charcoal suspension 30 min later. The third set of animals was administered 0, 0.05, 0.5, and 3 mg/kg of clonidine by oral gavage followed by administration of an activated charcoal suspension 90 min later. The amount of activated charcoal administered was 2 ml which is a common volume for this assay (Manara, Bianchi, Ferretti, & Tavani, 1986; Megens, Canters, Awouters, & Niemegeers, 1989). Thirty minutes after receiving the charcoal meal, rats were euthanized and the small intestine was removed. The length of the small intestine was measured by laying out the small intestine on a measuring tape and measuring the distance from the pyloric sphincter to the ileo-cecal valve. The distance traveled by the charcoal was measured from the pyloric sphincter to the most caudal edge of the charcoal.

2.4. Statistical analysis

Values for gastrointestinal transit time are expressed as a percent total intestinal length and were calculated in the following manner: The individual distance traveled by the charcoal in centimeters divided by the total length of the intestines in centimeters (pylorus to cecum) was evaluated for each rat. Mean values were calculated for each group and statistical comparisons were made between treatment groups using parametric statistics (one-way analysis of variance, Dunnett's t test). Statistical significance was considered to be $p<0.05$.

3. Results

3.1. Morphine

Gastrointestinal transit, expressed as percent total distance, was significantly greater in fasted (52.4 ± 4.2) versus fed (37.9 ± 1.7) rats dosed with vehicle (Fig. 1). In fasted rats, morphine did not have a significant effect on transit. In fed rats, 25 and 75 mg/kg morphine caused a significant decrease in transit. The percent total distance after administration of 25 and 75 mg/kg was 27.9 ± 1.5 and 24.3 ± 1.5, respectively.