Original Article

Lead Exposure Changes Gastric Motility in Rats: Role of Nitric Oxide (NO)

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Abstract

Background: Abdominal colic, constipation and delay in gastric emptying are symptoms of lead poisoning, but there is scant information about the effect of lead on gastric motility. In the present study, we investigated the effect of lead acetate on gastric motility in rats.

Methods: Animals were divided into nine groups (n=8); four groups were exposed to lead acetate solution (1%) for 1, 2, 3, and 4 weeks (Pb1, Pb2, Pb3, and Pb4 groups, respectively). Sodium acetate solution was given to another four groups for 1, 2, 3, and 4 weeks (Na1, Na2, Na3, and Na4 groups, respectively) and the control group had free access to tap water. Gastric motility was measured in the basal and acetylcholine (Ach)-stimulated states using a physiograph instrument. Nitric oxide metabolite of gastric tissue was determined by Griess micro-assay.

Results: There were no significant differences between basal and Ach-stimulated gastric motility in Pb1, Pb2, Na1, and Na2 groups. However, it was significantly greater in Pb3 and Pb4 groups when compared with Na3 and Na4 groups in both basal and Ach-stimulated states ($P<0.05$). In addition, nitric oxide metabolite of gastric tissue was more in all Pb groups in comparison with their Na counterparts ($P<0.05$).

Conclusion: We found that lead exposure could affect gastric motility via the nitric oxide pathway.

Keywords: gastric motility, lead, NO

Introduction

Lead is one of the most toxic metals for human body systems; it has a wide distribution in the environment[1] and induces a broad range of physiological, biochemical, and behavioral dysfunctions.[2] Lead is a soft, heavy, blue-gray metal naturally found in the earth's crust but spread throughout the environment by various human activities. In the past, lead also was used in house painting and gasoline. Lead is still present in batteries, solder, ammunition, pipes, unglazed pottery, folk medicine, and roofing materials. Overall, 85 – 90% of lead in the blood is bound to erythrocytes.[2] Lead accumulates in many body organs and causes damage to their tissues. In addition, it affects cardiovascular, hematopoietic, learning, memory, and the gastrointestinal (GI) system. Disorders such as constipation, vomiting, cramps, nausea, and abdominal colic are symptoms of lead poisoning, though the mechanisms of these effects have not been elucidated.[2,3]

Since gastric muscular layer contractions are important elements in mixing food with GI secretions to form a homogenous chymus, assisting in protein digestion, and emptying the gastric contents into the gut, therefore any disturbance in these movements and contractions can cause GI diseases. Although Ryden and Walsh have demonstrated that sub-chronic exposure to lead (4% for seven weeks) in rats slows gastric emptying,[4] there is little evidence concerning the effects of lead exposure on gastric motility and its possible mechanisms. Previous studies have shown that NO is involved in GI function. Endogenous NO plays an important role as a non-adrenergic, non-cholinergic neurotransmitter in the GI tract. NO is also involved in the secretion of fluids, electrolytes, and gastric acid[5,6] which can affect gastric motility. The aim of present study is to investigate the effects of lead exposure on gastric motility and its possible mechanism in rats.

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Accepted for publication: 21 June 2010

Materials and Methods

The procedure was done in accordance with the Guideline for the Care and Use of Laboratory Animals of Tehran University of Medical Sciences, Iran. Rats consumed lead acetate through drinking water.[1] Lead acetate (Merck, KGa 64271, Darmstadt, Germany; purity 99.5 – 100%) was dissolved in drinking water acidified with 5N hydrochloric acid to prevent precipitation of insoluble lead salts. Glucose (5%) was added to improve palatability. We dissolved sodium acetate (Merck, D-6100 Darmstadt, Germany; purity, 99.5 – 100%) in drinking water and added 5N HCl and glucose. These two solutions were macroscopically homogenous and clear.

Animals and experimental design

Male Wistar rats (200 – 250 g) purchased from the Animal House of the Medical School of Tehran University of Medical Sciences, Iran were maintained in a temperature-controlled environment, on a 12 hr light/dark cycle, and allowed free access to food and water. There were nine groups of animals (n=8): I) control group with free access to tap water and food; II) lead acetate groups (Pb1, Pb2, Pb3, and Pb4) treated with water containing 1% lead acetate[7] for 1,

2, 3, and 4 weeks, respectively. III) Sodium acetate groups (Na1, Na2, Na3, and Na4), treated with water containing 1 M sodium acetate similar to the Pb groups for 1, 2, 3, and 4 weeks, respectively.

