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Vincristine delays gastric emptying and gastrointestinal transit of liquid in awake rats

A.A. Peixoto Júnior[1,2], B.C.V. Teles[1], E.F.B. Castro[1], A.A. Santos[1], G.R. de Oliveira[1,2], R.A. Ribeiro[1], F.H. Rola[1] and F.A.A. Gondim[1,2]

[1]Departamento de Fisiologia e Farmacologia, [2]Hospital Universitário Walter Cantídio, Universidade Federal do Ceará, Fortaleza, CE, Brasil

Correspondence to: F.A.A. Gondim, Departamento de Fisiologia e Farmacologia, Universidade Federal do Ceará, Rua Cel. Nunes de Melo, 1127, 60430-270 Fortaleza, CE, Brasil
Fax: +55-85-3366-8333. E-mail: gondimfranc@yahoo.com

We evaluated the effects of vincristine on the gastrointestinal (GI) motility of awake rats and correlated them with the course of vincristine-induced peripheral neuropathy. Vincristine or saline was injected into the tail vein of male Wistar rats (180-250 g) on alternate days: 50 µg/kg (5 doses, N = 10), 100 µg/kg (2, 3, 4 and 5 doses, N = 49) or 150 µg/kg (1, 2, or 5 doses, N = 37). Weight and stool output were measured daily for each animal. One day after completing the vincristine treatment, the animals were fasted for 24 h, gavage-fed with a test meal and sacrificed 10 min later to measure gastric emptying (GE), GI transit and colon weight. Sensory peripheral neuropathy was evaluated by hot plate testing. Chronic vincristine treatments with total cumulative doses of at least 250 µg/kg significantly decreased GE by 31-59% and GI transit by 55-93%. The effect of 5 doses of vincristine (150 µg/kg) on GE did not persist for more than 1 week. Colon weight increased after 2 and 5 doses of vincristine (150 µg/kg). Fecal output decreased up to 48 h after the fifth dose of vincristine (150 µg/kg). Vincristine decreased the heat pain threshold 1 day after 5 doses of 50-100 µg/kg or after 3-5 doses of 150 µg/kg. This effect lasted for at least 2 weeks after the fifth dose. Chronic intravenous vincristine treatment delayed GE and GI transit of liquid. This effect correlated with the peak increase in colon weight but not with the pain threshold changes.

Key words: Constipation; Gastrointestinal transit; Peripheral neuropathy; Rat gastric emptying; Vincristine

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Introduction

The therapeutical use of *Vinca rosea* was recognized by indigenous people who used the infusion of leaves to control hemorrhage, for healing and cleansing of chronic wounds and as a mouthwash for toothache (1). Vincristine is a chemotherapeutic agent derived from *Vinca rosea* (Linn), which has been widely employed against hematological malignancies and solid tumors since the 1960's (2,3). However, its use is limited by a wide spectrum of negative systemic effects such as neurotoxicity, orthostatic hypotension, gastrointestinal (GI) complications, loss of appetite, alopecia, and hyponatremia (4).

Neurotoxicity, especially against the somatic and autonomic peripheral nervous system, is the most commonly reported systemic complication (5). Neurotoxicity manifests mainly as peripheral neuropathy with predominant sensory findings. In humans, autonomic involvement has

been widely reported (6). GI complications can be severe and include constipation, paralytic ileus and Olgivie's syndrome (7), in some cases leading to death due to bowel perforation (8). The impact of vincristine on GI motility and other autonomic functions has not been adequately studied in humans or animals (9,10). Acute intravenous (*iv*) administration of vincristine increases the intestinal myoelectrical activity (11) and delays GI transit in rats (12). Although the acute effect of vincristine on GI motility has been described, to our knowledge, there are no studies addressing the chronic effects of vincristine on rats, which may be significant, given the possibility of development of autonomic neuropathy (13).

Thus, the objectives of the present study were to determine the impact of acute and chronic *iv* vincristine treatment on the gastric emptying (GE) and GI transit of liquids in awake rats and to determine the relationship between these effects and the development of somatic neuropathy.

Material and Methods

Animals, drugs and experimental design

Experiments were performed on male Wistar rats weighing 200 to 250 g. All procedures and animal treatments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (DHEW Publication No. (NIH) 85-23, Bethesda, MD, USA) and were approved by the Institutional Review Board of the Universidade Federal do Ceará (#109/07).

