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Research Article

Assessment and Validation of Animal Models to Evaluate Topical Effects of Substances on Gastrointestinal Mucosa

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The *in situ* rabbit colon model is a sensitive and reproducible test to evaluate the topical effect of up to three substances applied to the colonic mucosa. Vibra-Tabs (doxycycline hyclate), Inderal (propranolol hydrochloride), and Slow-K (potassium chloride) were compared for topical effects in the Carlborg–Densert cat esophagus model, the Alphin–Droppleman cat gastric mucosa model adapted for dog intestine, and the rabbit colon. Because results were comparable in the all models, additional dosage formulations were subsequently tested only in the rabbit colon model. After exposure of the tissue to drugs, macroscopic and histologic effects were scored on four- and eight-point scales, respectively. In all three models, Vibra-Tabs and Inderal produced the highest macroscopic and histologic scores, although Slow-K was also irritating. In the rabbit colon model, potassium released from Slow-K and Micro-K Extencaps caused more irritation than from controlled-release GITS (KCl) (gastrointestinal therapeutic system KCl).

KEY WORDS: rabbit colon model; gastrointestinal irritation; animal model; gastrointestinal therapeutic system (GITS); potassium chloride; propranolol; doxycycline.

INTRODUCTION

Irritation of the gastrointestinal mucosa caused by oral therapeutic agents can occur through the systemic route and through topical contact. Animals models have been developed to assess the potential for topical irritation of the gastrointestinal mucosa caused by local contact of drugs. The Alphin–Droppleman model (1), using cat gastric mucosa, offers the advantage of simultaneous assessment of damage to adjacent areas of tissue caused by two or more agents. The Carlborg–Densert method (2), using cat esophagus, permits assessment of not only the acute response to irritating compounds, but also the progress of healing. However, in this model, the animals are kept alive for 3 to 4 days, potentially subjecting them to undue suffering. John and colleagues (3) reported the use of an *in vivo* pig model to study topical effects of drugs on the ileal mucosa. However, animals must be anesthetized 24 hr, and the model may record not only topical irritation but irritation produced by systemic drug levels.

Because oral sustained- and controlled-release dosage forms are likely to release a sizable fraction of drug in the lower portions of the intestine, attention has shifted from potential drug-induced irritation of the upper gastrointestinal tract to potential irritation in the small intestine and the colon. We modified the Alphin–Droppleman cat gastric mucosa model for rabbit colon and dog intestine to evaluate the potential for irritation of compounds in the lower gastrointestinal tract.

After initial findings that the rabbit colon model yielded results qualitatively equivalent to those of the dog intestine and Carlborg cat esophagus model, we studied the effects of several commercial products as well as GITS (KCl) (gastrointestinal therapeutic system KCl) in this model.

Our findings indicate that the rabbit colon model is a very sensitive and reproducible means of evaluating the topical effect of up to three substances simultaneously applied to the colonic mucosa.

MATERIALS AND METHODS

Agents Tested

Table I lists the agents tested and the animal models used.

Cat Esophagus Model

Nine female cats (2.4–3.9 kg) were quarantined and acclimated to laboratory conditions for a minimum of 2 weeks before use.

Anesthesia

The cats were anesthetized with an induction dose of sodium pentobarbital, 30 mg/kg iv, supplemented as needed.

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Table I. Agents Tested by Animal Model

Agent/dose	Manufacturer	Cat esophagus	Dog intestine	Rabbit colon
Vibra-Tabs, 100 mg (doxycycline hyclate)	Pfizer	X	X	X
Inderal, 80 mg (propranolol HCl)	Ayerst	X	X	X
Slow-K (KCl), 600 mg or 8 mEq/8 hr	CIBA	X	X	X
GITS (KCl) (GI therapeutic system KCl), 750 mg or 9 mEq/18 hr	ALZA/controlled release			X
Micro-K Extencaps (KCl), 600 mg or 8 mEq/8–10 hr	Robins			X
Artificial intestinal fluid (AIF)	U.S.P. formulation		X	X
Ringer's solution	Abbott Labs			X

Test Procedure

The animal was placed on its back, with the head elevated approximately 10° from the horizontal to reduce the possibility of aspiration and gastroesophageal reflux and to allow normal esophageal drainage into the stomach. Each animal received one dosage form. A silk suture was tied around the dosage form, which was then inserted into the lumen of the esophagus and positioned approximately 5 cm below the upper esophageal sphincter. The proximal (free) end of the suture was taped to the cat's lip.