We included the Na groups to determine if the probable differences were due to Pb^{2+} ion or acetate ion on gastric motility.

Surgical procedure and measurement of gastric motility

Animals had no food for 24 hr before the experiments but had free access to water. The rats were anesthetized with sodium thiopental (50 mg/kg, i.p.).[8] Tracheostomy was carried out and the esophagus was tied in the neck region to prevent reflux aspiration.[9] Then the abdomen was exposed via a midline incision and a silicon tube (2.5 mm diameter, 10 cm length) passed through the incision in the duodenum into the stomach and tied in the pyloric region. One end of the tube was inserted into the stomach and the other end connected to a three-way stopcock and pressure transducer system (Narco, RP1500, Houston, Texas; sensitivity, 0.01 mmHg). The pressure transducer was connected to a physiograph and gastric motility measured after 30 min to reach a steady state.[10]

It is known that injection of a solution (1.5 mL/100 g of body weight) into the stomach causes distention.[11] Thus, we introduced different volumes of saline (<1.5 mL/100 g) into the stomach to measure basal gastric pressure and chose a suitable volume (0.5 mL/100 g) after the dose-response study. Then, basal gastric motility was measured for 15 min. To measure stimulated-gastric motility, Ach (10^{-1} M i.p.) was administered followed by measurement of gastric motility by a physiograph (CPM Bio. System, Narco Inc., Houston, Texas; velocity 0.5 mm/sec, sensitivity, 0.5 mv/mm) for 15 min. The intra-gastric pressure recorded by a physiograph is an indicator of gastric motility.[12,13] Calibration of the physiograph was by standard mercury sphygmomanometer.

Blood lead and NO metabolite assay

Blood lead was measured using a Shimadzu atomic absorption spectrometer (AA-670G).[14] Gastric tissue and plasma samples were prepared to assay NO metabolite by the Griess micro-assay method.[15] In this method, reactive nitrogen intermediate (RNI) was measured in supernatant fluids as nitrites using the Griess reaction, after converting nitrates to nitrites with nitrate reductase treatment.[15] At the study end, all animals were killed by infusions of potassium chloride into their hearts.

Statistical analysis

All values were expressed mean±SE. Statistical analysis was performed with one-way analysis of variance and *post hoc* Tukey tests. $P<0.05$ was considered statistically significant.

Results

There were no significant differences between the blood lead level, plasma and stomach tissue NO metabolite concentration in the control and Na groups. In addition, the basal and stimulated-gastric motility were the same in these groups. Therefore, we compared the Pb groups to their Na counterparts, which showed that the changes seen in the lead groups should be due to Pb^{+2} ions, and not acetate ions.

Blood lead level was the same in all four Na groups. As seen in Figure 1, blood lead increased over time in the Pb groups [56.7±4.22 µg/dL (Pb1); 85.0±5.12 µg/dL (Pb2); 145.77±11.2 µg/dL (Pb3); and 265.0±21.4 µg/dL (Pb4); $P<0.05$]. The blood lead level was significantly more in the Pb groups than their Na counterparts ($P<0.05$; Figure 1).

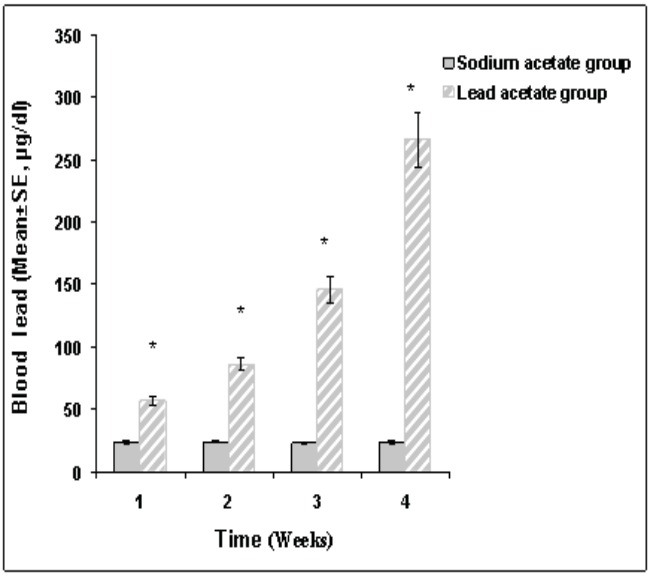

Figure 1. Blood lead levels in sodium acetate and lead acetate groups (n=8 in each group). *Significantly different from the sodium group counterpart ($P<0.05$).

