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Effects of a Bicarbonate–Alkaline Mineral Water on Digestive Motility in Experimental Models of Functional and Inflammatory Gastrointestinal Disorders

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SUMMARY

This study investigates the effects of Uliveto, a bicarbonate–alkaline mineral water, in experimental models of diarrhea, constipation and colitis. Rats were allowed to drink Uliveto or oligomineral water (control) for 30 days. Diarrhea and constipation were evoked by 16,16-dimethyl-prostaglandin E_2 ($dmPGE_2$) or loperamide, respectively. Colitis was induced by 2,4-dinitrobenzenesulfonic acid (DNBS) or acetic acid. Gastric emptying, small-intestinal and colonic transit were evaluated. $dmPGE_2$-induced diarrhea reduced gastric emptying and increased small-intestinal and colonic transit. In this setting, Uliveto water enhanced gastric emptying, and this effect was prevented by L-365,260 (gastrin receptor antagonist). Loperamide-induced constipation reduced gastric emptying, small-intestinal and colonic transit, and these effects were prevented by Uliveto water. L-365,260 counteracted the effects of Uliveto on gastric emptying, while alosetron (serotonin 5-HT_3 receptor antagonist) blunted the effect of Uliveto on colonic transit. Gastric emptying, small-intestinal and colonic transit were reduced in DNBS-induced colitis, and Uliveto water enhanced gastric emptying and normalized small-intestinal and colonic transit. Gastric emptying, small-intestinal and colonic transit were also reduced in acetic acid-induced colitis, and Uliveto increased both gastric emptying and small-intestinal transit. In conclusion, Uliveto water exerts beneficial effects on gastrointestinal motility in the presence of bowel motor dysfunctions. The effects of Uliveto water on gastric emptying depend on gastrin-mediated mechanisms, whereas the activation of serotonergic pathways accounts for the modulation of colonic functions.

Key words: Functional gastrointestinal disorders - Gastrin receptor - Inflammatory bowel disorders - Mineral water - Serotonin 5-HT_3 receptor

INTRODUCTION

Functional disorders of the digestive tract are characterized by a variety of symptoms that can significantly interfere with the quality of life, although not being associated with evident organic lesions. The most frequent functional digestive diseases include functional chronic dyspepsia, irritable bowel syndrome and idiopathic chronic constipation (1-3). At present, the mechanisms involved in the development of such disturbances are not completely understood. Nevertheless, it is thought that the development of functional digestive symptoms may depend on alterations of the nervous and hormonal mechanisms involved in the regulation of secretive, motor and sensory functions of the proximal and/or distal digestive tract (2-4).

Intestinal inflammatory diseases are also associated with alterations of digestive functions, such as motility and absorption/secretion processes, which may concur in the development of various symptoms, including diarrhea, changes in bowel habit, meteorism and abdominal pain (5). The pathophysiological mechanisms involved in the development of these symptoms are under active investigation, and it has been proposed that neuronal adaptive and plasticity processes, induced by inflammatory factors in the enteric nervous system, are responsible for digestive dysfunctions in the presence of inflammation (6, 7).

Pharmacological treatments of functional digestive disorders are aimed to interfere with the pathophysiological mechanisms responsible for symptoms. However, currently available drugs are often ineffective or allow only a partial remission of the disturbances. Previous evidence suggests that, in addition to pharmacological treatments, the use of mineral waters endowed with peculiar electrolyte compositions, together with modifications of lifestyle and alimentary behavior, could contribute favorably to the management of functional digestive syndromes (8, 9). It has been hypothesized that mineral waters can influence various digestive functions depending on their electrolyte content and, in support of

this view, previous studies have shown that mineral waters with high contents of bicarbonate and calcium can modulate the release of peptide hormones in the gastrointestinal tract (10-12). Accordingly, bicarbonate–alkaline waters promote gastric emptying in dyspeptic patients and evacuation frequency in patients with bowel constipation, and it has been proposed that prolonged and regular cycles of chrenotherapy with mineral waters could exert beneficial effects in patients with functional digestive disorders (8, 9, 11).

Although the available data suggest that chrenotherapy with mineral water might represent a valuable adjuvant tool for the treatment of functional digestive symptoms, convincing evidence concerning the pathophysiological basis underlying their favorable actions is lacking. In this respect, studies on preclinical models may help to clarify the mechanisms through which mineral waters can influence the regulatory pathways driving the secretive and motor functions of the gastrointestinal tract. Therefore, this study was designed to investigate the effects of a bicarbonate–alkaline mineral water (Uliveto®) on digestive motility in *in vivo* models of enteric motor dysfunction and bowel inflammation.

METHODS

Animals and experimental design

Male albino Wistar rats (220–250 g body weight) were used throughout the study. They were fed standard laboratory chow and tap water *ad libitum* and were not employed for at least one week after their delivery to the laboratory. The animals were housed, five in a cage, in temperature-controlled rooms on a 12-h light cycle at 22–24 °C and 50–60% humidity. Their care and handling were in accordance with the provisions of the European Community Council Directive 86–609, recognized and adopted by the Italian Government.

At the beginning of the experimental period, the animals were housed in single cages and allowed to drink either Uliveto water or a commercial oligomineral water (control water) *ad libitum* for 30 days. The respective chemico-physical properties of Uliveto and control oligomineral water are displayed in Table 1. During the period of exposure to the mineral waters, changes in both body weight and water intake were carefully monitored.

