Colonic transit studies with ^{99m}Tc-rhenium sulfide colloid in live rats. A preliminary study

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Keywords: Colonic transit – ^{99m}Tc-colloid – IBS – Rat – GIT imaging

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Received:
17 April 2008
Accepted revised:
1 May 2008

Abstract

Abnormal colonic motility is associated with clinical relevant conditions such as irritable bowel syndrome or constipation. Accurate assessment of colonic transit in an animal model would be useful in studying these conditions and screen potential drug candidates. The aim of this study was to assess if scintigraphic analyses could reliably evaluate total and segmental colonic transit as a measure of colonic motility of a non-absorbable radiotracer in rats. Normal Lewis rats (250-300 g) were given oral technetium-99m-rhenium sulfide colloid (15-20 MBq; 0.5 mL; n=4) followed by a rinse with water for injection (1.0 mL). Rats were fed and hydrated *ad libidum*. After 30 min, each rat was contained inside an 'imaging' tube then placed on a g-camera collimator. Whole body 5 min static images were acquired every 30 min up to 9 h, and then finally at 25 h. Region of interest analyses were applied to the caecum/proximal colon, sigmoidal loop and distal colon/rectum. The tracer entered into the colon at ~4 h, and the rats remained static to permit 'live' imaging. At 4 h the % whole body activity was: 51% caecum/proximal colon, 39% sigmoidal loop, 6% distal colon/rectum; at 8 h, 30% caecum/proximal colon, 13% sigmoidal loop, 7% distal colon/rectum. In the whole colon there was ≤1% of total activity present at 25 h, and the half clearance time was determined as 4.0 h. These results suggest this is a reliable technique of measuring regional colonic transit as a measure of colonic motility in normal rats. This methodology might be well suited to screen potential motility effects of drug candidates.

Hell J Nucl Med 2008; 11(2): 96-100 • *Published on line: 1 June 2008*

Introduction

The large intestine has been a widely studied organ in terms of its anatomy and physiology. Each of the segments perform specific functions: the caecum for absorption of water and electrolytes, the mid-colonic segments for propulsion of the contents, and the rectum for accumulation prior to expulsion of faeces from the body. The motor functions of the colon are different to the small intestine, with the continuous mixing and propulsion of the intraluminal material over durations of 24 h of more. Colonic motility (or transit) is a complex yet coordinated function that appears to be dependent on neuroendocrine and complex neuromuscular mechanisms.

Disorders of colonic motility include intractable constipation and faecal incontinence. Intractable constipation conditions include abnormal colon contractions, dysfunctional colonic nerves/muscles, anal sphincter and pelvic floor dysfunction causing obstructive defaecation. Faecal incontinence is a pelvic floor and anal sphincter motility disorder, characterized by poor bowel control and involuntary loss of stool. Diagnostic testing for colonic motility disorders include: (i) colonic transit studies by sequential X-rays of the patient over five days following oral ingestion of a cocktail of radioopaque markers - a test that may not accurately estimate regional retention; (ii) colonic motility using a manometric pressure tube to measure colon muscle strength and response. Patients with excessive contractions can be treated with pharmaceuticals, and those with intractable constipation may require surgical intervention (colectomy); (iii) motility of the anorectum and pelvis by anal/rectal manometry to measure muscle strength and nerve sensitivity; (iv) defaecography, or the radiological examination of anaorectal structures and pelvic floor during the elimination of rectal barium; (v) the size and integrity of anal muscle by endoscopic ultrasound following insufflation with air; (vi) pudenal nerve latency testing requiring nerve stimulation to identify anal sphincter nerve damage. The disadvantages of some tests are that they are physically invasive to the patient, and pain may be endured. Colonic transit studies that are performed following oral administration of radioactive tracers, such as indium-111 (^{111}In) and gallium-67 (^{67}Ga), can give an accurate as-

sessment of regional colonic transit [1] and have proved useful in patients with chronic constipation as well as in irritable bowel syndrome (IBS) and diarrhoeal disorders [2]. Nuclear medicine investigations have the advantage of being potentially less invasive than the existing procedures.