There were no significant differences in basal and stimulated gastric motility when comparing Pb1 and Pb2 groups with Na1 and Na2 groups (Table 1). However, as seen in Table 1, basal gastric motility were significantly more in the Pb3 and Pb4 groups, ($P<0.05$). Also stimulated gastric motility were significantly more in Pb3 and Pb4 groups than their Na counterparts ($P<0.05$, Table 1).

Table 1. Mean gastric motility in basal Ach-stimulated states in lead acetate and sodium acetate groups (n=8 in each group). *Significantly different from the sodium acetate and control groups ($P<0.05$).

Groups	State	
	Basal gastric motility (Mean±SE, mmHg/15 min)	Stimulated gastric motility (Mean±SE, mmHg/15 min)
Control	22.22±1.03	35.11±1.55
Sodium acetate (1 week)	22±1.05	34.87±1.88
Sodium acetate (2 weeks)	22.5±1.08	35.37±1.63
Sodium acetate (3 weeks)	22.12±0.8	35.37±1.54
Sodium acetate (4 weeks)	22.5±0.98	35.12±1.64
Lead acetate (1 week)	22.12±0.91	36.5±1.4
Lead acetate (2 weeks)	21.5±1.03	35.5±1.47
Lead acetate (3 weeks)	34.12±1.59*	55.25±1.19*
Lead acetate (4 weeks)	34.62±1.67*	56.62±1.13*

Figure 2 shows a significant increase in gastric tissue NO metabolite concentration in the Pb1, Pb2, Pb3, and Pb4 groups compared to their Na counterparts [41.12±1.65 (Pb1); 48.25±1.55 (Pb2); 59.26±1.28 (Pb3); 68.8±1.9 (Pb4); 22.72±0.15, (Na1); 22.02±0.13 (Na2); 22.76± 0.22 (Na3); and 22.85±0.21 (Na4), µmol/g wet weight tissue]. However, the amount of plasma NO metabolite concentration was the same in all animals (Figure 3).

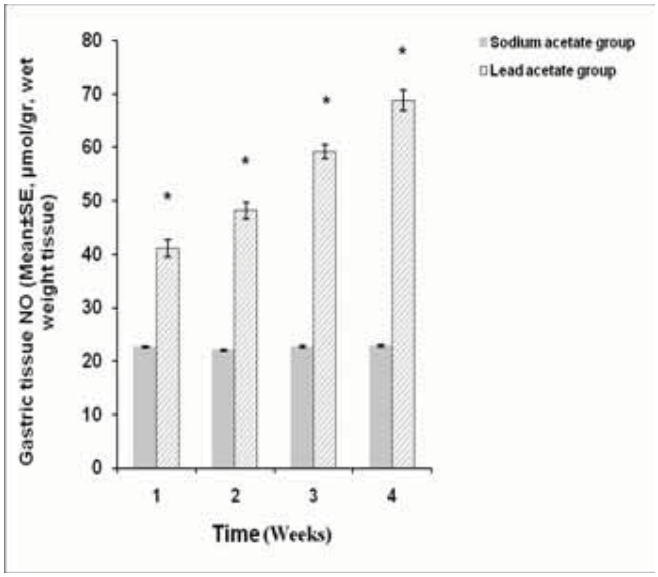

Figure 2. Gastric tissue NO metabolite concentration in sodium acetate and lead acetate groups (*n*=8 in each group). *Significantly different from the sodium group counterpart (*P*<0.05).

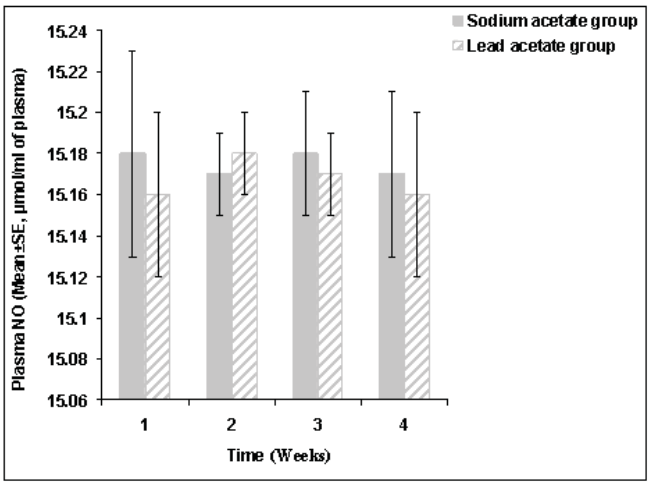

Figure 3. Plasma NO metabolite levels in sodium acetate and lead acetate groups (*n*=8 in each group).