A first set of experiments was performed to examine the effects of Uliveto water on the gastrointestinal motor activity in experimental models of diarrhea and constipation, as reported below. In a second set of experiments, the effects of Uliveto water on the digestive motor activity were assessed in models of colitis induced by 2,4-dinitrobenzenesulfonic acid (DNBS) or acetic acid. In both series of experiments, gastrointestinal functions were evaluated by measuring gastric emptying as well as small-intestinal and colonic transit. In all experiments, animals were maintained in cages provided with wire-net bottoms to prevent coprophagy and were deprived of food 24 h before the beginning of evaluations. Free access to water was allowed until 1 h before starting the experimental procedures. The experimental designs are summarized in Figure 1.

TABLE 1. Chemico-physical properties of Uliveto water and oligomineral water (control) used in the present study.

	Uliveto water	Control water
HCO_3^-	650 mg l^{-1}	180 mg l^{-1}
Ca^{++}	169 mg l^{-1}	57 mg l^{-1}
Cl^-	75 mg l^{-1}	7.7 mg l^{-1}
F^-	1 mg l^{-1}	0.14 mg l^{-1}
Li^+	0.2 mg l^{-1}	0.1 mg l^{-1}
Mg^{++}	32.8 mg l^{-1}	3.7 mg l^{-1}
NO_3^-	6.5 mg l^{-1}	1.2 mg l^{-1}
K^+	8.1 mg l^{-1}	0.46 mg l^{-1}
Na^+	87 mg l^{-1}	4.6 mg l^{-1}
SO_4^{--}	111.4 mg l^{-1}	8.5 mg l^{-1}
SiO_2	7.3 mg l^{-1}	3.7 mg l^{-1}
CO_2	1265 mg l^{-1}	130 mg l^{-1}
pH (20 °C)	6.2	7.8
Dry residue (180 °C)	860 mg l^{-1}	175 mg l^{-1}

To examine the putative mechanisms involved in the effects of Uliveto water on gastrointestinal motility, gastric emptying and small-intestinal and colonic transit were evaluated, in separate set of experiments, in animals treated with L-365,260 (antagonist of gastrin CCK-2 receptors, 5 μmol/kg, i.p.) or alosetron (serotonin 5-HT_3 receptor antagonist, 1 mg/kg, i.p.) administered 15 min before the beginning of experiments.

Induction of diarrhea

Experimental diarrhea was induced by administration of 16,16-dimethyl-prostaglandin E_2 ($dmPGE_2$, 500 μg/kg, i.p.) as reported by Aikawa and Karasawa (13). Each animal was observed for 90 min after $dmPGE_2$ administration. The fecal output was scored by

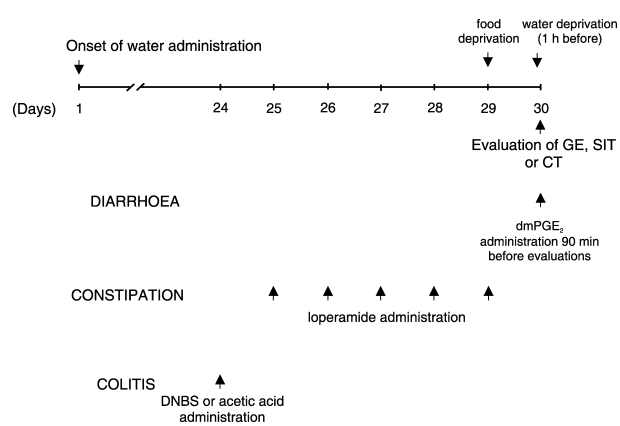

FIG. 1. Diagram showing the design and the course of experimental procedures on rat models of diarrhoea, constipation, and colitis. GE, gastric emptying; SIT, small intestinal transit; CT, colonic transit.

arbitrary criteria as follows: 0, hard stools or no stools; 1, normal stools; 2, wet but formed stools; 3, unformed stools; 4, severe watery diarrhea. The mean score was assumed as an index of diarrhea severity. At the end of 90-min observation, animals were subjected to evaluation of gastric emptying and small-intestinal and colonic transit, as reported below.

Induction of experimental constipation

Induction of experimental constipation was performed in accordance with the method reported by Shimotoyodome et al. (14). During the last five days of treatment with mineral water, animals were injected with loperamide (1.5 mg/kg, s.c., twice daily). At the end of the treatment, animals were subjected to evaluation of gastric emptying and small-intestinal and colonic transit, as reported below. The evacuated feces were collected, dried and weighed to estimate the water content of the stools, which is expressed as percentage.

Induction of colitis by DNBS

Experimental colitis was induced in accordance with the method previously described by Fornai et al. (15). Briefly, during anesthesia with diethyl ether, 30 mg of DNBS in 0.25 ml of 50% ethanol was administered intrarectally via a polyethylene PE-60 catheter inserted 8 cm proximal to the anus. Control rats received 0.25 ml of saline. Animals underwent subsequent experimental procedures 6 days after DNBS administration, to allow a full development of histologically evident colonic inflammation.

Induction of colitis by acetic acid

The induction of colitis was performed as previously described by Blandino et al. (16). During a short anesthesia with diethyl ether, animals were subjected to intracolonic instillation of 4% acetic acid (1 ml/rat, pH 2.3) by means of a polyethylene PE-60 catheter inserted 8 cm proximal to the anus. After 30 s, the acid solution was removed, and the colonic lumen was flushed with 1.5 ml of phosphate buffered saline. Animals were subjected to subsequent experimental procedures 6 days after treatment with acetic acid to allow a full development of histologically evident colonic inflammation.

