Open Access **Baghdad Science Journal** **P-ISSN: 2078-8665**
Published Online First: March, 2023 **E-ISSN: 2411-7986**

DOI: https://dx.doi.org/10.21123/bsj.2023.7594

Lead acetate deteriorates the improvement effect of L-arginine and tetrahydrobiopterin on endothelin-1 receptors activity in rat aorta

Zhikal Omar Khudhur [1]* ***Ismail Mustafa Maulood*** [2]

[1]Department of Biology, Faculty of Education, Tishk International University, Erbil, Iraq
[2]Department of Biology, College of Science, University of Salahaddin, Erbil, Iraq
*Corresponding author: zhikal.omer@tiu.edu.iq
E-mail address: ismail.maulood@su.edu.krd

Received 25/6/2022, Revised 3/9/2022, Accepted 4/9/2022, Published Online First 20/3/2023

Abstract:

Endothelin-1 (ET-1) is a potent vasoconstrictor hormone that has been identified as an important factor responsible for the development of cardiovascular dysfunctions. ET-1 exerts its vasoconstrictor activity through two pharmacologically distinct receptors, ETA and ETB that are found in vascular smooth muscle cells (VSMCs) and the vasodilator activity through an ETB receptor located on endothelial cells. This study aimed to show the impact of 1µM L-arginine (LA), 100µM tetrahydrobiopterin (BH_4), and their combined effect on ET-1 activity in both lead-treated and lead-untreated rat aortic rings. This means, investigating how endothelial dysfunction reverses the role of nitric oxide precursor and cofactor. In this study, Rat aortic rings have been pre-incubated with BH_4, LA and their combination. Subsequently, the aortic rings were pre-incubated with 200µM N-Nitro-L-arginine methyl ester (L-NAME) and 0.5µM BQ-123. Then, the vascular response to cumulative doses of rat ET-1 was analyzed in each of the above-mentioned groups (LA, BH_4, LA & BH_4, L-NAME, BQ-123), in the presence and absence of lead acetate 1µM Pb $(C_2H_3O_2)_2$. ET-1 efficacy and potency were significantly decreased in the presence of LA, BH_4, and LA and BH_4 combination in the untreated group, while it significantly increased in the presence of lead. In the second trial of experiments ET-1 efficacy markedly decreased in BQ-123- incubated cells in both lead-treated and-untreated aortic rings. In the presence of lead, the efficacy of ET-1 was raised with the use of L-NAME. In conclusion, LA and BH_4 can be considered pharmacological agents to alter the potency of ET-1-induced vasoconstriction and concomitantly lower blood pressure.

Keywords: Endothelin-1, Endothelial dysfunction, L-arginine, Lead acetate, Nitric oxide synthase, Tetrahydrobiopterin.

Introduction:

Endothelin is a prominent vasoconstrictor that is synthesized and released by vascular endothelial cells, that was first found by Yanagisawa, Inoue[1, 2] ET is known to have a role in leading to many cardiovascular diseases[3]. Jankowich et al [4] described the association of ET-1 with hypertension, cardiac remodeling, aging, chronic kidney disease, pulmonary hypertension and diabetes[5]. It has been demonstrated that endothelin-1 (ET-1) induces pulmonary vasoconstriction through the activation of Ras homolog family member A[6].

Until now, three isoforms of ET have been identified, namely ET-1, ET-2, and ET-3[7]. ET-1 is the most prominent type of ET and it is produced mainly by endothelial cells, but also by several other cell types in the cardiovascular system. ET-1 exerts its vasoconstrictor effect by activating the G protein ETA and ETB receptors that can be found in (VSMCs). ET-1 exerts the vascular relaxation effect through activation of ETB receptor on endothelial cells. Primary, ET-1 engages endothelial nitric oxide synthase (eNOS) that increases nitric oxide (NO) bioavailability[8].

Under normal conditions, there is a harmony between the free radical and the antioxidant system in the body, which are both essential to maintain vascular functions[9]. However, during endothelial dysfunctions the production of ET increases, while NO quantity decreases, which is referred to as "an imbalance between vasodilating and vasoconstricting substances produced by (or acting on) endothelial cells"[10, 11].

Lead is a common environmental contaminant that can produce several acute and chronic diseases. According to various populational studies, there is a significant association between lead exposure and the risk of development of cardiovascular diseases particularly hypertension[12]. It has been published that the exposure of endothelial cells to lead increases the vascular reactivity to phenylephrine (PE)[13]. However, dropping the vascular reactivity has been identified in different studies[14]. Lead exposure is involved in cardiovascular disease in various mechanisms, according to certain studies, which are stimulating oxidative stress, restrictive NO accessibility, rising the secretion of endothelin, modifying the renin-angiotensin system, disturbing vascular smooth muscle Ca2+ signaling, falling endothelium-dependent vasorelaxation, and changing the vascular response to vasoactive agonists. Furthermore, lead exposure has been linked to different cellular disruptions in the heart such as injury in endothelial cells, delaying endothelial repair, restricting endothelial cell growth, suppressing the production of proteoglycan, and accelerating vascular smooth and muscle cell proliferation[15].

Nitric oxide production needs two main substrates: LA, which acts as a NO precursor, and a cofactor named BH_4, which is essential for eNOS dimerization (coupling)[3]. BH_4 is an essential cofactor of a set of enzymes that are of central metabolic importance, i.e. the hydroxylases, ether lipid oxidase, and the three nitric oxide synthase (NOS) iso-enzymes. Also, BH_4 acts as an antioxidant and neutralizes RNS and ROS[16]. According to our knowledge, no published evidence is yet available to explain how BH_4 and LA combination affects ET-1 receptors activity in the intact and lead-treated aortic rings. Therefore, the goal of this study is to see how BH_4, LA and their combination affect ET-1 efficacy in lead-treated aortic rings.

