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Effects of vincristine and monosodium glutamate on gastrointestinal motility and visceral sensitivity

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Abstract

Background: Chemotherapy-induced adverse effects are an unresolved nightmare. In preclinical studies in rats, the food additive monosodium glutamate (MSG) improved some of the side effects caused by cisplatin, but its effects in other models of chemotherapy-treated animals are not well known. The aim of this study was to test if MSG may improve some of the adverse effects induced by vincristine in rats.

Methods: Young male Wistar rats were exposed or not to MSG ($4\,g\,L^{-1}$) in drinking water from week 0 till 1 week after treatment (week 3). Rats received two cycles of five daily intraperitoneal (*ip*) injections (Monday to Friday, weeks 1 and 2) of either saline ($2\,mL\,kg^{-1}$) or vincristine ($0.1\,mg\,kg^{-1}$). Gastrointestinal motility was measured *in vivo* by radiological methods after the first and tenth *ip* administrations. On week 3, the threshold for mechanical somatic and colorectal sensitivity was recorded using Von Frey filaments applied to the paws and an intracolonic balloon, respectively. Finally, samples of the terminal ileum and distal colon were histologically evaluated in sections.

Key Results: Vincristine reduced body weight gain, food intake, and upper gastrointestinal transit, caused somatic (but not visceral) hypersensitivity and increased the thickness of the submucosal and muscle layers of the small intestine. In vincristine-treated animals, MSG partially prevented gastrointestinal dysmotility and reduced visceral sensitivity but did not improve structural alterations of the small intestine.

Conclusions & Inferences: MSG could be used as an adjuvant to conventional treatments to improve some gastrointestinal dysfunctions caused by chemotherapy.

KEYWORDS
gastric emptying, intestinal transit, monosodium glutamate, peripheral neuropathy, vincristine, visceral pain

1 | INTRODUCTION

Cancer is the second leading cause of death per year in the United States[1] and Spain,[2] and the sixth worldwide.[3] One of the most common anticancer treatments is chemotherapy. Vincristine is an antineoplastic drug commonly used in the treatment of different tumors, but it is associated with many side effects.

The most important adverse effect of vincristine is cumulative peripheral neuropathy, which is dose-dependent.[4,5] Paresthesia, loss of tendon reflexes and progressive weakness are the most common clinical features, although autonomic dysfunctions, including gastrointestinal (GI) disturbances, may also occur.[6–9] In fact, 30%–40% of patients receiving vincristine may develop GI complications. The earliest symptoms may include colicky abdominal pain and constipation. In addition, enteric neuropathy has also been reported to occur with this antineoplastic drug.[4,10] Since constipation is the most widely recognized manifestation, colonic dysmotility has received the most attention. Patients treated with vincristine may also develop symptoms indicative of upper GI dysmotility, including anorexia and nausea, or even extreme symptoms such as paralytic ileus. In fact, paralytic ileus occurs in 3%–12% of patients and can be fatal in up to 30% of patients.[11] In experimental animals, the acute effects of single vincristine administration on GI motor function have been evaluated radiographically,[12] and the effects of repeated vincristine administration have also been assessed by radiographic and fluoroscopic techniques.[4]

Another adverse effect of chemotherapy that has received less attention than somatic pain (associated with peripheral neuropathy) is visceral pain. Visceral pain is difficult to localize, radiates to superficial structures, and is often accompanied by nausea, vomiting, and other manifestations.[13] Of the 40% of the population who experience this type of pain, 28% are cancer patients, in whom it may be associated with metastasis or antineoplastic treatment.[14] Visceral pain has recently been shown to occur in the rat shortly after administration of paclitaxel, another antineoplastic drug,[15] whereas, after acute administration of cisplatin, a decrease in responses to intracolonic mechanical stimuli has been observed.[16] It is not known what changes in visceral sensitivity may be caused by repeated administration of vincristine.

Monosodium glutamate (MSG) is one of the most consumed food additives, commonly used by the food industry because it provides a specific flavor called "umami" (tasty).[17] The name of this additive in food products is E-621.[18] High doses of MSG cause toxicity, such as increased oxidative stress and metabolic syndrome or impaired liver and kidney function. However, administration of doses below 2 g kg^{-1}, or short-term administration using water or food as a vehicle is insufficient to induce those toxic effects.[17] Interestingly, MSG reversed the peripheral neuropathy caused by cisplatin and partly improved the GI dysmotility produced in the rat by this drug,[19] and glutamate ameliorated vincristine-induced thermal hypersensitivity (one sign of peripheral neuropathy) in rats,[20] but its potential to prevent or palliate other adverse effects of vincristine, including those affecting the GI tract, has not been evaluated. Thus, the aim of this work was to test whether the supplementation of MSG to the diet may alleviate the adverse effects that vincristine induces in rats.

Key points

- Chemotherapy produces many adverse effects. Gastrointestinal dysmotility, neuropathic pain, and gut histological damage were induced by repeated vincristine in rats.
- The food additive monosodium glutamate (MSG) included in drinking water prevented some of the gut motility disturbances induced by vincristine and decreased colorectal sensitivity in rats treated with this antitumoral drug.
- The use of dietary MSG could be useful in the context of cancer chemotherapy.

2 | MATERIALS AND METHODS

The experiments were carried out at Universidad Rey Juan Carlos (URJC; Madrid, Spain) and were designed and performed according to the EU Directive for the Protection of Animals Used for Scientific Purposes (2010/63/EU) and Spanish regulations (Law 32/2007, RD 53/2013 and order ECC/566/2015) and were approved by the Animal Ethics Committee at URJC and Comunidad Autónoma de Madrid (PROEX 061/18). Every effort was made to minimize animal pain and discomfort as well as to reduce the number of animals used.

2.1 | Animals

Seventy-seven male Wistar rats were obtained from the Veterinary Unit of URJC and divided into two cohorts. In cohort 1, 31 rats (315–320 g, $n = 7$–8/treatment group) were used for the X-Ray and histological experiments; in cohort 2, 46 rats (248–290 g, $n = 11$–12/treatment group) were used for the tactile and visceral sensitivity studies. Animals were housed in groups (3–4/cage) in standard transparent cages under environmentally controlled standard conditions with a 12 h light/12 h dark cycle (lights on at 8 am). Animals had free access to standard laboratory rat chow (Harlan Laboratories Inc.) and sterile tap water.

2.2 | Experimental protocol

Rats received two cycles of five daily (Monday to Friday) intraperitoneal (*ip*) injections of saline (2 mL kg^{-1}) or vincristine (0.1 mg kg^{-1})[4] during 2 consecutive weeks. Half of the rats were exposed to MSG (4 g L^{-1}) in drinking water from week 0 till 1 week after treatment, week 3. This dose of MSG, corresponding to approximately 0.45 g kg^{-1} day^{-1} (which in turn corresponds to 5.1 g per day for a 70 kg man[21]), was previously shown to prevent the development of neuropathic pain and partly improved the GI dysmotility induced by cisplatin in the rat,[19,22] without eliciting significant toxic effects.[21] Thus, the experimental groups were: saline + water (S + W), saline + MSG (S + MSG), vincristine + water (VC + W) and vincristine + MSG (VC + MSG).

The body weight of the animals and the intake of drinking water/MSG and of freely available food were recorded once a week. All analyses were performed as described below by experienced researchers, blinded to the treatments received by the animals (see Figure 1A for a general overview of the experimental protocol).

2.3 | Gastrointestinal motor function (radiographic study)

Gastrointestinal motor function was studied radiographically without prior fasting in cohort 1, as previously described.[23] After the first and tenth vincristine or saline administration, 2.5 mL of a barium sulfate (Barigraf®, 2 g mL^{-1}, $t° = 22°C$) suspension was intragastrically administered. Plain facial radiographs (20 ms) were obtained using a CS2100 (Carestream Dental) digital X-ray apparatus (60 kV, 7 mA) with a focus distance manually fixed to 50 ± 1 cm. Immobilization of the rats in a prone position was achieved by placing them inside hand-made, transparent plastic tubes, which were adjusted to the size of the rat. Habituation to these restraint devices prior to the commencement of the study did not significantly alter GI motility.[23] X-rays were recorded on Carestream Dental T-MAT G/RA film (15 × 30 cm) housed in a hand-made cassette provided with a regular intensifying screen, immediately and 1, 2, 4, 6, and 8 h (T0–T8) after contrast administration. The film cassette was located directly beneath the restraining tube. A rectangular metallic block (3 × 1 × 1 cm) was positioned aside the plastic tube in which the rat was placed, so the metallic block could serve as a reference for morphometric and densitometric analyses (see below). While taking the radiographs, the qualified investigator remained at least 2 m away from the X-ray source. Films were developed in a Kodak X-omat 2000 automatic processor. Alterations in gut motility were semiquantitatively determined from the images by assigning a compounded value to each region of the GI tract considering the following parameters: percentage of the GI region filled with contrast (0–4); intensity of contrast (0–4); homogeneity of contrast (0–2); and sharpness of the GI region profile (0–2). Each of these parameters was scored and a sum (0–12 points) was made. The X-ray images were digitized, and the size and density of contrast were analyzed for the stomach, caecum, and fecal pellets, with the aid of an image analysis system[24] (Image J 1.38 for Windows, National Institute of Health, USA, free software: http://rsb.info.nih.gov/ij/).