The rats were anesthetized with ether and treated with vincristine, which was slowly injected intravenously into the tail vein every other day. Sham animals received equivalent amounts of saline according to their weight.

Vincristine sulfate (Tecnocris™, Eurofarma/Zodiac Produtos Farmacêuticos S/A, Brazil, 1 mg/mL) was diluted with saline to concentrations of 15, 30, and 45 µg/mL. Then, vincristine was administered by *iv* injection into the tail vein at doses of 50, 100, or 150 µg/kg every other day. A subgroup of rats (N = 5) received 5 doses of 50 µg/kg vincristine (total cumulative dose of 250 µg/kg), four other subgroups received either 2 (N = 6), 3 (N = 6), 4 (N = 5), or 5 doses of 100 µg/kg vincristine (N = 9; total cumulative dose of 200-500 µg/kg), and the three remaining subgroups received 1 (N = 5), 2 (N = 6) or 5 doses of 150 µg/kg vincristine (N = 7; total cumulative dose of 150-750 µg/kg). This protocol was similar to the model described by Authier et al. (14) to study vincristine-induced sensory peripheral neuropathy in rats.

The thermal latency threshold and fecal output were measured at different times before and during the treatment with *iv* vincristine. One day after the last administration of vincristine, we evaluated the colonic weight, GE, and GI transit of liquids.

Two subgroups of rats treated with 5 doses of 150 µg/kg vincristine were maintained for 1 (N = 6) or 2 weeks (N = 5), with food and water *ad libitum*. In these 2 groups, the thermal latency threshold was assessed 24 h prior to the measurement of GE and GI transit. The animals were also fasted for 24 h prior to the assessment of GE and of GI transit.

GE and GI transit measurements

The animals were fasted for 24 h prior to the GE and GI transit measurements, but water was allowed *ad libitum* until 2 h before the measurements. We used a modification of the technique described by Reynell and Spray (15) for GE and GI transit measurements, which we have previously utilized (16,17). First, 1.5 mL of the test meal containing a nonabsorbable marker (0.5 mg/mL phenol red solution in 5% glucose) was given orally by gavage. After 10 min, the animals were killed by cervical dislocation. The stomach and small intestine were exposed by laparotomy, quickly clamped at the pylorus, cardia and terminal ileum, and then removed. The stomach and small intestine from the gastroduodenal junction to the cecum were carefully stretched along a meter-stick on a plain table top and divided into the following four consecutive segments: stomach, proximal (40% of small intestine), middle (30% of small intestine), and distal small intestine (last 30%).

Each segment was placed in a measuring cylinder and its volume was measured by adding 100 mL 0.1 N NaOH. They were cut in small pieces and homogenized for 30 s. The suspension was allowed to settle for 20 min at room temperature and 10 mL of the supernatant was centrifuged for 10 min at 2800 rpm (about 1400 *g*). Proteins in 5 mL of the homogenate were precipitated with 0.5 mL trichloroacetic acid (20% w/v) and centrifuged for 20 min at 2800 rpm (about 1400 *g*), and 3 mL from the supernatant was added to 4 mL 0.5 N NaOH. The absorbance of the sample was read at 560 nm. A standard dilution curve was obtained in each experiment relating the concentration of phenol red in 0.1 N NaOH to absorbance at 560 nm. The linear coefficient of the standard dilution curve (α) was established and used to determine the concentration of the solution read at 560 nm and the amount of phenol red recovered from each segment.

The percent recovery of phenol red in each segment was determined according to the following equation: % recovery in segment = amount of phenol red recovered in the segment / total amount of phenol red recovered from all four segments x 100.

Fecal output and colon weight

Animals were placed in separate Bowman cages with

food and water available *ad libitum* except when GE and GI transit studies were scheduled. Weight and stool output were measured daily for each animal. Colon weight was measured after sacrifice to assess the presence of increased fecal retention.

Thermal latencies

Thermal threshold assessment was performed using the hot plate method described by Eddy and Leimbach (18), which was modified by O'Callaghan and Holtzman (19) to determine paw withdrawal latencies before and after the administration of different doses of vincristine or saline. Rats were placed on a hot plate surface (I.I.T.C. Life Science, USA) with a heat source to maintain a constant temperature of 51 ± 0.5°C. A typical response used to define the threshold consisted of a discomfort reaction, i.e., licking of the hind paws or jumping. The latency in time until reaction was recorded manually with a chronometer and a cut-off time of 20 s was used. Hyperalgesia to heat was defined as a decrease in withdrawal latency.