Further studies are necessary to understand the physiology and molecular controls of the gastrointestinal tract [3], particularly for treating disorders of this complex organ at the clinical level. The literature is sparse of pre-clinical diagnostic studies, and some of those that have used rats include gastric emptying of live parabiotic animals during continuous voluntary food intake [4], gastric intestinal transit using a charcoal meal [5], technetium-99m (^{99m}Tc)-pertechnetate to detect Meckel's diverticulum [6], and the influence of serotonin on colonic transit using ^{99m}Tc-diethylenetriaminepentaacetic acid (^{99m}Tc-DTPA) with carbon-14-polyethylene glycol 4000 [7]. The aim of this particular study was to evaluate the feasibility of imaging 'live' normal rats with a non-absorbable tracer, and defining anatomical segments of the colon for a semi-quantitative analysis of colonic transit as a marker of colonic motility.

Methods

General

Rhenium Sulphur Colloid Kit was obtained from the local manufacturer (RAH Radiopharmacy; Adelaide; Australia). The Kit consists of four separate non-radioactive components: (10% Gelatine solution [10x 1 mL]; Thiosulphate solution [19.2 mg potassium perrhenate and 80.0 mg sodium thiosulphate in 8.0 mL of water for injection]; 1.0 M HCl [8.0 mL]; and Basic Phosphate Buffer [280.0 mg sodium dihydrogen phosphate dihydrate in 8.0 mL of 1.0 M sodium hydroxide]). Sodium ^{99m}Tc-pertechnetate was obtained from a ^{99}Mo/^{99m}Tc-generator (Gentech; Australian Radioisotopes; Sydney; Australia). Experiments were performed in quadruplicate unless stated otherwise.

Radiotracer preparation

Preparation of ^{99m}Tc-rhenium sulfide colloid included adding ^{99m}Tc-pertechnetate (500 MBq/0.5 mL saline), HCl (1.0 M; 0.5 mL), then thiosulphate solution (0.5 mL) to one vial containing 10% gelatine. The reaction vial was then heated in a boiling water bath for 3-5 min, and the brown liquid was allowed to cool to room temperature. After neutralizing the dispersion with Basic Phosphate Buffer (0.5 mL), ^{99m}Tc-rhenium sulfide colloid was used within 30 min of preparation in the animal studies. ^{99m}Tc-rhenium sulfide colloid with >95% radiochemical purity was used in the study, as tested by paper instant thin layer chromatography/saline [8].

Animal studies

Experiments performed with the rats, complied with "The Australian Code of Practice for the Care and Use of Animals for Scientific Purposes NHMRC" and according to a protocol approved by the Animal Ethics Committee of the Institute of Medical and Veterinary Sciences, Adelaide. Standard rodent feed was provided in the form of pellets ('Meat Free Rat & Mouse'; Specialty Feeds; Glen Forrest; Australia) containing the following ingredients: wheat, lupins, barley, soya meal, fish meal, mixed vegetable oils, canola oil, salt, calcium carbonate, dicalcium phosphate, magnesium oxide, with vitamin and trace mineral premixes. Pellets contained 19.0% protein, 4.6% total fat, 4.8% crude fibre and 71.6% carbohydrate that is equivalent to 13.5 MJ/kg digestible energy.

Preliminary physiological distribution

One normal Lewis rat (male; 250 g) was marked with a texta pen on the fur exposed to an anaesthetic (halothane; 1 mL) in a large sealed jar for ~2 min until early narcolepsy was visible. Beyond the jar, the rat was administered with ^{99m}Tc-rhenium sulfide colloid (3 MBq; 0.5 mL) by oral gavage using a plastic syringe (3 mL) and a blunt, rounded-end stainless steel syringe needle (10 cm length x 0.1 cm internal diameter) inserted 4.5 cm down from the mouth. The bolus administration was followed by a rinse with water for injection (1.0 mL) using the same apparatus. The lightly anaesthetized rodent was allowed to recover alone in its original cage. After 5 h, the rat was euthanased (halothane; 1 mL), the gastrointestinal tract was excised and then the following organs or segments were harvested: stomach, duodenum, jejunum, ileum, caecum, sigmoidal/distal colon and rectum. Rat faeces were also collected from the cage. The samples were counted in a large volume gamma counter linked to a multichannel analyser (Model 3100; Canberra Industries Inc; USA) over a ^{99m}Tc-window (70-210 keV). The proportion of organ uptake was calculated as sample counts divided by total measured counts or injected dose (id). All values were background corrected.

