SHORT COMMUNICATION

Gastric emptying, small intestinal transit and fecal output in dystrophic (*mdx*) mice

Flavia Mulè · Antonella Amato · Rosa Serio

Abstract Duchenne muscular dystrophy (DMD), which results from deficiency in dystrophin, a sarcolemma protein of skeletal, cardiac and smooth muscle, is characterized by progressive striated muscle degeneration, but various gastrointestinal clinical manifestations have been observed. The aim was to evaluate the possible impact of the dystrophin loss on the gastrointestinal propulsion in *mdx* mice (animal model for DMD). The gastric emptying of a carboxymethyl cellulose/phenol red dye non-nutrient meal was not significantly different at 20 min from gavaging between wild-type and *mdx* mice. The intestinal transit and the fecal output were significantly decreased in *mdx* versus normal animals, although the length of the intestine was similar in both animals. The present results provide evidence for motor intestinal alterations in *mdx* mice in in vivo conditions.

Keywords Duchenne muscular dystrophy · *mdx* mouse · Gastric emptying · Intestinal transit · Fecal output

Introduction

Duchenne muscular dystrophy (DMD) is an X-linked neuromuscular disorder that affects one in 3300 live male births, characterized primarily by progressive striated muscle degeneration. In DMD and in the *mdx* mouse (animal model for DMD) there is a mutation in the gene encoding dystrophin, a protein localized on the cytoplasmatic side of the sarcolemma in skeletal, cardiac and smooth muscular fibers [1, 2]. Comprehensive understanding of the mechanisms leading from the absence of dystrophin to the muscular degeneration is still debated [3, 4]. They include: fragile membranes, aberrant cell signaling, increased oxidative stress, recurrent muscle ischemia, abnormal Ca^{2+} influx [3] and loss of nitric oxide (NO) synthase (NOS) function [5, 6].

Different degrees of dystrophic involvement have also been observed in *mdx* smooth muscle of the digestive tract [7, 8] and various clinical manifestations, such as bloating, feeling of fullness and constipation, have been reported in DMD patients [9–13] or in other forms of muscular dystrophy [14, 15]. Severe and even fatal cases of acute gastric dilatation and intestinal pseudo-obstruction have been reported and associated with histological evidence of smooth muscle fibrosis throughout the gastrointestinal tract [9, 16, 17]. However, despite postmortem evidence of significant gastrointestinal smooth muscle degeneration in DMD [9, 16, 17], little attention has been paid in studying its importance. Some patients with DMD suffer from constipation, but colonic motility, a possible factor responsible for the genesis of constipation, has not been studied. The constipation in these patients has been related to their immobility and weakness of their abdominal wall musculature.

Although numerous in vitro studies have indicated that *mdx* mice experience gastric and intestinal contractility disturbances [18–22], mainly attributed to an impairment of NO [19, 21–26], so far investigations on the motor activity of the gut in *mdx* mouse in vivo conditions have not been performed yet. Therefore, the aim of the present study was to evaluate the possible impact of the dystrophin loss on the gastrointestinal propulsion in *mdx* mice to assess the presence of motor disturbances. In this view,

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gastric emptying, small intestinal transit time and fecal output were determined in *mdx* mice in comparison with control animals.

Materials and methods

Animals

The experimental procedures employed in the present study were in accordance with internationally accepted principles for care of laboratory animals (E.E.C. Council Directive 86/609, OJ L358; 12 December 1987). Eighteen male normal (C57BL/10SnJ) and eighteen dystrophic mice (*mdx* mutants; C57BL/10Sn-DMD/J supplied by Jackson Laboratory, Bar Harbor, ME) (wild-type 12–18 months old, 24–32 g; *mdx* 12–18 months old, 25–34 g) were kept under controlled environmental conditions ($22 \pm 1°C$, $55 \pm 15\%$ relative humidity, 12-h light). Tap water and standard laboratory rodent chow (Mucedola, Settimo Milanese, Milan, Italy) were provided ad libitum; however, mice were deprived of food 24 h before the start of the experiments, except as otherwise stated.

Gastric emptying and intestinal transit

We assessed gastric emptying and small intestinal transit in 24-h fasted mice according to the method of phenol red as performed by earlier workers [27]. Briefly, the animals (normal and *mdx* mice) received by gavage 0.3 ml of test meal and were euthanized by cervical dislocation immediately ($t = 0$) or 20 min after gavaging. The test meal consisted of a non-nutrient meal of 50 mg phenol red in 100 ml 1.5% carboxymethylcellulose (CMC), which was constantly stirred and held at 37°C. Under laparatomy, the stomach and the small intestine were excised after ligature of the pylorus and the cardias. The stomach was cut into pieces and homogenized with its contents in 25 ml of 0.1 N NaOH. The homogenate was allowed to settle for 1 h at room temperature, and 8 ml of the supernatant was added to 1 ml of 33% of trichloroacetic acid to precipitate proteins. After centrifugation (3000 rpm for 30 min at 4°C), 2 ml of 2 N NaOH were added to the supernatant, and the amount of phenol red was determined from the absorbency at 560 nm. This correlates with the concentration of phenol red in the stomach, which in turn depends on the gastric emptying. The gastric emptying (GE) rate was derived as $GE = (1 - X/Y)100$ where X is absorbance of phenol red recovered from the stomach of animals killed 20 min after test meal. Y is the mean absorbance of phenol red recovered from the stomachs of animals killed at 0 min following test meal.

