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Lead Exposure Activating NF-kB Pathways Through Reactive Oxygen Species Increasing Cellular Permeability

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Abstract. Lead exposure is a significant problem nowadays. There are millions death due to lead exposure per year, and most of them are due to the effect from lead exposure such as, idiopathic intellectual disability, cardiovascular disease, and chronic kidney diseases etc. By activating reactive oxygen species (ROS), it functions as an environmental toxin that affects a variety of biological systems and ecosystems, resulting in diseases and inflammation. It is estimated that exposure to lead causes millions of deaths yearly and long-term health problems, especially in children. These experiments show that lead causes oxidative stress through reactive oxygen species (ROS), which affects inflammatory pathways and cellular functions such as ERK1/2 and NF-kB. These pathways display crosstalk when oxidative stress activates them, which may affect inflammatory responses and cell permeability. This emphasizes how important lead exposure is in impairing cellular health and function. Where this study aims to find out the relationship between reactive oxygen species, Nf-kB pathways, and cellular permeability, and therefore obtaining 8 possible results that indicates the possibilities in the relationship between ROS, Nf-kB, and cell permeability. While obtaining a perfect result can help use in mechanism insight, pathway research, public health, and therapeutic targets.

Keywords: Lead, Nf-kB pathway, Reactive oxygen species, cellular permeability, environmental toxic

1. Introduction

In April 25, 2014, in Flint, Michigan the government decides to switched its drinking water supply from the Detroit system to the Flint River in order to save money. This inadequate water treatment causes significant water quality and health issues that like rashes, hair loss, and itchy skin on the Flint residents due to the high lead exposure in such water [1].

Lead (Pb) is heavy metal that is only composed by one type atom and has resulted in environmental degradation and health issues because of its poisonous. It is an environmental toxin that impacts the gastrointestinal, cardiovascular, neurological, hematologic, renal systems, and the ecosystems etc. Lead was found as a harm in the human body as lead exposure causing diseases and inflammation due to the activation of reactive oxygen series causing death [2]. According to data, lead exposure in human body was estimated causing almost millions of people dying each year with an increase on long term health problems in children [3]. Where there are multiple experiments done

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about lead inducing oxidative stress via reactive oxygen species (ROS). When increasing concentration and manipulating in lead can cause stress responses in activation of ROS, generating enzymes and inhibiting production of NO causing an increase in ROS [4]. Where Reactive oxygen species (ROS) are molecules with at least one oxygen atom and one or more unpaired electrons that are able to survive by their own [5]. The appearance of reactive oxygen species in human body is one of the most well-known causations of many inflammatory diseases. Reactive oxygen species directly oxidize biological macromolecules like proteins and lipids etc. damaging and dysfunction tissues causing inflammation. Additionally, ROS also cause inflammation by activating inflammatory pathways through oxidative stress such as the erk1/2 and nf-kb pathways. ERK1/2 signaling pathway is a key signaling channel that controls various kinds of cellular process [6].Through reactive oxygen species and oxidative stress ERK pathways causes degranulation of neutrophils and can cause inflammation issues. NF-kB pathway a similar pathway with the ERK1/2 pathway, is a proinflammatory signaling pathway that appears in almost all animal cell type that controls the DNA transcription, cytokine production, and cell survival. Both of these pathways can be activated in human body through oxidative stress and stimuli. Furthermore, the activation of these pathways has crosstalk and interaction. This means that activation of one influence the other, so it is possible that lead activating the NF-kB pathway can cause cell permeability like the ERK pathway causing degranulation [7]. Cell permeability is the ability of a cell membrane to control flow in and out of substances and chemicals. Increasing of cell permeability can cause inflammation disease and might also affect the cell functioning. Furthermore, theoretically activation of NF-kB can cause cellular permeability through oxidative stress [8].

1.1. Research question

Since Pb can induce oxidative stress via reactive oxygen species(ROS) and use the ERK ½ signaling pathways, can it also activates NF-κB and causes changes in cellular permeability?

1.2. Hypothesis

Since ERK1/2 pathway through reactive oxygen species and oxidative stress causing degranulation, I predict that increasing concentration and treatment duration with Pb can activate NF-κB pathway and increase reactive oxygen species and increased cell permeability.

2. Material and methods

Activation of NFkb is measured by phospho-IKB western blots, reactive oxygen species measured by thermo total reactive oxygen species assay kit, permeability measured by propidium iodide. Positive control is Fenretinide and negative control is PBS.

