

Lead Exposure Changes Gastric Acid Secretion in Rat: Role of Nitric Oxide (NO)

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Received: 12 Jul. 2009; Received in revised form: 23 Dec. 2009; Accepted: 15 Mar. 2010

Abstract- Sub chronic exposure to lead in rats slows gastric emptying, but little is known about the effects of lead on gastric secretion. This study was designed to investigate the effects of lead on gastric acid secretion and its possible mechanisms in rats. Lead acetate was dissolved in drinking water in a concentration of 1%. Sodium acetate-containing water with a molar concentration similar to lead was also prepared. We had nine groups of animals (n=8); four of them were exposed to lead 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). Gastric secretion was collected by washout technique and its acid output was measured in the basal (Basal Acid Output, BAO), vagotomy (Vagotomized Acid Output, VAO), and vagally stimulated (Vagally Stimulated Acid Output, VSAO) states using titrator instrument. Nitric oxide (NO) metabolite of gastric tissue was determined by Griess micro assay method to evaluate the possible mechanism of lead effect on gastric secretion. VSAO was significantly less in Pb1 and Pb2 groups than Na1 and Na2 ones respectively (1.75 ± 0.17 , 2.10 ± 0.30 vs. 5.79 ± 0.20 , 6.18 ± 0.27 $\mu\text{mol}/15\text{min}$) ($P=0.001$, $P=0.001$). BAO was significantly more in Pb3 and Pb4 groups than Na3 and Na4 ones respectively (2.77 ± 0.37 , 2.80 ± 0.31 vs. 1.73 ± 0.16 , 1.79 ± 0.34 $\mu\text{mol}/15\text{min}$) ($P=0.01$, $P=0.02$), but it was the same after vagotomy. VSAO was more in Pb3 and Pb4 groups than their Na counterparts ($P=0.001$, $P=0.0001$). NO metabolite of gastric tissue was more in all Pb groups in comparison to their Na counterparts ($P=0.0001$). In this study, it seems that lead exposure, via NO mechanism, has different effects on acid secretion. Nitric oxide in small and large amounts decrease and increase gastric acid secretion, respectively.

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Acta Medica Iranica, 2011; 49(1): 3-8.

Keywords: Lead; Nitric oxide; Gastric acid; Rats

Introduction

Lead exposure is still one of the most important industrial and environmental hazards, due to its widespread distribution and ubiquitous exposure in human populations. Lead is a metal which has been associated with human activities for 6000 years (1). In recent years, increasing attention has been drawn to the effects of metals and their compounds on human and animal health (2). Lead accumulates in various organs and tissues. Overall, 85-90% of lead in the blood is bound to erythrocytes (2). It is excreted through glomerular filtration in the kidney, probably followed by partial tubular reabsorption, and also through bile,

pancreatic juice, and feces. Also, one of the most often examined early effects of lead, with mild clinical symptoms, is hypertension, which has been proven both in humans and animals (3, 4). Gastrointestinal complications such as constipation, vomiting, cramps, nausea, and abdominal colic are signs of lead poisoning, though the mechanisms of these effects have not been elucidated (5).

Previous studies have demonstrated that sub chronic exposure to lead in rats slows gastric emptying (6), but little is known about the effects of lead poisoning on gastric secretion. The purpose of this study was to investigate the effects of lead on gastric acid secretion and its possible mechanisms in rats.

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Materials and Methods

The procedures were in accordance with the guidelines for the care and use of laboratory animals of Tehran University of Medical Sciences, Tehran, Iran. Lead acetate was administered in drinking water to rats (7). The acetate form (Merck® KGAA in 64271 Darmstadt, Germany, purity: 99.5-100%) was dissolved in distilled water acidified with hydrochloric acid 5N to prevent precipitation of insoluble lead salts. Glucose (5% w/v) was added to improve palatability. We also made sodium acetate solution by dissolving this salt (E. Merck®, D-6100 Darmstadt, F.R. Germany, purity: 99.5-100%) in drinking water and adding HCl 5N and glucose. These two solutions were macroscopically homogenous and clear. Male Wistar rats, 200-250g in weight, were used in this study, and were purchased from animal house of medical school of Tehran University of Medical Sciences, Tehran, Iran. They were maintained in a temperature-controlled environment and on a 12:12 hour light: dark cycle with free access to water and standard chow containing lucern, bran, red meat powder, fish meat powder, multivitamins, vitamin C, wheat, oats and corn, produced in pellet form by Razi Vaccine & Serum Research Institute, Tehran, Iran. The animals were divided into the following 9 groups (n=8):

