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Small Intestinal Transit Does Not Adequately Represent Postoperative Paralytic Ileus in Rats

Hyun-Tai Lee, Suk-Jae Chung, and Chang-Koo Shim
Department of Pharmaceutics, College of Pharmacy, Seoul National University, Seoul 151-742, Korea

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Small intestinal transit (SIT) has often been regarded as an index of pathophysiological state of postoperative ileus (PI) in rats. The reliability of SIT as an index of PI was examined in the present study. PI was induced via abdominal surgery (i.e., laparotomy with evisceration and manipulation) in rats. For one group of PI-induced rats, SIT of a charcoal test meal was measured. When necessary, the physical state (i.e., severity and site of distension) of the gastrointestinal (GI) tract in each rat was visually examined. For another group of PI-induced rats, abdominal X-ray radiographs were obtained after introducing the barium sulfate suspension. The abdomen was then opened and the physical state of the GI tract was visually examined. The SIT was decreased in most of the PI-induced rats, and the GI distension was observed, with substantial intersubject variations, in all of the PI-induced rats. However, no linear relationship was evident between the SIT and the severity of GI distension (e.g., at 20 h after PI induction). Instead, the severity and site of GI distension could be monitored by the X-ray radiology. Therefore, the use of SIT as an index of PI should be substantially limited.

Key words: Postoperative ileus, Small intestinal transit, X-ray, Distension

INTRODUCTION

Ileus is defined as the functional inhibition of propulsive bowel activity, irrespective of pathogenetic mechanisms, including a dilation of the gastrointestinal (GI) tract and the accumulation of ingesta, secretions, and gas within the GI lumen. Ileus is known to occur after all laparotomy and intra-abdominal operative procedures, and such a condition is generally referred to as postoperative ileus (PI) (Livingston and Passaro, 1990). Some of the remarkable characteristics of clinical PI include the absence of passage of flatus and stool, diminished bowel sounds, and abdominal distension (Benson *et al.*, 1994). PI is further classified into non-paralytic and postoperative paralytic ileus (PPI), based on the duration of the ileus. Non-paralytic ileus is an uncomplicated ileus that is resolved spontaneously within 2-3 days, while PPI indicates a complicated ileus that is not spontaneously resolved, and persists for more than 3 days after abdominal surgery (Bungard and Kale-Pradhan, 1999). Non-paralytic ileus most likely results from the temporary inhibition of extrinsic motility regulation and is more severe in the colon, while PPI affects all segments of the bowel and probably results from further inhibition of local, intrinsic contractile systems (Livingston and Passaro, 1990).

The treatment of PI is particularly important because of its considerable contribution to the postoperative discomfort of patients, who require nasogastric suction or even relaparotomy in many cases. In addition, it influences the time point of hospital discharge, increasing the length of hospital stay and, thus, health costs (Livingston and Passaro, 1990). Despite major advances in our understanding and treatment of diseases in many areas of medicine, relatively few improvements have been made in the area of PI. As a result, no specific therapies for the treatment of PI are currently available, and thus, PI still remains an important clinical problem for many patients who have undergone abdominal surgery, which should be properly managed (Bungard and Kale-Pradhan, 1999).

In order to screen therapeutics that can resolve PI, the establishment of an appropriate animal model is crucial. In addition, reliable and efficient methods to estimate the efficacy of the therapeutics are also required. For the animal model, abdominal surgery of rats, which is followed by laparotomy with evisceration and manipulation,