In two animals an area just proximal to and around the dosage form was perfused *in situ* with normal saline solution, 0.2 ml/hr, delivered by a small-gauge catheter attached to a syringe mounted in a Harvard infusion pump. The catheter was inserted into the lumen of the esophagus approximately 2 cm above the dosage form.

After 8 hr of exposure to the test agent, the suture was cut just proximal to the upper esophageal sphincter and the test article was allowed to pass through the gastrointestinal tract, except for GITS, which were recovered from the esophagus for residual drug analysis. The animals were then allowed to recover in an individual cage with food and water provided ad libitum. Three days later each animal was euthanized.

Evaluations

The esophagus, stomach, and upper part of the duodenum were removed and opened. The esophageal area exposed to the test agent and any other regions of the upper gastrointestinal tract that showed macroscopic pathology on visual examination were excised, photographed, and placed in 10% buffered formalin for histological evaluation. The tissue was graded macroscopically on a four-point scale (Table II). For histological evaluation, fixed specimens were embedded in paraffin, stained with hematoxylin and eosin, mounted on slides, and histologically graded on an eight-point scale (Table II). Both the macroscopic and the histologic grading scales are those used by Carlborg and Densert (2). All grading was done in a blinded fashion by an independent pathologist.

Rabbit Colon and Dog Intestine Models

Male and female New Zealand White rabbits (2–4 kg) and mongrel dogs (15–20 kg) were used. At least 10 animals of each species were acclimated to laboratory conditions for a minimum quarantine period of 2 weeks for rabbits and 1 week for dogs. All animals were fasted for 18–24 hr before the study.

Anesthesia

In rabbits, anesthesia was induced with xylazine, 14–18 mg/kg im, and ketamine HCl, 80–90 mg/kg im, and maintained by intermittent iv administration of pentobarbital sodium, 13 mg/ml, as required. Dogs were anesthetized with iv pentobarbital sodium, 28–31 mg/kg, and maintained by intermittent iv pentobarbital, 65 mg/ml, as required.

Surgical Preparation of the Rabbit

The colon of the rabbit was exposed with a 10-cm midline abdominal incision, and a segment immediately distal to the cecum was isolated by placing two ligatures around the colon 15–20 cm apart. The isolated segment of colon was separated from the remaining intestine by cutting just inside each ligature, leaving intact only the nerves and blood vessels supplying the segment. A longitudinal incision was made along the antimesenteric border of the entire length of the segment to preserve the blood supply directly beneath the mucosa. The isolated segment was then clamped into a three-chambered cell (Fig. 1A) so that it formed the floor of each chamber (Figs. 1B–D). Each cell measured 7.0 cm^2.

In several preliminary studies, the rabbit stomach was used. The stomach was exposed with a 10-cm midline incision and a well-vascularized section on the greater curvature was isolated from the surrounding stomach by cutting along the lesser curvature, leaving the vasculature intact. The isolated segment was then clamped into a three-chambered cell. However, the small size of the rabbit stomach and local bleeding, which led to difficulty in fitting the tissue into the chamber, made macroscopic evaluation of the effects of agents on the muscosa difficult. Therefore, this model was not pursued.