- A- Control group with free access to tap water and food.
- B- Lead acetate groups (Pb groups), treated with water containing lead acetate 1% (8) for 1, 2, 3, and 4 weeks, named as Pb1, Pb2, Pb3, and Pb4 groups, respectively.
- C- Sodium acetate groups (Na groups), treated with water containing sodium acetate with a molar concentration similar to Pb groups for 1, 2, 3, and 4 weeks, named as Na1, Na2, Na3, and Na4 groups, respectively.

We included the Na groups to determine if the probable differences in gastric acid secretion and other parameters are due to Pb²⁺ ion or acetate ion.

Before performing the experiences, the animals were deprived of food for 24 hours, but had a free access to water. Anesthesia was induced by intraperitoneal injection of 50mg/Kg b.w. of sodium thiopental (9). After induction of general anesthesia, tracheostomy was carried out and esophagus was tied in the neck region to prevent reflux aspiration (10). Then, the vagus nerve was isolated on both sides of neck for approximately 1cm and anchored by a string and finally covered with cotton soaked in normal saline (11). Then, laparotomy was carried out by a midline incision and a silicon tube

with an external diameter of 2.5mm was introduced into the stomach via the duodenum. Gastric juice was collected by washout technique (12). For measuring basal acid output (BAO), 1ml of normal saline solution was entered into the stomach, after 15 minutes another 1ml was added and the stomach content was aspirated for acid titration using titrator instrument (West Germany) by 0.001N sodium hydroxide solution. Then the vagus nerve was tightly ligated and cut in the neck. After 15 minutes, gastric content was collected with the same technique to determine vagotomized acid output (VAO). In order to measure vagal stimulated acid output (VSAO), the right vagus nerve was stimulated by a stimulator (V=15v, F=5Hz, W=1msec) for 15 minutes and then acid secretion was measured (13). The voltage and frequency of stimulation were chosen in a way that no cardiovascular problem would arise (13).

Blood lead was measured using Shimadzu® atomic absorption spectrometer AA-670G (14).

Stomach tissue and plasma samples were prepared to assay nitric oxide (NO) metabolite using Griess micro assay method (15). Values were reported as Mean ± SE. We used paired T-test to compare the amount of acid output in vagotomy or vagally stimulated states with the basal state in each group. In order to see if any difference exists in acid output between all groups, ANOVA was performed, and then to discriminate exactly which differences had caused ANOVA to be significant, Tukey was done. $P < 0.05$ was considered statistically significant.

Results

There were no significant difference between the blood lead level, plasma and stomach tissue NO metabolite concentration in the control and Na groups (Table 1). Also, the BAO, VAO, and VSAO were the same in all these groups (Table 2). Therefore, we compared the Pb groups to their Na counterparts. This shows that the changes seen in the lead groups should be due to Pb²⁺ ions, and not to the acetate ion. Whole blood lead level was the same in all four Na groups. Although blood lead increases with time in Pb groups, it is not significantly different in Pb1 and Pb2 animals (Figure 1). The same is true about Pb2 and Pb3 groups. But the blood lead differences between Pb1 and Pb3, Pb1 and Pb4, and Pb3 and Pb4 are statistically significant (56.50 ± 4.65 (Pb1), 134.77 ± 12.21 (Pb3), 225.42 ± 33.53 (Pb4), $\mu\text{g/dl}$) ($P < 0.05$). The blood lead level was significantly more in Pb groups than their Na counterparts ($P < 0.001$) (Figure 1).

