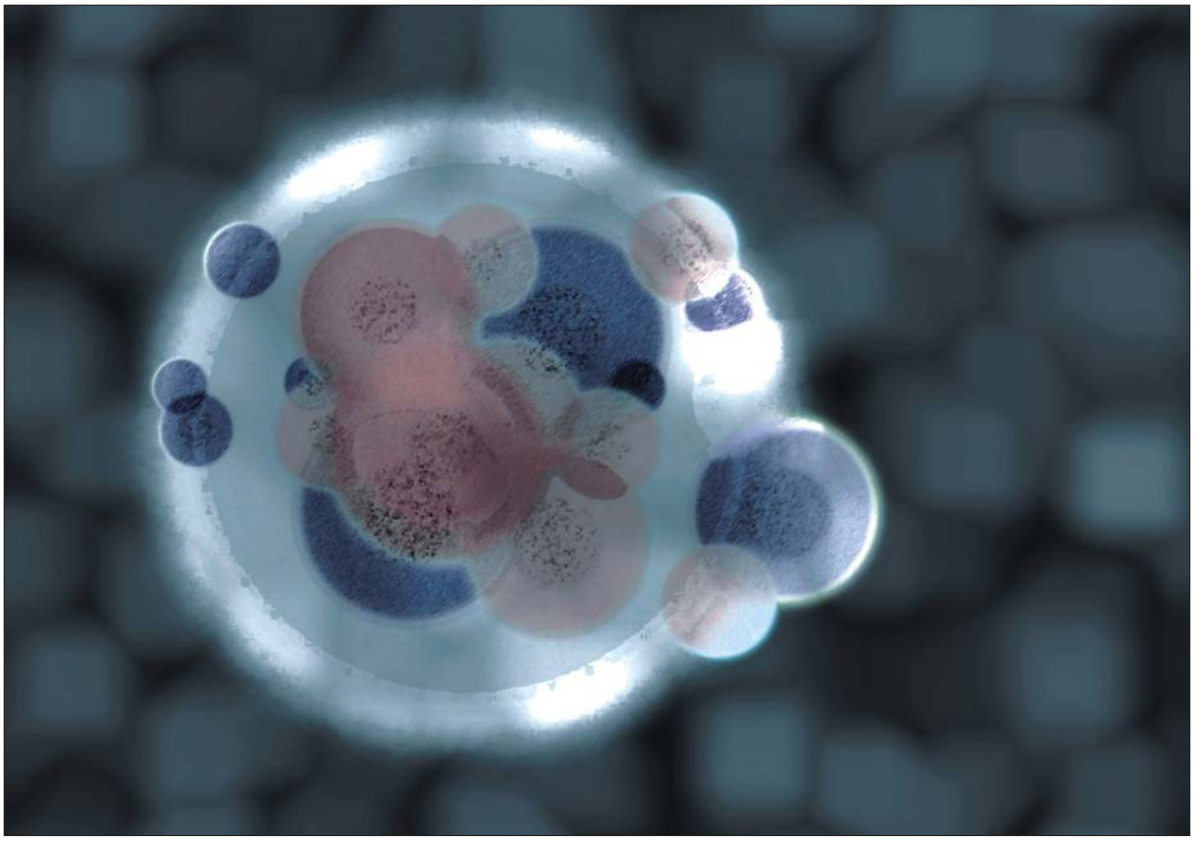

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Potentiation of Gastric Ulceration by Experimental Lead Exposure in Rats

[1]S.B. Olaleye, [1]Y. Raji, [1]S.A. Onasanwo, [1]P. Erigbali,
[1]S.O. Oyesola, [2]A. Odukanmi, [2]I.O. Omotosho and [1]R.A. Elegbe
[1]Department of Physiology, [2]Department of Medicine,
College of Medicine, University of Ibadan, Ibadan, Nigeria