Materials and Methods:

Animals and aortic rings preparation

25 Male albino rats weighing 200-300 gm were divided into 5 cages and kept in prescribed conditions from February/ 2019 to September/ 2020, according to the laboratory animal care guide.

Intraperitoneal injection of a mixture of ketamine: xylasine 90 mg/kg: 10 mg/kg, has been used to anesthetize rats[17]. The thoracic aorta was immediately extracted, and cleaned from adipose tissues in a cold Krebs solution, then cut into four segments with lengths 2-2.5mm.

The aortic rings were suspended in the organ bath (Automatic organ Bath-Pan lab, Harvard apparatus USA, AD Instrument Power lab 8/35-Australia) that is filled with 10 ml of Krebs solution (mM/L NaCl,119; $MgSO_4$, 1.2; $CaCl_2$, 1.5; $NaHCO_3$; KCl, 4.7; KH2PO4 1.2; glucose 11.1.) that kept up at 37 °C and aerated with a mixture of 95% O2 and 5% CO2. In this, study each aorta was cut into four segments and each sample size represent one segment.

The aortic rings were allowed to equilibrate for 60 to 90 minutes, through that time the Krebs solution was replaced every 15 minutes. The function of the aortic ring was determined through the use of (60mM) KCl, after the response had reached a plateau of the maximum contraction by KCl, aortic rings were washed with a fresh medium and re-equilibrated for at least 30 minutes before adding any vasoactive substances. In denuded aortic rings, the endothelium was removed. Then each ring was contracted by 1µM PE and relaxed by 10µM of acetylcholine (ACh) as denudation assessment. ACh is used for endothelial function assessment because an artery with a healthy endothelium responds to acetylcholine by releasing nitric oxide that translates into vasodilation. While, In the presence of artery denudation from the endothelium or if the action of the nitric-oxide synthase enzyme is blocked, the artery responds to acetylcholine with vasoconstriction due to the stimulation of smooth muscle muscarinic receptors not counteracted by the nitric oxide of endothelial origin

Chemicals

Endothelin-1 was purchased from Bachem-Switzerland; BH_4 from Nootropic- USA; BQ-123 from Selleckchem- USA; Pb $(C_2H_3O_2)_2$ from Merck-Germany; LA and L-NAME were provided by Scharlab- Spain.

Experimental protocol

Prepared aortic rings used to evaluate the effect of lead acetate on vascular rings to ET-1 response in two trials. Cumulative concentration doses of ET-1 ($5*10^{-11}$-10^{-7}) were applied in both Pb $(C_2H_3O_2)_2$(1µM)-treated and untreated rings. 1µM of Pb $(C_2H_3O_2)_2$ was prepared by adding 3.3 mg of Pb $(C_2H_3O_2)_2$ to 10 mL of distilled water, which produce 1mM, and diluted to 1 µM. To assess the influence of endothelium on the response to ET-1, cumulative doses of ET-1 were used in denuded and intact aortic rings.

Experiment 1: The role of LA, BH_4, and their combination in the ET-1 elicited contractile response was investigated in the presence and absence of Pb

$(C_2H_3O_2)_2$. The intact aortic rings were incubated for 45 minutes with the mentioned substances before the generation of the ET-1 concentration-response curves. Then, to find out the role of Pb $(C_2H_3O_2)_2$ on vascular reactivity in response to ET-1, other aortic rings were incubated with 1µM of Pb $(C_2H_3O_2)_2$ for 45 minutes before using LA, BH_4, and their combination.

Experiment 2: To evaluate the effect of different vasoactive substances in ET-1 elicited vascular contraction, aortic segments were incubated with 200µM L-NAME, and 0.5µM of BQ-123. These drugs were added 20 minutes before the concentrated doses of ET-1 were applied, in the absence and presence of 1µM Pb $(C_2H_3O_2)_2$. Finally, 10µM ACh was added to lead-treated groups to check the endothelial dysfunction (ED) induced by lead acetate.

Statistical analysis

The contractions stimulated by 60mM KCl were observed and recorded as percentages (%KCl). By means of Graph Pad Prism, the concentration-response curves of ET-1 were fitted nonlinearly. To differentiate the effect materials in both control and lead-treated groups of aortic segments, all results were expressed as the differences area under curves (dAUC). Means ± standard error of the mean (SEM) were used for the expression of all data. Independent Students t-test was applied to compare dAUC and potency difference (pD_2) between groups. To investigate the distinction between the study groups and control, a two-way analysis of variance (ANOVA) was used, followed by Sidak multiple comparison tests for each mean comparison, Dunnett-test was for comparing the studied groups with the control, and Tukey-test as the pD_2 comparison between groups. P-Value ≤ 0.05.

Results

The prepared aortic rings were exposed to cumulative doses of ET-1 ($5*10^{-11}$-10^{-7}M). At the end of each experiment on lead acetate-treated aortic rings, impairment of the endothelial has occurred resulting in a lower response to ACh.

The effect of LA, BH_4, and their combination on vascular response to ET-1.

1µM of L-arginine was applied to analyze the effect of NO precursor on vascular reaction to ET-1. LA significantly reduced the efficacy and potency of ET-1 in untreated groups Fig.1A. While, in the presence of Pb $(C_2H_3O_2)_2$, LA noticeably boosted the vasoconstriction response to ET-1 in table 1. According to the dAUC graph Fig. 1A, a significant alteration in vascular response to ET-1 was observed with the presence of lead during LA pre-incubation.