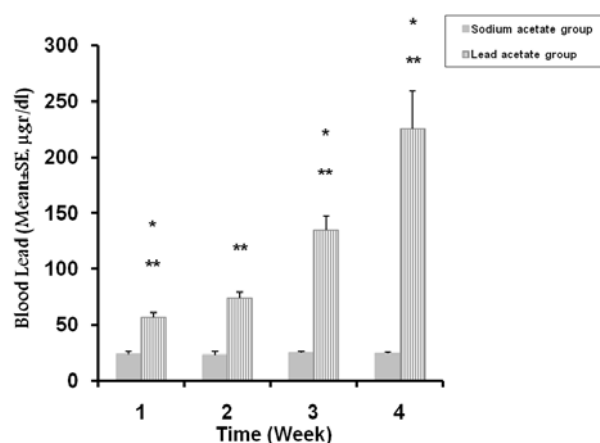
Table 1. Blood lead level, stomach tissue NO concentration, and plasma NO concentration in control and sodium acetate groups (n=8 in each group).

Groups	Blood lead level ($\mu\text{g/dl}$)	Stomach tissue NO ($\mu\text{mol/gr.wet weight tissue}$)	Plasma NO concentration ($\mu\text{mol/ml}$)
Control	23.84 \pm 2.36	22.85 \pm 0.18	15.09 \pm 0.10
Sodium acetate (1 week, Na1)	23.93 \pm 2.48	22.92 \pm 0.13	15.18 \pm 0.23
Sodium acetate (2 weeks, Na2)	23.18 \pm 2.97	22.76 \pm 0.26	15.17 \pm 0.22
Sodium acetate (3 weeks, Na3)	24.86 \pm 1.16	23.25 \pm 0.43	15.16 \pm 0.14
Sodium acetate (4 weeks, Na4)	24.15 \pm 1.33	22.85 \pm 0.36	15.17 \pm 0.22

The mean BAO and VAO were the same in Pb1 animals compared to Na1 and control ones. So was the condition in Pb2 and Na2 groups (Table 2). But VSAO was significantly less in Pb1 and Pb2 groups in comparison to Na1 and Na2 groups, respectively (1.75 ± 0.17 (Pb1), 5.79 ± 0.20 (Na1), 2.10 ± 0.30 (Pb2), 6.18 ± 0.27 (Na2) $\mu\text{mol/15min}$) ($P < 0.0001$) (Table 2).

On the other hand, BAO was significantly more in Pb3 and Pb4 groups than Na3 and Na4 ones, respectively (2.77 ± 0.37 (Pb3), 1.73 ± 0.16 (Na3), 2.80 ± 0.31 (Pb4), 1.79 ± 0.34 (Na4), 1.59 ± 0.11 (control) $\mu\text{mol/15min}$) ($P < 0.05$). But the VAO was the same in these animals (Table 2). Also VSAO was significantly more in Pb3 and Pb4 groups than their Na counterparts (10.16 ± 0.69 (Pb3), 5.56 ± 0.51 (Na3), 9.08 ± 0.84 (Pb4), 5.56 ± 0.20 (Na4), $\mu\text{mol/15min}$) ($P < 0.001$) (Table 2). As was expected in all groups, the acid output significantly decreased after vagotomy and increased with vagal stimulation compared to the basal state (Table 2). There was a significant increase in stomach tissue NO metabolite concentration in Pb1, Pb2, Pb3 and Pb4 groups compared to their Na counterparts (41.22 ± 0.97 (Pb1), 41.27 ± 1.95 (Pb2), 47.06 ± 1.00 (Pb3), 47.91 ± 1.26 (Pb4), 22.92 ± 0.13 (Na1), 22.76 ± 0.26 (Na2), 23.25 ± 0.43 (Na3), 22.82 ± 0.36 (Na4) $\mu\text{mol/gr}$

wet weight tissue) ($P < 0.001$) (Figure 2), but the amount of plasma NO metabolite concentration was the same in all these animals (Figure 3).

**Figure 1.** Whole blood lead levels in lead acetate and sodium acetate groups (n=8 in each group).

* $P < 0.05$ Comparison of blood lead level between lead acetate groups (Pb1, Pb2, Pb3).

** $P < 0.001$ Comparison of blood lead level in lead acetate and sodium acetate groups.

Table 2. Gastric acid output in basal, vagotomized, and vagally stimulated states in lead acetate and sodium acetate groups (n=8 in each group).