2.4 | Assessment of mechanical sensitivity

Mechanical sensitivity was assessed in cohort 2 by measuring the withdrawal threshold to calibrate Von Frey hairs (2–60 g; Bioseb Instruments, USA) after treatment finalization, on week 3 (as previously described[4,19]). Rats were placed individually on an elevated iron mesh in a clear plastic cage and allowed to adapt to the testing environment for at least 10 min 2–3 days before the assessment. Each stimulus was applied to the plantar surface of each hind paw for 1–2 s and repeated five times with 1–3 s intervals. When at least three out of five trials (60%) evoked paw withdrawal, the force applied by that hair was considered as the tactile threshold. Mechanical allodynia was defined as a significant decrease in tactile threshold evoked by mechanical stimuli.

2.5 | Assessment of colorectal sensitivity

Colorectal sensitivity was measured in cohort 2 as previously described.[16,25,26] Briefly, after sedation with Sedator® (medetomidine hydrochloride, 1 mg kg^{-1}, *ip*), a 10 cm longitudinal line was drawn over the linea alba of the abdomen. Transverse lines were drawn every 2 cm to better visualize the contractions during the recordings. Then, fecal material was gently removed from the rectum and a 5 cm long latex balloon lubricated with Vaseline was inserted through the anus into the colon so that the tip of the balloon was 7 cm inside the colorectum. The catheter to which the balloon was connected was fixed to the tail of the rat with Parafilm®, to avoid its expulsion. Sedation was reverted with Revertor® (atipamezole hydrochloride, 0.66 mg kg^{-1}, *ip*). After waking up (normally in <5 min), the rat behavior was recorded using a video camera (iPad; Apple, Madrid, Spain) located 30 cm

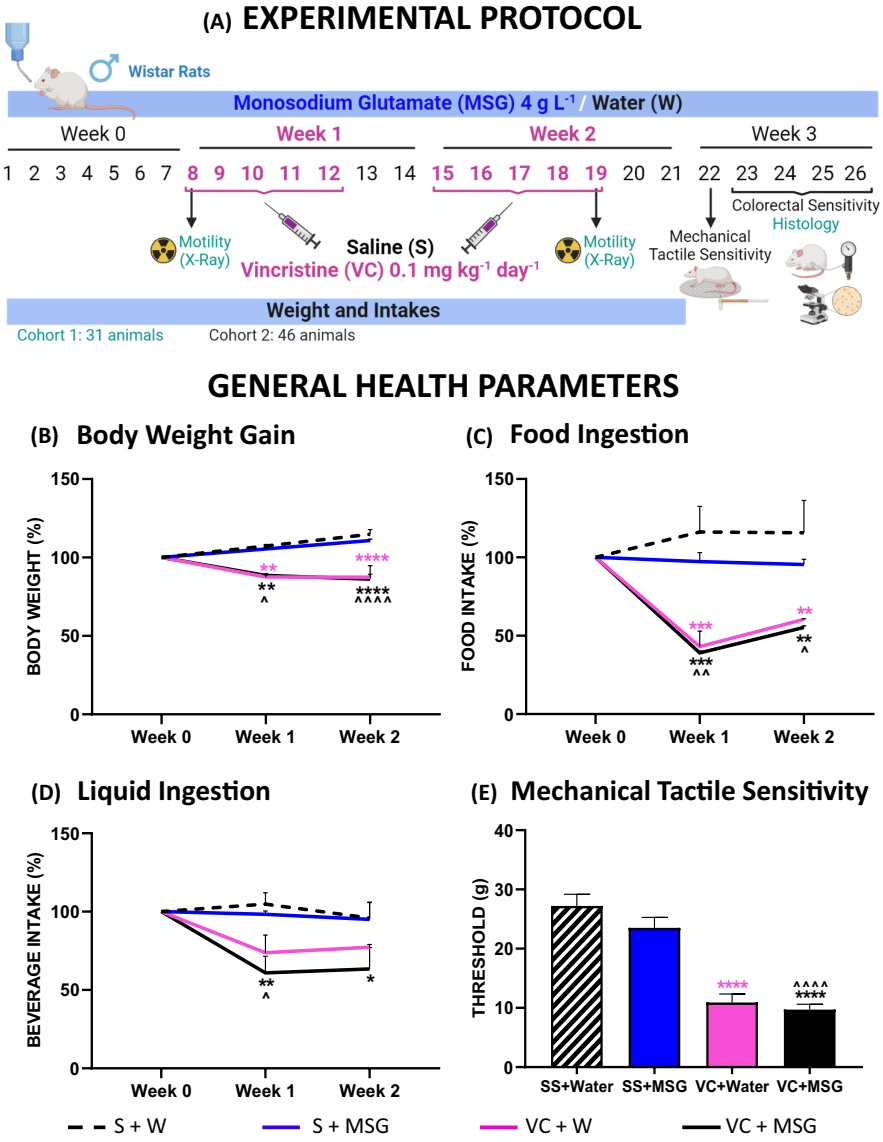


FIGURE 1 Experimental protocol and effect of vincristine and monosodium glutamate (MSG) on general health parameters in the rat. As shown in A (experimental protocol) rats were intraperitoneally administered with saline (2.5 mL kg^{-1}) or vincristine (0.1 mg kg^{-1}) daily for 10 days (Monday to Friday, weeks 1–2) and exposed or not to MSG (4 g L^{-1}) in drinking water from week 0 to week 3 in two different cohorts. Cohort 1: Gastrointestinal motility was measured by radiological methods after barium sulfate (2.5 mL, 2 g mL^{-1}) administration immediately after the first and the tenth intraperitoneal administration of the drug; 1 week after treatment (week 3) histological samples were embedded in paraffin for histological processing. Cohort 2: Mechanical tactile (Von Frey filaments) and colorectal sensitivity (tonic intracolonic stimulation using an inflatable balloon) was recorded on week 3. Body weight gain (B), food ingestion (C), and liquid ingestion (D) were measured from week 0 to week 2. In E, calibrated Von Frey filaments were applied to the hind paws and the withdrawal threshold was recorded on week 3 (cohort 2). Experimental groups were: S + W (saline + water: dotted line, $n = 8$–11), S + MSG (saline + MSG: blue line, $n = 7$–12), VC + W (vincristine + water: pink line, $n = 8$–11) or VC + MSG (vincristine + MSG: black line, $n = 8$–12). Data represent the mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$ versus S + W; $^{\wedge}p < 0.05$, $^{\wedge\wedge}p < 0.01$, $^{\wedge\wedge\wedge\wedge}p < 0.0001$ versus S + MSG (two-way ANOVA followed by Tukey's post hoc test in B, C, and D; Kruskal–Wallis test followed by Dunn's multiple comparison test in E).

below the recording cage floor. The first 5 min were only used to confirm the normal behavior of the rat after recovery from sedation and were discarded; thereafter, the pressure of the intracolonic balloon was gradually increased using a sphygmomanometer. Tonic stimulation was applied: the intracolonic pressure was increased from 0 to 75 mmHg, in steps of 15 mmHg every 5 min, and finally returned to 0 mmHg again (for each pressure value, a single stimulus was applied and maintained for 5 min).

2.6 | Histopathological analysis

Histological changes were analyzed after treatment finalization in cohort 1. After euthanasia, samples (2 cm long) were obtained from the terminal ileum and distal colon of 4–10 animals per experimental group, fixed in buffered 10% formalin and embedded in paraffin. Sections of 5 μm were stained with hematoxylin–eosin (H/E), Van Gieson's staining, and Periodic acid-Schiff (PAS) staining. They were studied under a

Zeiss Axioskop 2 microscope equipped with the image analysis software package AxioVision 4.6 to calculate the morphometric parameters. The analysis was made in triplicate in 5 random fields measured in 20–40× objective microphotographs per section and specimen.

Histological damage was evaluated in ileum sections stained with H/E using criteria adapted from Saccani et al. (2012).[27] A numerical score of 0–9 was assigned to each section considering the general loss of mucosal architecture (graded 0–3, absent to severe), the extent of inflammatory cell infiltrate (graded 0–3, absent to transmural), crypt abscess formation (0–1, absent or present), goblet cell depletion (0–1, absent or present) and muscular layer thickness (0–1, normal to reduced).

The thickness of both muscle layers was measured. The percentage of goblet cells per villi was counted. Submucosa thickness was also measured after Van Gieson's staining.

Histological damage was also evaluated in colonic sections stained with H/E using a semiquantitative score system[28] in which the following features were graded: damage of epithelium (0–3 normal to severe destruction), inflammatory cell infiltration (0–4 absent to severe), separation of muscular layer (0–2 normal to severe), and goblet cell depletion (0–4 no depletion to complete depletion). The total score for histological damage (0–13) was the sum of the different scores.

2.7 | Compounds and drugs

Barium sulfate (Barigraf®AD, Juste SAQF, Madrid, Spain) was suspended in tap water. Vincristine was purchased from Abcam (Cambridge, UK) and dissolved in saline. MSG was purchased from Manuel Riesgo (Madrid, Spain).