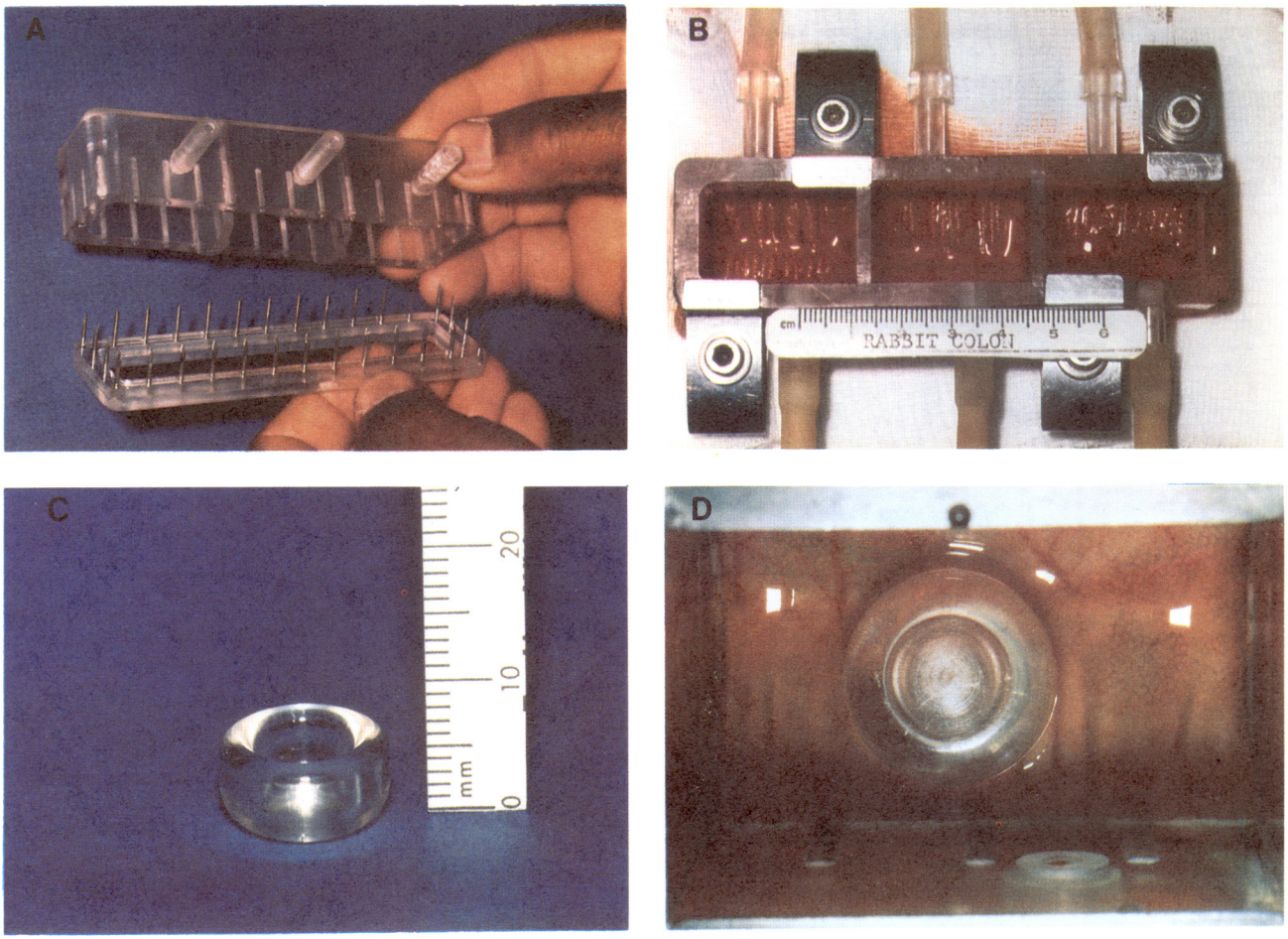

Fig. 1. (A) Three-chambered test cell. Each chamber measures 3.2 × 2.0 × 2.5 cm. (B) Rabbit colonic mucosa clamped into a three-chambered test cell. (C) Polyurethane cup for testing compounds. (D) Polyurethane cup positioned on rabbit mucosa.

Surgical Preparation of the Dog

A section of the jejunum was prepared in the same manner as rabbit colon.