Abstract: In the present study, the effects of long-term, low level (Lo-Pb) and high level (Hi-Pb) exposure of rats to lead on total gastric juice secretion and experimental ulceration were studied. Rats were exposed to low (0.01%; 100 ppm) or high (0.5%, 5,000 ppm, HiPb) levels of lead for a period of 15 weeks. The formation of ulcers was induced by hypothermic stress, the administration of indomethacin and the application of an HCl/Ethanol mixture. Exposure of animals to lead significantly increased gastric lesions produced by HCl/Ethanol mixture and indomethacin but not those induced by restraint stress. Both the LoPb and HiPb treatments significantly increased gastric acidity and reduced gastric juice volume. The results underscore the role of cumulative lead exposure in the aetiology of gastric ulcers in high lead areas.

Key words: Lead toxicity, ulcer, gastric, rats

INTRODUCTION

Increased incidence of gastric ulceration has been reported in many countries (Elegbe and Bamgbose, 1976a; Sato *et al*., 1985). Although stress has been implicated as being responsible for the observation, the possibility of the involvement of other factors such as dietary intake of potential ulcerogens should not be ruled out (Ibironke *et al*., 1997).

Lead, one of the oldest metals known to mankind, is released into the atmosphere from paints, car exhausts, etc. Although Lead is important industrially, it is considered as one of the most hazardous trace elements which contaminate the environment. In man, the main routes of exposure to lead are via ingestion with food and other products and via inhalation (Goyer and Rhyme, 1973; Anetor *et al*., 2003). Substantial exposure of individuals to lead also occurs in the workplace (Schroeder and Tipton, 1968; Al-Saleh, 1999).

Lead intoxication has been shown to affect mainly the haemopoeitic, Skeletal, nervous and renal systems (Stowe *et al*., 1973; Oberly *et al*., 1995; Ronis *et al*., 2001). From m available literature, it appears there is a dearth of information on the role of environmental pollutants in the aetiology of ulcer. We present here, findings on increased incidence of experimental ulcers in rats exposed to both high and low-level lead.

MATERIALS AND METHODS

The study was carried out between March and August 2004 in the Department of Physiology, College of Medicine, University of Ibadan, Nigeria in March 2004.

Chemicals: Lead acetate was obtained from BDH chemicals Ltd, Poole. England. Indomethacin (Indocid®) was obtained from Strides, Belgium. All other reagents were of analytical grade and were obtained from the British Drug Houses, Poole, UK.

Animals: Fifty four young male albino rats of the Wistar strain (80-90 g) were obtained from the small animal house, College of Medicine, University of Ibadan, Nigeria in March 2004 and randomly divided into three group, with adequate matching of weight. They were kept in wire meshed cages and fed with commercial rat pellets (Ladokun Feeds Ltd., Ibadan, Nigeria) and allowed water *ad libitum*.

Lead treatment: The animals were exposed to lead and the high lead group (HiPb) was given 5,000 ppm lead acetate in drinking water daily while the low lead group (LoPb) received 1000 pm lead acetate in drinking water. The control animals received only drinking water. Fifteen weeks after the onset of lead treatment, ulcer was induced as follows:

Corresponding Author: Dr. S.B., Olaleye, Department of Physiology, College of Medicine, University of Ibadan, Ibadan, Nigeria
Tel: +234-02-8100826, 08023255893

Stress-induced gastric lesions: Stress induced gastric lesions were obtained by the method described by Rao *et al.* (1997). Briefly, following a 36 h fast, rats were placed inside a ventilated refrigerator maintained at 3±1°C. After 4, the animals were sacrificed, the stomachs excised, washed in normal saline to remove any debris, pinned on a corkboard and examined for the severity of intraluminal bleeding.