Assessment of colitis

Animals were euthanized, and the severity of intestinal inflammation was evaluated macroscopically and histologically, in accordance with the criteria previously reported by Wallace and Keenan (17), as modified by Barbara et al. (18). The macroscopic criteria were based on the following: presence of adhesions between colon and other intraabdominal organs, consistency of colonic fecal material (indirect marker of diarrhea), thickening of colonic wall, presence and extension of hyperemia and macroscopic mucosal damage (assessed with the aid of a ruler). Microscopic criteria were assessed by light microscopy on hematoxylin- and eosin-stained sections obtained from whole-gut specimens taken from a region of inflamed colon immediately adjacent to the gross macroscopic damage and fixed in cold 4% neutral formalin diluted in phosphate buffered saline (PBS). Histological criteria included degree of mucosal architecture changes, cellular infiltration, external muscle thickening, presence of crypt abscess and goblet cell depletion. All parameters of macroscopic and histological damage were recorded and scored for each rat by two observers blinded to the treatment.

Evaluation of gastric emptying

Gastric emptying was assessed as previously described (11). A solution of red phenol (0.6 g/l) was used as liquid meal. Three milliliters of prewarmed (37 °C) test meal were instilled directly into the gastric lumen by a polyethylene orogastric catheter. Fifteen minutes later, the rats were sacrificed, their stomachs were rapidly removed, and the luminal content was collected by gravity into graduated tubes. The stomach was then rinsed with 3 ml of saline solution (154 mM NaCl), and the resulting washing solution was added to the recovered gastric content. The phenol red concentration in the mixture was then measured by a spectrophotometer at 560 nm, following the addition of 0.1 N NaOH, and the total amount of the dye marker recovered from the gastric lumen was calculated. Gastric emptying was expressed as the volume of dye-marker solution emptied over a period of 15 min and expressed as milliliters per 15 min.

Evaluation of small-intestinal transit

Evaluation of small-intestinal transit was performed as reported by Blandizzi et al. (7). Two milliliters of a charcoal suspension (10% charcoal in 12.5% arabic gum) was injected into the gastric lumen by a polyethylene orogastric catheter. After 25 min, animals were killed by cervical dislocation, and the small intestine was quickly removed avoiding stretching. The small-intestinal transit was then evaluated by comparing the distance travelled by the charcoal meal from the pyloric sphincter with the total length of the small intestine from the pyloric sphincter to the ileocecal junction.

Evaluation of colonic transit

Colonic transit was assessed in accordance with the method reported by Negri et al. (19). At the time of the experiment, animals were subjected to a short anesthesia with diethylether. The abdomen was then opened through laparotomy, 1 ml of blue Evans solution (5% in 1.5% methocel) was injected into the proximal colon, and the incision was quickly sutured. Two hours later, animals were sacrificed and the colon was removed, avoiding stretching. Colonic transit was assessed by

comparing the distance travelled by the dye marker with the total length of the colon from the cecum to the anus.

Determination of tissue myeloperoxidase

Myeloperoxidase (MPO) levels in colonic tissues were determined as previously reported by Pacheco et al. (20) and assumed as a quantitative index to estimate the degree of mucosal infiltration by polymorphonuclear cells. Briefly, colonic samples (300 mg) were homogenized three times (30 s each) at 4 °C with a polytron homogenizer (Cole Parmer Homogenizer, Vernon Hills, Illinois, USA) in 1 ml of ice-cold 50 mM phosphate buffer (pH 6.0) containing 0.5% of hexadecyltrimethylammonium bromide to prevent the pseudoperoxidase activity of hemoglobin as well as to solubilize membrane-bound MPO. The homogenate was sonicated for 10 s, frozen–thawed three times and spun by centrifugation for 20 min at 18,000*g*. The supernatant was then recovered and used for the determination of MPO using a kit for enzyme-linked immunosorbent assay (Bioxytech, Oxis International, Portland, Oregon, USA). The results were expressed as nanograms of MPO per 100 mg of tissue.

Drugs and reagents

Loperamide hydrochloride, 16,16-dimethylprostaglandin E_2, 2,4-dinitrobenzenesulfonic acid, hexadecyltrimethylammonium bromide (Sigma chemicals, St Louis, Missouri, USA) alosetron and L-365,260 (kindly provided by Merck Research Laboratories, Rahway, New Jersey, USA) were used. Loperamide and 16,16-dimethylprostaglandin E_2 were dissolved and administered in sterile 0.9% NaCl. Alosetron and L-365,260 were dissolved in dimethylsulphoxide and administered upon dilution in sterile 0.9% NaCl.

Statistical analysis

Results are given as mean ± standard error of mean (SEM). The significance of differences was evaluated by one-way ANOVA followed by Student-Newman-Keuls test. In all cases, *p*-values lower than 0.05 were considered significant, and *n* indicates the number of animals.