Test Procedure

After completion of the surgical preparation for either rabbit or dog, the test cell was placed in a level position on top of the abdomen. Ringer's formulation or artificial intestinal fluid (AIF) was circulated through each test chamber at 37°C at one of two perfusion rates: 2.2 ml/min for comparison of single-unit tablets or other solid dosage forms and 0.2 ml/min for comparison of multiunit capsules with single-unit tablets. The lower rate assured that the multiunit dosage forms remained on the mucosa for the duration of the experiment.

The perfusion solution was circulated through all chambers at 2.2 ml/min for 1 hr before starting any study. Each agent tested was then placed directly on the tissue in a separate chamber, and each chamber was perfused at one of the above rates throughout the test period.

For drug comparisons, each compound was formulated in solution or suspension with Klucel and placed in a 250-ml polycarbonate cup (Fig. 1C). The cup was placed open side down directly on the tissue in the test chamber (Fig. 1D).

Evaluations

In most cases the tissue was exposed to the test drug for 3 hr. However, several substances were left in place for 8 hr to evaluate progression of the lesions. At the conclusion of each study, the tissues were removed from the animal, photographed, and evaluated both macroscopically and histologically (Figs. 2A–D). Samples were evaluated macroscopically for degree of visible irritation according to a modification of the Carlborg–Densert scale (Table II). The area (0–7.0 cm^2) of mucosal irritation was determined and multiplied by the maximum score in the area to yield an index of irritation (0–27.0) reflecting both the severity of the response and the extent of the area within the test chamber affected by an agent. For histologic assessment, the scale of evaluation and method of preparation were the same as described for studies of cat esophagus.

RESULTS

Local irritation of several commercial drug formulations was evaluated macroscopically and histologically in