Immediately after the excision of the stomach, the whole small intestine was grossly freed from its mesenteric attachments, and its length (from the pyloric sphincter to the ileocecal junction) was measured. The intestine was opened at the level of the front of the test meal, which was revealed by a few drops of 0.1 N NaOH. The rate of intestinal transit was expressed as the ratio between the distance travelled by the test meal and the total length of intestine.

Fecal excretion

Fecal excretion was assessed in mice placed individually in grid-floor cages (size $26 \times 44 \times 22$ cm) and left there to become acclimatized to their environment for 3 days before the experiment. During this period, the animals were fed normal chow and supplied water ad libitum. The day of the experiments, food was withdrawn, and fecal pellet output was then monitored. The pellets discharged by each animal during a period of 8 h and 24 h were collected, counted and weighed immediately (wet weight) and after drying (24 h at 46°C) (dry weight). Any difference on intestinal secretion or reabsorption of fluids was scored as the ratio of wet to dry fecal weight.

Statistical analysis

All results are expressed as means $\pm$ SE; n refers to tissues obtained from different animals. Statistical analysis was carried out using GraphPad (Prism) software. Differences between animals of each type were compared by Student's t test for unpaired data or for multiple comparison analysis of variance (ANOVA) followed by Bonferroni t test. A P value less than or equal to 0.05 was considered to be statistically significant.

Results

The gastric emptying of a carboxymethyl cellulose/phenol red dye non-nutrient meal was not significantly different at 20 min from gavaging, being $45.6 \pm 3.5\%$ ($n = 9$) in wild-type and $49 \pm 6.9\%$ ($n = 9$) ($P > 0.05$) in *mdx* mice (Fig. 1a). Intestinal transit rate, expressed as the ratio between the distance traveled by the phenol red meal and the total length of the small intestine, was significantly decreased in *mdx* ($43.8 \pm 5\%$; $n = 9$) versus wild-type animals ($62.2 \pm 6.8\%$; $n = 9$) ($P \leq 0.001$) (Fig. 1b). However, the length of small intestine was not significantly different between the two groups of animals (34.3 ± 2.3 and 32.6 ± 0.8 cm, in *mdx* and wild type, respectively; $n = 9$; $P \geq 0.05$).

Fig. 1 Gastric emptying and small intestinal transit 20 min after gavage of 0.3 ml of phenol red meal in wild-type (*WT*) and dystrophic (*mdx*) mice. Values are mean ± SE of *n* = 9 animals. *P* < 0.001; unpaired Student's *t* test
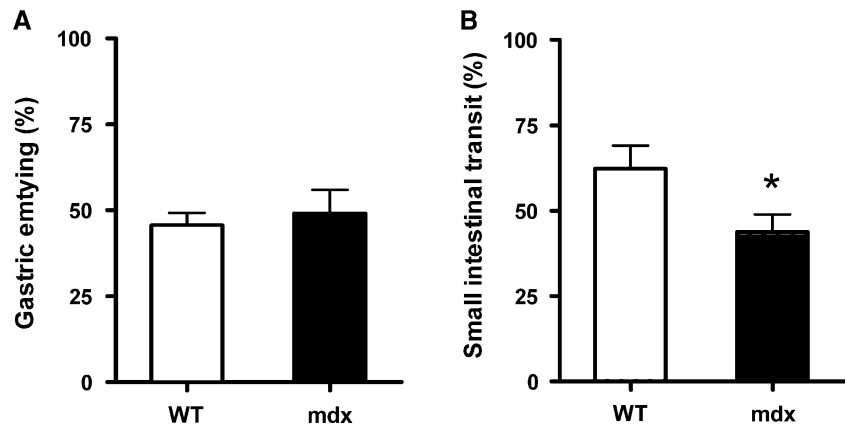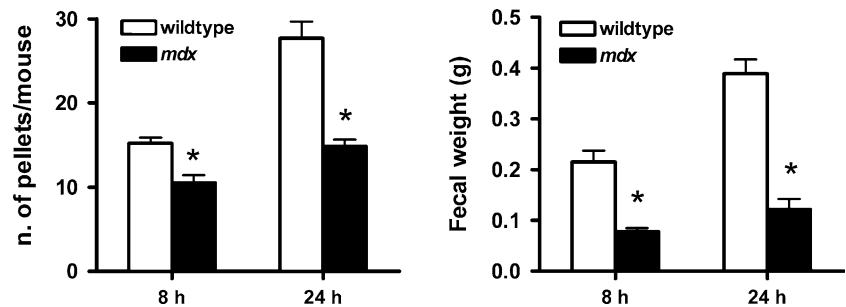