Preparation and dose administration

Six normal Lewis rats (male; 250-300 g) in a single cage stocked with pellet food and water, were each familiarized with a cardboard 'imaging' tube (8 cm diameter x 20 cm length; one closed end) for at least 48 h. The tube contained a square support on the closed end for stability on flat surfaces (Fig. 1). One rat at a time was marked with a texta pen on the fur and then anaesthetized as above. The rat was administered with ^{99m}Tc-rhenium sulfide colloid (15-20 MBq; 0.5 mL) by oral gavage as above. The lightly anaesthetized rodent

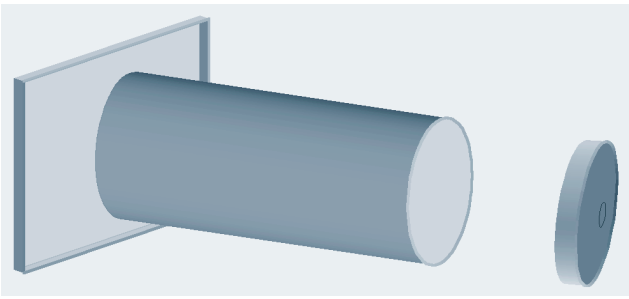

Figure 1. Imaging tube with lid. With a rat in the tube, its tail is passed through the lid hole. The lid is engaged, and a firmly held tail restrains the rat. The square support prevents rolling.

was placed back in the cage containing the other rats and allowed to recover. Animals were allowed to interact, feed and drink *ad libidum* before and in between imaging sessions.

Scintigraphic imaging procedure

At the time of imaging, the marked rat was placed inside the imaging tube, its tail was inserted through a hole in the lid, and the lid was secured shut onto the tube. Whilst holding the tail, the loaded tube was placed on the face of a collimator of a gamma camera (Starcam 300M; GE, USA). The tail was held firmly against the lid to encourage restraint of the rat during the entire period of image acquisition. Commencement of imaging occurred if the rodent remained stable for an initial 30 sec. Whole body static anterior images of 5 min were then obtained every 30 min up to 4 h for n = 2 rats, as well as in the 'main study' from 4 to 9 h and at 25 h for n = 4 rats. An acquisition was aborted for that time point if the rat moved erratically within the tube.

Image analysis

For each scan, a best fit region of interest (ROI) analysis was used to obtain the counts per segment of gastrointestinal tract of the rodent. The regions used were the caecum/proximal colon, sigmoidal loop, distal colon/rectum and a composite or whole colon. All values were background corrected and results reported as mean ± standard deviation. The % region counts were calculated as a proportion of the total initial counts (whole body) to generate a time-activity curve. A curve of natural log of % region counts versus time was generated for the whole colon and each segment to derive the respective slope (clearance rate constant), the half clearance time and regression coefficient (r^2). The half clearance time was calculated as the 0.693 divided by the clearance rate constant. Whilst scanning a rat, a cobalt-57-spot marker was placed next to the nose and another next to the rectum, providing reference points of animal size. An annotated diagram of a typical rat body was placed on an image using the reference points, to highlight the location of radioactive anatomical features as regions of interest.

Results

Preliminary physiological distribution

Results of the quantitative uptake of ^{99m}Tc-rhenium sulfide colloid in the rat gastrointestinal tract are shown in Table 1 below. At 5¼ h, 2% was found in the stomach, 4% in the small intestine, most (90%) radioactivity was in the large intestine with some faecal excretion (4%). These data indicated for the imaging part of the study, scans should commence at a time prior to any faecal excretion and earlier than 5¼ h after administration.

Rodent preparation for imaging

Trial runs involving a mock injection scenario, revealed the rats required familiarity with the imaging tube and the lid for at least 2 days in the cage, just in advance of the study. This prerequisite step allowed training of each rodent to accept the lid on the tail, and to adopt a passive, motionless state for 5-8 min during confinement inside the tube. All of the lightly anaesthetized rats recovered within 5 min, and they behaved with regular vitality amongst the other rodents.

Table 1. *Organ distribution of ^{99m}Tc-colloid in the gastrointestinal tract of a rat after an oral dose.*

Organ	% id ^{99m}Tc-colloid at 5 1/4 h
stomach	2
duodenum	0
jejunum	1
ileum	3
caecum	46
sigmoidal /distal colon	34
rectum	10
faeces	4
Total	**100**

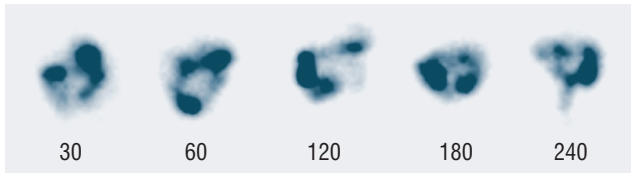

Figure 2. *In vivo* distribution of ^{99m}Tc-rhenium sulfide colloid in rat #2 at 30, 60, 90, 120, 180 and 240 min after oral administration.