RESULTS

Animals with diarrhea

In normal animals treated with control or Uliveto water, the diarrhea severity index accounted for 1.30 ± 0.2 and 1.45 ± 0.3, respectively. The administration of $dmPGE_2$ induced a significant increase in the diarrhea index with similar values in both groups of animals (3.2 ± 0.7 and 3.9 ± 0.5, respectively) (Fig. 2). In normal rats, Uliveto water intake was associated with a significant increase in gastric emptying (+30% *vs.* control water; $p < 0.05$). The enhancing effect of Uliveto water

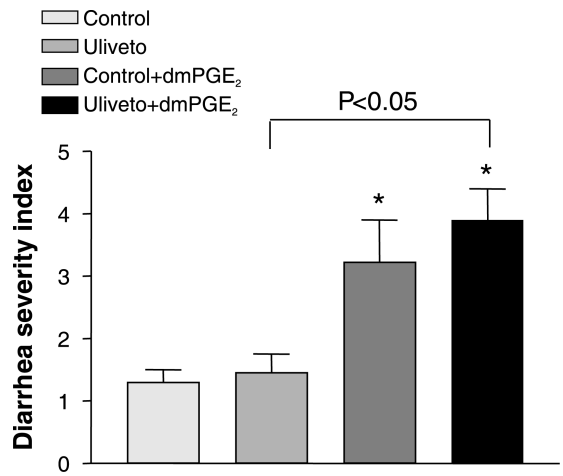

FIG. 2. Effects of control or Uliveto water on the severity of diarrhea under normal conditions and in animals treated with 16,16-dimethylprostaglandin E_2 ($dmPGE_2$). Each column represents the mean ± SEM obtained from 8–10 animals. *$P < 0.05$, significant difference vs. control water group.

no longer occurred in animals pretreated with L-365,260, whereas in animals treated with control water the CCK_2 receptor antagonist was without effect (Fig. 3A). In animals with $dmPGE_2$-induced diarrhea, gastric emptying was significantly decreased. Likewise, in animals treated with Uliveto water, $dmPGE_2$ administration was associated with a significant reduction of gastric emptying (−28% *vs.* Uliveto alone; $p < 0.05$) (Fig. 3A). However, when compared with control water, Uliveto water induced a significant increase in gastric emptying (+34% *vs.* control; $p < 0.05$). In animals treated with control water, L-365,260 did not modify gastric emptying, whereas the enhancing action exerted by Uliveto water was prevented by the CCK_2 receptor antagonist (Fig. 3A). Small-intestinal transit in normal animals exposed to control water did not differ significantly from that observed in the Uliveto water group (63.6 ± 6.4% and 71.5 ± 5.3%, respectively). The induction of diarrhea generated a significant and similar increment of small-intestinal transit in both groups (Fig. 3B). In normal rats receiving control water, colonic transit accounted for 61.5 ± 4.3%. Uliveto water determined a significant increase in colonic transit. The induction of diarrhea was associated with a significant increase in the colonic transit of animals exposed to control water or Uliveto water (Fig. 3C).

Animals with constipation

In normal rats drinking control water, fecal excretion accounted for 3.7 ± 0.4 g/die, while in the Uliveto water group a significant increase in this parameter was recorded (+38% *vs.* control; $p < 0.05$). The induction of constipation by loperamide in animals exposed to control water caused a significant decrease in fecal excretion. In this setting, fecal excretion in the Uliveto water

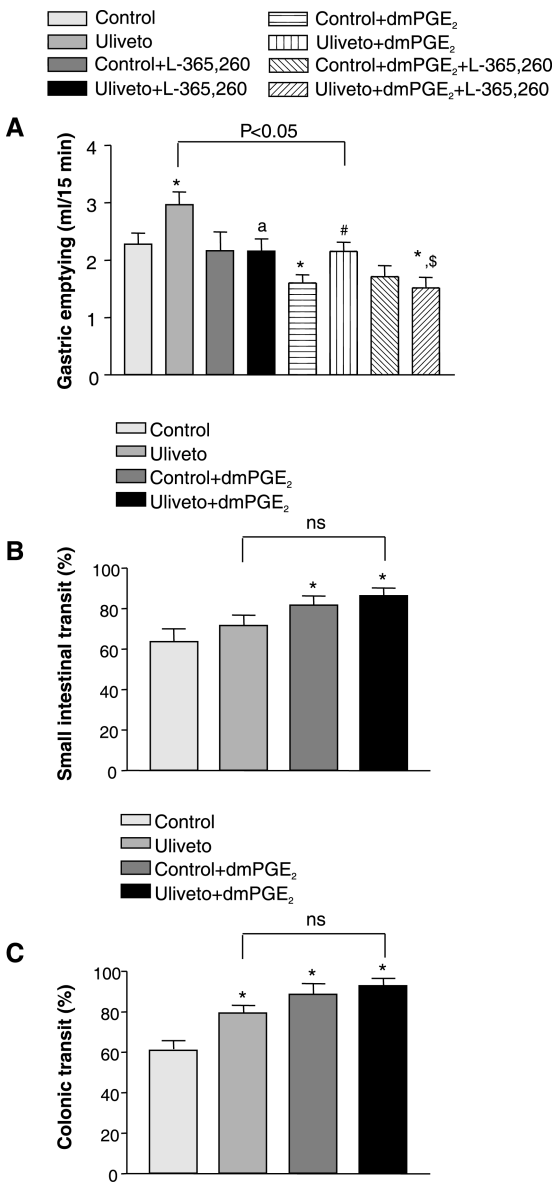

FIG. 3. Effects of control or Uliveto water, either alone or in combination with L-365,260, on gastric emptying (A) in normal rats or in animals with experimental diarrhea induced by 16,16-dimethylprostaglandin E_2 ($dmPGE_2$). Effects of control or Uliveto water on small intestinal transit (B) or colonic transit (C) in normal rats or in animals with experimental diarrhea. Each column represents the mean ± SEM value obtained from 8–10 animals. $^{*}P < 0.05$, significant difference vs. control water; $^{\#}P < 0.05$, significant difference vs. control water+$dmPGE_2$; $^{\$}P < 0.05$, significant difference vs. Uliveto water+$dmPGE_2$; $^{a}P < 0.05$, significant difference vs. Uliveto water.