2.8 | Statistical analysis

Normality and homogeneity were assessed by Shapiro–Wilk comparisons (using Prisma 8.0.2, GraphPad Software Inc., La Jolla, CA, USA) and Levene's test (using IBM SPSS Statistics 27.0 statistical software, Chicago, USA), respectively. Graphs were obtained using Prisma 8.0.2 and data were presented as the mean values ± SEM. To compare the normally distributed data, one- or 2-way ANOVA was used, followed by post hoc Bonferroni or Tukey's multiple comparison tests; in the case of not normally distributed data, the Kruskal–Wallis test followed by Dunn's multiple comparison test was performed. Values of $p < 0.05$ were considered significantly different.

3 | RESULTS

3.1 | General health parameters

Body weight progressively increased over time in the S + W (control) and S + MSG groups (Figure 1B). Vincristine-treated animals (VC + W and VC + MSG) lost weight (around 13% in the first week and 22% in the second week, Figure 1B) with statistically significant differences from the control group. MSG did not significantly modify the effect of vincristine on body weight.

Throughout the study, the average daily food and fluid intake of the control group was about 23 g and 35 mL (per rat), respectively. Mean food intake in the S + MSG group was similar to that of the control group, but in both vincristine-treated groups, it decreased to 50% in the first week and 40% in the second week (Figure 1C), with statistically significant differences from the control group. The mean fluid intake of the S + MSG group was similar to the control group, but the fluid intake in the vincristine-treated group decreased by 30% without statistically significant differences compared to the control group (Figure 1D), and MSG could not reverse this (but even tended to worsen it), with statistically significant differences in weeks 1 and 2 compared to the control group.

One week after *ip* treatment finalization, the mechanical sensitivity threshold was approximately 23–27 g in control and MSG animals, but in vincristine-treated animals it was around 10 g, indicating the presence of mechanical allodynia, which was not prevented by MSG supplementation (Figure 1E).

3.2 | Gastrointestinal motor function

3.2.1 | Semiquantitative analysis

After the first administration of saline in the control group (S + W), gastric emptying was progressive and only a low amount of barium was still visible in the stomach 8 h after gavage (Figure 2A). The amount of barium in the small intestine reached its maximum in just 1 h, remained at a similar value until 2 h and then progressively decreased, and this part of the gut was practically empty of barium by 8 h (Figure 2B). Barium began to fill the caecum 2 h after intragastric administration, reached the maximum content at 4 h and then slightly reduced this value (Figure 2C). The colorectum (Figure 2D) started to have barium contents at 4 h and filled progressively until 8 h after intragastric administration. When this experiment was performed after the 10th administration (week 2) (Figure 3A–D), similar curves were obtained for the control group.

After the first administration of saline, MSG in drinking water did not cause any effect, except for a slight significant reduction of small intestinal filling at T1 and T2, and a slight nonsignificant acceleration of colorectal filling at 6 h compared to control animals (Figure 2D). However, after the last administration, MSG tended to slightly accelerate gastric emptying with no effect on the rest of the GI organs (Figure 3D).

After its first administration, vincristine delayed gastric emptying (Figure 2A) compared with the control group. Emptying of the small intestine (Figure 2B), and filling of the caecum (Figure 2C) and colorectum (Figure 2D) were not significantly different from the control group. The effect of vincristine on GI motility was more intense after the tenth administration (Figure 3A–D). Thus, compared with the control group, vincristine delayed gastric and small

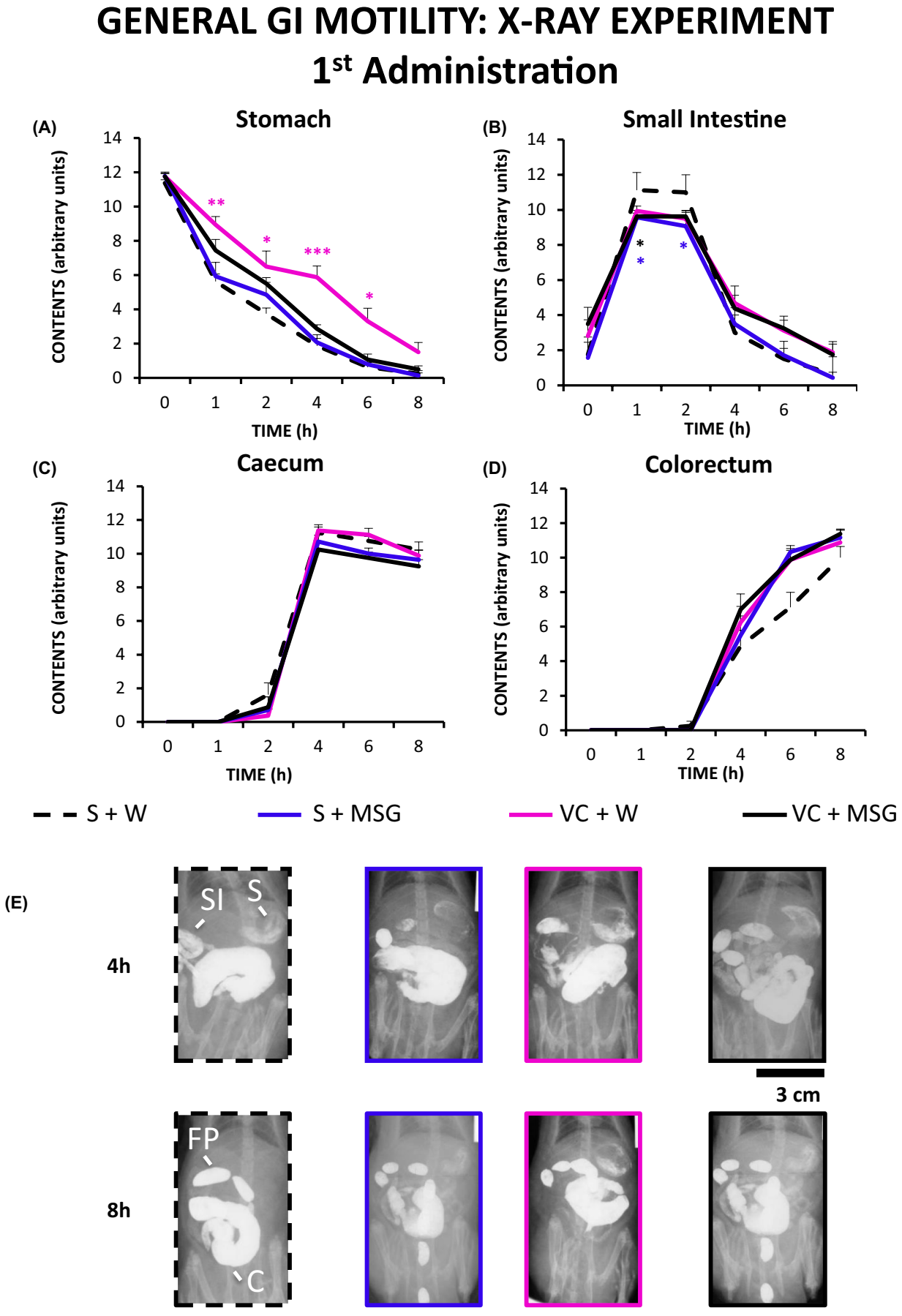


FIGURE 2 Effect of vincristine and monosodium glutamate (MSG) on gastrointestinal motility in the rat after vincristine first administration. The rats were intraperitoneally administered with saline (2.5 mL kg^{-1}) or vincristine (0.1 mg kg^{-1}) daily for 10 days (Monday to Friday, weeks 1–2) and exposed or not to MSG (4 g L^{-1}) in drinking water from week 0 to week 3. Gastrointestinal motility was measured by radiological methods (see text) in stomach (A), small intestine (B), caecum (C) and colorectum (D). Barium sulfate (2.5 mL, 2 g mL^{-1}) was intragastrically administered immediately after the first intraperitoneal administration and X-rays were taken 0, 1, 2, 4, 6, and 8 h after barium administration. Experimental groups were: S + W (saline + water: dotted line, $n = 8$), S + MSG (saline + MSG: blue line, $n = 7$), VC + W (vincristine + water: pink line, $n = 8$) or VC + MSG (vincristine + MSG: black line, $n = 8$). Data represent the mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ versus S + W (one-way ANOVA followed by Tukey's post hoc test or Kruskal–Wallis test followed by Dunn's multiple comparison test as appropriate). (E) Representative radiographic images obtained for the different experimental groups at 4 and 8 h after contrast administration. C, caecum; FP, fecal pellets (in colorectum); S, stomach; SI, small intestine. Scale bar: 3 cm.

intestine emptying, delayed caecum filling and tended to slightly accelerate colorectum filling (in a similar manner than after the first administration).

Interestingly, MSG improved vincristine-induced gastric dysmotility after the first administration (Figure 2A) but did not significantly modify the vincristine effect in the remaining GI regions (Figure 2B–D). After the last administration, MSG completely abolished vincristine-induced gastric dysmotility (Figure 3A). Although the emptying phase of the small intestine was not different from that obtained for the animals treated only with vincristine (Figure 3B), the filling phase of the caecum (Figure 3C) in VC + MSG animals was accelerated after the tenth administration with respect to the control and vincristine groups. On the contrary, VC + MSG tended to slightly accelerate colorectal filling (Figure 3D) but without statistically significant differences with any of the other groups.