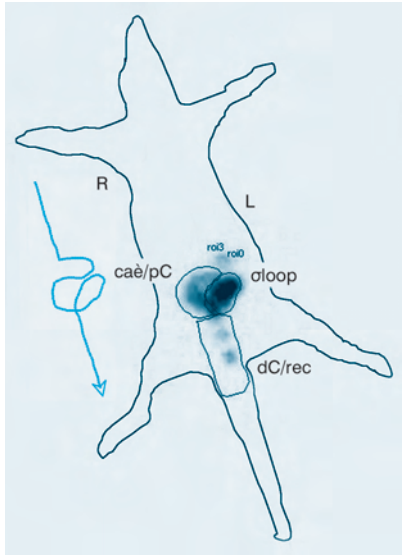

Figure 3. Regions of the gastrointestinal tract of a rat including the caecum (cae)/proximal colon, sigmoidal (s) loop, distal colon (dC)/rectum (rec), and radioactivity migration pathway.

Scintigraphic imaging procedure

Rats were well behaved during the scanning times, exhibiting restraint whilst in the tube on almost every occasion and more compliance with the later time points. Scan acquisitions that were aborted due to erratic movement occurred on 2 out of 49 scanning time points. Early data for n=2 rats identified the radiotracer entered into the colon at ~4 h post administration and no activity was excreted. Static images every 30 min of a typical rat are shown in Figure 2. The gastrointestinal images resulted in sufficient resolution of the anatomical features.

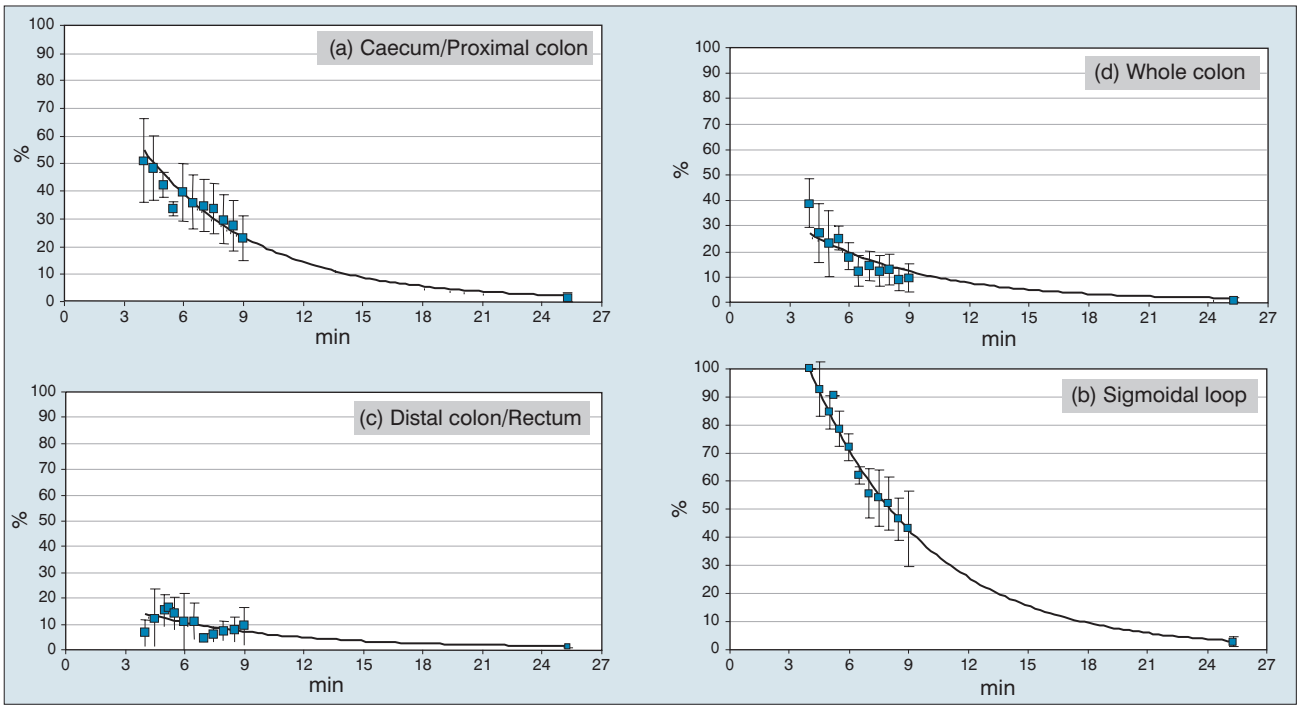

Figure 4. Percentage of ^{99m}Tc-rhenium sulfide colloid at a time after oral administration to normal rats (n=4), segmentally in the caecum/proximal colon (a), sigmoidal loop (b), distal colon/rectum (c), and in the whole colon (d).