Gastric lesions were evaluated by examining the inner surface with a magnifying lens. Mucosal lesions were independently assessed and scored by two observers using the method of Rao *et al.* (1997) as follows: 0 = no ulcers (normal stomach); 1 = up to 5 petechial hemorrhages with erosions of depth 1mm 3 = up to 10 petechial hemorhages with erosions of depth 1 mm; 4 = up to 10 petechial hemorrhages with erosion of depth above 1mm. Indomethacin-Induced gastric lesions.

Indomethacin (Strides, Belgium) dissolved in 1% N- methyl acetamide was administered orally (40 mg kg^{-1}) to 36 h fasted rats (Elegbe and Banigbose, 1976b). Four hours later, the animals were killed by ether overdose. The stomachs were opened along the greater curvature, washed in normal saline to remove any debris and pinned on a corkboard for ulcer scoring.

Ulcers were independently assessed and scored by two observers using the method of Rao *et al.* (1997) as described above.

HCl/Ethanol induced ulceration: Thirty six hours fasted rats were given 1.0 mL of HCl/ethanol mixture containing 0.15N HCl in 70% V/V ethanol (Anadan *et al.*, 1999). After administering the ulcerogen, all the three groups underwent surgery under sodium pentobarbitone anaesthesia (60 mg kg^{-1}; i.p) according to Brodie (1966). Gastric lesions were evaluated by as described for the other ulcer models above. Index of ulceration was calculated as the total lesion lengths divided by the number in each group (Cho and Ogle, 1978).

The 4 h gastric juice collection was drained into a graduated test tube and centrifuged at 2000 rpm for 10 min. The supernatant volume and pH were recorded. The total acid content of the gastric juice was also determined by titration to pH 7.0 with 0.05 N NaOH, using phenolphthalein as indicator. The protein content was also estimated as described by Lowry *et al.* (1951).

Estimation of gastric barrier mucous: The gastric barrier mucous was estimated by the method of Corne *et al.* (1974). Briefly, the excised stomachs from the rats were soaked for 2 hours in 0.1% Alcian blue dissolved in buffer solution containing 0.1 M sucrose and 0.05 M Sodium acetate (pH adjusted to 5.8 with hydrochloric acid). After washing the stomach twice in 0.25 M sucrose (15 and 45 min), the dye complexed with mucous was eluted by immersion in 10 mL aliquots of 0.5 M $MgCl_2$ for 2 h. The resulting blue solution was shaken with equal volumes of diethyl ether and the optical density of the aqueous phase measured at 605 nM using a spectrophotometer.

Using a standard curve, the absorbance of each solution was then used to calculate the various concentration of dye and the weight of dye (expressed in mg). The weight of the dye was then expressed over the weight of the stomach.

Statistical analysis: The Pre and Post-treatment changes in body weights were compared using the Paired t-test, while the Analysis of Variance was used wherever applicable to assess statistical differences between groups. $p<0.05$ was considered as being statistically significant.

RESULTS

Body weight changes and final blood lead: Table 1 shows at the mean weights of the control animals increased from an initial value of 87.9±2.4 to 114.5±0.4 g at the end of the study ($p<0.001$), the weights of the low-lead treated groups significantly increased from 85.6±1.9 to 101.6±2.8 g ($p<0.05$). No significant change in weight was observed in the high lead treated group (88.1±2.1 to 93.8±3.4 g, $p>0.05$). The blood lead levels at the end of the treatments are also shown in Table 1.

Effect of lead on gastric acid output: The gastric volume, pH and acid output content of the gastric juice of control animals are compared with the low-level and high-level lead treatments in Table 2-4. The lead-exposed rat had reduced gastric juice volume, pH and total protein and a significant increase in gastric acid output in both the HCl/Ethanol as well as the indomethacin models.

Effect of lead on gastric mucosal lesions: The ulcer scores in Control and Lead exposed rats are shown in Fig. 1. Ulcer formation induced by HCl/Ethanol was significantly increased in animals exposed to lead (Fig. 1a), the ulcer scores being 2.82±0.33 mm for the LoPb group ($p<0.01$) and 3.80±0.45 mm for the HiPb group ($p<0.002$) when compared with the untreated control group (2.04±0.66 mm).