3.2.2 | Quantitative analysis

Figures 4 and 5 show data from the morphometric and densitometric study of the stomach, caecum, and fecal pellets. These figures complement the information provided by the semiquantitative study, particularly regarding the maximum values of organ size and barium density within each organ. Thus, the maximum size of the stomach (immediately after barium intragastric administration, 0 h) was around 460–500 mm^2 in all groups in the first radiographic session, without statistically significant differences among them (Figure 4A). In contrast, vincristine tended to decrease the size of the stomach at a 0 h time point after the last administration, with statistically significant differences in the case of VC + MSG with respect to the control group. Gastric emptying was progressive in all groups, and curves representing it overlapped all along the study except at 8 h, where barium was still apparent in vincristine-treated animals after both first and tenth administrations, to a similar degree (Figure 4A,A′). The maximum density in the stomach was similar to the control after the 1st vincristine administration but slightly increased after the 10th administration, although the difference did not reach statistical significance (Figure 4B,B′). In accordance with the semiquantitative study, MSG improved this parameter at T4-T8 in both X-ray sessions (Figures 2 and 3).

The maximum size of the caecum, reached at 4–8 h after barium, was around 600–650 mm^2 in the first X-ray session for control and S + MSG groups (Figure 4C,C′), but after the 10th administration, S + MSG rats had a bigger caecum (around 730 mm^2) than the control group, with statistically significant differences at 6 h. In contrast, vincristine significantly reduced the caecum size to 460–500 mm^2 in the first X-ray session and to 250–330 mm^2 in the second X-ray session, after the 10th administration. Although MSG tended to increase the maximum caecum size in vincristine-treated animals, the differences were not statistically significant between the two groups of animals treated with the antitumoral drug (Figure 4C,C′). After the first administration, the maximum density of the caecum (Figure 4D,D′) overlapped in all groups, but after the last administration, vincristine increased the density of this organ and MSG reverted this effect.

The number of fecal pellets progressively increased from 2 to 8 h in all groups after both administrations. Compared to the control group, all groups had a larger number of fecal pellets within the colon with statistically significant differences at 4–8 h after the first administration and only at 8 h after the fifth (Figure 5A,A′). After the first administration, similar values of a maximum area of around 60–70 mm^2 were reached in all groups (Figure 5B). After the tenth administration, fecal pellets of the control and S + MSG treated groups were increased to around 80 mm^2. Repeated treatment with vincristine decreased the maximum size of fecal pellets to around 45–50 mm^2, with statistically significant differences compared with the control group, and the combination VC + MSG tended to increase the maximum size of fecal boluses, which reached around 60 mm^2 at 4 h after barium administration (Figure 5B′). The maximum density of the fecal pellets in all groups was the same compared to the control group after the first administration (Figure 5C), but, in contrast, after its tenth administration, vincristine, irrespective of MSG exposure, caused the fecal pellets to have a significantly higher maximum density than those of the control and S + MSG groups (Figure 5C′).

3.3 | Visceral mechanical sensitivity

The number of abdominal contractions per minute in the control group (Figure 6A) showed a progressive increase in response to a progressive increase of the intracolonic pressure, reaching a maximum of 8 contractions per minute at 75 mmHg. The number of abdominal contractions in the S + MSG and VC + W groups overlapped with the control group, suggesting that none of the two treatments alone was able to modify the response to the

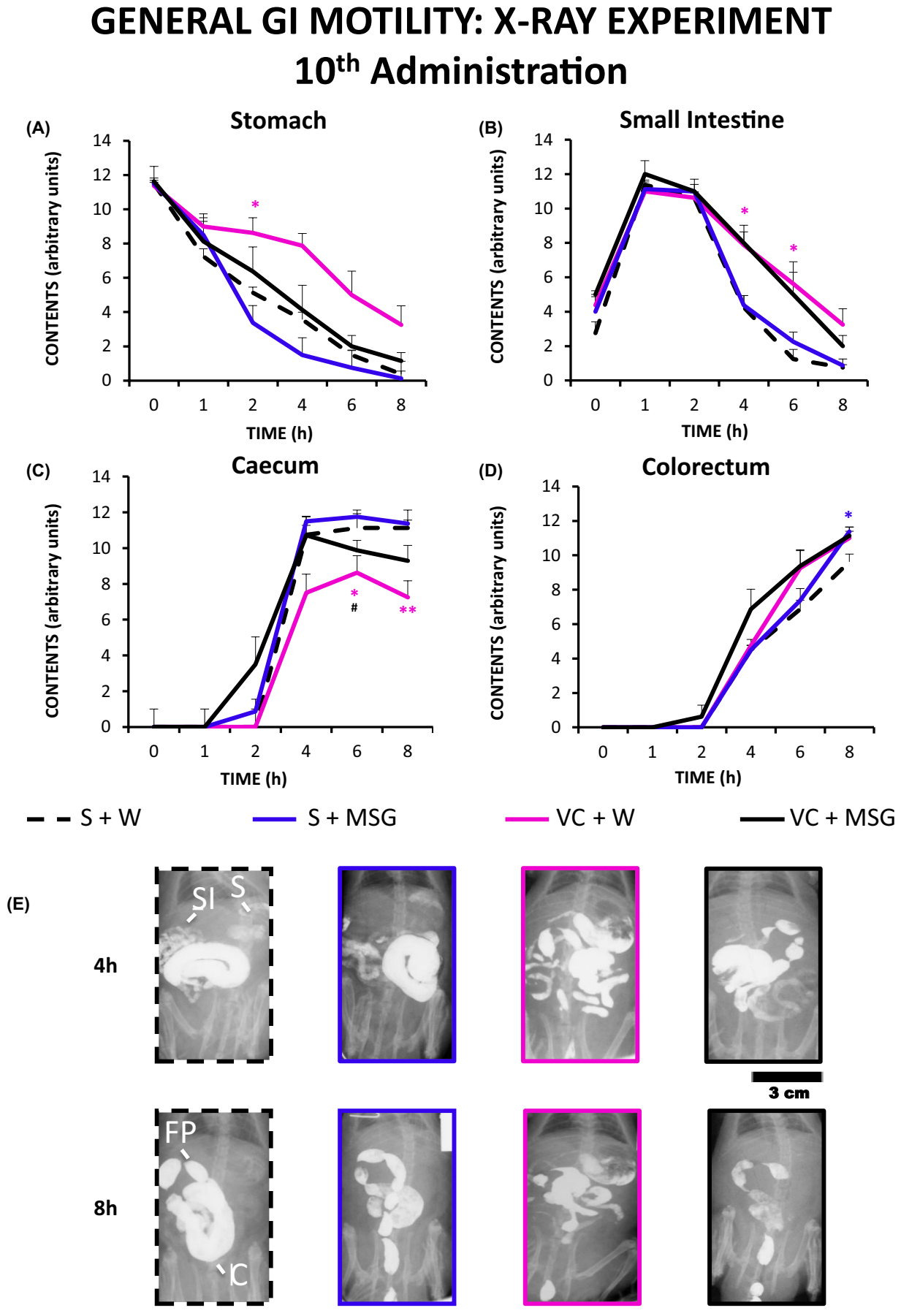


FIGURE 3 Effect of vincristine and monosodium glutamate (MSG) on gastrointestinal motility in the rat after vincristine tenth administration. The rats were intraperitoneally administered with saline (2.5 mL kg^{-1}) or vincristine (0.1 mg kg^{-1}) daily for 10 days (Monday to Friday, weeks 1–2) and exposed or not to MSG (4 g L^{-1}) in drinking water from week 0 to week 3. Gastrointestinal motility was measured by radiological methods (see text) in stomach (A), small intestine (B), caecum (C) and colorectum (D). Barium sulfate (2.5 mL, 2 g mL^{-1}) was intragastrically administered immediately after the tenth intraperitoneal administration and X-rays were taken 0, 1, 2, 4, 6, and 8 h after barium administration. Experimental groups were: S + W (saline + water: dotted line, $n = 8$), S + MSG (saline + MSG: blue line, $n = 7$), VC + W (vincristine + water: pink line, $n = 8$) or VC + MSG (vincristine + MSG: black line, $n = 8$). Data represent the mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$ versus S + W; $\#p < 0.05$ versus VC + W (one-way ANOVA followed by Tukey's post hoc test or Kruskal–Wallis test followed by Dunn's multiple comparison test as appropriate). (E) Representative radiographic images obtained for the different experimental groups at 4 and 8 h after contrast administration. C, caecum; FP, fecal pellets (in colorectum); S, stomach; SI, small intestine. Scale bar: 3 cm.

intracolonic mechanical stimulation. However, in the VC + MSG group, the response decreased to a maximum of 6 contractions per minute without statistically significant differences compared to the VC + W group. The duration of the contractions (Figure 6B) was around 2.7–3.5 s at all stimulation pressures in all experimental groups, without any statistically significant differences among them. Regarding the percentage of time in contraction (Figure 6C), in the control group, it progressively increased with pressure application, with a maximum of 44% at 75 mmHg. Again, in the groups exposed to only one treatment (MSG or vincristine), the response was similar to that of the control animals, without statistically significant differences. Remarkably, the combined treatment with VC + MSG decreased the percentage of time in contraction by a maximum of 33% at 75 mmHg. Statistically significant differences with control animals were reached at 0 and 60 mmHg, but there were no differences with the MSG-only treated group. The most interesting result was when this group was compared with that treated with vincristine only: in this case, statistically significant differences were found at 45 and 60 mmHg.