Correspondence to: Chang-Koo Shim, Ph.D., Department of Pharmaceutics, College of Pharmacy, Seoul National University, San 56-1, Shinlim-dong, Kwanak-gu, Seoul 151-742, Korea
E-mail: shimck@plaza.snu.ac.kr

has been used as a method to induce PI. For the estimation of the efficacy of potential therapeutics, a number of experimental systems have been proposed. These have focused mainly on the examination of gastric emptying (Barquist *et al.*, 1992; Martínez *et al.*, 1999), gastric motility (Huge *et al.*, 1998; Zittel *et al.*, 2001b), small intestinal transit (SIT) (Holzer *et al.*, 1986; Pairet and Ruckebusch, 1989; De Winter, 1997; De Winter, 1999), small intestinal motility (Sagrada *et al.*, 1987; Rivière *et al.*, 1993), colonic transit (Zittel *et al.*, 1998), and colonic motility (Zittel *et al.*, 2001a) in rats. Many of these studies have focused on early alterations in motility that take place within hours. However PI in humans often lasts for several days or more, and is usually called PPI. The pathogenetic mechanisms of PPI appear to be different from those of simple PI that result in early motility changes only within hours after abdominal surgery (Kalff *et al.*, 2000).

In the present study, the issues of whether or not SIT varies as a function of time, and what information on PPI can be obtained from SIT were examined. Charcoal meal is commonly used as a test meal in the methods of measuring SIT (Holzer *et al.*, 1986; Pairet and Ruckebusch, 1989) and the transit rate of the test meal in the small intestine is utilized as an index of PI. However, the issue of whether or not the SIT in the test meal method adequately represents the degree of PI with the time elapse after abdominal surgery has not been equivocally proved. To accomplish this, either an abdominal X-ray radiology with introducing the barium sulfate suspension, or visual observation following the surgical opening of the abdomen was conducted.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (Animal Center for Pharmaceutical Research, Seoul National University, Seoul, Korea) weighing 260-320 g were used in all experiments. Rats were maintained under conditions of controlled temperature (22±2°C), humidity (55±5%), and illumination (light on 7 AM to 7 PM). The institutional guidelines for the care and use of laboratory animals were followed throughout the study, and an approval for the research protocol was obtained from the institutional committee of Seoul National University for animal research. Animals were deprived of food for 20 h before experimentation but were allowed free access to tap water.

Induction of PI

Rats were anesthetized by an intraperitoneal injection of ketamine hydrochloride (Ketalar®, 50 mg/kg, Yuhan Corporation, Seoul, Korea). Ileus was induced by laparotomy followed by evisceration and manipulation (palpation of the bowel) using slight modifications of reported methods (Holzer *et al.*, 1986; De Winter *et al.*, 1997). The abdomen was shaved, disinfected and the peritoneum was then opened. The cecum and small intestine were pulled out of the abdominal cavity and gently massaged by the fingers, starting from the cecum up to the duodenal end of the small intestine. The cecum and small intestine were palpated 3 times during the 10-min exposure and replaced in the abdominal cavity, and the midline incision was closed by running sutures. The whole procedure was performed within 20 min. After the operation, the rats were placed into individual cages and allowed to recover for specified time periods (e.g. 1, 5, 10, 20, and 30 h). Rats that had not been subjected to abdominal surgery served as the control group.

Assay of SIT and optical examination of the physical state of the GI tract

For one group of rats that had received abdominal surgery (PI induction), GI propulsion was assessed by measuring SIT following the oral administration of a charcoal meal preparation with minor modification of previously reported methods (Holzer *et al.*, 1986; Pairet and Ruckebusch, 1989). The charcoal meal was freshly prepared by mixing sodium carboxymethyl cellulose (Dong-A Pharm. Co., Ltd., Seoul, Korea) and carbon black (Shinyo Pure Chemicals Co., Ltd., Osaka, Japan) in distilled water to yield final concentrations of 1 and 10% (w/v), respectively. The suspension was stirred continuously and prewarmed to body temperature. The test meal (1 ml) was then administered intragastrically through an orogastric tube. After a period of 30 min, the rats were killed by cervical dislocation, and the transit front of the charcoal meal in the small intestine was marked. SIT was expressed as the percentage of the distance traveled by the visually detected marker divided by the total length of the small intestine. Simultaneously, the physical state of the GI tract was optically examined and was ranked arbitrarily for the severity of GI distension. In an attempt to quantitatively evaluate the distension, 0, 1, 2, and 3 points were scored for cases of no, mild, moderate, and severe distension, respectively. The observer was blinded to the treatment groups.