Withdrawal latencies were determined at baseline, before vincristine (N = 5) or saline (N = 5) administration and 24 h after the first, third and fifth doses. The subgroups of rats treated with 5 doses of 150 µg/kg vincristine or saline were observed 1 and 2 weeks after the last dose.

General toxicity

We also evaluated the presence of general toxicity by determining the amount of weight loss, diarrhea, alopecia, piloerection, and death.

Statistical analysis

The results are reported as means ± SEM. One-way analysis of variance (ANOVA) and Bonferroni's test were used to compare the differences in phenol red recovery, stool and colon weight and the thermal latencies between the various groups. Thereafter, two-way ANOVA was performed to determine whether the GI motility changes induced by vincristine were dose-dependent. Differences were considered to be statistically significant at $P < 0.05$.

Results

Effect of chronic *iv* vincristine treatment on gastric and gastrointestinal phenol red recovery

Table 1 shows that phenol red recovery in the stomach was significantly increased by chronic vincristine treatment by 31-59% and gastrointestinal phenol red recovery increased by 55-93%. As can be seen in Table 1, the minimal total cumulative dose of vincristine necessary to induce GI motility inhibition was 250 µg/kg. In rats treated with one dose of 150 µg/kg vincristine and with two doses of 100 µg/kg vincristine, the phenol red recovery in the stomach and small intestine was similar to that observed in the control group. However, as can be seen in Figure 1A, chronic administration of higher doses of *iv* vincristine (5 doses of 50 µg/kg; 3, 4, and 5 doses of 100 µg/kg; 2 and 5 doses of 150 µg/kg) significantly increased phenol red recovery in the stomach 1 day after the last dose ($P < 0.05$). Two-way ANOVA revealed that the effect of vincristine on the GE or GI transit of liquid was not dose-dependent.

Table 1. Effect of intravenous vincristine administration on the dye recovery in the stomach, proximal, middle and distal small intestine (when present) of awake rats, 10 min after gavage administration of 1.5 mL of a 0.5 mg/mL phenol red solution in 5% glucose, 1 day after 5 doses of vincristine 50, 100, or 150 µg/kg.

Treatment	Vincristine					Saline				
	Stomach	Proximal	Middle	Distal	N	Stomach	Proximal	Middle	Distal	N
50 µg/kg										
5 doses	63.9 ± 3.0*	23.1 ± 1.4	12.8 ± 3.3*	0.0 ± 0.0*	5	43.7 ± 6.2	27.1 ± 3.4	28.1 ± 4.4	0.9 ± 0.1	5
100 µg/kg										
2 doses	53.0 ± 5.1	30.7 ± 2.9	16.1 ± 5.2	0.0 ± 0.0	6	48.6 ± 1.9	30.0 ± 1.5	21.3 ± 1.8	0.0 ± 0.0	8
3 doses	54.5 ± 2.9*	27.8 ± 2.0	17.6 ± 4.4	0.0 ± 0.0*	6	38.1 ± 6.6	30.2 ± 3.3	23.4 ± 6.8	8.1 ± 1.9	5
4 doses	57.8 ± 1.0*	22.8 ± 1.8	19.2 ± 1.7	0.0 ± 0.0*	5	44.0 ± 4.5	20.3 ± 2.1	24.7 ± 2.1	10.8 ± 4.3	5
5 doses	62.2 ± 3.7*	30.0 ± 2.3	7.2 ± 2.2*	0.4 ± 0.4*	9	43.7 ± 6.2	27.1 ± 3.4	28.1 ± 4.4	0.9 ± 0.1	5
150 µg/kg										
1 dose	44.7 ± 3.2	35.0 ± 1.0	16.5 ± 1.8	3.6 ± 1.6	5	45.0 ± 3.1	23.8 ± 2.0	23.7 ± 2.5	7.3 ± 1.9	6
2 doses	69.3 ± 4.4*	29.2 ± 3.2	1.4 ± 1.4*	0.0 ± 0.0	6	48.6 ± 1.9	30.0 ± 1.5	21.3 ± 1.8	0.0 ± 0.0	8
5 doses	69.3 ± 3.3*	26.8 ± 2.5	4.1 ± 1.3*	0.3 ± 0.3	7	43.7 ± 6.2	27.1 ± 3.4	28.1 ± 4.4	0.9 ± 0.1	5

Data are reported as means ± SEM. N = number of animals in each experimental subgroup. *$P < 0.05$ *vs* saline (ANOVA and Bonferroni's test).

