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Effects of Isoflurane on gastrointestinal motility after brief exposure in rats

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Abstract

In pre-clinical studies, investigation of oral formulations often necessitates the use of general anesthesia to facilitate deposition of material directly into the stomach. Since the effectiveness of intestinal drug absorption is dependent on gastric emptying (GE) and intestinal motility, drugs that influence either will also influence drug absorption. This study investigated gastrointestinal motility in rats after brief exposure to Isoflurane (ISO) general anesthesia for orogastric gavage. The use of metochlopramide was also evaluated.

Twenty-five fasted rats were induced with brief ISO anesthesia (<6 min). Rats were gavaged a gelatin capsule (8 mm (L) × 2.0 mm (o.d.)) containing 9 mg of activated charcoal powder (gastrointestinal marker) and rapidly recovered. Gavage was performed using a 15 cm feeding device with a soft hollow tip to hold the capsule. Study included three groups (60 and 120 min recovery, metochlopramide pre-treatment with 60 min recovery) and control. Animals were sacrificed for exposure and examination of the gastrointestinal tract following the allocated recovery period.

Gastrointestinal transit of charcoal was reduced approximately 50% 120 min after brief ISO anesthesia. Metochlopramide pre-treatment did not increase gastrointestinal propulsion despite increased GE. These data warrant consideration in intestinal drug absorption studies where ISO is the anesthetic of choice.

Keywords: Anesthesia; Gavage; Gastrointestinal; Isoflurane; Motility; Metochlopramide

1. Introduction

The rate at which an orally administered compound travels to the intestines is dependent on gastric empty-

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ing since drug absorption from the stomach is usually negligible. The rate of drug absorption is also dependent on intestinal propulsion or transit time (Boyce and Palmer, 1975; Perry et al., 1993). Besides those critical factors, large intra- and inter-individual variability in GE rates has been reported in human and animal studies that will also affect drug absorption (Reilly and Nimmo, 1984; Nimmo, 1989; Reppas et al., 1998; Peachey et al., 2000; Carriere et al., 2001; Soppimath et al., 2001).

In pre-clinical studies using small animals, the pharmacokinetic and pharmacodynamic investigation of oral formulations often necessitates the use of general anesthesia to facilitate deposition of material directly into the stomach (orogastric gavage) without causing injury (Torres-Molina et al., 1996; Morishita and Takayama, 2001). Since the effectiveness of intestinal drug absorption is dependent on gastric emptying (GE) and intestinal motility, anesthetics that influence either will also influence drug absorption (Wright et al., 1982). Furthermore, certain drug formulations such as protein-based drugs (e.g. oral insulin formulations) must be protected from the harsh environment of the stomach before they can be absorbed into the small intestine (Morishita et al., 2004). Therefore, a prerequisite to performing studies of this nature is the understanding of potential anesthetic effects on GE and motility of the gut. A delay in gastric emptying with or without changes in intestinal motility from general anesthesia may alter drug kinetics, or even render those drugs ineffective.

In this laboratory, Isoflurane (ISO) is the preferred anesthetic for most small and large animal procedures. This inhalation agent provides for a rapid induction, preservation of cardiac output, and the ability to maintain a safe level of surgical anesthesia using spontaneous ventilation. A literature search was performed using the Medline database (1966–May 2004) to determine whether any studies have investigated the ISO effects on gastrointestinal motility. The results of this literature search revealed no published studies specifically reporting on the effects of ISO on gastric emptying or intestinal motility. A study by Hall et al. (1995) investigated gastric myoelectric and motor activity in dogs after 6 MAC hours of ISO anesthesia. They observed a significant decrease in the gastric motility index 18 h post ISO anesthesia. Another study by Seyde and Longnecker (1984) examined the hemodynamic effects of ISO in normal and hemorrhaged rats and reported no significant ISO effects on gastrointestinal and hepatic blood flow in normal rats. The former study provided information on gastric motility in dogs while the latter studied hemodynamic characteristics of the GI system after ISO general anesthesia; however, neither gastric emptying nor intestinal motility were investigated after brief ISO exposure for short procedures.