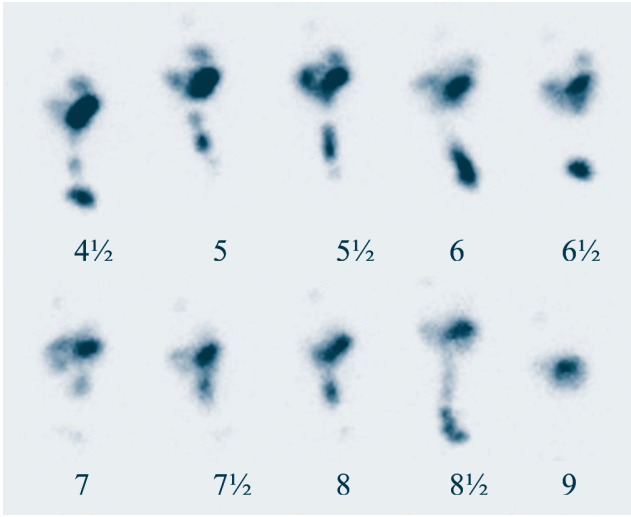

Figure 5. *In vivo* distribution of orally administered ^{99m}Tc-rhenium sulfide colloid at 4½, 5, 5½, 6, 6½, 7, 7½, 8, 8½ and 9 h in rat #1.

Colonic motility was subsequently examined in n=4 rats from 4 h onwards (main study). Figure 3 shows the radioactive migration pathway, and the caecum/proximal colon, sigmoidal loop and distal colon/rectum as regions of interest within a rat body.

The delayed intestine activity is shown as graphs for all rats (Fig. 4) and as typical images (Fig. 5). Of the total activity in the intestine an exponential decrease was observed, mainly due to the emptying pattern of the caecum/proximal colon and sigmoidal loop. The statistical parameters and half clearance times are summarized in Table 2. The best fit data set are reflected in the higher regression coefficient values. Of the set, the distal colon/rectum gave a variable emptying pattern over the 4-25 h duration and it resulted in the lowest r^2 value. At 25 h ≤ 1% of ^{99m}Tc-rhenium sulfide colloid was present in the whole colon, and the half clearance time of this organ was determined as $t_{½}$ = 4.04 h.

Table 2. *Half clearance time of ^{99m}Tc-colloid and other parameters in segments of the colon.*

Organ	Constant (clearance rate)	Half clearance time (h)	r^2
caecum/proximal colon	-0.1741	3.98	0.991
sigmoidal loop	-0.1713	4.05	0.943
distal colon/rectum	-0.1525	4.55	0.842
whole colon	-0.1716	4.04	0.998

Discussion

The aim of this study was the assess the feasibility of colonic transit measurements utilizing orally administered ^{99m}Tc-colloid as a transit marker. The initial part of this study commenced with the quantitative organ assay (non-imaging) for the purpose of estimating the time of entry of radioactivity into (and exit from) the large intestine. At the randomly selected time of 5 h, the non-absorbable radiotracer was predominantly found in the colon, with minor faecal excretion. Based on these results, a scanning protocol of rodents would need to commence earlier, at a time prior to any excretion, so that the whole body counts could be measured as well as to identify the radiotracer migration pathway. Furthermore, the high retention in caecum and distal colon indicated that the protocol was likely to extend for some time beyond 5¼ h, and this in

turn would affect the timing of when the dose should be administered and the end point of scanning.

The technical demands of the study required an early morning start of the investigators to administer the oral ^{99m}Tc-colloid dose, and then commence image acquisitions every 30 min thereafter until a feasible end point of 9 h. The early images up to 4 h after administration, provided significant information for the main part of the study. First, the direction of movement of the solid emptying marker could be observed in relation to the whole body outline (Fig. 3) and that it was a non-absorbable tracer. Second, the image quality for resolving anatomical features was assessed to be good enough to identify segments, considering that the large intestine changes shape due to segmental and propagated activities internally [9], even though a rat retains a static posture. The frequency of static images obtained every 30 min, did yield enough data to clarify the direction of radiotracer movement. Third, the time taken for the marker to reach the distal colon and prior to faecal excretion was 4 h, indicating that acquisition of images for the main study should be initiated at 4 h.