X-ray radiological observations

To another group of PI-induced rats, 5 ml of 24% (w/v) barium sulfate suspension (Solotop®, Tae Joon Pharm. Co., Ltd., Seoul, Korea) was introduced through an orogastric tube, and GI distension was examined at 30 min after this administration by radiological visualization of the abdomen. Abdominal radiographs were obtained on dental X-ray films (Agfa Dentus M2 Comfort, 5×7 cm, Heraeus Kulzer Inc., South Bend, IN) using an intra-oral X-ray system (Novelix ETX™ with a CCX digital timer and a scissor arm, Trophy Radiologie, Paris, France). GI distension could be detected visually by observing the trace of barium sulfate suspension on the abdominal radiograph.

In order to compare the X-ray radiographs between the PI-induced rats and patients with PPI, an abdominal X-ray radiograph obtained from a patient (Shim, one of the authors in the present report), who was suffering from PPI after abdominal surgery to remove a colon tumor, was utilized. The X-ray radiograph was obtained at the Seoul National University Hospital during the hospitalization period of the patient, using a standard method which does not need contrast media.

Statistics

Values were expressed as the mean±SD of repeated experiments. Statistical analysis of the results was carried out using a one-way analysis of variance (ANOVA) followed by unpaired Student's *t* test. Probability values <0.05 were regarded as statistically significant.

RESULTS

Effect of food-deprivation and ketamine anesthesia on SIT

Effect of food-deprivation on the SIT of the charcoal meal in rats was first examined for the control rats (i.e., rats that had received no abdominal surgery). No differences in the SIT for a 20 (65.1±4.2%, n=11), 40 (63.1±4.4%, n=10), and 50 h (60.9±5.3%, n=9) deprivation were observed. Thus, food-deprivation for a 20-h period was adopted in the subsequent studies. The effect of ketamine anesthesia (50 mg/kg i.p. as ketamine hydrochloride) on the SIT was also examined in the control rats after 20 h of food-deprivation. No differences between the conscious rats (65.1±4.2%, n=11) and the ketamine-anesthetized rats (64.2±3.4%, n=13) were observed either. Thus, all of the subsequent measurements of SIT were conducted under these conditions (i.e., food-deprivation for 20 h and anesthesia, using a ketamine hydrochloride dose of 50 mg/kg).

SIT as a function of time after PI induction

The SIT of the charcoal meal in the control rats following 20 h of food-deprivation and ketamine anesthesia was 64.2±3.4% (n=13, Fig. 1A). The SIT was substantially reduced in all groups of PI-induced rats. It was 28.1±5.8 (n=11), 15.3±9.7 (n=17), 12.9±13.1 (n=15), 16.4±19.6 (n=23), or 20.1±18.7% (n=18), when measured at 1, 5, 10, 20, and 30 h, respectively, after abdominal surgery (i.e., laparotomy with evisceration and manipulation). A substantial intersubject variation in the SIT was found among the rats, which increased as the time period after PI induction increased. For example, the SIT values at 1 h were between 19.8 and 37.6% (Fig. 1B), while those for 20 h were between 0 and 66.7% (Fig. 1E). However, the variation at 30 h (Fig. 1F) remained similar to that at 20 h, suggesting that nearly steady state is reached at 20 h after PI induction.