3.4 | Histological analysis

Figures 7 and 8 show the histological representative images and quantitative analysis of H/E-stained sections of the intestinal wall, respectively. No general damage was observed after MSG, vincristine, or their combination in the small intestine (Figures 7A–D and 8A) or the distal colon (Figures 7Q–T and 8B). However, in the small intestine, MSG, vincristine and the combined treatment affected several aspects of the intestinal wall. MSG increased the thickness of the submucosa (Figures 7I–L and 8E) and the muscle layers (Figures 7E–H and 8C,D), although this increase was not statistically significant in the case of the longitudinal muscle layer (Figure 8C). Vincristine also increased the thickness of the three components of the gut wall in a significant manner compared with control animals (Figure 8C–E). When MSG was used in vincristine-treated animals, the effects were similar to those obtained for animals treated with vincristine alone (Figure 8D). Finally, compared with control animals, the percentage of goblet cells per villi was not altered in the small intestine after vincristine and/or MSG administration (Figures 7M–P and 8F).

4 | DISCUSSION

This is the first study in which the effects of repeated vincristine on colorectal sensitivity have been evaluated in rats. As expected, vincristine produced alterations in general health parameters, GI motility, and somatic sensitivity. However, it did not alter colorectal sensitivity. On the other hand, we confirmed that the food additive MSG does not produce alterations in these parameters on its own. Although MSG could not improve the chemotherapy-induced alterations of general health parameters, somatic sensitivity, and gut wall structure, it partially prevented GI motor dysfunctions altered by vincristine treatment. Curiously, MSG reduced colorectal sensitivity when combined with vincristine.

4.1 | General health parameters

In accordance with other published studies and our previous results, chronic administration of vincristine reduced body weight gain and food intake.[4,29–31] It also reduced fluid intake, which was not observed in our previous study.[4] MSG, alone or combined with vincristine, did not modify body weight or food and liquid intake, in agreement with previous results in control rats[21] or rats treated with another antineoplastic drug, cisplatin.[19]

Chronic vincristine administration induced mechanical allodynia (a sign of peripheral neuropathy) when the Von Frey test was applied 1 week after treatment finalization, also in agreement with previous reports.[4,30,31] In this study, MSG could not prevent the development of neuropathic pain caused by vincristine. Although our result is in accordance with studies in humans,[32] it is in contrast with a study in rats by Boyle et al.,[20] probably due to methodological differences between both preclinical studies. Interestingly, MSG administration was neuroprotective in animals treated with cisplatin.[19,22,33] Platinum-based anticancer agents induce neuronal damage by oxidative stress,[34] and MSG may improve the endogenous antioxidant profile and glutathione (GSH) levels and reduce lipid peroxidation (malondialdehyde, MDA)[35] with an indirect effect on microtubules.[33] In contrast, vincristine damages the microtubule structure,[36] and it is possible that MSG could not counteract this effect.

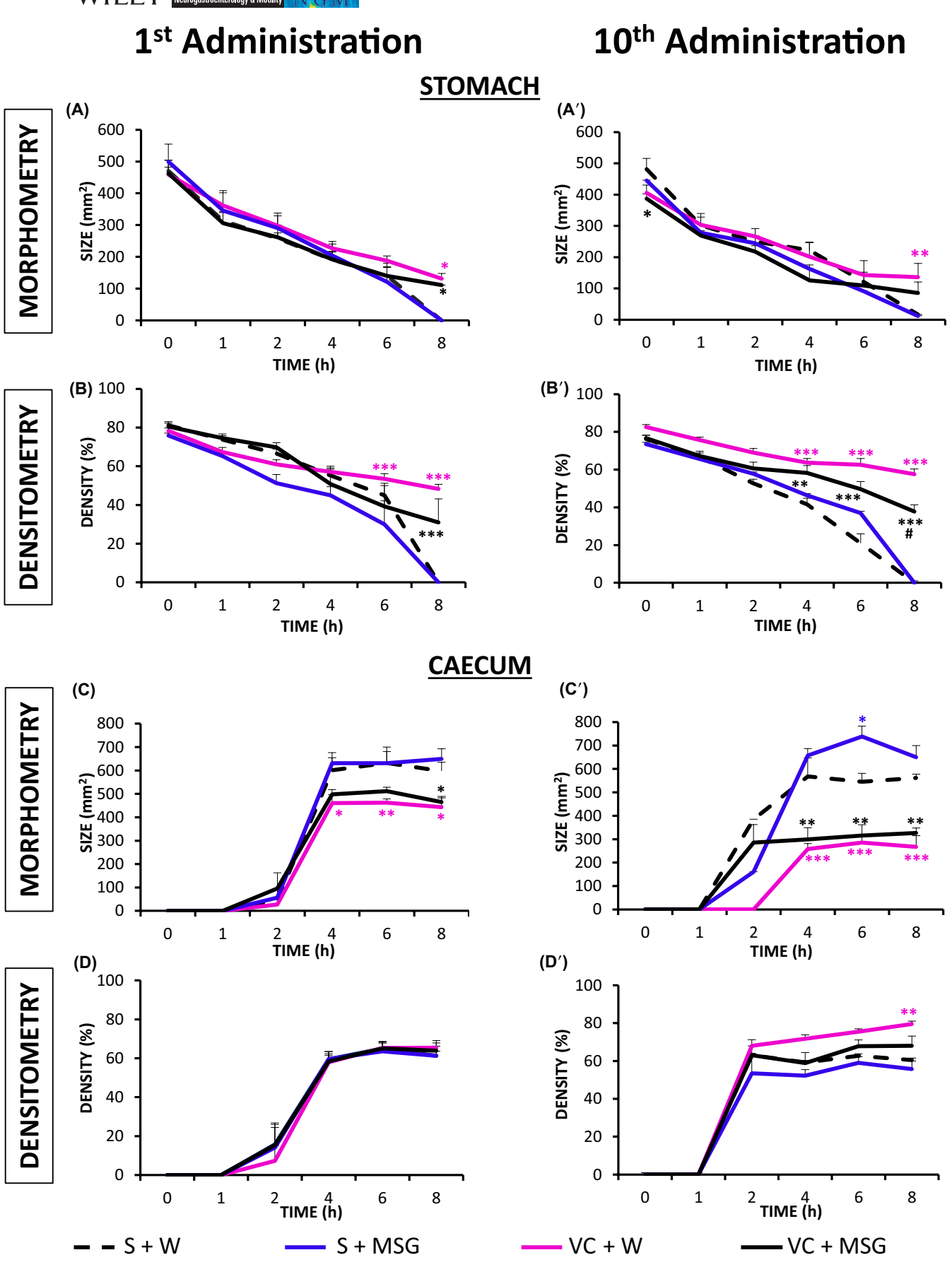


FIGURE 4 Morphometric and densitometric analysis of the effect of vincristine and monosodium glutamate (MSG) on the rat stomach and caecum. The rats were intraperitoneally administered with saline (2.5 mL kg^{-1}) or vincristine (0.1 mg kg^{-1}) daily for 10 days (Monday to Friday, weeks 1–2) and exposed or not to MSG (4 g L^{-1}) in drinking water from week 0 to week 3. Barium sulfate (2.5 mL, 2 g mL^{-1}) was intragastrically administered and X-rays were taken 0, 1, 2, 4, 6, and 8 h after contrast, immediately after the first (A, B, C, D) or the tenth (A′, B′, C′, D′) administration. Gastric size (A, A′), gastric density (B, B′), caecum size (C, C′), and caecum density (D, D′) were analyzed with the aid of an image processor (Image J). Experimental groups were: S + W (saline + water: dotted line, $n = 8$), S + MSG (saline + MSG: blue line, $n = 7$), VC + W (vincristine + water: pink line, $n = 8$) or VC + MSG (vincristine + MSG: black line, $n = 8$). Data represent the mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ versus S + W; $\#p < 0.05$ versus VC + W (two-way ANOVA followed by Tukey's *post hoc* test).

4.2 | Gastrointestinal motor function

In a previous study, we evaluated the effects of vincristine on general GI motor function[4] using the same dose and pattern of administration as here. Consistent with the mentioned study, vincristine reduced gastric emptying after the first administration, an effect that was also observed after the last administration but did not produce gastric distension. This effect on gastric emptying was similar to that of repeated cisplatin.[19] One of the most important differences between these drugs is that cisplatin is highly emetogenic in the clinic and, in rodents, not capable of vomiting, it causes gastric distension and pica (parameters correlated with the emetogenic potential of this drug).[4,19,23,37] In contrast, vincristine causes much less nausea and vomiting[38,39] and does not cause gastric distension[4] or pica[40] in rodents. In addition, in contrast to cisplatin,[19,23,41] the delay of gastric emptying induced by vincristine is not considered an early event (i.e., takes longer to be observed).[4,12,42,43]

Chemotherapy is thought to induce mainly acute GI motility disturbances through mucosal damage or the release of certain substances, such as serotonin[16,41,44] (which justifies the use of serotonin antagonists as antiemetics[41,45]) or even endocannabinoids, as suggested in a radiographic study of the acute effects of vincristine on GI transit.[12] Other symptoms, such as delayed emesis, may be associated with the release of substance P and the consequent activation of NK_1 receptors.[38,46] However, the persistence of symptoms after treatment with vincristine and other antitumoral drugs may be due to neurotoxicity affecting the innervation of the GI tract, including a direct effect on the vomiting center to induce gastric dysmotility[34] and the development of an enteric neuropathy.[4,29,34,47,48] This may also contribute to the aggravation of gastric dysmotility observed at the end of vincristine treatment.