Table 1. The maximum response (Emax) and the potency difference (pD_2) to ET-1 in the presence and absence of Pb $(C_2H_3O_2)_2$ in rat aortic rings

Groups	Untreated			Pb.treated		
	No	Emax (%KCL)	pD_2	No	Emax (%KCL)	pD_2
Control	7	182.2 ± 6.441	-8.278 ± 0.0705	8	185.9 ± 13.68	-7.585 ± 0.1021
LA	5	139.8 ± 9.455*	-7.887± 0.1114***	5	237.1 ± 14.39*	-8.03 ± 0.1084*
BH_4	7	129.3 ± 12.29**	-8.141 ± 0.1837$^{\#\#}$	5	160.5 ± 10.05	-8.227 ± 0.1201** ##
LA + BH_4	5	143.6 ± 4.605*	-7.606 ± 0.0451*** ## ¤¤¤	5	183.9 ± 5.968	-7.846 ± 0.056

The studied groups were compared with each other (ANOVA was applied with Tukey test). *, **, *** represents statistical differences at $P \leq 0.05$, $P \leq 0.01$ $P \leq 0.001$versus the corresponding control group, ## Significant differences between groups vs LA group at $p \leq 0.01$, and ¤¤¤ Significant differences between groups vs BH_4 group at $p \leq 0.001$.

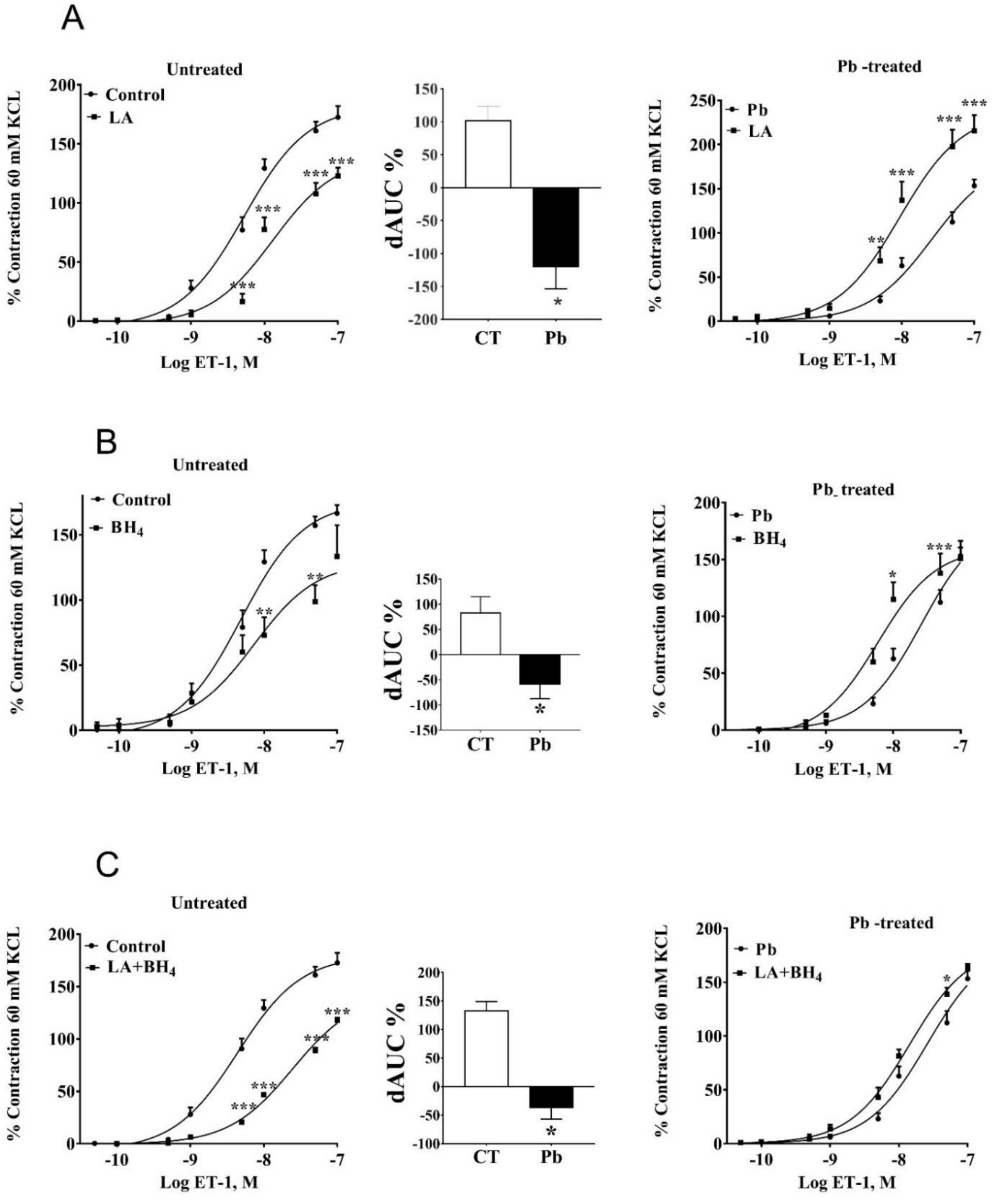

Figure 1. (A) Effect of 1µM LA, (B) Effect of 100 µM BH_4, and (C) Effect of LA and BH_4 combination on the vasoconstrictor responses to ET-1 in Pb $(C_2H_3O_2)_2$ treated and untreated aortic rings. The inset graph shows dAUC. The asterisks; *, **, * represent statistical differences at P<0.05, P<0.01 P<0.001versus the corresponding control group.**

100µM of BH_4 was applied to determine the influence of lead acetate on vascular actions and the possible role of the NOS cofactor. Fig. 1B showed significant reductions in both efficacy and potency in the absence of lead acetate, while in the lead acetate treated group the concentration-response curve shifted to the left with significant rises in ET-1 potency and maximum response. The dAUC shown in Fig. 1B represented the highly significant change in the presence of lead acetate that can alter vascular behavior.