Fig. 2 Fecal pellet output (number and total weight) in wild-type (*WT*) and dystrophic (*mdx*) mice. Values are mean ± SE of *n* = 6 animals. *P* < 0.001; ANOVA followed by Bonferroni *t* test

Fecal output, monitored by counting and weighing fecal pellets excreted over a period of 8 or 24 h by each animal, was significantly reduced in *mdx* animals (Fig. 2). In fact, over 24 h, the fecal pellet number was 27.7 ± 1.9 and 14.8 ± 0.8 (*n* = 6; *P* ≤ 0.001), and the stool weight was 388 ± 28 and 122 ± 20 mg (*n* = 6; *P* ≤ 0.001) in wild-type and *mdx* mice, respectively. However, the feces produced by *mdx* mice had a ratio of wet to dry fecal weights not significantly different from wild-type mice (Fig. 3).

Discussion

This study provides evidence that *mdx* mice show alterations in the gastrointestinal propulsion, with a significant delay in the small intestinal transit and a decreased amount of stools excreted, and it suggests that loss of dystrophin has important in vivo effects, at least on intestinal motility.

Because changes in gastrointestinal contractility in vitro have been attributed to an impairment of NO [19, 21–26], it seems plausible to associate the reduction in the motor small and large intestinal activity to the defective production/release of NO, which increases resistance to flow and decrease transit. In fact, reduced nitrergic relaxation at the level of the small intestine leads to delayed intestinal transit as manifested from studies with NOS-inhibitors in different species illustrating the essential role of NO in intestinal peristalsis [28–30]. On the other hand, the
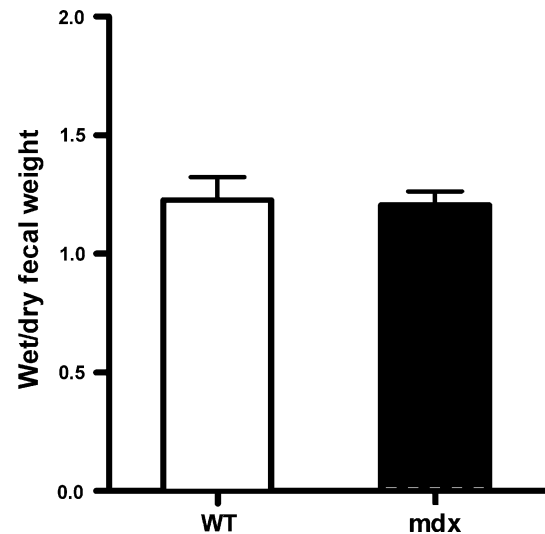

Fig. 3 Fecal water content expressed as ratio of wet to dry fecal weight in wild-type (*WT*) and dystrophic (*mdx*) mice. Values are mean ± SE of *n* = 6 animals

possibility that the difference in small intestinal transit between normal and *mdx* mouse may be due to differences in the length of their respective small intestines can be ruled out, because the length, as measured in our experiments, was not significantly different between the two groups of animals.

Furthermore, in our experiments the fecal excretion was reduced in *mdx* mice compared to the wild-type animals.

Because stool output can be considered an index of colonic propulsion, our results suggest a decreased motor activity in the large intestine, as observed for the small intestine. On the other hand, the normalized amount of fluids present in fecal output from *mdx* mice was not significantly different from wild-type mice, which could suggest that there is not an altered exchange of fluids from gut and lumen in dystrophic mice. Therefore, smooth muscle involvement of the colon, besides immobility and weakness of abdominal wall muscles, might explain the high frequency of constipation that has been reported in DMD patients [16, 31].

However, the gastric emptying rate was not delayed in *mdx* mice, although also in the stomach an impairment of nitric oxide has been reported [21]. This could be explained assuming that reduced antropyloroduodenal contractility due to the decrease of NO is countered by increased proximal gastric tone, which tends to accelerate gastric emptying of liquids. Indeed, an increase in the gastric tone has been reported previously in *mdx* mice [21]. Moreover, it has been reported that inhibition of NO synthesis by L-NNA did not slow gastric emptying [31]. However, because there is a striking difference between the emptying of liquids and solids from the stomach, our technique does not allow us to rule out the possibility that the gastric emptying of a solid meal can be delayed in *mdx* mice. Further experiments are needed to clarify this point.

Changes in intestinal motility could also be the consequence of perturbation of intracellular calcium homeostasis. In fact, an increased influx of Ca^{2+} through L-type voltage-sensitive channels appears to be responsible for sustained mechanical tone in colonic circular muscle from dystrophic mice [32]. In addition, because down regulation of tachykinergic NK2 receptors in *mdx* smooth muscle cells has been reported [33], a dysfunction of the excitatory neural control could also be involved in the reduced motility observed in in vivo conditions.

In conclusion, our results provide evidence for motor functional alterations also in in vivo conditions, confirming that intestinal preparations from the *mdx* mouse are a good model available to study the pathogenic mechanisms associated with DMD.

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