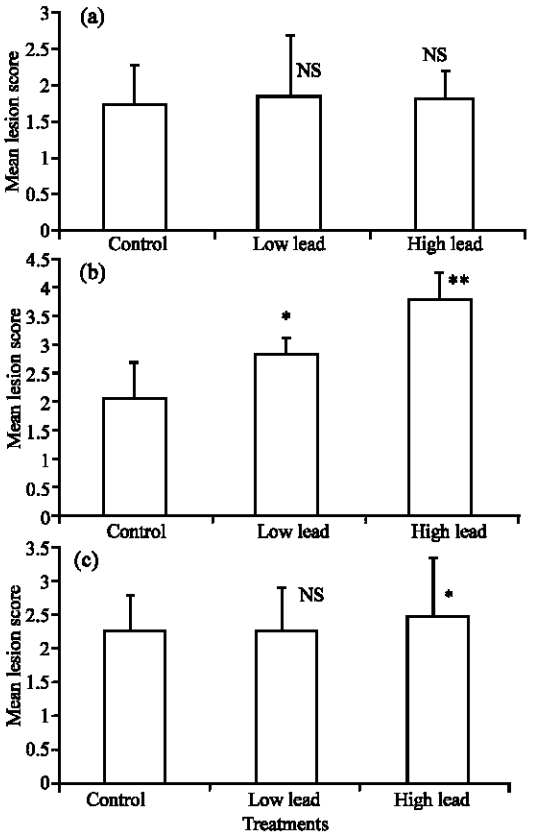

Fig. 1: Effect of long term, low and high level lead treatments on gastric mucosal lesions induced by restraint stress (a), HCl/Ethanol mixture (b) and indomethacin (c). Each bar represents mean±SEM of 8 rats in each group. *p<0.05; **p< 0.01; NS = Not Significant

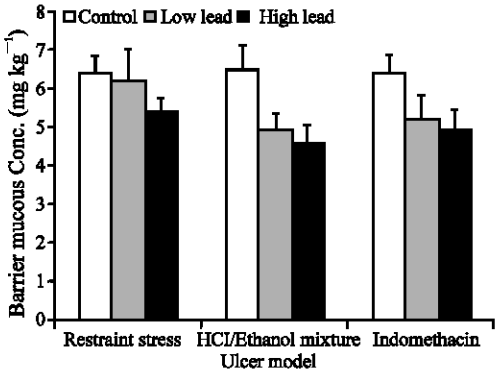

Fig. 2: Gastric barrier mucous in control and ulcerated rats exposed to high and low levels of lead

High lead treatment also increased ulcer scores in the indomethacin model from a control value of 2.20±0.24 to 2.58±0.18 in the high lead treated group (p<0.05).

Table 1: Final blood lead (Pb) levels and weight gain in untreated (control)and Lead-treated animals

			Body weights changes (g)		
Groups	Treatments	Final blood Pb (μg dL^{-1})	Initial	Final	Gain (%)
1	Control	39.4±1.55	21.9±0.3	30.2±0.4	37.9
2	Low-lead	75.42±5.24*	20.9±0.5	24.8±0.3	18.7
3	High-lead	101.55±6.08**	22.8±0.3	23.6±0.2	3.5

* p<0.05; **p < 0.01 (c.f. control)

Table 2: Effect of HCl/Ethanol induced gastric ulceration on the gastric juice profile of untreated (control) and Lead-treated animals

Groups	Treatments	Gastric juice volume (mL)	pH	Acid output (×10^4 mmoL/ 4 h)	Total protein (mg g^{-1})
1	Control	11.32±0.02	2.65±0.1	7.16±0.48	10.65±1.14
2	Low-lead	1.06±0.01	2.36±0.02	8.40±0.23*	7.91±1.02
3	High-lead	1.02±0.01	2.05±0.06	19.00±0.33**	6.88±1.15**