The results revealed that aortic rings pre-incubation with LA and BH_4 combination causes a significant decrease in the maximum response, as well as a highly significant decrease in potency to ET-1 in the absence of lead acetate in comparison to control, LA and BH_4 groups. While no statistical change was observed in ET-1 potency and efficacy in the lead acetate-treated group as shown in Table. 1, the dAUC showed a highly significant change in the vascular response to ET-1, under exposure to both LA and BH_4 and in the existence of LA Fig. 1C.

Table 2. The maximum response (Emax) and the potency difference (pD_2) to ET-1 in the presence and absence of Pb $(C_2H_3O_2)_2$ in rat aortic rings

Groups	Untreated			Pb- treated		
	No	Emax (%KCL)	pD_2	No	Emax (%KCL)	pD_2
Control	7	182.2 ± 6.441	-8.278 ± 0.0705	8	185.9 ± 13.68	-7.585 ± 0.1021
L-NAME	5	243 ± 10.62	-8.019 ± 0.0789	5	234.1 ± 6.686*	-8.217 ± 0.05373
BQ-123	4	105 ± 6.96*	-5.756 ± 1.146***	5	168.6 ± 7.8**	-5.919 ± 0.4859***
Denudation	5	208.9 ± 8.036	-8.479 ± 0.08498	5	185.3 ± 6.22	-8.328 ± 0.07034*

The studied groups were compared with each other (ANOVA was applied with Dunnet test). * Significant differences between groups vs control group at $p \leq 0.05$, ** Significant differences between groups vs control group at $p \leq 0.01$, *** Significant differences between groups vs control group at $p \leq 0.001$.

Role of endothelial NOS inhibitor on vascular responsiveness to ET-1.

200µM of L-NAME has been performed to explore the effects of lead acetate on ET-1 vascular actions and the probable impact of eNOS. The result showed that inhibiting eNOS noticeably increases the vasoconstriction response of ET-1 with no significant changes in potency and efficacy in the lead-untreated group. On the contrary, in the presence of Pb $(C_2H_3O_2)_2$, L-NAME exhibited a highly significant rising effect of vascular ET-1 efficacy, although, ET-1 potency remained significantly unchanged. According to the dAUC graph shown in Fig. 2A, L-NAME exerts a higher impact in the presence of lead acetate.

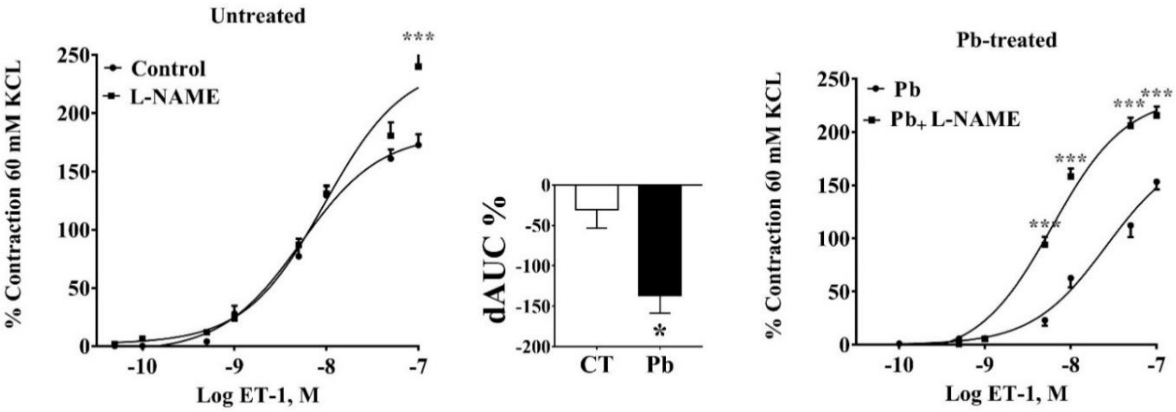

Figure 2. Effect of 200µM L-NAME on the vasoconstrictor responses to ET-1 in Pb $(C_2H_3O_2)_2$ treated and untreated aortic rings. The inset graph shows dAUC. The asterisks; *, **, * represent statistical differences at P<0.05, P<0.01 P<0.001versus the corresponding control group.**

Role of ETA receptor antagonist on vascular responsiveness to ET-1.

0.5µM of BQ-123 was used to investigate the role of lead acetate on ET-1 vascular actions and the role of the ETA receptor. The result, in the presence and absence of AC, showed that BQ-123 significantly reduced the maximum response and potency in Table. 2. The dAUC graph in Fig. 3 showed the great effect of lead on the vascular response to ET-1 as compared with the control group.