At 4 h, 51±12% of the initial dose was present in the caecum/proximal colon, and this level decreased by about half to 23±8% at 9 h. From Figure 4, inclusive of the 25 h time point, the radiotracer was cleared from the caecum/proximal colon in exponential fashion. Likewise there was a similar clearance from the sigmoidal loop segment over the same period. Their similar half clearance times of 4 h indicated colonic motility of the large intestine is influenced by functions of the caecum/proximal colon and sigmoidal loop. Since these segments can be characterized by their $t_{½}$ values, it seems feasible that a comparison of colonic motility could be made using this technique between rodents that are normal versus those with inflammatory bowel diseases. This laboratory is currently evaluating a model of transient colitis in rats, and also plans to apply this model in such a comparison in the future.

The distal colon/rectum gave an irregular ^{99m}Tc-colloid transit pattern because the radioactivity was rapidly expelled from the body as faeces, in between the scanning occasions. The distal colon in humans is able to shift large quantities of contents and the physiological function has been associated with high amplitude propagated contractions [9]. Even during scanning of rats, the propulsion of contents was visible as evidenced from the variable radioactivity distributed along the segment (see Fig. 5 image at 8½ h).

In conclusion, it is feasible to scan live normal rats instilled with ^{99m}Tc-colloid over a 4-9 h period, in a setting that best equates to clinical conditions. From this, the utility of non-absorbable ^{99m}Tc-colloid in the model conveniently avoids using longer half life isotopes such as ^{111}In and ^{67}Ga. Segments of the large intestine were clearly distinguishable on the images, including the caecum/proximal colon, sigmoidal loop and distal colon/rectum. A semi-quantitative analysis of segments on the image gave reproducible data, and particularly for the whole colon the half clearance time was determined as 4 h, a far lower value to normal $t_{½}$ in humans of 28±12 h [2]. Although rats and humans have vastly different diets [10], the colonic anatomy of both species has similar regions such as the caecum, sigmoidal loop and rectum. This investigation is a prerequisite to a wider study of the colonic motility of rats in response to specific drugs or disease conditions.

Bibliography

1. Bellen JC, Chatterton BE, Penglis S, Tsopelas C. Gallium-67 complexes as radioactive markers to assess gastric and colonic transit. *J Nucl Med* 1995; 36: 513-517.
2. Bartholomeusz D, Chatterton BE, Bellen JC et al. Segmental colonic transit after oral ^{67}Ga-citrate in healthy subjects and those with chronic idiopathic constipation. *J Nucl Med* 1999; 40: 277-282.
3. Tsopelas C, Penglis S, Ruszkiewcz A, Bartholomeusz FDL. Scintigraphic imaging of experimental colitis with technetium-99m-infliximab in the rat. *Hell J Nucl Med* 2006;9:85-89.
4. Van der Velde P, Koslowsky I, Koopmans HS. Measurement of gastric emptying during and between meal intake in free-feeding Lewis rats. *Am J Physiol Regulatory Integrative Comp Physiol* 1999; 276: 597-605.
5. de-Oliveira GR, Gondim FA, da-Graça JR et al. Acute blood volume expansion delays the gastrointestinal transit of a charcoal meal in awake rats. *Braz J Med Biol Res* 1998; 31: 835-840.
6. Taylor AT, Alazraki N, Henry JE. Intestinal concentration of ^{99m}Tc-pertechnetate into isolated loops of rat bowel. *J Nucl Med* 1975; 17: 470-472.
7. Oosterbosch L, von der Ohe M, Valdovinos MA et al. Effects of serotonin on rat ileocolonic transit and fluid transfer in vivo: possible mechanisms of action. *Gut* 1993; 34: 794-798.
8. Tsopelas C. Particle size analyses of ^{99m}Tc-labelled and unlabelled rhenium sulfide and antimony trisulphide colloids intended for application in lymphoscintigraphic studies. *J Nucl Med* 2001; 42: 467-475.
9. Bassotti G, de Roberto G, Castellani D et al. Normal aspects of colorectal motility and abnormalities in slow transit constipation. *World J Gastroenterol* 2005; 11: 2691-2696.
10. Rao SS, Kavelock R, Beaty J et al. Effects of fat and carbohydrate meals on colonic motor response. *Gut* 2000; 46: 205-211.