State	Basal acid output (BAO)	Vagotomized acid output (VAO)	Vagally stimulated acid output (VSAO)
Groups	(Mean \pm SE, $\mu\text{mol/15min}$)	(Mean \pm SE, $\mu\text{mol/15min}$)	(Mean \pm SE, $\mu\text{mol/15min}$)
Control	1.59 \pm 0.11	1.19 \pm 0.10	5.38 \pm 0.17
Sodium acetate (1week, Na1)	1.4 \pm 0.12	0.95 \pm 0.09	5.79 \pm 0.20
Sodium acetate (2weeks, Na2)	1.58 \pm 0.15	1.24 \pm 0.12	6.18 \pm 0.27
Sodium acetate (3weeks, Na3)	1.73 \pm 0.16	1.36 \pm 0.17	5.56 \pm 0.51
Sodium acetate (4weeks,Na4)	1.79 \pm 0.34	1.46 \pm 0.10	5.6 \pm 0.20
Lead acetate (1 week, Pb1)	1.42 \pm 0.11	1.07 \pm 0.08	1.75 \pm 0.17 ***
Lead acetate (2 weeks, Pb2)	1.53 \pm 0.11	1.07 \pm 0.07	2.1 \pm 0.30 ***
Lead acetate (3 weeks, Pb3)	2.77 \pm 0.37 *	1.86 \pm 0.33	10.16 \pm 0.69 **
Lead acetate (4 weeks, Pb4)	2.8 \pm 0.30 *	1.95 \pm 0.24	9.08 \pm 0.84 **

* $P < 0.05$ Comparison of BAO in Pb3 and Pb4 with Na3 and Na4 groups.

** $P < 0.001$ Comparison of SVAO in Pb3 and Pb4 with Na3 and Na4 groups.

*** $P < 0.0001$ Comparison SVAO in Pb1 and Pb2 with Na1 and Na2 groups.

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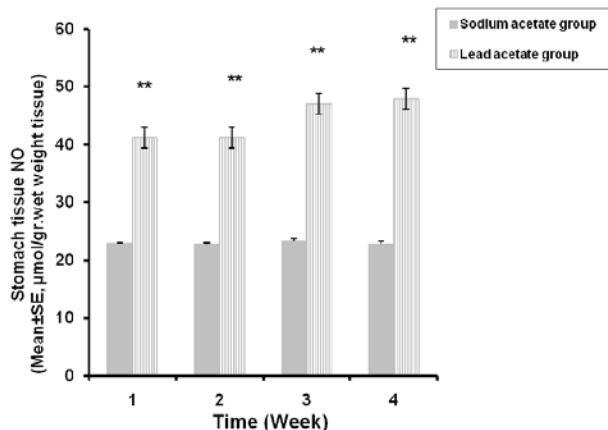


Figure 2. Stomach tissue NO metabolite concentration in sodium acetate and lead acetate groups (n=8 in each group). ** $P < 0.001$ comparison of stomach tissue NO metabolite concentration in lead acetate and sodium acetate groups.

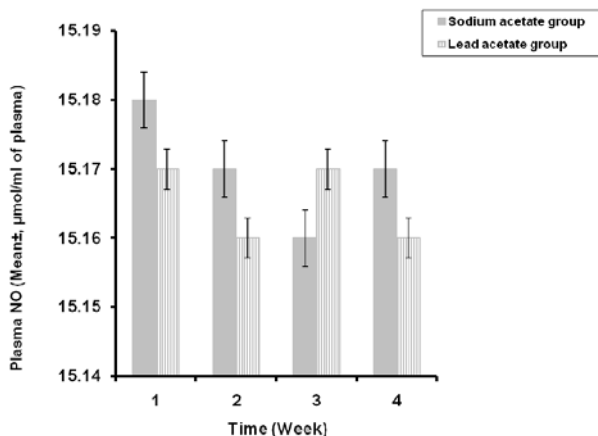


Figure 3. Plasma NO metabolite levels in lead acetate and sodium acetate groups (n=8 in each group).

Discussion

There were no significant differences in the blood lead level, gastric acid secretion, plasma and stomach tissue NO metabolite concentration among control and Na animals (Table 1, 2). Therefore we can assume that the changes seen most probably is due to Pb^{2+} ion effect, not the acetate ion.

A significant decrease in acid output was seen in vagotomized rat, compared to the basal state in all groups (Table 2). This shows that the basal vagal tone has an important role in acid secretion.