*p<0.05 (c.f. control)

Table 3: Effect of indomethacin induced gastric ulceration on the gastric juice profile of untreated (control) and Lead-treated

Groups	Treatments	Gastric juice volume (mL)	pH	Acid output (× 10^4 mmol/4 h)	Total protein (mg g^{-1})
1	Control	1.30±0.01	2.59±0.08	7.35±0.33	10.72±0.65
2	Low-lead	1.11±0.01	2.20±0.04	6.77±0.27	7.33±0.82*
3	High-lead	1.02±0.01	2.15±0.10	13.04±0.15**	6.40±0.14**

*p<0.05; *p<0.01 (c.f. control)

Table 4: Effect of stress-induced gastric ulceration on the gastric juice profile of untreated (control) and Lead-treated animals

Groups	Treatments	Gastric juice volume (mL)	pH	Acid output (× 10^4 mmol/4 h)	Total protein (mg g^{-1})
1	Control	1.30±0.04	2.62±0.10	7.26±0.82	10.35±0.54
2	Low-lead	1.06±0.01	2.59 ±0.83	7.42±0.81*	9.01±0.53
3	High-lead	1.02±0.01	2.55±0.74	8.35±1.03*	8.97±0.93

*p<0.05 (c.f. control)

However, there was no significant difference between ulcer scores in control and the low-lead treated animals.

When compared with the control, the formation of gastric ulcers induced by cold restraint stress was not significantly affected by Lead treatment (Fig. 1a-c).

Effect of lead on gastric barrier mucous in rats with experimental gastric ulceration: Figure 2 shows the effect of exposure of rats to lead on gastric barrier mucous. The mean value of gastric barrier mucous in control (unexposed) animals was 6.45±0.93 mg kg^{-1}. In the three models of experimental ulceration used, gastric barrier mucous was significantly decreased by exposure levels, there was no significant change in the barrier mucous in the restraint stress model of rats to high lead levels. For the rats exposed to low lead levels, there was no significant change in the barrier mucous in the restraint stress model.

DISCUSSION

The results of this study show that chronic exposure of rat to lead increased the formation of gastric ulcers induced by a HCl/ethanol mixture and indomethacin but not by cold restraint stress. The high plasma lead levels of rat treated with lead leaves little doubt that the animals actually had a supra-normal level of lead. Although the nervous system, bone and kidney are the organs of primary sensitivity to the effects of lead, exposure, the focus of the present study was to define the role lead plays in aetiology of ulcer.

Gastrointestinal wall integrity is known to be controlled by two opposing forces (a) defensive forces of the stomach-mucus barrier and (b) aggressive force -acid secreted, (Johnson, 1967; Corne *et al*., 1974). Thus ulceration of the gastric mucosa occurs when there is a surge in the aggressive forces or a reduction in the defensive forces or both. The results of the present study show that the mucous barrier was significantly reduced by excessive lead exposure. Also, the increase in total acid content of the gastric juice in this study suggest that both the defensive and aggressive forces may be altered by excessive exposure to lead. This assertion however warrants further investigation.

Many studies have implicated the generation of reactive oxygen radicals and lipid peroxidation in the genesis of ethanol-induced gastric lesions (Rao *et al*., 1997; Soll, 1990). On the other hand, indomethacin-induced ulceration is believed to be precipitated by inhibition of prostaglandin synthesis (Vane, 1971; Desai *et al*., 1997). The findings in the present work suggest that lead promotes ulcer formation by HCl/ethanol mixture through a prostaglandin dependent mechanism, probably involving free-radical Scavenging actions. Further studies to assess the role of anti-oxidant enzymes in the pathogenesis of gastric lesions produced by chronic lead exposure are going on in our laboratory.

In conclusion, the results of the present study show that there is potentiation of ulcer formation in the stomach of lead-exposed animals

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