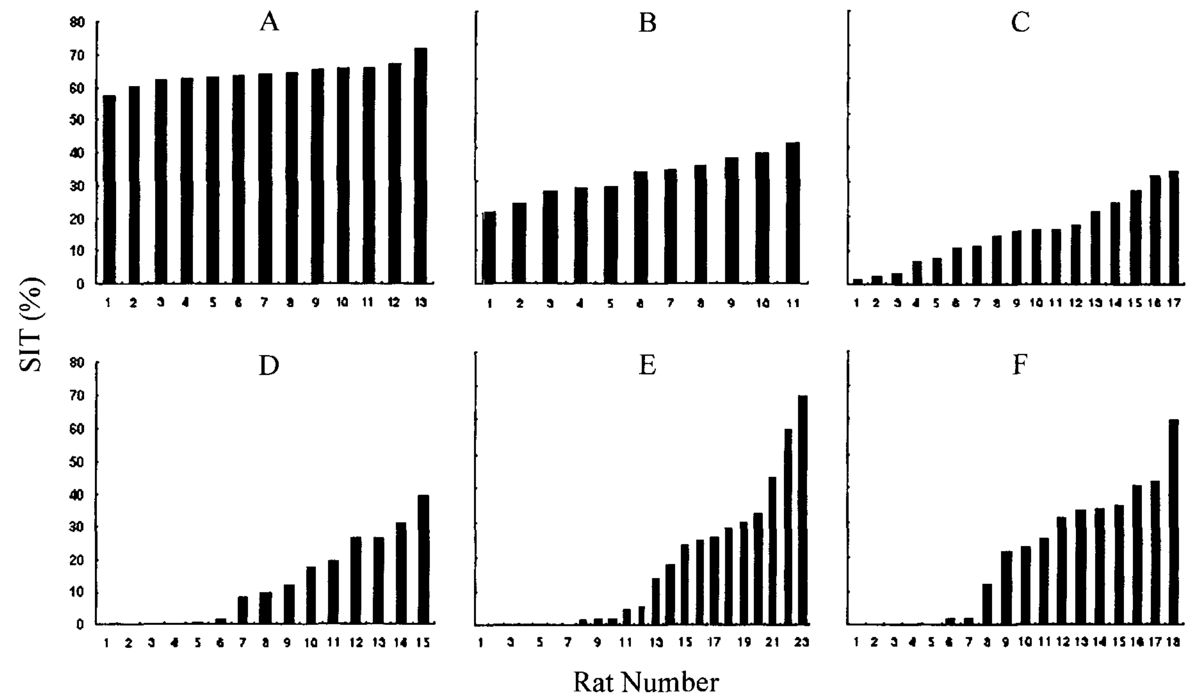


Fig. 1. The respective SITs in the control (A) and PI-induced rats at 1 (B), 5 (C), 10 (D), 20 (E), and 30 h period (F) after abdominal surgery. Data are arranged in a row, and bars show the SIT values for each rat.

Relation between SIT and optical observation

In order to elucidate whether the SIT represents the degree of distension, both the SIT and the severity of distension were measured in 36 individual rats (23 rats at 20 h after PI induction and 13 control rats). The SIT of the charcoal meal varied in the range of 0-66.7% for the PI-induced rats (16.4±19.6%, n=23) and 57.5-71.7% (64.2±3.4%, n=13) for the control rats. After measuring the SIT, the distension of the stomach (gastric distension) and small intestine (small intestinal distension) was examined for each of the rats after opening the abdomen. Distension was not observed in all of the control rats, but most of the PI-induced rats showed some distension either in the stomach or in the small intestine with considerable intersubject variation. Of the 23 PI-induced rats tested, 9, 9, 4, and 1 rats exhibited severe, moderate, mild, and no gastric distension, respectively, while 5, 8, 8, and 2 rats exhibited severe, moderate, mild, and no small intestinal distension, respectively.

Fig. 2 shows the plots between the SIT of the charcoal meal and the severity point of distension in the stomach or small intestine. If the SIT represents the severity of distension, an inverse proportional relationship between the SIT and the severity of distension would be expected. It is obvious from Fig. 2, however, that no such relationships were found, either for the stomach or the small intestine, indicating that no relationship is evident between the SIT and the abdominal distension or PI. The reason for this is that nearly normal SIT values were obtained when the distension was located in the lower part of the GI tract.