group was significantly higher than in the control water group (Fig. 4A). The administration of loperamide to animals receiving Uliveto water induced a significant reduction of fecal excretion (−37% *vs*. Uliveto alone; $p < 0.05$). Pretreatment of normal or constipated animals with alosetron prevented the enhancing actions of Uliveto water, whereas alosetron did not modify fecal excretion in animals treated with control water (Fig. 4A). The fecal water content was increased in animals receiving Uliveto water, either in the absence or in the presence of experimental constipation, and the enhancing effects of Uliveto water were completely prevented by alosetron (Fig. 4B). Under these conditions, alosetron did not affect fecal water content in animals treated with control water (Fig. 4B). In normal rats, Uliveto water promoted a significant increase in gastric emptying (+30% *vs*. control; $p < 0.05$). The enhancing effect of Uliveto water was prevented by L-365,260, whereas in the control water group L-365,260 was without effects (Fig. 5A). In control water animals with experimental constipation, gastric emptying was significantly reduced. Under these conditions, Uliveto water induced a significant increase in gastric emptying, and this enhancing action was counteracted by L-365,260 (Fig. 5A). In animals receiving Uliveto water, the administration of loperamide elicited a significant reduction in gastric emptying (−57% *vs*. Uliveto alone; $p < 0.05$). In the presence of experimental constipation, L-365,260 did not affect gastric emptying in animals

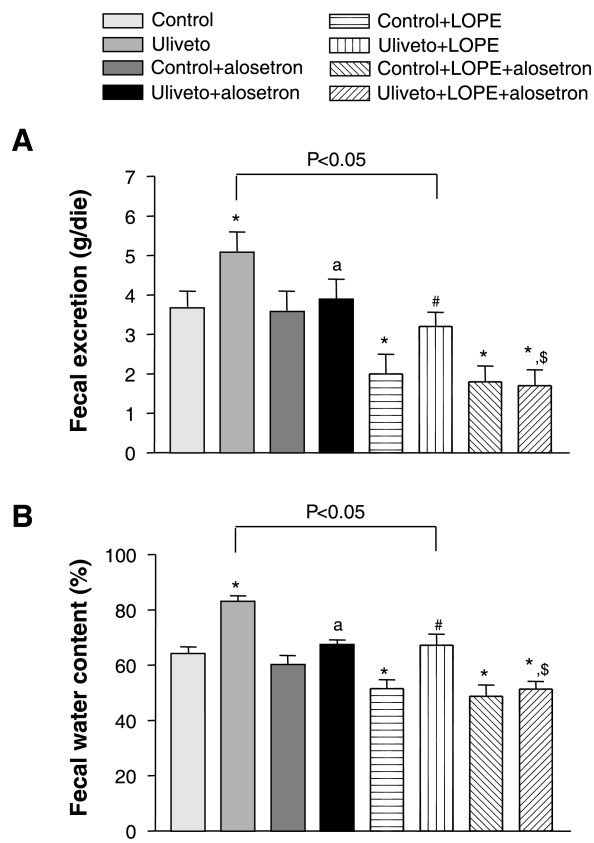

FIG. 4. Effects of control or Uliveto water, either alone or in combination with alosetron, on fecal excretion (A) and fecal water content (B) in normal animals or in rats with experimental constipation induced by loperamide (LOPE). Each column represents the mean ± SEM obtained from 8–10 animals. $^{*}P < 0.05$, significant difference vs. control water; $^{\#}P < 0.05$, significant difference vs. control water+LOPE; $^{\$}P < 0.05$, significant difference vs. Uliveto water+LOPE; $^{a}P < 0.05$, significant difference vs. Uliveto water.