Effect of chronic *iv* vincristine treatment on colonic function

Vincristine treatment with doses of 150 µg/kg decreased the daily fecal output from 3.4 ± 0.4 to 1.1 ± 0.4 g after the first dose ($P < 0.05$). This effect persisted after repeated administration of vincristine, and 48 h after 5 doses of vincristine (150 µg/kg) the stool output was still significantly decreased to 1.0 ± 0.4 g (Figure 1C), $P < 0.05$.

The colon weight of rats sacrificed 24 h after the fifth dose of 50 µg/kg vincristine was 2.2 ± 0.4 g, similar to control values of 2.4 ± 0.1 g (Figure 1B). The colon weight of rats submitted to 3 or 4 doses of 100 µg/kg vincristine was also similar to that of control animals: 2.8 ± 0.2 and 2.9 ± 0.1 g, respectively; $P > 0.05$.

As can be seen in Figure 1B, animals treated with two doses of 150 µg/kg vincristine and 5 doses of 100 or 150

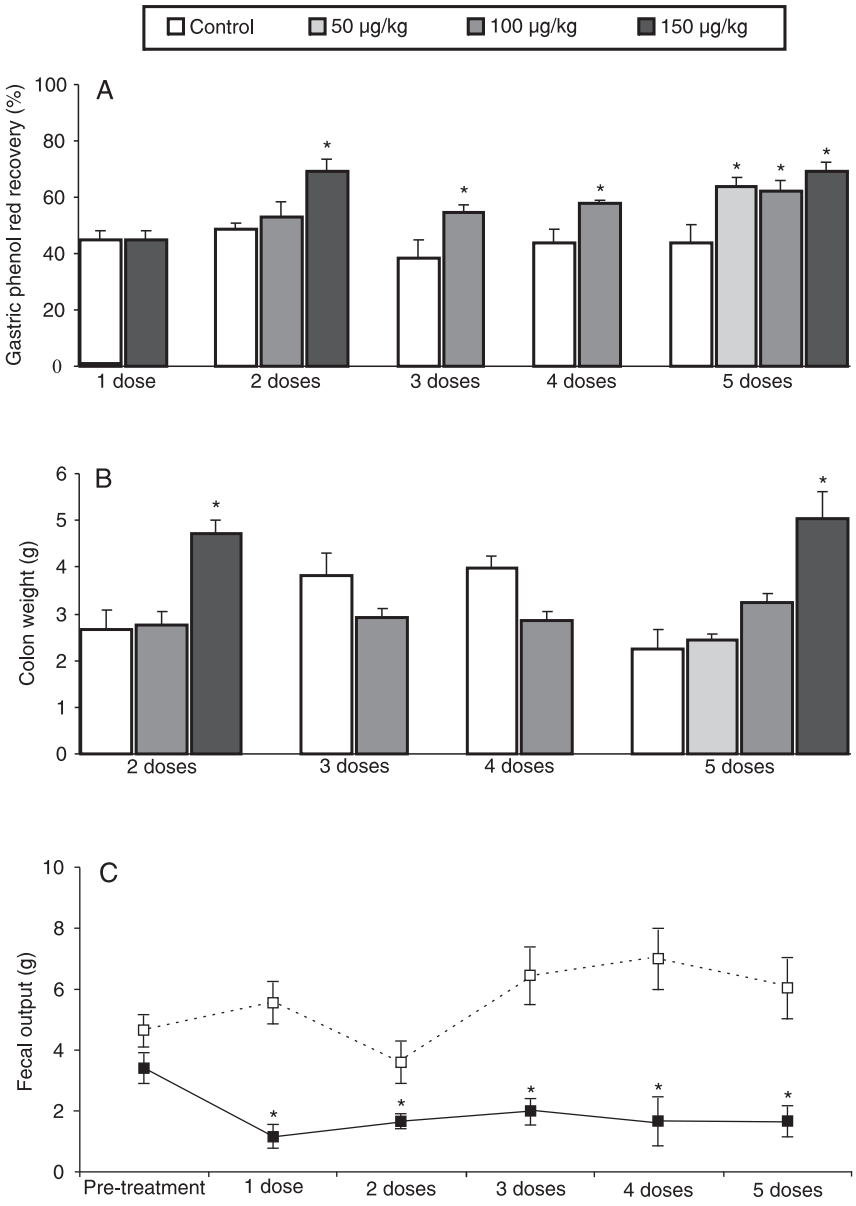

Figure 1. Effect of vincristine administered on alternate days on phenol red recovery (A), colon weight (B) and fecal output (C). Measurements were made 1 day after 5 doses of 50 µg/kg vincristine administered *iv*; 2, 3, 4, and 5 doses of 100 µg/kg, and 1, 2 and 5 doses of 150 µg/kg. Data are reported as means ± SEM, N = 5-9. *$P < 0.05$ *vs* respective control (Student-Newman-Keuls test).