The purpose of this study was to assess gastrointestinal motility in rats after brief exposure to ISO general anesthesia. The use of metochlopramide, a gastrointestinal prokinetic agent, was also evaluated.

2. Methods

After approval from the Thomas Jefferson University Institutional Animal Care and Use Committee, 25 male Sprague–Dawley rats obtained from Charles River Laboratories (Wilmington, MA) were fasted for 24 h with water available ad libitum. The animals' cages were fitted with raised wire bottoms to prevent coprophagy. The study was conducted in the early morning without reversal of the animal's photoperiod. Induction of general anesthesia was performed by placing the animals in an induction chamber using a 3% ISO concentration in 100% oxygen. Animals were ready for the orogastric gavage procedure within 6 min after being placed into the induction chamber. Immediately after induction of ISO general anesthesia, the rats were fed a small gelatin capsule (8 mm (L) × 2.0 mm (o.d.), Shionogi, Japan) containing 9 mg of activated charcoal powder used as a gastrointestinal marker. Orogastric gavage was performed using a 15 cm feeding device with a soft polyethylene hollow tip (3 mm (o.d.)) serving to hold the oval capsule that protruded 1 mm from the tip, thereby forming a rounded extremity at the head of the device (Fig. 1). The feeding device was constructed using an epidural needle modified to accommodate a hollow polyethylene tip. The animal's tongue was retracted to the side and the device was gently advanced into the esophagus, past the esophageal sphincter. A metal stylette (cut 1 mm shorter than the device) was then advanced through the needle to cause the capsule release into the stomach. This maneuver was followed by a slow 1 mL injection of sterile water (pH 6.0–6.5) through the device to promote rapid dissolving of the capsule. The orogastric gavage pro-

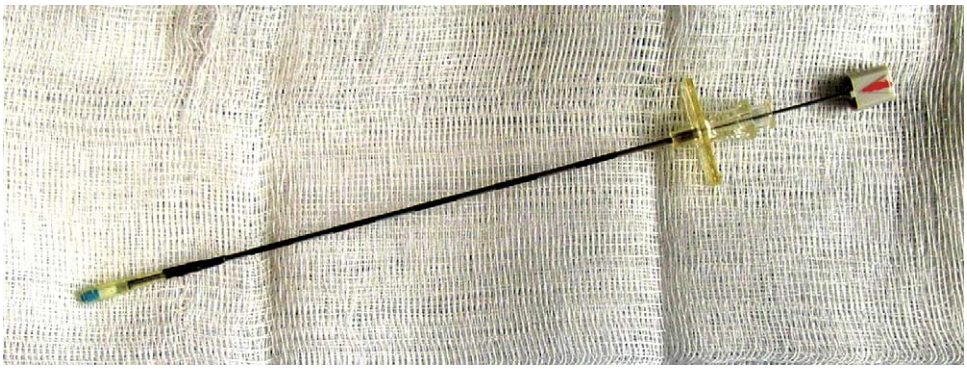

Fig. 1. Illustration of the pill pusher device constructed by the investigator for feeding the charcoal capsule to a rat. The device is composed of an epidural catheter with a plastic hollow tip cemented to the end of the catheter. The device is gently advanced into the esophagus past the esophageal sphincter and the capsule is released into the stomach after depressing the metal stylette.

cedure took approximately 10 s and was performed by the same investigator skilled in the technique.

The study was designed to include one control and three treatment groups as follows: Group 1 animals (control $n = 4$) were anesthetized with 3% ISO for induction, fed the capsule, and anesthesia was maintained for 120 min using ISO at a 1.5% concentration in 100% oxygen. All animals breathed spontaneously with an SpO2 in the 96–100% range measured at the hind paw using a Nellcor® (Tyco International Ltd.) pulse oximeter. Animals were then sacrificed using an intracardiac injection of 1 mL beuthanasia solution. A laparotomy was performed for exposure and examination of the gastrointestinal tract. Group 2 animals ($n = 6$) were anesthetized, fed the capsule, and anesthesia was discontinued within 6 min from the start of induction. The animals were recovered and remained awake for 1 h without access to food or water after which time they were sacrificed for exposure and examination of the gastrointestinal tract. Group 3 animals ($n = 9$) received the same treatment as Group 2 but the animals were awake for 2 h. Group 4 animals ($n = 6$) were administered metochlopramide, 0.2 mg/kg by intramuscular injection 20–30 min prior to induction of anesthesia. Those animals were then fed the capsule, rapidly recovered, and remained awake for 1 h before being sacrificed. The handling of the animals and experimental procedures were consistent across all groups only varying anesthetic duration, recovery time, and pre-treatment with metochlopramide.