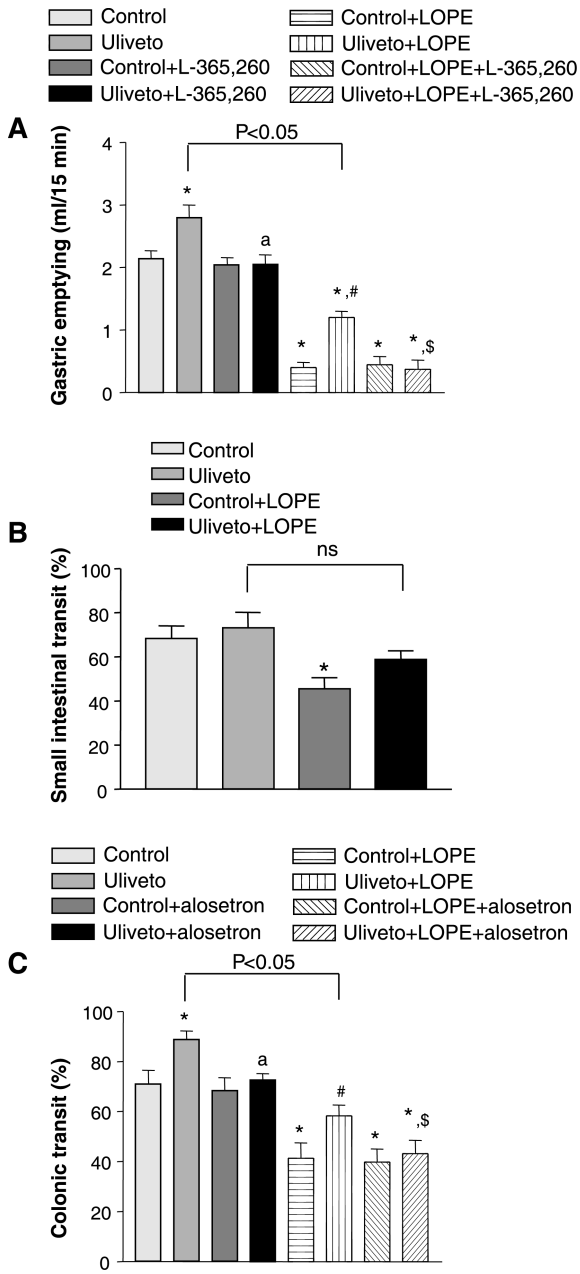

FIG. 5. (A) Effects of control or Uliveto water, either alone or in combination with L-365,260, on gastric emptying in normal animals or in those with experimental constipation induced by loperamide (LOPE). (B) Effects of control or Uliveto water on small intestinal transit in normal rats or in animals with experimental constipation. (C) Effects of control or Uliveto water, either alone or in combination with alosetron, on colonic transit in normal rats as well as in animals with loperamide-induced constipation. Each column represents the mean ± SEM obtained from 8–10 animals. $^{*}P < 0.05$, significant difference vs. control water; $^{\#}P < 0.05$, significant difference vs. control water+LOPE; $^{\$}P < 0.05$, significant difference vs. Uliveto water+LOPE; $^{a}P < 0.05$, significant difference vs. Uliveto water.

treated with control water (Fig. 5A). Small-intestinal transit in normal animals exposed to Uliveto water did not differ significantly from that measured in control water animals. The induction of constipation by loperamide significantly delayed small-intestinal transit in the control water group, whereas this parameter was not affected significantly in the Uliveto water group (Fig. 5B). In rats drinking Uliveto water, the colonic transit was significantly increased, either in the absence or in the presence of experimental constipation. The enhancing effects exerted by Uliveto water were completely prevented by alosetron (Fig. 5C). Administration of loperamide to animals receiving Uliveto water induced a significant delay in colonic transit (−34% *vs.* Uliveto alone; $p < 0.05$). Colonic transit was not affected by alosetron in animals drinking control water, either in the absence or in the presence of experimental constipation (Fig. 5C).

Assessment of colitis

Six days after DNBS or acetic acid treatment, the distal colon of animals treated with control water appeared thickened and ulcerated with evident areas of transmural inflammation. Adhesions were often present, and the bowel was occasionally dilated. Histologically, colitis was evident as granulocyte infiltration extending throughout the mucosa and submucosa, sometimes involving the muscular layer. Both macroscopic and microscopic damage scores were significantly increased in animals with colitis exposed to control water, and they were not affected by treatment with Uliveto water (Table 2).

Evaluation of tissue myeloperoxidase

In normal animals treated with control water, colonic myeloperoxidase levels accounted for 3.75 ± 1.4 ng/100 mg. In colonic specimens obtained from normal animals treated with Uliveto water, myeloperoxidase levels were similar to those observed in the control water group (3.21 ± 1.7 ng/100 mg) (Table 2). The induction of colitis with DNBS or acetic acid induced a marked increase in colonic MPO levels, both in animals treated with control water and those receiving Uliveto water. No significant difference was observed between the control water and Uliveto water groups (Table 2).

Animals with colitis induced by DNBS

In normal rats, Uliveto water significantly increased gastric emptying (+27% *vs.* control; $p < 0.05$) (Fig. 6A). In control water animals, induction of colitis by DNBS caused a reduction in gastric emptying. In the presence of colitis, the intake of Uliveto water was associated with a significant increment of gastric emptying in comparison with control water (Fig. 6A). However, in inflamed animals treated with Uliveto water, gastric emptying did not vary significantly when compared with normal animals exposed to Uliveto water. Under normal conditions, the small-intestinal transit was not affected by Uliveto water. In the presence of DNBS-induced colitis, the small-intestinal transit was significantly

TABLE 2. Effects of control or Uliveto water on experimental colitis.

	Control	Uliveto	Control+DNBS	Uliveto+DNBS	Control+acetic acid	Uliveto+acetic acid
Macroscopic damage	1.56 ± 0.27	1.41 ± 0.32	11.08 ± 1.61*	10.76 ± 1.06*#	4.47 ± 1.30*	4.21 ± 1.45*#
Microscopic damage	1.19 ± 0.31	1.09 ± 0.46	6.88 ± 1.74*	7.21 ± 1.49*#	4.11 ± 1.23*	4.78 ± 1.23*#
Myeloperoxidase (ng/100 mg)	3.75 ± 1.4	3.21 ± 1.7	16.9 ± 3.9*	18.4 ± 4.2*#	15.1 ± 2.9*	16.3 ± 4.4*#

Macroscopic and microscopic scores of colonic damage and colonic myeloperoxidase levels were estimated under normal conditions or after induction of experimental colitis with DNBS or acetic acid. Each value represents the mean obtained from 8 to 10 animals ± SEM.
*Significant difference from values obtained in control water group is $p < 0.05$.
#Significant difference from values obtained in Uliveto water group is $p < 0.05$.

reduced in control water but not the Uliveto water group (Fig. 6B). In normal animals, colonic transit was significantly increased in the Uliveto water group. In rats with DNBS-induced colitis, the colonic transit was significantly decreased in control water but not the Uliveto water group. The induction of colitis in animals treated with Uliveto water was associated with a significant reduction in colonic transit (−31% *vs*. Uliveto alone; $p < 0.05$) (Fig. 6C).