MSG activates glutamate sensors in the stomach and intestine, stimulating GI tract motility.[21,49–51] In particular, oral MSG accelerates gastric emptying,[49,50] and we noted this effect after 3 weeks of MSG-only administration in this study. In our previous study, MSG in drinking water tended to improve cisplatin-induced gastric dysmotility after five administrations of the antitumoral drug.[19] Interestingly, MSG improved vincristine-induced gastric dysmotility already after the first drug administration. The improvement of gastric dysmotility by MSG can be due to vagal activation,[21,51] or maybe to the regeneration of gastric damage or to its neuroprotective effect.[19,20,22,33]

Vincristine also affects the motility of the small and large intestines.[4,6–9,11,12,29] Small intestinal motility was only affected after the last administration of the drug, with a delayed emptying phase that could be due to delayed gastric emptying and/or impaired intestinal contractility. Indeed, other authors reported altered myoelectric activity, increased tone, and spasmogenic actions in the small intestine, caused by this and other vinca alkaloids.[52,53] Our previous studies using acute or repeated vincristine administration demonstrated that this chemotherapeutic agent directly affected the small intestinal architecture[4,12] and the myenteric neuronal population.[4] Accordingly,[4] repeated treatment with vincristine increased the thickness of the different layers of the gut wall, namely, the submucosa, and the longitudinal and circular smooth muscle layers. The increase in submucosal thickness could be due to inflammation or an increase in mucosal permeability, while the increase in muscle thickness suggests that vincristine produces hypertrophy, perhaps underlying the increased myoelectrical activity. Although vincristine could induce inflammation in this tissue in the same way as other chemotherapeutic compounds,[34,54–56] no significant inflammation was observed, but this should be confirmed by specific studies of immunocyte proliferation.

Only minor changes in the small intestinal motility (a reduced plateau at 1–2 h, with no impact on the general pattern of filling and emptying of this organ), induced by MSG alone or in combination with vincristine, were detected radiographically. These results are consistent with our previous studies using MSG.[19,21] MSG alone did not modify the histological appearance of the ileum, and Cai et al.[57] did not find a deleterious effect in murine intestinal organoid growth patterns, although no studies on intestinal wall thickness have been conducted. In contrast with our previous results in cisplatin-treated animals,[19] MSG did not normalize the thickness of the submucosa in the small intestinal wall. Interestingly, Shang et al.[58] demonstrated that the release of glutamine from macrophages into the muscle microenvironment drives muscle regeneration. Some authors report that the glutamate/GABA-glutamine cycle is affected in pathological conditions,[59] but no one has investigated whether vincristine modifies this cycle. These results suggest that oral MSG administration might normalize intestinal muscle bulk by providing the glutamate needed for macrophages to release glutamine, although we did not observe any significant difference in the thickness of the muscle layers.

Vincristine delayed the filling of the caecum after the last administration of the drug, probably due to the delayed emptying of the small intestine, which is consistent with our previous results.[4] In addition, vincristine reduced the size of the caecum[4] after the first administration and this effect became more severe after repeated administration, which was accompanied by an increase in the density

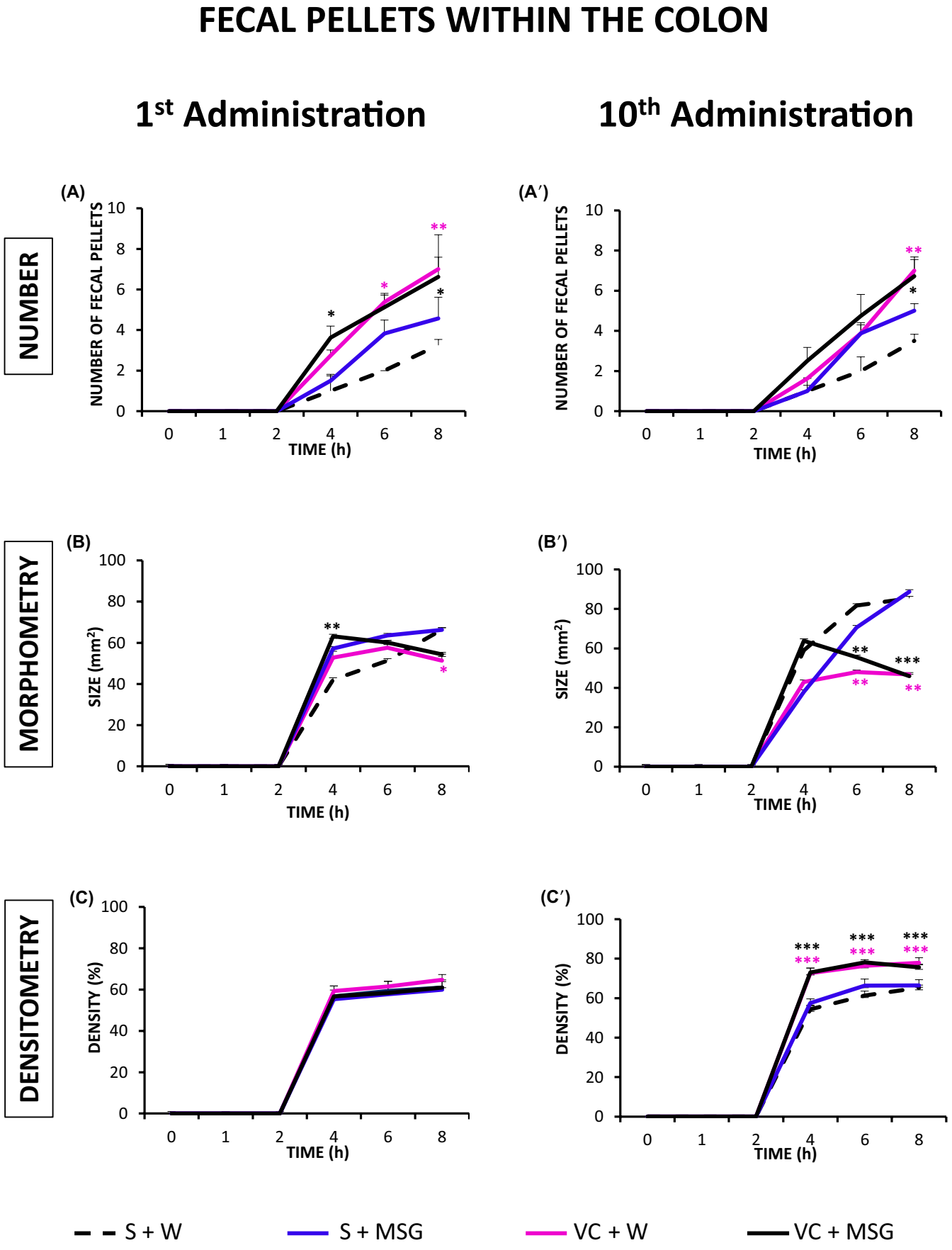


FIGURE 5 Quantitative, morphometric, and densitometric analysis of the effect of vincristine and monosodium glutamate (MSG) on the rat fecal pellets. The rats were intraperitoneally administered with saline (2.5 mL kg^{-1}) or vincristine (0.1 mg kg^{-1}) daily for 10 days (Monday to Friday, weeks 1–2) and exposed or not to MSG (4 g L^{-1}) in drinking water from week 0 to week 3. Barium sulfate (2.5 mL, 2 g mL^{-1}) was intragastrically administered and X-rays were taken 0, 1, 2, 4, 6, and 8 h after contrast, immediately after the first (A, B, C) or the tenth (A′, B′, C′) administration. Fecal pellets were counted (A, A′), and their size (B, B′) and density (C, C′) were analyzed with the aid of an image processor (Image J). Experimental groups were: S + W (saline + water: dotted line, $n = 8$), S + MSG (saline + MSG: blue line, $n = 7$), VC + W (vincristine + water: pink line, $n = 8$) or VC + MSG (vincristine + MSG: black line, $n = 8$). Data represent the mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ versus S + W (two-way ANOVA followed by Tukey's *post hoc* test).