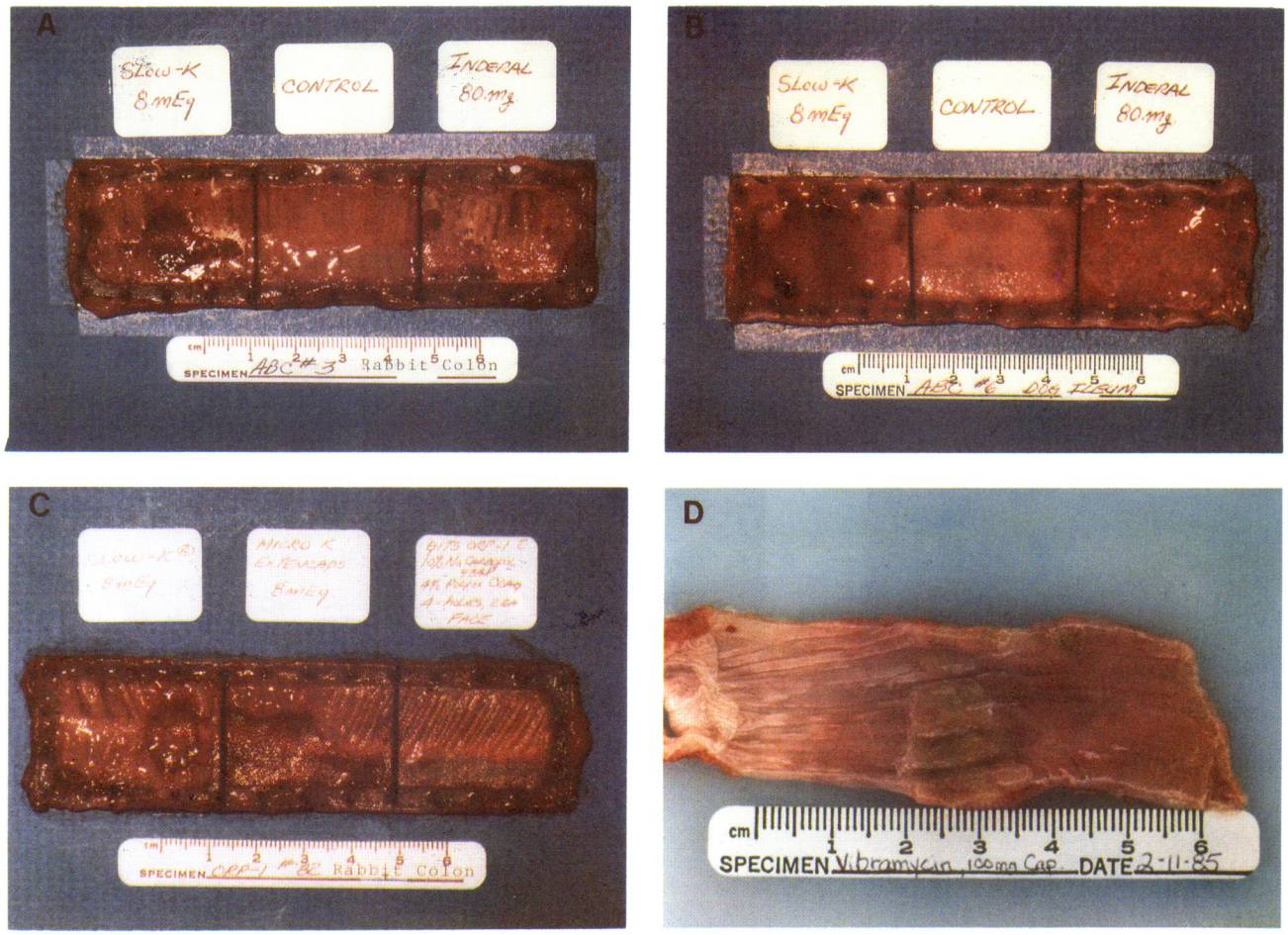

Fig. 2. (A) Effect of potassium chloride and propranolol on rabbit colonic mucosa after an 8-hr exposure. (B) Effect of potassium chloride and propranolol on dog ileum after an 8-hr exposure. (C) Effect of Slow-K, Micro-K, and GITS (KCl) on rabbit colonic mucosa after a 3-hr exposure. (D) Effect of doxycycline on cat esophagus after an 8-hr exposure.

three animal models (Table III). In all three models, macroscopic and histologic scores were highest and most consistent for Vibra-Tabs (doxycycline hyclate) and Inderal (propranolol) tablets, although Slow-K (KCl) was also irritating. For Vibra-Tabs, macroscopic scores on dog intestine were somewhat lower than scores on rabbit colon or cat esophagus. For Slow-K, cat esophagus scores were somewhat lower than those in the rabbit and dog models. In general, however, the qualitative appearance of the tissue was consistent in all models.

Ringer's solution and AIF were tested as controls for up to 8 hr on rabbit colon and dog intestine, and no topical irritation was noted (Table IV). In an unpublished study of 27 rabbits, GITS dosage form placebos showed essentially no irritation when placed on colonic mucosa for 6 hr. Table V presents macroscopic irritation results in the rabbit colon model for GITS (KCl), a controlled-release form of potassium, and for Slow-K, a slow-release form. At 3 and 8 hr, GITS was less irritating, with an index averaging close to 1, as opposed to Slow-K's 14.

Significantly, irritation does not appreciably increase after 3 hr. Indeed, in preliminary studies, potassium began causing irritation after about 30 min of continuous exposure that progressed in severity up to—but not after—3 hr. After 3 hr, only small increases in affected area increase the final irritation index value; at no site did scores increase for degree of visible tissue damage.

The rabbit colon model is also useful for comparing the effects of single-tablet dosage forms with those of multiunit forms. Table VI shows that Slow-K and Micro-K Extencaps caused nearly equal local irritation. The controlled-release GITS form causes less irritation than either Slow-K or Micro-K Extencaps.

DISCUSSION

This study clearly demonstrates that the rabbit colon model has the potential to quantify topical irritation of the gastrointestinal mucosa. It is sensitive and reproducible and permits evaluation of up to three substances simultaneously. The time of exposure is easily varied, and the progression of some events may be observed as they occur. Results with controls show that tissues remain viable at least 8 hr.