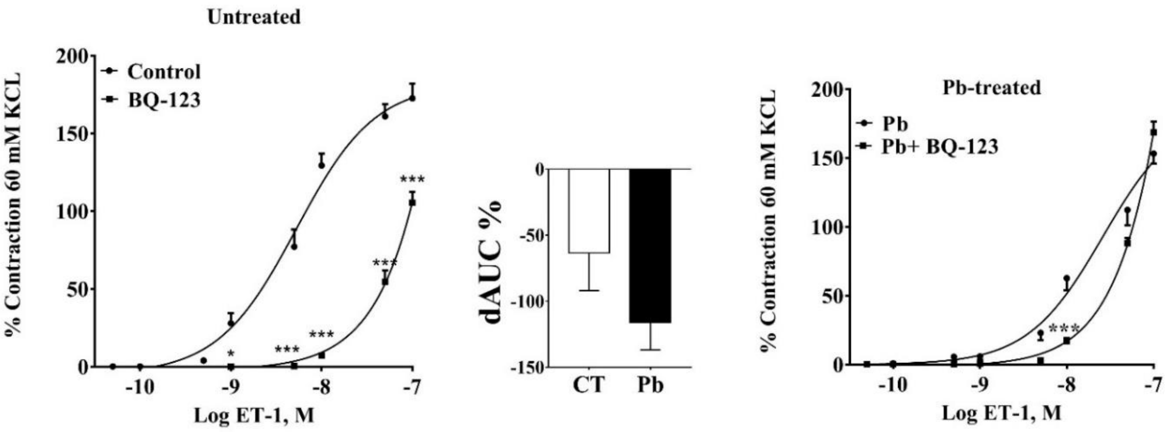

Figure 3. Effect of 0.5µM BQ-123 on the vasoconstrictor responses to ET-1 in Pb $(C_2H_3O_2)_2$ treated and untreated aortic rings. The inset graph shows dAUC. The asterisks; *, **, * represent statistical differences at P<0.05, P<0.01 P<0.001versus the corresponding control group.**

The role of endothelium layer on vascular responsiveness to ET-1.

To determine the influence of the endothelial layer on the vascular response of aortic rings to ET-1, cumulative doses of ET-1 were applied to intact and denuded rat aortic rings. ET-1 reactivity following endothelial damage (denudation) did not undergo alterations of the maximum response in both lead-treated and untreated groups, whereas the potency significantly rises in the lead-treated group but remain unchanged in the untreated group as shown in Table. 2. The dAUC values demonstrate a similar attenuating effect in the presence and absence of lead acetate as shown in Fig. 4.

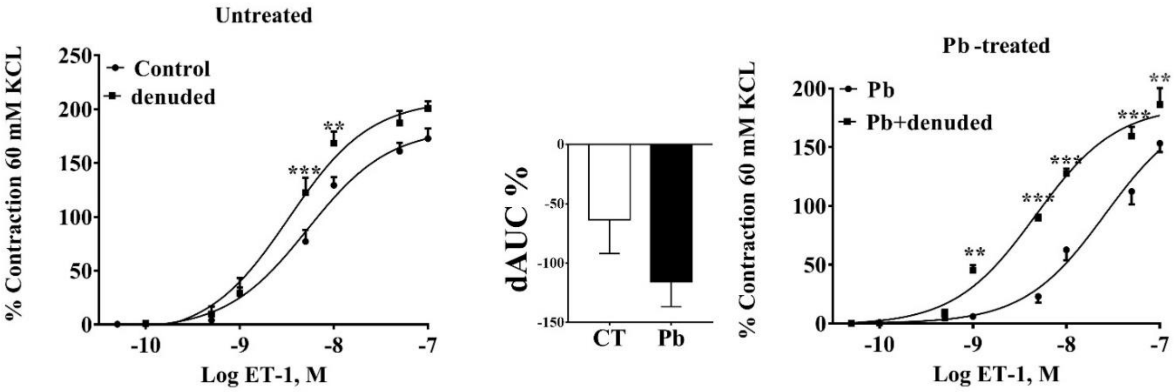

Figure 4. Effect of endothelial layer on the vasoconstrictor responses to ET-1 in Pb $(C_2H_3O_2)_2$ treated and untreated aortic rings. The inset graph shows dAUC. The asterisks; *, **, * represent statistical differences at P<0.05, P<0.01 P<0.001versus the corresponding control group.**

Discussion:

The most important finding of this study revealed that acute lead exposure to isolated rat aortic rings deteriorates the improvement effect of LA and BH_4. From the present results, we found that lead acetate reduces ET-1 vascular reactivity which is most probably associated with oxidative stress and hence ED. Although, the exact mechanism by which lead acetate induces ED is not fully understood, the rational reason for our finding may be related to the elevation of hydrogen peroxide and subsequent alteration of K^+ channel [18]. As demonstrated by this study, pre-incubation of LA, which acts as a substrate for NO production, directly influences NO levels as observed by the decreases of the vascular response to ET-1 in the absence of lead acetate. NO represents a potent vasodilator of the cyclic guanosine monophosphate (cGMP) pathway [19]. In addition, basal NO production can modulate ET-1 activity through endothelial ET-B receptor activity [20]. Interestingly, according to the dAUC shown in Fig.1A, LA elicited the vascular response to ET-1 in the presence of lead and shifted the ET-1 dose-response curve to the left, because LA probably enhances the ROS production in the presence of lead. ROS may uncouple the eNOS-catalyzed reduction of molecular oxygen from the oxidation of L-arginine, resulting in the paradoxical production of the ROS superoxide anion instead of the reducing NO. Alternatively, ROS may react with NO directly, reducing its bioavailability[21]. Furthermore, the result suggests that LA in lead acetate-treated aortic rings may deteriorate the vascular reactivity of ET-1 as recorded by the study of Rosano[22], that concluded that the rise of ROS generation increases ET-1 gene expression[23].

ROS may uncouple the eNOS-catalyzed reduction of molecular oxygen from the oxidation of L-arginine, resulting in the paradoxical production of the ROS superoxide anion instead of the reducing NO. Alternatively, ROS may react with NO directly, reducing its bioavailability

In the same manner, BH_4 markedly decreases the vascular responses to ET-1 by enhancing NO bioavailability [3, 24], whereas BH_4 increases the vascular reactivity to ET-1 in the presence of lead. Reports associated with the impact of BH_4 on ET-1 potency are very limited. Also, according to our knowledge, this is the first study that provides evidence that BH_4 enhances vascular reactivity in lead-treated and untreated isolated aortic rings. It is well established that lead induces the release of free radicles that potentially oxidize BH_4 to BH_2; indeed, $ONOO^-$ produced by lead acetate can oxidize BH_4 spontaneously within minutes at physiologically relevant Concentration[25].