To evaluate the gastrointestinal parameters of interest, the gastrointestinal tract was rapidly removed after a laparotomy was performed under ISO general anesthesia using 3% ISO with the stomach excised 2–3 mm above the lower esophageal sphincter. Gastrointestinal fluid was evacuated using a 1 mL syringe and stomach volume measured in 50 μL increments by aspiration of the gastrointestinal fluid from the lower esophageal sphincter. Three drops of gastrointestinal fluid were then expelled onto pH paper for the measurement of pH. The stomach and intestines were placed on a white gauze surface and dissected open to expose the entire intestinal surface (Fig. 2). The intestines were carefully examined by the same investigator to determine the location of charcoal deposition. Charcoal deposition in the gastrointestinal tract could easily be observed with the naked eye and was quantitated as the measured distance (cm) from the pyloric sphincter to the leading edge of the charcoal stained area (Borella and Lippmann, 1980).

2.1. Statistical analyses

The sample size for this study was determined by power analysis for a one-way ANOVA with three treatment levels and the criterion for significance alpha set at 0.05. A sample size of six animals per treatment group yielded a power of 80% assuming a minimum detectable difference of 50% between means (10, 15 and 22 cm). Charcoal distances (leading edge) in the intestines were analyzed by ANOVA. Once significant main effects were obtained the Tukey post hoc test was used to identify differences between groups. Data were analyzed using Systat software (Systat Software Inc.,

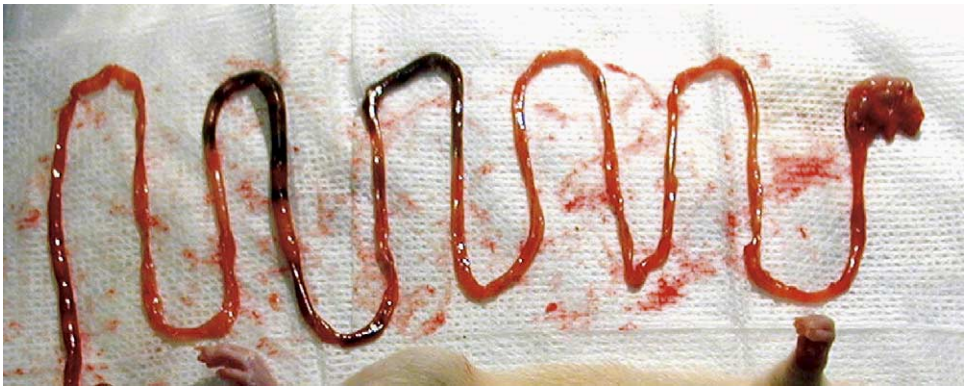

Fig. 2. Illustration of gastrointestinal tract exposure after laparotomy for quantitative measurement of distance traveled by charcoal marker. The presence of charcoal was visually identified by longitudinal dissection of the exposed intestines.

Richmond, CA) and presented as means ± standard deviation. Statistical significance was set at $p < 0.05$.