Until now, investigating the gastrointestinal irritation of compounds has relied upon oral administration in various animal models, use of expensive laboratory animals, or ap-

Table II. Scales for Macroscopic and Histologic Evaluation of Animal Models

Macroscopic evaluation: cat esophagus

0 = normal mucosa
1 = redness, edema, or slight erosion
2 = superficial ulceration
3 = deep ulceration
4 = perforation

Macroscopic evaluation: rabbit colon and dog intestine

0 = mucosa normal
1 = barely perceptible color change
2 = mucosa slightly red or pale to white; area definable
3 = mucosa red/purple or white; possible surface erosion
4 = mucosa very red/dark purple or white to gray; necrotic in appearance/frank ulceration

Histologic evaluation: all models

0 = essentially normal mucosa; mild changes induced by experimental procedures not test article related
1 = increased number of inflammatory cells in lamina propria: inflammation only in tunica mucosa
2 = erosion of tunica mucosa with or without increased numbers of inflammatory cells in lamina propria; inflammation in tunica mucosa
3 = same as 2, with inflammation in tunica submucosa
4 = same as 3, with inflammation in circular muscle layer of tunica muscularis
5 = same as 4, with inflammation in longitudinal muscle layer of tunica muscularis
6 = ulceration of tunica mucosa with or without increased numbers of inflammatory cells in lamina propria; inflammation in tunica submucosa
7 = same as 6, with inflammation in circular muscle layer of tunica muscularis
8 = same as 7, with inflammation in longitudinal muscle layer of tunica muscularis

Table IV. Biopsy of Rabbit Colon Control Chamber Mucosa Immediately After Surgical Preparation, After a 1-hr Equilibration Period and After 3 and 8 hr of Exposure to AIF ($N = 2$)

	Rabbit colon 1		Rabbit colon 2	
	Macro	Histo	Macro	Histo
Surgical preparation	0	0	0	0
1-hr equilibration	0	0	0	1
3-hr exposure	0	0	0	0
8-hr exposure	0	0	0	0

plication of the agent to human buccal mucosa (4). Most of these models allowed assessment of only one compound at a time and, thus, did not offer the advantage of direct comparison of different presentations of the same compound on adjacent tissue sites in the same animal. Additionally, the Carlborg–Densert method (2) using cats subjected the animals to undue suffering to make it possible to follow the progression of the lesion.

It is important that the effects seen in the rabbit colon model qualitatively reflect topical tissue irritation seen at other gastrointestinal sites: cat esophagus and dog intestine. Occasional quantitative variations between the models may occur, as in tests of Vibra-Tabs and Slow-K showing lower irritation indices for dog intestine and cat esophagus than for rabbit colon (Table III). In this study, the variations may have occurred because only a small number of tests were done (to minimize the use of animals). However, differences may also reflect the greater sensitivity of the rabbit colon model.

The rabbit colon model provides a practical, inexpensive, and rapid means of assessing the potential of compounds for topical irritation of gastrointestinal mucosa. It requires only 3 hr of exposure, and the results are represen-

Table III. Macroscopic and Histologic Evaluations of the Effects of Various Drugs on Cat Esophagus, Rabbit Colon, and Dog Intestine After 8 hr of Exposure

		Test scores					
		Cat esophagus		Rabbit colon		Dog intestine	
Drug	Test no.	Macro	Histo	Macro	Histo	Macro	Histo
Doxycycline hyclate (Vibra-Tabs, 100 mg)	a	3.0	4.0	4.0	3.0	4.0	1.0
	b	3.0	4.0	4.0	3.0	4.0	1.0
	c	3.0	4.0	3.0–4.0	2.0	3.0	0.0
	d	—	—	3.0–4.0	1.0	3.0	0.0
Propranolol HCl (Inderal, 80 mg)	a	2.0	4.0	4.0	2.0	2.5	4.0
	b	3.0	4.0	4.0	4.0	2.0	2.0
	c	3.0	4.0	4.0	3.0	2.5	2.0
	d	—	—	4.0	4.0	0.0	0.0
Potassium chloride (Slow-K), 8 mEq/600 mg	a	0.0	0.0	3.0	5.0	4.0	4.0
	b	1.0	1.0	4.0	4.0	4.0	2.0
	c	1.0	2.0	3.0	2.0	4.0	4.0
	d	—	—	4.0	4.0	4.0	4.0