The vasoconstrictor effect of ET-1 is markedly reduced in lead untreated aortic rings in the presence of LA and BH_4 combinations. However, the same result was not obtained in the presence of lead. The present finding is consistent with Milewski study, which recorded that LA and BH_4 are the primary substrates in the generation of NO [26]. Moreover, bonding both BH_4 to eNOS rises the enzymatic turnover of LA[27] and enhances the stability of the active eNOS dimer [28]. In addition, another study found that pre-incubation of both LA and BH_4 stimulated a significant increase in ACh-induced relaxation [29].

However, in lead-treated aortic rings, LA and BH_4 in combination significantly increase the vascular responsiveness to ET-1, as previously described, due to two logical reasons. Firstly, it is well known that lead-induced ROS production decreases NO bioavailability[24], hence ET-1 vasoconstrictor activity increases. Secondly, the oxidation of BH_4 to BH_2 exerts allosteric action to stabilize the active dimeric form of eNOS [30].

Several studies demonstrated that NO is an important relaxing factor in conductance arteries[31]. Therefore, in order to investigate the influence of NO on the vascular response to ET-1 in isolated aortic rings, we applied the non-specific NOS inhibitor L-NAME. The obtained results indicated that L-NAME increases the reactivity of ET-1 in both lead-treated and untreated groups although its impact is greater in the presence of lead, as illustrated in dAUC in Fig. 2. L-NAME reduces NO availability and it is well established that NO reduces the vasoconstrictor activity of ET-1 [32], hence NO reduction by L-NAME in isolated aorta potentiates the ET-1 vasoconstriction. On the other hand, the enhancement of ET-1 potency by lead is mainly due to the overproduction of ROS[24]. Additionally, D'Angelo recorded that an increase in ROS may affect downstream ET receptor activation [33].

It is not surprising that blocking the ETA receptor by BQ-123 in the absence and presence of lead significantly reduced the vascular sensitivity to ET-1. It has been confirmed that vasoconstriction activity of ET-1 generally depends on this receptor subtype[34] and ETA antagonists are able to fully reverse an established ET-1 constrictor response[35].

In the present study, the removal of the endothelial layer potentiated the vasoreactivity of ET-1, this verifies that the endothelial-independent vasoconstriction of the peptide is through ETA and ETB on VSMCs. This finding is supported by the study of Schiffrin[36] , that demonstrated that ET-1 exerts endothelial-independent vasoconstriction. In the impaired endothelial layer induced by lead, the efficiency of ET-1 was increased possibly through the induction of oxidative stress that further potentiated ET-1 maximum response by removing NO availability and eNOS activity[37].

Conclusion:

In conclusion, our results demonstrated for the first time that pre-incubation of LA and BH_4 alone and in their combination reverse the potency of ET-1-induced vasoconstriction. These effects are markedly changed by lead acetate pre-incubation to ET-1. The results also indicated that the potency of ET-1 partially depends on NO and prostacyclin pathways. Moreover, and according to the data obtained using ETA receptor antagonist (BQ-123) and denudation of the aortic rings suggested that changes in ET-1 reactivity are mostly due to its ET-A receptor which is highly located on VSMCs. In summary, LA and BH_4 may be considered pharmacological tools to modulate the potency of ET-1-induced vasoconstriction and concomitantly lower blood pressure.

Authors' declaration:

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for re-publication attached with the manuscript.
- The author has signed an animal welfare statement.
- Ethical Clearance: The project was approved by the local ethical committee in Tishk International University, Iraq.
- **Ethics approval:** Experimental design accepted by the ethics committee and animal care committee in the College of Science, Salahaddin University with the ethic reference number 45/144.

Authors' contributions statement:

ZK Carried out the experiment, data acquisition and writing the manuscript. Statistical analysis, conception and design of the study was done by IM.

Limitation of the study

There are two major limitations in this study that could be addressed in future research. First, because of time and cost we couldn't estimate ET A and B receptors' gene expression from the isolated aorta by using RT-PCR or western blotting. Second, we highly recommended using different potassium channel blockers which may associate with the LA signal transduction pathway which produces vasodilators in the presence of BH_4.

References:

1. Yanagisawa M, Inoue A, Ishikawa T, Kasuya Y, Kimura S, Kumagaye S, et al. Primary structure, synthesis, and biological activity of rat endothelin, an endothelium-derived vasoconstrictor peptide. Proc Natl Acad Sci U S A. 1988; 85(18): 6964-7.
2. Kostov K. The Causal Relationship between Endothelin-1 and Hypertension: Focusing on Endothelial Dysfunction, Arterial Stiffness, Vascular Remodeling, and Blood Pressure Regulation. Life (Basel). 2021; 11(9): 1-16
3. Khudhur ZO, Maulood IM. L-arginine and tetrahydrobiopterin modulate endothelin-1A receptor activity in isolated rat aorta. Zanco J pure Appl Sci. 2019; 31(2): 101-8.
4. Jankowich M, Choudhary G. Endothelin-1 levels and cardiovascular events. Trends Cardiovasc Med. 2020; 30(1): 1-8.
5. Khidhir SM, Mahmud AM, Maulood IM. Association of Endothelin-I and A symmetric Dimethylarginine Levels with Insulin Resistance in Type-2 Diabetes Mellitus Patients. Baghdad Sci J. 2022; 19(1): 0055-.
6. Sugimoto K, Yokokawa T, Misaka T, Kaneshiro T, Yamada S, Yoshihisa A, et al. Endothelin-1 Upregulates Activin Receptor-Like Kinase-1 Expression via Gi/RhoA/Sp-1/Rho Kinase Pathways in Human Pulmonary Arterial Endothelial Cells. Front. Cardiovasc. Med. 2021; 8: 1-8
7. Torres Crigna A, Link B, Samec M, Giordano FA, Kubatka P, Golubnitschaja O. Endothelin-1 axes in the framework of predictive, preventive and personalised (3P) medicine. EPMA J . 2021; 12(3): 265-305.
8. Masaki T, Sawamura T. Endothelin and endothelial dysfunction. Proc Jpn Acad Ser B Phys Biol Sci. 2006; 82(1): 17-24.
9. Citi V, Martelli A, Gorica E, Brogi S, Testai L, Calderone V. Role of hydrogen sulfide in endothelial dysfunction: Pathophysiology and therapeutic approaches. J Adv Res. 2021; 27: 99-113.
10. McElwain CJ, Tuboly E, McCarthy FP, McCarthy CM. Mechanisms of Endothelial Dysfunction in Pre-eclampsia and Gestational Diabetes Mellitus: Windows Into Future Cardiometabolic Health? Front Endocrinol (Lausanne). 2020; 11(655): 1-19
11. Khaleel FM, N-Oda N, A Abed B. Disturbance of Arginase Activity and Nitric Oxide Levels in Iraqi Type 2 Diabetes Mellitus. Baghdad Sci J. 2018; 15(2): 189-91.
12. Tsoi MF, Lo CWH, Cheung TT, Cheung BMY. Blood lead level and risk of hypertension in the United States National Health and Nutrition Examination Survey 1999–2016. Sci Rep. 2021; 11(1): 3010.
13. Silveira EA, Lizardo JH, Souza LP, Stefanon I, Vassallo DV. Acute lead-induced vasoconstriction in the vascular beds of isolated perfused rat tails is endothelium-dependent. Braz J Med Biol Res. 2010; 43(5): 492-9.
14. Fiorim J, Ribeiro Júnior RF, Silveira EA, Padilha AS, Vescovi MV, de Jesus HC, et al. Low-level lead exposure increases systolic arterial pressure and endothelium-derived vasodilator factors in rat aortas. PLoS One. 2011; 6(2): e17117.
15. Jiang X, Xing X, Zhang Y, Zhang C, Wu Y, Chen Y, et al. Lead exposure activates the Nrf2/Keap1 pathway, aggravates oxidative stress, and induces reproductive damage in female mice. Ecotoxicol Environ Saf. 2021; 207: 111231.
16. Kietadisorn R. Drainage versus defense: The management of vascular leakage in cardiovascular diseases. 2018. Thesis, Maastricht University]. Ridderprint BV. https://doi.org/10.26481/dis.20180523rk .
17. Linsenmeier RA, Beckmann L, Dmitriev AV. Intravenous ketamine for long term anesthesia in rats. Heliyon. 2020; 6(12): e05686.
18. Khan M, Rolly NK, Al Azzawi TNI, Imran M, Mun B-G, Lee I-J, et al. Lead (Pb)-Induced Oxidative Stress Alters the Morphological and Physio-Biochemical Properties of Rice (Oryza sativa L.). Agronomy. 2021; 11(3): 409.
19. Dao VT, Elbatreek MH, Deile M, Nedvetsky PI, Güldner A, Ibarra-Alvarado C, et al. Non-canonical chemical feedback self-limits nitric oxide-cyclic GMP