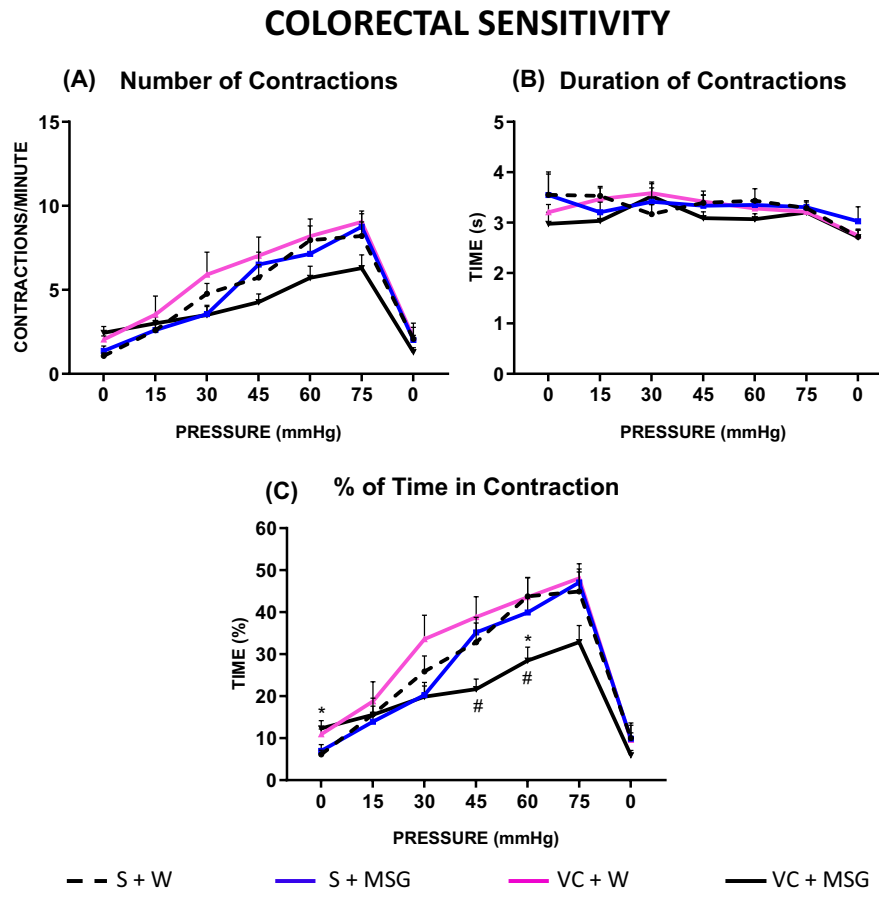


FIGURE 6 Effect of vincristine and monosodium glutamate (MSG) on colorectal sensitivity in the rat. The rats were intraperitoneally administered with saline (2.5 mL kg^{-1}) or vincristine (0.1 mg kg^{-1}) for 10 days (Monday to Friday, weeks 1–2) and exposed or not to MSG (4 g L^{-1}) in drinking water from week 0 to week 3. Colorectal sensitivity was recorded 1 week after treatment (week 3). Animals were subjected to tonic mechanical intracolonic stimulation using an inflatable balloon. Pressure was increased from 0 to 75 mmHg, in steps of 15 mmHg every 5 min, to finally return to 0 mmHg again; for each pressure value, a single stimulus was applied and maintained for 5 min. Number of contractions per minute (A), duration of contractions (B), and percentage of time contracting the abdomen (% of time in contraction) (C) were measured. Experimental groups were: S + W (saline + water: dotted line, $n = 11$), S + MSG (saline + MSG: blue line, $n = 12$), VC + W (vincristine + water: pink line, $n = 11$) or VC + MSG (vincristine + MSG: black line, $n = 12$). Data represent the mean ± SEM. $^{*}p < 0.05$ versus S + W; #$p < 0.05$ versus VC + W (one-way ANOVA followed by Tukey's post hoc test or Kruskal–Wallis test followed by Dunn's multiple comparison test as appropriate).

of the contents. These results suggest that, in animals treated with vincristine, less and drier contents arrive from upstream regions and the caecum is less distended, accordingly, or that, as in the small intestine, the thickness of the muscle layers is bigger, and the caecum can distend less. Curiously, in animals receiving both vincristine and MSG, caecum filling was significantly improved after the last administration, in the same way as with cisplatin.[19] The effect of MSG in this and the other GI organs may be due to the umami receptors, present along the GI tract,[60–63] although other mechanisms, such as its neuroprotective effect, cannot be ruled out.[19,20,22,33]

In contrast with our previous results,[4] vincristine apparently increased colorectum motility in both X-ray sessions. To clarify this result, we evaluated the number, size, and density of the fecal pellets within the colon. On the first day of treatment, vincristine

increased the number of fecal pellets. Although these pellets reached the same density, their maximum size was significantly smaller than in the control group. After the last administration of the drug, vincristine increased the number of fecal pellets too, but the pellets were much smaller and denser than those of the control group, which is consistent with the data shown in the caecum. This result suggests that since the first administration, vincristine-induced constipation with retention of feces inside the colon, which is in accordance with other studies using colonic propulsion measurements.[4,29] Constipation could be due to the smaller size of the fecal pellets, leading to a decrease in mechanical stimulation of the colon wall,[64] to an effect on the muscle ability to contract, associated with mechanical alterations of the muscle (as in the small intestine) or to the enteric neuropathy caused by

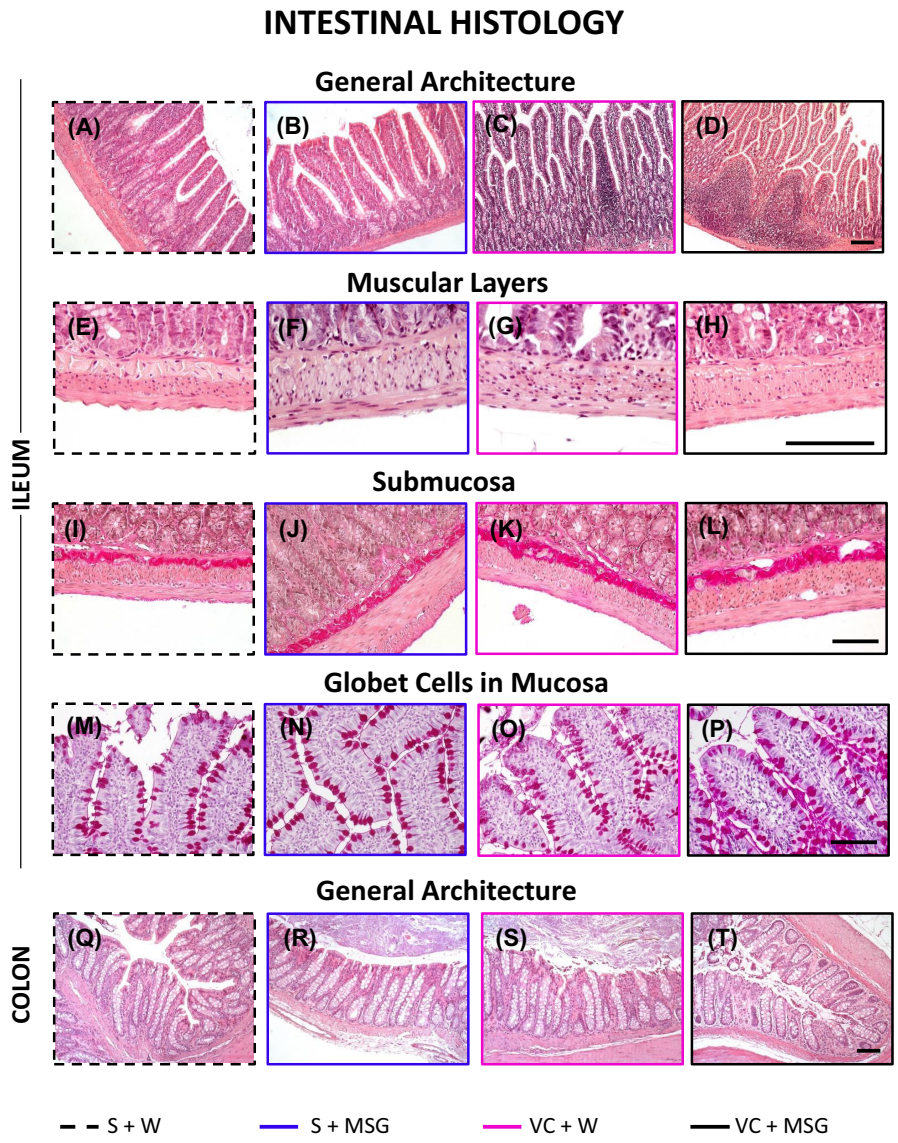
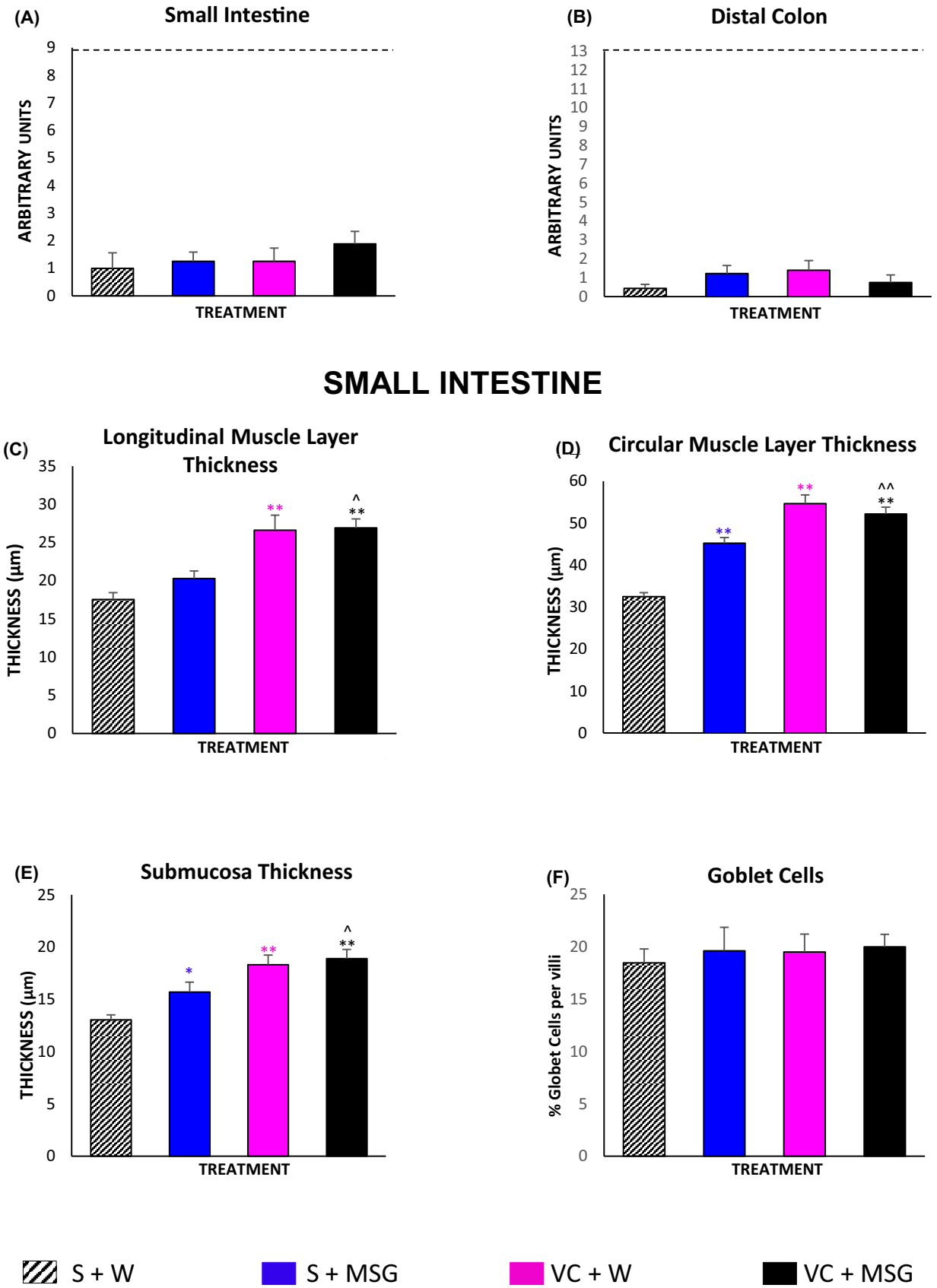