Table V. Comparison of the Irritation from GITS KCl and Slow-K on Rabbit Colonic Mucosa After 3 and 8 hr

Test no.	Area[a]	Score	Index	Test no.	Area[a]	Score	Index
	GITS KCl, 3 hr				Slow-K, 3 hr		
a	0.18	2.5	0.45	a	7.59	3.5	26.57
b	0.36	1.5	0.54	b	3.46	3.5	12.11
c	0.12	2.0	0.24	c	4.18	3.0	12.54
d	0.60	3.0	1.80	d	2.52	3.0	7.56
e	0.60	3.0	1.80	e	3.23	3.5	11.31
f	0.42	2.5	1.05	f	6.18	3.5	21.63
				g	3.30	3.0	9.90
				h	4.48	3.5	15.68
				i	4.05	3.5	14.18
				j	2.42	3.0	7.26
Mean	0.38	2.4	0.98		4.14	3.3	13.87
SD	0.20	0.6	0.69		1.63	0.3	6.11
	GITS KCl, 8 hr				Slow-K, 8 hr		
a	0.40	3.0	1.20	a	1.68	4.0	6.70
b	0.31	3.0	0.90	b	6.93	4.0	27.70
c	0.15	3.0	0.50	c	1.10	3.0	3.30
d	1.20	1.5	1.80	d	3.08	4.0	12.30
				e	2.88	3.0	8.60
				f	6.93	4.0	27.70
Mean	0.52	2.6	1.10		3.77	3.7	14.38
SD	0.47	0.7	0.55		2.56	0.5	10.72

[a] Are in cm^2

tative of those in other animal species. Ideally, an *in vitro* model would be preferable for assessing these effects; in its absence the rabbit colon model, because of its intact nerve and blood supply and thin layer of tissue, is probably the best alternative at present.

It is not known how well the reactions of rabbit colon correspond to those of human gastrointestinal tissue for all drugs, but results with this model confirm clinical findings with potassium chloride, an irritating drug known to produce ulcers in human intestinal mucosa (5–8). The model is very sensitive and can be used to compare different products, although it probably overestimates the amount of contact a drug has with the human mucosa because of normal residence times and motility in the gastrointestinal tract. In addition, the rabbit colon model offers a humane alternative to the cat esophagus model for screening drugs and for designing the least irritating dosage forms before attempting human clinical studies.

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Table VI. Crossover Study of the Irritation from Slow-K, 8 mEq (600 mg), Micro-K Extencaps (600 mg), and a Prototype GITS (KCl) (750 mg) on Rabbit Colonic Mucosa After 3 hr of Exposure During Perfusion at 0.21 ml/min

	GITS (KCl) prototype (750 mg)			Slow-K (600 mg)			Micro-K (600 mg)		
Experiment no.	Area (cm^2)	Score	Index	Area (cm^2)	Score	Index	Area (cm^2)	Score	Index
78	0.32	2.5	0.8	3.05	3.0	9.2	4.32	3.5	15.1
79	0.41	2.5	1.0	6.72	3.5	23.5	6.72	3.0	20.2
80	0.28	3.0	0.8	5.12	3.5	17.9	3.92	3.0	11.8
81	3.84	2.5	9.6	6.72	3.5	23.5	6.72	3.5	23.5
82	0.04	2.5	0.1	1.80	3.5	6.3	4.04	3.5	14.1
83	0.72	3.5	2.5	3.97	3.0	11.9	4.16	3.0	12.5
84	0.48	3.0	1.4	3.20	3.5	11.2	0.99	3.0	3.0
Mean	0.87	2.8	2.3	4.37	3.4	14.8	4.41	3.2	14.3
SD	1.33	0.4	3.3	1.89	0.2	6.9	1.95	0.3	6.6

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