signaling in health and disease. Sci Rep. 2020; 10(1): 10012.
20. Koyama Y. Endothelin ETB Receptor-Mediated Astrocytic Activation: Pathological Roles in Brain Disorders. Int J Mol Sci. 2021; 22(9): 4333.
21. Schulz E, Gori T, Münzel T. Oxidative stress and endothelial dysfunction in hypertension. Hypertens Res. 2011; 34(6): 665-73.
22. Rosanò L, Spinella F, Bagnato A. The importance of endothelin axis in initiation, progression, and therapy of ovarian cancer. Am J Physiol Regul Integr Comp Physiol. 2010; 299(2): R395-404.
23. Rafnsson A, Matic LP, Lengquist M, Mahdi A, Shemyakin A, Paulsson-Berne G, et al. Endothelin-1 increases expression and activity of arginase 2 via ETB receptors and is co-expressed with arginase 2 in human atherosclerotic plaques. Atherosclerosis. 2020; 292: 215-23.
24. Bendall JK, Douglas G, McNeill E, Channon KM, Crabtree MJ. Tetrahydrobiopterin in cardiovascular health and disease. Antioxid Redox Signal. 2014; 20(18): 3040-77.
25. Feng Y, Feng Y, Gu L, Liu P, Cao J, Zhang S. The Critical Role of Tetrahydrobiopterin (BH4) Metabolism in Modulating Radiosensitivity: BH4/NOS Axis as an Angel or a Devil. Front Oncol. 2021; 11: 720632.
26. Milewski K, Czarnecka AM, Albrecht J, Zielińska M. Decreased Expression and Uncoupling of Endothelial Nitric Oxide Synthase in the Cerebral Cortex of Rats with Thioacetamide-Induced Acute Liver Failure. Int J Mol Sci. 2021; 22(13): 6662.
27. Costa D, Benincasa G, Lucchese R, Infante T, Nicoletti GF, Napoli C. Effect of nitric oxide reduction on arterial thrombosis. Scand Cardiovasc J. 2019; 53(1): 1-8.
28. Guerby P, Tasta O, Swiader A, Pont F, Bujold E, Parant O, et al. Role of oxidative stress in the dysfunction of the placental endothelial nitric oxide synthase in preeclampsia. Redox Biol. 2021; 40: 101861.
29. Jiang J, Valen G, Tokuno S, Thorén P, Pernow J. Endothelial dysfunction in atherosclerotic mice: improved relaxation by combined supplementation with L-arginine-tetrahydrobiopterin and enhanced vasoconstriction by endothelin. Br J Pharmacol. 2000; 131(7): 1255-61.
30. Gonçalves DA, Jasiulionis MG, Melo FHMd. The Role of the BH4 Cofactor in Nitric Oxide Synthase Activity and Cancer Progression: Two Sides of the Same Coin. Int J Mol Sci. 2021; 22(17): 9546.
31. Shimokawa H, Godo S. Nitric oxide and endothelium-dependent hyperpolarization mediated by hydrogen peroxide in health and disease. Basic Clin Pharmacol Toxicol. 2020; 127(2): 92-101.
32. Rapoport RM. Acute nitric oxide synthase inhibition and endothelin-1-dependent arterial pressure elevation. Front Pharmacol. 2014; 5: 57.
33. D'Angelo G, Loria AS, Pollock DM, Pollock JS. Endothelin activation of reactive oxygen species mediates stress-induced pressor response in Dahl salt-sensitive prehypertensive rats. Hypertension. 2010; 56(2): 282-9.
34. Miyauchi T, Sakai S. Endothelin and the heart in health and diseases. Peptides. 2019; 111: 77-88.
35. Maguire JJ, Davenport AP. Endothelin receptors and their antagonists. Semin Nephrol. 2015; 35(2): 125-36.
36. Schiffrin EL. Does Endothelin-1 Raise or Lower Blood Pressure in Humans?. Nephron. 2018; 139(1): 47-50.
37. Stefanov G, Briyal S, Pais G, Puppala B, Gulati A. Relationship Between Oxidative Stress Markers and Endothelin-1 Levels in Newborns of Different Gestational Ages. Front Pediatr. 2020; 8: 279.

Open Access Baghdad Science Journal P-ISSN: 2078-8665
Published Online First: March, 2023 E-ISSN: 2411-7986

تأثيرالأسيتات الرصاص على تدهور نشاط الارجنين و تيتراهيدروالبايوبترين فى نشاط مستقبلات الاندوثيلين-1 في الشريان الأبهر للفئران

زيكال عمر خضر[1] اسماعيل مصطفى مولود[2]

1قسم علوم الحياة، كلية التربية، جامعة تيشك الدولية، أربيل، العراق
2قسم علوم الحياة، كلية العلوم، جامعة صلاح الدين، أربيل، العراق

الخلاصة:

يعتبر هرمون الأندوثلين-1 مضيق قوي للأوعية تم تحديده كعامل مهم مسؤول عن الإصابة باضطرابات القلب والأوعية الدموية. يظهر ET-1 فعاليته كمضيق للأوعية من خلال مستقبلين متمايزين دوائيًا هما ETA وETB الموجودين على خلايا العضلات الملساء الوعائية (VSMCs)، والنشاط الموسع للأوعية من خلال مستقبل ETB الموجود على الخلايا البطانية. تهدف هذه الدراسة إلى إظهار تأثير كل من ال 1µM L-arginine (LA) و 100µM tetrahydrobiopterin (BH_4) وتأثيرهما المشترك على نشاط ET-1 في كل من الحلقات الأبهرية للجرذان المعالجة وغير المعالجة بالرصاص. وهذا يعني التحقيق من مدى تاثير الخلل البطاني على دور أكسيد النيتريك والعامل المساعد .حيث تم احتضان الحلقات الأبهرية في هذه الدراسة للجرذان مسبقًا باستخدام BH_4 و LA بالإضافة لتأثيرهما المشترك. بعد ذلك، تم حضن الحلقات الأبهرية مسبقًا باستخدام N-Nitro-L-arginine methyl ester (L-NAME) 200µMو0.5µM .BQ-123 بعد ذلك حيث تم الكشف عن الاستجابة الأوعية الدموية للجرعات التراكمية من ET-1 في كلا المذكورتين أعلاه (LA, BH_4, LA & BH_4, L-NAME, BQ-123) بوجود وغياب خلات الرصاص 1µM Pb($C_2H_3O_2$)2 . حيث تم ملاحظة انخفاض ملحوظ في الفعالية وقوة التأثير لل ET-1 بوجود تركيبة LAو BH_4 و LA و BH_4 في المجموعة غير المعالجة ، بينما زادت بشكل واضح بجود الرصاص. ، بينما زادت بشكل ملحوظ في وجود الرصاص. وفي المجموعة الثانية من التجربة، انخفضت فعالية ET-1 بشكل ملحوظ في الخلايا المحتضنة BQ-123 في كل من الحلقات الأبهرية المعالجة بالرصاص والغير المعالجة. بوجود الرصاص، تمت زيادة فعالية ET-1 باستخدام ال L-NAME . نستنتج من هذا ، يمكن اعتبار LA و BH_4 من العوامل الدوائية التي لها تأثير على فاعلية ET-1 في تضيق الأوعية الدموية المتزامن مع انخفاض ضغط الدم.

الكلمات المفتاحية: الأندوثلين-1، اعتلال الظهارية الداخلية ، الأرجنين ، خلات الرصاص، مخلقة اوكسيد النتريك، تيتراهيدرو البيوبتيرين.