3. Results

Summary data for the four groups are presented in Table 1. The 24 h fasting period with animals housed on raised cage bottoms was effective in creating stomach conditions resulting in presence of only clear fluid, and easily identifiable charcoal stained areas with a leading edge. No capsule remains could be identified upon examination of the stomach and intestines confirming that all capsules had dissolved in the stomach. ANOVA showed statistically significant differences among groups in gastric volume and pH. Post hoc analysis revealed significantly ($p < 0.05$) higher gastric volume and pH in Group 1 compared to Group 4 animals. Charcoal movement out of the stomach was not observed in all animals following the awake period post ISO anesthesia. The distribution of animals that experienced any charcoal movement out of the stomach (positive respondents) in each group was: 50.0%, 83.0%, 78.0%, and 83.0% for Groups 1, 2,

Table 1
Measured gastrointestinal parameters after brief isoflurane exposure in rats

	Group 1 ($n = 4$) ISO 120 min, control	Group 2 ($n = 6$) Awake 60 min	Group 3 ($n = 9$) Awake 120 min	Group 4 ($n = 6$) Metochlopramide Pre Rx, awake 60 min
Rat weight (gm)	268.50 ± 81.21	234.50 ± 15.63	242.77 ± 13.54	198.83 ± 5.84
Stomach volume (μL)	425.0 ± 95.7	183.3 ± 160.2	308.3 ± 241.1	83.33 ± 25.8*
Stomach fluid pH	5.00 ± 1.63	3.08 ± 1.24	4.22 ± 1.75	2.17 ± 0.98*
Positive respondents (% rats with any charcoal propulsion out of stomach)	50.0	83.0	78.0	83.0
Charcoal propulsion in respondent subset (cm from pylorus)	20.0 ± 15.6	16.0 ± 12.1†	52.0 ± 6.0*	21.0 ± 7.7†
Charcoal propulsion in all rats (cm from pylorus)	15.0 ± 16.2	9.0 ± 10.8	24.0 ± 26.7	17.5 ± 11.0
Gastrointestinal rate of propulsion (approximated) (cm/min)	0.17	0.27	0.43	0.35

Group 1 (control): rats were anesthetized with Isoflurane for 120 min. Group 2: rats were awake for 60 min after brief Isoflurane anesthesia. Group 3: rats were awake for 120 min after brief Isoflurane anesthesia. Group 4: rats received, pre-treatment with metochlopramide and were awake for 60 min following brief Isoflurane anesthesia.

* $p < 0.05$ compared to Group 1 (control).

† $p < 0.05$ compared to Group 3.

3, and 4, respectively. This distribution of respondent animals was not statistically significant. ANOVA was performed on the subset of positive respondents to assess whether those animals experienced differences in gastrointestinal propulsion across the four treatments. Statistical significance was achieved between the groups ($p = 0.002$) in the subset analysis and Tukey post hoc analysis was performed revealing Group 3 animals (awake 120 min) to have significantly increased charcoal movement post ISO anesthesia. In that subset of animals ($n = 4$), charcoal stained distances ranged from 46 to 60 cm from the pylorus to the distal ileum (Table 1). Although 83% of the metochlopramide-treated animals (Group 4) did demonstrate charcoal movement out of the stomach following a 60 min awake period post ISO anesthesia, the measured distances from the pylorus to the charcoal leading edge in those animals were similar to Groups 1 and 2 subsets.

4. Discussion

The results of this study showed that brief ISO exposure for induction of general anesthesia had a time dependent effect on post anesthesia gastrointestinal motility measured by the charcoal technique. Approximately 20% of the animals in the three treatment groups showed significant interruption of GE, with marker remaining in the stomach and no marker observed in the intestines, while the remaining 80% showed movement of the marker out of the stomach and into the intestinal tract. In the latter 80% (referred to as positive respondents), the marker traveled more distally into the ileum in animals awake for 120 min after anesthesia (Group 3), in contrast to the 60 min post anesthesia measurements (Group 2) showing most of those animals with marker reaching the duodenum and jejunum. Interestingly, continuous ISO administration (control animals) did not completely interrupt GE based on the presence of marker in the intestines of two of the control animals who remained anesthetized for 120 min (1.2 MAC hours). In those two animals, charcoal leading edges were identified in the proximal ileum at a mean distance of 29.0 ± 1.4 cm from the pylorus.

The mean stomach volume was generally more elevated in control animals compared to all other groups although statistical significance was only reached between control and metochlopramide-treated animals. Gastric volume in metochlopramide-treated animals was reduced approximately 80% compared to control ($p < 0.05$), which was indicative of an increased GE rate following metochlopramide pre-treatment.