Animals with colitis induced by acetic acid

In normal rats, Uliveto water significantly increased gastric emptying (+28% *vs*. control; $p < 0.05$) (Fig. 7A). In animals with colitis induced by acetic acid, gastric emptying was reduced (−36% *vs*. control; $p < 0.05$). In this setting, Uliveto water significantly enhanced gastric emptying, when compared with that in animals exposed to control water. In animals receiving Uliveto water, the induction of experimental colitis did not modify

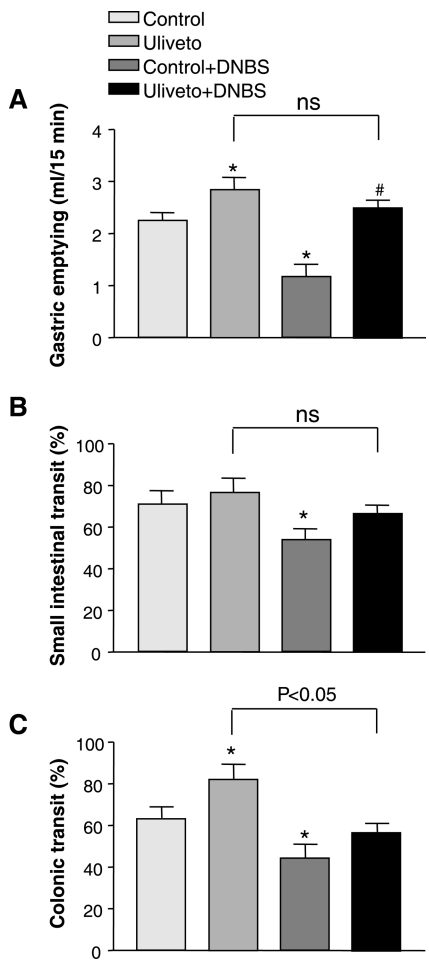

FIG. 6. Effects of control or Uliveto water on gastric emptying (A), small intestinal transit (B), or colonic transit (C) in normal animals or in rats with colitis induced by 2,4-dinitrobenzenesulfonic acid (DNBS). Each column represents the mean ± SEM obtained from 8–10 animals. *$P < 0.05$, significant difference vs. control water; #$P < 0.05$, significant difference vs. control water+DNBS.

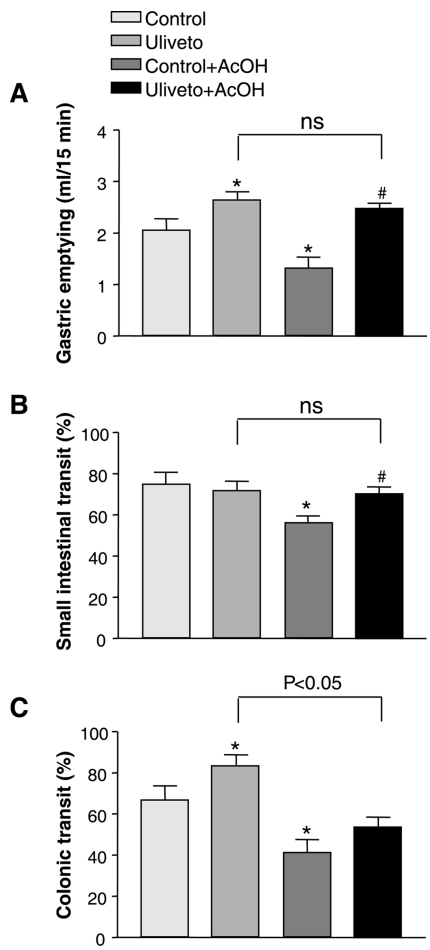

FIG. 7. Effects of control or Uliveto water on gastric emptying (A), small intestinal transit (B), or colonic transit (C) in normal animals or in rats with colitis induced by acetic acid (AcOH). Each column represents the mean ± SEM obtained from 8–10 animals. *$P < 0.05$, significant difference vs. control water; #$P < 0.05$, significant difference vs. control water+AcOH.

gastric emptying as compared to animals treated with Uliveto water alone (Fig. 7A). The induction of colitis by acetic acid elicited a significant reduction in the small-intestinal transit of animals exposed to control water. In the Uliveto water group, the transit delay associated with acetic acid-induced colitis no longer occurred. Treatment of animals receiving Uliveto water with acetic acid did not exert any significant effect on small-intestinal transit upon comparison with Uliveto water alone (Fig. 7B). Colonic transit was significantly decreased in the presence of experimental colitis, and Uliveto water did not exert significant influences. In animals receiving Uliveto water, the induction of experimental colitis induced a significant reduction in colonic transit (-37% *vs.* Uliveto alone; $p < 0.05$) (Fig. 7C).