µg/kg exhibited a significant increase in colon weight, i.e., 4.0 ± 0.2, 3.2 ± 0.1, and 5.0 ± 0.5 g, respectively (P < 0.05).

Effect of chronic *iv* vincristine treatment on thermal pain thresholds

Vincristine treatment significantly (P < 0.05) reduced paw withdrawal latencies after 5 doses of 50 µg/kg (from 11.3 ± 1.3 to 5.7 ± 0.6 s) or 100 µg/kg (from 9.8 ± 1.2 to 4.7 ± 0.5 s). This effect also occurred after 3 doses of 150 µg/kg (12.2 ± 0.7 *vs* 9.2 ± 1.0 s) and was more intense after 5 doses of vincristine at 150 µg/kg (6.5 ± 0.5 s, P < 0.05). After 1 dose of 150 µg/kg vincristine, there was no significant reduction in paw withdrawal latencies: 12.2 ± 0.7 *vs* 10.8 ± 1.4 s.

The effects of lower doses of vincristine (1 and 3 doses of 50 µg/kg and 1 and 3 doses of 100 µg/kg) on paw withdrawal latencies also showed a trend (P > 0.05) to a reduction when compared to baseline latencies: 11.3 ± 1.3 *vs* 7.8 ± 1.5 and 10.6 ± 2.3 s for the 50 µg/kg group and 9.8 ± 1.2 *vs* 5.3 ± 0.7 and 6.1 ± 0.6 s for the 100 µg/kg group.

Effect of chronic *iv* vincristine treatment on GE, GI transit and colonic function

The effect of vincristine on the GE and GI transit of liquid was not present in rats treated with 5 doses of 150 µg/kg and sacrificed 1 or 2 weeks after the last dose (Table 2; P > 0.05).

Stool output returned to baseline levels 96 h after the fifth dose of 150 µg/kg vincristine (6.8 ± 2.5 g; P > 0.05). However, as can be seen in Table 2, the colonic weight was still increased in the subgroup of animals sacrificed 1 week after 5 doses of 150 µg/kg vincristine: 4.2 ± 0.3 g (P > 0.05). However, in the subgroup of animals sacrificed 2 weeks after the fifth dose of 150 µg/kg vincristine, the colonic weight was similar to that of the control group: 3.5 ± 0.4 g (P > 0.05).

The decrease in paw withdrawal latencies was present 1 or 2 weeks after 5 doses of 150 µg/kg vincristine (12.8 ± 1.2 *vs* 6.9 ± 1.0 s and 12.8 ± 1.2 *vs* 5.2 ± 0.4 s; P > 0.05), but was not present in control animals: 12.9 ± 1.4 *vs* 8.6 ± 0.6 and 12.9 ± 1.4 *vs* 8.4 ± 0.4; Table 2; P > 0.05.

Two rats treated with 5 doses of vincristine 150 µg/kg (N = 5) developed diarrhea. Transient weight loss (17.4%) due to vincristine treatment was also observed (215.4 ± 4.9 *vs* 177.9 ± 7.9 g; P > 0.05), but with prompt weight recovery 8 days after vincristine was discontinued (196.3 ± 11.0 g; P > 0.05). Alopecia and death due to vincristine administration did not occur. Transient piloerection was also observed in some animals treated with the higher doses of vincristine (150 µg/kg).

Discussion

GI motility changes are an important adverse effect of vincristine administration, reducing the quality of life of patients submitted to vinca-alkaloid chemotherapy (4). Constipation, paralytic ileus and dysmotility of the upper GI tract are common in these patients (7,8). However, few studies have examined changes of GI motility in the proximal gut and the relationship between peripheral neuropathy and GI dysmotility (9). Our results demonstrated that chronic vincristine treatment delayed the GE and GI transit of liquid in awake rats. The minimum total cumulative dose of vincristine, which could induce these GI motility changes was 250 µg/kg. Total cumulative doses above 250 µg/kg produced a similar delay in GE and GI transit and therefore the overall effect of vincristine was not dose-dependent. This delay in GE and GI transit was not demonstrable 1 or 2 weeks after the treatment with 5 doses of 150 µg/kg vincristine was completed.