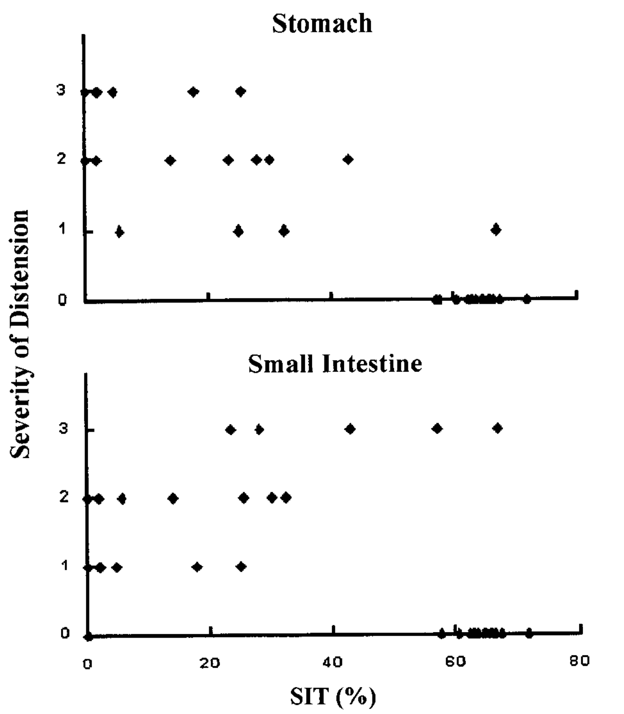
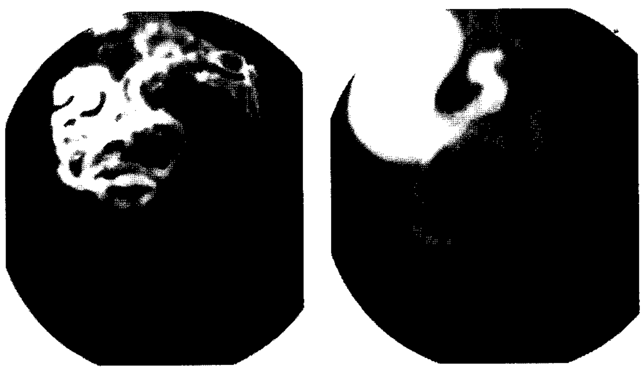


Fig. 2. Relationships between the SIT values and the severity of distension in the stomach (upper figure) and small intestine (lower figure) for the 23 PI-induced and 13 control rats. The severity was classified into normal, mild, moderate, and severe, and numerically scored as 0, 1, 2, and 3, respectively.

X-ray radiographs

In the preliminary studies, we couldn't find the image of GI tract (or abdominal distension) in all of the abdominal radiographs taken for the rats without introducing contrast media. Therefore, X-ray radiographs were taken for the control (i.e., no abdominal surgery, fasted for 20 h) and PI-induced rats (i.e., abdominal surgery, fasted for 20 h) at 30 min after introducing the barium sulfate suspension as contrast media. No distension of the GI tract was observed in the radiographs for all of the control rats (n=15), as exemplified in Fig. 3A, whereas some distended small bowel was observed for all of the rats 1 h after PI induction (n=15), as exemplified in Fig. 3B. The transition front of the barium sulfate suspension remained at the upper site of the GI tract in the PI-induced rats compared with the controls, indicating an interruption of SIT by PI induction, which is consistent with the decrease of SIT in the PI-induced rats (Figs. 1A and 1B).

The homogeneous pattern of the GI distension was observed among the abdominal radiographs obtained from the rats 1 h after PI induction (data not shown). The variation in the severity and site of distension among rats, however, increased substantially at 20 h after abdominal surgery (n=20), as exemplified for 3 rats in Fig. 4. Significant intersubject variation in the radiographs appears to be consistent with the increased intersubject variation in the SIT with the time elapse (Fig. 1). The radiological study in all of the rats, regarding the severity and site of distension of the GI tract, was in good agreement with visual observations that performed under abdominal opening.



A B

Fig. 3. Examples of abdominal radiographs obtained from an untreated control (A) and a PI-induced rat (B, 1 h after abdominal surgery).