The stomach tissue concentration of NO metabolites is significantly more in Pb- treated animals than Na acetate-treated ones, but blood NO metabolite concentration is the same in all groups. At least three

isoforms of the enzyme NO synthase (NOS) have been known: neuronal NOS (nNOS), inducible NOS (iNOS), and endothelial NOS (eNOS) (16). It is shown that rat parietal cells express nNOS, which means endogenous NO, acting as an intracellular signaling molecule, may participate in regulation of gastric acid secretion (16). Also, about 50% of the nerves in the enteric nervous system contain nNOS (16). Although it has been shown that lead exposure causes a reduction in plasma NO level (17), we did not find any respective any changes in our animals. This could be due to different lead content our exposure time. We may conclude that NO production is locally increased in the stomach, probably via lead-induced nNOS (but not eNOS) expression or activity.

There was no difference in BAO or VAO in Pb1 and Pb2 groups compared to their Na counterparts and control animals, but the VSAO is significantly decreased in Pb1 and Pb2 animals than control group, and Na1 and Na2 ones, respectively, though VSAO is much more than the basal state in each group. It has been shown that NO inhibits histamine-induced gastric acid secretion (18) via increasing intraparietal cell levels of Cyclic guanosine monophosphate (c-GMP) (19). Moreover, there is some evidence that NO decreases gastrin-induced histamine release from enterochromaffin-like (ECL) cells through a pathway in which it activates guanylate cyclase (20). The calcium response to gastrin is biphasic in ECL cells, with an initial transient increase followed by a plateau (20). There is evidence that the plateau phase is essential for exocytosis (21). NO may affect the plateau phase that is mediated by the L-type Ca channel; in fact, it can rise intracellular c-GMP and then reduce gastrin-induced calcium influx by inhibiting the activity of L-type Ca channels (20). The results of this study indicate that endogenous NO is related to the mechanism of vagally stimulated gastric acid secretion (22). It is thought that acetylcholine released by high frequency stimulation, such as 5 Hz, (like our study) acts not only on ECL cells but also directly on parietal cells (22). It is possible that NO inhibits vagally-mediated acid secretion by suppressing neuronal activity of the vagus nerve (23). Similarly, it can be postulated that NO may inhibit ECL secretion of histamine and/or histamine-induced parietal cell secretion. Accordingly, due to direct action of Ach on parietal cells, VSAO is significantly more than the basal state in Pb1 and Pb2 treated animals, meanwhile it is much less than the Na-treated animals.

In Pb3 and Pb4 groups BAO was significantly more than their Na and control animals, but in vagotomized

rat, the amount of acid secretion returned to a value similar to Na and control groups. This finding suggests an increased basal vagal tone in Pb3 and Pb4 due to lead intoxication. The VSAO was also significantly increased compared to their respective Na3 -Na4 ones. The amount of VSAO was more than the BAO in all groups. In all these findings together with increased stomach tissue NO metabolite in Pb groups, lead us to assume that NO increment probably owing to lead exposure causes increased basal and stimulated vagus nerve tone & subsequent acetylcholine release or effects on target cells.

There is some evidence that NO acts presynaptically to facilitate vagal neurotransmission via a presynaptic L-type Ca channels opening followed by increased presynaptic calcium influx and vesicular release of acetylcholine in the heart (24). NO generated by NOS in parasympathetic ganglia has also a modulatory role in facilitating the release of acetylcholine and the subsequent heart response (25). A similar mechanism may be involved in the stomach.

It has been shown that both endogenous and exogenous NO via intracellular c-GMP enhances histamine release from gastric histamine-containing cells, probably ECL cells (26, 27). Vagal stimulation (5Hz) mentioned earlier causes NO-induced Ach release which in turn directly acts on parietal cell & indirectly on ECL cells (22).

It should be mentioned that NO has a dual effect on gastric acid secretion. Haseb K, *et al.* have shown that small amounts of NO have a stimulatory effect on gastric ECL cells resulting in increased acid secretion, while a large amount of NO has an inhibitory effect on parietal cells leading to decreased gastric secretion (26, 27), but we saw an opposite result, i.e. In conclusion, according to our findings, NO in small amounts caused a decrease, and in large amounts caused an increase in gastric acid secretion, particularly when vagally stimulated. This discrepancy may be due to lead exposure. However, this hypothesis needs to be more elucidated.

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