Kaneko et al. (11) evaluated the effect of vincristine on the gastric antral motility of rats and observed that one dose of 750 µg/kg vincristine acutely increased gastric motility 2 h after its injection, suggesting a presynaptic cholinergic activation. However, decreased gastric motility was observed on the second day after the injection. Sninsky (12) reported similar findings: increased myoelectric activity of the small intestine 2 h after vincristine administration, with a return to control levels within 6 h after injection and a markedly reduced action-potential activity after 3 days.

In the present study, we determined whether doses

Table 2. Phenol red recovery, colon weight, and thermal latency 1 day and 1 and 2 weeks after 5 doses of 150 µg/kg vincristine or saline.

	Control/pre-treatment	One day	One week	Two weeks
Gastric phenol red recovery (%)	57.8 ± 3.7	69.3 ± 3.3*	60.5 ± 3.6	55.9 ± 4.0
Colon weight (g)	2.4 ± 0.1	5.0 ± 0.5*	4.2 ± 0.3*	3.5 ± 0.4
Thermal latency (vincristine)	12.8 ± 1.2	7.2 ± 0.8*	6.9 ± 1.0*	5.2 ± 0.4*
Thermal latency (saline)	12.9 ± 1.4	9.5 ± 0.6	8.6 ± 0.6	8.4 ± 0.4

Data are reported as means ± SEM, N = 5-7. *P < 0.05 *vs* control (ANOVA and Bonferroni's test).

that induce somatic peripheral neuropathy can cause changes in GI motility. For this purpose, we employed the animal model of sensory neuropathy previously reported by Authier et al. (14). The reduction of paw withdrawal latencies detected by hot plate testing in vincristine-treated, but not in control animals, demonstrated that our animals developed thermal hyperalgesia, which is consistent with the clinical expression of sensory neuropathy.

Our findings suggest that the GE and GI transit delay after vincristine administration is not correlated with the temporal course of the sensory neuropathy. Somatic neuropathy (thermal hyperalgesia) was quickly induced by lower doses of vincristine and persisted until 2 weeks after the last dose of 150 µg/kg, while the delay of GE and GI transit was transient, with recovery after 2 weeks in the same group. These results suggest that the GI motility changes induced by chronic vincristine treatment are not due to the presence of neuropathic pain induced by vincristine, which could potentially enhance adrenergic activity. Moreover, we have also observed that guanethidine treatment does not prevent the development of the GI motility changes induced by vincristine (Peixoto AA Jr, Gondim FAA, unpublished results).

The most common effects of vincristine on the gastrointestinal tract in humans are constipation, abdominal pain and paralytic ileus (20). GI dysfunction and bladder disturbances may result from autonomic nerve damage (21). However, the exact pathological and neurophysiological mechanisms responsible for the GI motility changes after vincristine treatment have not been established (4). Similar to our animal model, GI and urinary disturbances induced by vincristine in humans usually start after the development of peripheral neuropathy (21). Mitolo-Chieppa et al. (22) studying the effect of vincristine on gastric vagus branches in rats, described a progressive inhibition of nerve activity with increased vincristine doses. These previous studies suggest that the GI motility changes observed in our animal model may be due to autonomic dysfunction induced by vincristine. Preliminary experiments using this protocol revealed baroreflex activity changes, which further reinforce the idea of a vincristine-induced autonomic neuropathy leading to GE and GI transit delays (Peixoto AA Jr, Gondim FAA, unpublished results).

Similar to humans, vincristine-induced fecal retention was reproduced in our animal model. This reduction in fecal output was associated with increased colon weight. Similar to humans, this effect was transient in rats, with recovery of fecal output 4 days after five doses of vincristine and normal colon weight 2 weeks after treatment. Since the GI motility changes were present when the fecal impaction and decreased colon weight were more evident, it is tempting to speculate that these changes might have played a role in the upper GI motility dysfunction after vincristine treatment, similar to that which we have previously observed in rats after high spinal cord injury (16,17,23).

The present results provide the first evidence that vincristine delays gastric emptying and transit, which indicates chronic dysautonomic changes in the GI tract in experimental models.

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