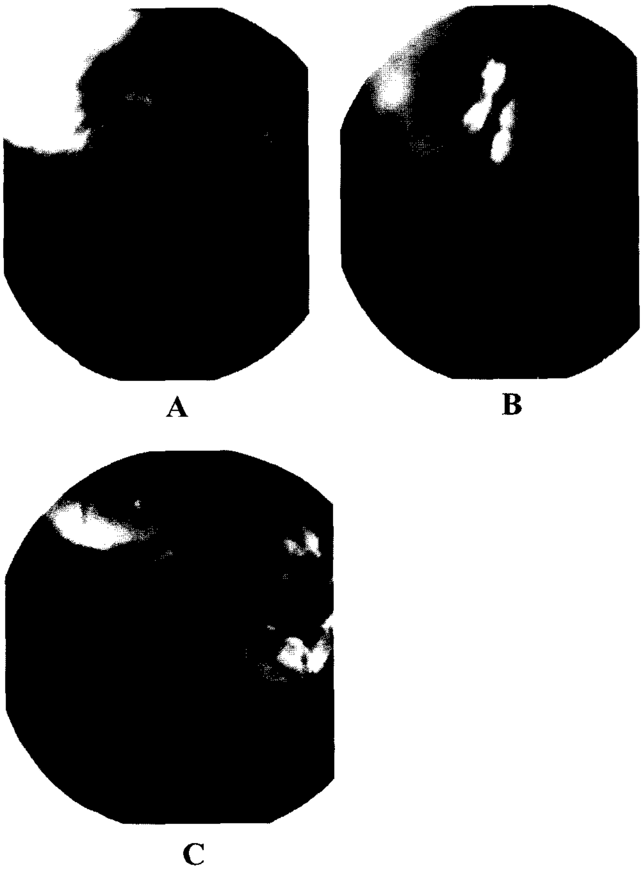


Fig. 4. Examples of abdominal radiographs obtained from the rats 20 h after PI induction showing gastric obstruction (A), partial distension of the small bowel (B), and considerable dilation in the duodenal and jejunal loops (C).

Fig. 5. An abdominal radiograph obtained from a patient with PPI 6 days after abdominal surgery. Arrows indicate the sites of the seriously dilated intestine caused by the accumulation of gas within the GI lumen.

The abdominal distension observed in radiographs taken for the rats (e.g., Fig. 4) was similar in the patterns to that for a patient with PPI (Fig. 5), suggesting that the animal model used in the present study (i.e., rats at 20 h after PI induction) properly reflects the clinical PPI.

DISCUSSION

SIT has often been regarded as an index of PI in the early postoperative stage. Smaller SIT seems to indicate a more severe degree of PI (Holzer *et al.*, 1986; Pairet and Ruckebusch, 1989; De Winter *et al.*, 1997; De Winter *et al.*, 1999). In the present study, we examined the issue of whether the SIT adequately represents the degree of PI regardless of the time elapse after abdominal surgery. In the control and PI-induced rats, the SIT was estimated by measuring the GI transit distance of the charcoal meal for a 30 min period, followed by a visual examination of the GI tract after abdominal opening. The degree of PI was estimated by the visual observation of the severity of abdominal distension. More reproducible SIT values were obtained as its measurement was performed at earlier stage after abdominal surgery (e.g., at 1 hr). Acceleration of SIT at this early stage by certain xenobiotics, however, may not necessarily represent an improvement or recovery of PPI by the compounds, because PPI appears to be distinct in mechanisms from simple PI at the early stage. The results, as are obvious in Fig. 2, strongly indicate that the SIT value may be associated with the position of distension in the GI tract, but does not adequately represent the severity of GI distension in rats. In the present study, we examined the SIT of the charcoal meal, but a similar conclusion is likely to be obtained for the Evans blue or other test meals. The severity of abdominal distension could be clearly assessed by the X-ray radiographic method. X-ray radiology does not need abdominal opening in the estimation of GI distension, but, instead, oral administration of contrast media (e.g., barium sulfate suspension) should be preceded before the radiology. Collectively, we conclude that the SIT, as determined by the charcoal meal method, provides only limited information (i.e., only on the site of GI distension) relative to PPI.

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