The increase in GE after metoclopramide treatment, however, did not result in significantly increased charcoal movement throughout the intestines compared to the other groups. This observation may be partly explained by metoclopramide's effectiveness as a prokinetic agent of the upper gastrointestinal tract but having little or no effect on intestinal peristalsis (Ponte and Nappi, 1981; Schmidt and Jorgensen, 1984; Adelhoj et al., 1985; Nemeth and Gullikson, 1989; Greiff and Rowbotham, 1994). Other physiological effects must also be considered as reported in the earlier studies of Summers et al. (1970) and Poulakos and Kent (1973) who demonstrated that in unanesthetized fasting rats intestinal propulsion is independent of GE once the intestinal marker has reached the duodenum.

Considering the lack of data on the effects of ISO on the gastrointestinal system, we took the liberty to compare our results to those of Borella and Lippmann (1980) who have studied movement of administered particles in the gastrointestinal tract of awake unanesthetized Sprague–Dawley rats. Those authors reported intestinal propulsion of Amberlite pellets reaching mean distances of 102.0 ± 3.1 cm at 120 min post administration. The mean distance of 24.0 ± 27 cm reported in the present study represents approximately a 75% decrease in intestinal propulsion, or approximately a 50% decrease when comparing the same data (mean = 102.0 cm) to the intestinal distances measured in our "respondent" animals subset at 120 min (mean = 52.0 ± 6 cm). Our findings are, therefore, indicative of a reduction in gastrointestinal propulsion after a brief ISO general anesthetic in spite of the approximately three-fold increase observed in charcoal movement (Table 1) when doubling the awake period from 60 to 120 min. Based on available published data on gastrointestinal transit time of non-absorbable material in awake rats (Borella and Lippmann, 1980; Tuleu et al., 2001) it can be estimated that normal gastrointestinal transit time for non-digestible particles in a rat is approximately 1 cm/min. Our calculated total transit time of 0.43 cm/min (respondent subset) after brief ISO inhalation for induction of general anesthesia still represents approximately a 57% decrease compared to normal gastrointestinal transit time in a rat. We do not

believe that activity or stress levels could have affected our results as those variables remained constant. Once awake, the animals were returned to their cages and allowed free access to water with stimulation kept to a minimum. It was noted that the rats' activity levels were low and none of the animals ingested water during the 60 and 120 min recovery periods.

Our literature search did not reveal any studies in the rat reporting on ISO's gastrointestinal effects. Longnecker et al. investigated the hemodynamic effects of ISO general anesthesia, and other anesthetic agents, in Sprague–Dawley rats under normovolemic and hypovolemic conditions. They reported no change in gastrointestinal blood flow during 120 min of ISO anesthesia compared to awake animals, even under hypovolemic conditions. They, however, did not measure gastrointestinal propulsion, which was beyond the scope of their work. The only other study that examined ISO's effects on the gastrointestinal tract was by Hall et al., who hypothesized that ISO general anesthesia (6 MAC hours) would have no significant effect on the electrical and mechanical response of the canine stomach 18 h post anesthesia. Although their negative results are not surprising considering ISO's elimination half-life, the study demonstrated that gastrointestinal studies could be performed the day after ISO anesthesia in dogs.

The present data, therefore, provide evidence that after a brief ISO general anesthetic, most rats will demonstrate some decrease in gastrointestinal function with significant improvement after 120 min post anesthesia. Based on these results, and data available in normal rats, this decrease amounts to approximately a 50% reduction in the distance traveled by the ingested non-absorbable charcoal particles. Therefore, in a fasting rat administered 1 mL of water after orogasric gavage of a charcoal capsule, approximately 120 min were necessary to demonstrate propulsion of charcoal material into the distal small intestine after a brief ISO general anesthetic. Metochlopramide-treated animals had increased GE following brief ISO general anesthesia; however, this did not translate into increased charcoal propulsion into the small intestine. Charcoal particles only reached the jejunum, therefore, showing no benefit of pre-treatment with metochlopramide.

In conclusion, the delay in the transit of material into the small bowel of a rat, combined with the variability in intestinal propulsion, should be considered in intestinal drug absorption studies where ISO is the anesthetic of choice.

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