FIGURE 7 Effect of vincristine and monosodium glutamate (MSG) on the rat small intestine and distal colon: representative histological images. Rats were intraperitoneally administered with saline (2.5 mL kg^{-1}) or vincristine (0.1 mg kg^{-1}) for 10 days (Monday to Friday, weeks 1–2) and exposed or not to MSG (4 g L^{-1}) in drinking water from week 0 to week 3. Histological samples were embedded in paraffin, sectioned and stained with H/E, Van Gieson's staining, and PAS staining. A–D: general architecture of the ileum (H/E). E–H: muscular layers of the ileum (H/E). I–L: submucosa of the ileum (Van Gieson's trichrome staining). M–P: goblet cells in the ileal mucosa (PAS staining). Q–T: general architecture of the colon (H/E). Bar: 100 µm.

FIGURE 8 Effect of vincristine and monosodium glutamate (MSG) on the rat small intestine and distal colon wall: histological analysis. The rats were intraperitoneally administered with saline (2.5 mL kg^{-1}) or vincristine (0.1 mg kg^{-1}) daily for 10 days (Monday to Friday, weeks 1–2) and exposed or not to MSG (4 g L^{-1}) in drinking water from week 0 to week 3. At the end of the experiment (week 3), histological samples were embedded in paraffin, sectioned and stained with H/E, Van Gieson's staining, and PAS staining. Top panel: general damage of ileum (A) and distal colon (B). Dotted lines on the OY axis in each graph indicate the maximum achievable damage, according to the corresponding semiquantitative score (see text for further details). Bottom panel shows specific parameters measured in the ileal wall: longitudinal muscle layer thickness (C), circular muscle layer thickness (D), submucosa thickness (E), and percentage of goblet cells per villi (F). Experimental groups were: S + W (saline + water: black striped bar, $n = 6$–7), S + MSG (saline + MSG: blue bar, $n = 8$–10), VC + W (vincristine + water: pink bar, $n = 4$–5) or VC + MSG (vincristine + MSG: black bar, $n = 7$–9). Bars show mean values ± SEM. *$p < 0.05$, **$p < 0.01$ versus S + W; ^$p < 0.05$, ^^$p < 0.01$ versus S + MSG (one-way ANOVA followed by Bonferroni *post hoc* test in A, C, D, E and F; Kruskal–Wallis test followed by Dunn's multiple comparison test in B).

HISTOLOGICAL DAMAGE OF THE INTESTINE



this drug.[4,10] Indeed, vincristine treatment induces changes in the enteric nervous system[4] in the same way as other antineoplastic drugs, such as oxaliplatin,[65,66] cisplatin[48,55] and 5-fluorouracil.[56] Gao et al.[67] demonstrated that vincristine causes injury to colonic myenteric neurons by stimulation of M1-type macrophages through increased phosphorylation of p38-MAPK and ERK1/2, resulting in an increased expression of proinflammatory factors (IL6, IL-1β, and TNFα). Interestingly, Kawada et al.[68] demonstrated that, in patients treated with vinca alkaloids, it is more effective to use magnesium oxide plus lubiprostone (a chloride channel activator[69]) than a stimulant laxative. Stimulant laxatives are considered effective when myenteric neurons remain functional,[68,70] but in patients treated with vincristine the myenteric neurons are dysfunctional.[4,10] In addition, pellet retention inside the intestine could favor increased water absorption and lead to their increased density, which might be associated with changes in the expression of aquaporins, as happens after treatment with opioids.[71,72] Finally, other mechanisms may also be involved, such as endocannabinoid release, leading to activation of the CB_1 cannabinoid receptor and GI motility inhibition.[12] Whatever the case may be, although MSG can increase colonic motility,[21,60] in this study it did not improve colonic dysmotility induced by vincristine, despite being able to accelerate gastric emptying, suggesting a differential effect (and mechanism) in both organs in this model.

4.3 | Colorectal sensitivity

In this study in rats, vincristine did not produce any significant alteration in visceral sensitivity as assessed by tonic intracolonic mechanical stimulation, which is in contrast to other chemotherapeutic agents.[14–16] This may be due to the type of stimulation, as it has been shown in both, rats[25,73,74] and humans,[75] that phasic stimulation appears to be more powerful than tonic stimulation in producing abdominal contractions and might be capable of causing clearer effects.

López-Miranda et al.[21] suggested that MSG alone increased the contractility of the colon in response to an intracolonic balloon distention, and it could consequently produce colic pain. Under our experimental conditions, MSG alone did not increase visceral sensitivity compared to control rats but, interestingly, in combination with vincristine, it reduced the responses to intracolonic mechanical stimulation. This result may be due to an effect on colonic smooth muscle contraction since MSG decreased rat uterine visceral smooth muscle contractile activity.[76] In contrast to other reports,[19,21] Mondal et al.[76] suggested that MSG may inhibit the smooth muscle contraction frequency by stimulating nitric oxide synthase (NOS) and increasing the production of NO in the cell body of nitrergic neurons. Interestingly, nNOS-immunoreactive neurons are increased in the colonic myenteric plexus of rats treated with vincristine,[4] possibly leading to a decrease in the strength and frequency of colonic contractions in the presence of MSG. Alternatively, MSG may act at the level of the cerebellum or the hypothalamus, as suggested by some authors that demonstrated that the unilateral microinjection of L-glutamate into the cerebellar fastigial nucleus[77] or the hypothalamus paraventricular nucleus[78] attenuated chronic visceral hypersensitivity. This would need the gut mucosa and the blood–brain barrier being more permeant in vincristine-treated rats than in control animals, to allow enhanced access of MSG to the systemic circulation and the brain, respectively. This possibility requires further investigation.

5 | CONCLUDING REMARKS

This is the first study to assess the alterations caused by vincristine on visceral sensitivity and to evaluate the effect of dietary MSG on this and other effects induced by this antitumoral drug in the rat. Although vincristine or MSG alone did not significantly modify the response to mechanical intracolonic stimulation, the combined treatment seemed to decrease colonic sensitivity. In addition, MSG partially improved gastrointestinal dysmotility, but not peripheral neuropathy induced by vincristine. Remarkably, these effects were obtained even with a dose of MSG that was lowered by a vincristine-induced reduction in fluid intake, suggesting that MSG could be more efficacious if the complete dose was secured (i.e., by intragastric administration).

MSG can improve some dysfunctions caused by chemotherapy and could be used as an adjuvant to conventional treatments for these effects.

AUTHOR CONTRIBUTIONS

Raquel Abalo designed the study. Yolanda López-Tofiño, Gema Vera, Esperanza Herradón, Laura López-Gómez, and José A. Uranga performed the experiments, and Yolanda López-Tofiño, Francisca de Sosa, and José A. Uranga analyzed the data. Yolanda López-Tofiño, Gema Vera, José A. Uranga, and Raquel Abalo wrote the manuscript. Visitación López-Miranda and Kulmira Nurgali contributed essential intellectual input. Raquel Abalo obtained financial support. All authors reviewed and approved the final version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

No competing interests declared.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding authors upon reasonable request.

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