Discussion

The present study showed no significant differences in blood lead level, gastric motility, and plasma and gastric tissue NO metabolite concentrations among the control and Na groups. Therefore, we can assume that the changes seen in these parameters in lead-treated animals should be due to the Pb^{2+} ion, not the acetate ion.

In this study, no significant differences existed in basal and stimulated gastric motility in the Pb1 and Pb2 groups compared to their Na counterparts, though stimulated gastric motility was more than the basal state in all groups. However, basal and stimulated gastric motility were significantly more in the Pb3 and Pb4 groups than their Na counterparts. This finding may suggest an increase of basal vagal tone due to lead intoxication for three and four weeks. In addition, a significant increase in stimulated gastric motility occurred in the Pb3 and Pb4 groups compared to the Na3 and Na4 groups, respectively. The amount of stimulation was more than the basal state in all groups.

On the other hand, the gastric tissue concentration of NO metabolites was significantly more in Pb-treated animals than Na acetate-treated ones, but blood NO metabolite concentration was the same in all groups. At least three isoforms of the enzyme NO synthase (NOS) exist: inducible NOS (iNOS), endothelial NOS (eNOS) and neuronal NOS (nNOS) of which about 50% of the nerves in the enteric nervous system contain nNOS.[16] It has been shown that rat parietal cells express nNOS. This finding suggests that endogenous NO may participate in the regulation of gastric motility by acting as an intracellular signaling molecule.[16] Although it has been shown that lead exposure (100 ppm in drinking water for 12 weeks) causes a reduction in plasma NO level[17] but we did not find any changes in plasma NO metabolite concentration. This could be due to the difference in lead concentration and/or exposure duration. An assumption is that NO production increases locally in the stomach, probably via induction of nNOS expression or activity.

Walsh and Ryden have shown that concentrations of lead sufficient to induce renal and hematologic toxicity in rats do not substantially affect GI transit.[3] Waksh and coworkers revealed that lead acetate inhibited smooth muscle contractility of the ileum in rats.[18]

Our findings showed higher amounts of gastric tissue NO metabolite concentration in the Pb groups than their Na counterparts. This result has lead us to assume that the NO increment (probably due to lead exposure) caused increased basal and stimulated vagus nerve tone and was due to increase of acetylcholine effects on muscular cells in the stomach. Evidence exists that NO acts presynaptically to facilitate vagal neurotransmission via a pathway that ultimately leads to increased phosphorylation of presynaptic L-type Ca^{+2} channels. This pathway causes increased presynaptic calcium influx and vesicular release of acetylcholine in the heart.[19] In this study, there may be an increment of Ca^{+2}, which causes increasing contractility and gastric motility. Also NO generated by NOS in parasympathetic ganglia may play a modulator role in facilitating the release of acetylcholine and the subsequent heart response.[20] A similar mechanism may be involved in the stomach.

In lead treated animals, NO increases in gastric tissue following lead exposure. NO may act via one of the following probable mechanisms on gastric motility: 1) NO may increase muscle fiber contractility and/or the contractile elements, and 2) it may increase gastric blood supply of gastric tissue,[21] which in turn leads to muscle fiber contractions in gastric tissue. Therefore, in the present study, gastric tissue NO concentration increased in lead-treated animals but its amount increased more in the Pb3 and Pb4 groups than in the Pb1 and Pb2 groups. On the other hand, gastric motility in basal and Ach-stimulated states increased insignificantly in the Pb1 and Pb2 groups, unlike the increase in gastric tissue NO concentration. Gastric motility increased significantly in the Pb3 and Pb4 groups with increased gastric tissue NO concentration. It seems that increased gastric motility due to gastric tissue NO concentration may depend on NO concentration and may be a delayed response. Thus, the increase of gastric muscle fibers and its activities and increased blood flow due to increased NO require more time. However, further studies should evaluate these suggestions.

In conclusion, we found that lead exposure could affect gastric motility via the nitric oxide pathway.

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