DISCUSSION

Functional disorders of the gastrointestinal tract are characterized by a variety of symptoms, and their occurrence appears to be independent from the presence of evident organic lesions or be a consequence of past inflammatory bowel disorders (21, 22). Current drug therapies usually allow a partial relief of symptoms, and it has been suggested that the use of mineral water might represent a support in the management of functional disorders, depending on their peculiar chemico-physical composition (8, 9). Based on these premises, this study was performed to evaluate the effects of a bicarbonate–alkaline mineral water (Uliveto) in experimental models of intestinal motor disorders and inflammatory bowel disease. The results indicate that, under normal conditions, Uliveto water promotes gastric emptying and colonic transit. In the presence of experimental digestive disorders, functional motor alterations were observed and, in these settings, Uliveto water exerted positive influences on such disturbances. In particular, Uliveto water was able to normalize the rate of gastric emptying in animals with diarrhea, and it induced an increase in gastric emptying and colonic transit in rats with loperamide-induced constipation, with a concomitant increase in fecal output and fecal water content. The induction of colitis in two experimental models was associated with a significant increase in colonic macroscopic and microscopic damage scores, as well as colonic myeloperoxidase levels. In the presence of colonic inflammation, a reduction in gastrointestinal motor activity was observed, and Uliveto water was able to significantly improve gastric emptying and normalize bowel transit.

The promoting effects of Uliveto water on gastric emptying in normal animals has been previously reported (11), whereas its enhancing effects on delayed gastric emptying associated with experimental digestive disturbances represent a novel observation. Our data reflect clinical findings on the effects of calcium/bicarbonate rich mineral waters on delayed gastric emptying associated with functional dyspepsia. In a study performed by Bortolotti et al. (23), the effects of a mineral water, with a high content of calcium and bicarbonate, on gastric emptying were assessed by scintigraphy in a group of patients with idiopathic dyspepsia and compared with that in normal subjects. The treatment with mineral water induced a significant increase in gastric emptying in both normal and dyspeptic patients, indicating a beneficial effect of this water on functional dyspepsia. A more recent study evaluated the effects of supplementation with high-salt mineral water on gastric emptying in patients with functional dyspepsia. In this trial, patients who received mineral water showed an improvement in gastric emptying, suggesting that the consumption of mineral water could be effective in the management of functional dyspepsia (24).

To examine the mechanisms through which Uliveto water ameliorates gastric emptying, we performed experiments in animals pretreated with the selective gastrin/CCK-2 receptor antagonist L-365,260. Under these conditions, the enhancing effects of Uliveto water no longer occurred, suggesting that the stimulant effects of Uliveto water on gastric emptying involve the release of endogenous gastrin. These results are in accordance with the findings reported by Bertoni et al. (11), who demonstrated that the administration of Uliveto water to normal rats induced a significant enhancement of gastric emptying and that this effect was prevented by gastrin/CCK-2 receptor blockade. The effects exerted by this mineral water might depend on its peculiar high content of calcium ions, which is thought to be responsible for gastrin release stimulation and subsequent increase in gastric emptying, as previously hypothesized by Bertoni et al. (11).

The investigation of the effects exerted by Uliveto water on intestinal motor activity revealed a scarce influence on small-intestinal transit, both under normal conditions and in the presence of experimental digestive dysfunctions, whereas significant effects were observed at the colonic level. In particular, animals with experimental constipation treated with control water displayed a significant reduction in both fecal excretion and fecal water content, whereas in those receiving Uliveto water a significant enhancement of both parameters was observed. Moreover, Uliveto water was able to increase the rate of colonic transit in animals with constipation, suggesting that this mineral water can exert beneficial effects on evacuation disturbances induced by constipation. Clinical data support our observations, since in a study by Gasbarrini et al. (8), mineral water supplementation in patients with constipation induced a significant enhancement of intestinal transit.

The effects exerted by Uliveto water at colonic level were blunted by the selective antagonist of serotonin 5-HT_3 receptors alosetron, suggesting an involvement of endogenous serotonin in the promoting actions elicited by this mineral water. Previous reports have shown that gastrocolonic reflexes are activated by antral mechanoreceptor and duodenal chemoreceptor stimulation

(25, 26) and that serotonin plays a predominant role in the occurrence of such responses by activating 5-HT_3 receptor subtypes (27). The exact mechanisms through which Uliveto water activates serotonergic pathways have not been demonstrated. However, previous reports indicate that the stimulation of duodenal osmoreceptors by hypertonic solutions is able to promote neuronal reflexes in humans (28, 29), and, therefore, it is likely that the high content in mineral salts of Uliveto water accounts for the stimulation of these osmoreceptors and the subsequent activation of colonic reflexes. In line with these arguments, Polushina (30) demonstrated that the administration of a mineral water, that is characterized by a high content of calcium bicarbonate, to rats elicited a significant increase in plasma serotonin levels, indicating a possible role of this mediator in the stimulation of colonic sensory/motor activity.

In conclusion, this study indicates that Uliveto water exerts beneficial effects on gastrointestinal motility functions, both under normal conditions and in the presence of bowel motor alterations. The effects of Uliveto water on gastric emptying depend on the recruitment of gastrin-mediated mechanisms, whereas the activation of serotonergic pathways seems to account for the positive modulation colonic functions.

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