2.1. Toxins

Lead solution needs to be prepared. Lead salt: Lead acetate; Solvent: PBS (phosphate- buffered saline); Concentration: 0.01mg/L, 0.05mg/L, 0.1mg/L, 0,5mg/L; Treatment duration: 5hr, 10hr, 15hr

2.2. Cell culture

Human bronchial epithelial cell line BEAS-2B cells are cultivated in an appropriate medium supplemented with antibiotics, growth factors, and fetal bovine serum (FBS). The cells are then kept

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at 37°C in 5% CO_2 humidified air until they reach 70–80% confluence. The prepared cell is then incubated in lead solutions.

2.3. Phospho-IKB western blots

(Concentration: 0.01mg/L, 0.05mg/L, 0.1mg/L, 0,5mg/L; Treatment duration: 5hr, 10hr, 15hr)

Phospho-IKB western blots is the method used to detect the appearance of Phospho-IKB. The activation of the NF-Kb pathway was primarily associated with its inhibitor protein called IKB protein. Which western blot was used by this:

Beas 2b cells were incubated in a cultural medium, and in a 24 well plates with the existence of pb acetate for 10 hours. Wash the cells using ice cold PBS. Lyse the cells using lysis buffer that contains protease and phosphatase inhibitors. Protein extraction reagent kit was used to extract total proteins and bicinchoninic acid kit was used for protein concentration. Protein separation was done by electrophoresis. The separated protein from the gel was then transport to a PVDF cell membrane. 5% nonfat milk in TBS-T was used to block the membrane. The membranes were then incubated with primary antibodies in 4°C overnight. TBS-T was used to wash the unbound primary antibodies on the membrane. Then do the same for secondary antibodies for 1 hours in room temperature. The primary antibodies are phospho-IκBα (Ser32/Ser36) antibody and IκBα antibody [9, 10].

2.4. Thermo total reactive oxygen species assay kit

(Concentration: 0.01mg/L, 0.05mg/L, 0.1mg/L, 0,5mg/L; Treatment duration: 5hr, 10hr, 15hr)

Thermo total reactive oxygen species assay kit is used for the measuring the Reactive oxygen species due to the lead exposure.

Expose Beas 2b cells in lead for 10 hours. Include a control group that won't be exposed in lead. Wash the cells then resuspend them by the assay buffer provided in the thermo total reactive oxygen assay kit. Add the dichlorofluorescin diacetate/ROS detection reagent in the cell suspension. ROS detection reagent was oxidized by the reactive oxygen species activated in the cell and this led to the composition of fluorescent product. Then transfer the sample to a microplate for fluorescence measurements. Use a microplate reader for data analysis. At last, calculate the relative fluorescence units for comparison [11, 12].

2.5. Propidium iodide

(Concentration: 0.01mg/L, 0.05mg/L, 0.1mg/L, 0,5mg/L; Treatment duration: 5hr, 10hr, 15hr)

Propidium iodide is a fluorescent molecule that bonds to the DNA causing characteristics in cells to determine the cell permeability.

Take Beas-2B cells and incubated in Pb compounds for 10 hours in a cultural medium. Take the Beas-2B out and wash it with phosphate buffer saline (PBS). Take the cell and resuspend it in a PI solution (Propidium iodide, RNase A, PBS). Incubate the cell after the resuspend into a dark environment for 20 minutes in room temperature. Uses the FACS analysis for determination. Analyze the cell using a FACS instrument. Excite the cell with a 488 nm laser and identify the fluorescence emission. Calculate the PI fluorescence intensity.

2.6. Statistical method

Statistical method is significant in an experiment since we need to evaluate and analyze the results we have obtain or get. The functions of using a statistical analysis are the following: Data

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interpretation, hypothesis testing, significance assessment, comparison, variation explanation, decision makings etc. All of these benefits help us to analyze our results.

Students T test:

The student's t-test looks at the means of the two groups to see if there is a significant difference between them. It is helpful for data that is regularly distributed or for small sample data. There are paired t-tests for the same group at different dates as well as independent t-tests for distinct groups. To determine significance, you calculate the means, variability, and t-value and compare them to a critical value. For the results that are significant it indicates that the result has a great effect. The results that are insignificant means that the replicates are happen in chance but not frequently happened [13].

ANOVA:

Analysis of variance is a statistical method used to find the statical significance. It is similar with t test but is used in a larger sample size and compares more means [14].

Replicates:

The experiment would have 4 replicates for each treatment groups.

3. Results

3.1. Possible result 1: fully support hypothesis

The exposure of lead increases cell permeability through the activation of NF-kB pathways and ROS.

In the Beas 2B cell there is a high concentration of the phospho-IκBα (Ser32/Ser36) antibody and IκBα antibody activated through lead which means that there is an a activation of NF-kB pathway. The sample that was incubated in lead has a significant difference in relative fluorescence units in comparison with the control group. Furthermore, the Pb increases cell permeability by FACS for propidium iodide compared to the control group and the PI fluorescence intensity increases.

3.2. Possible result 2: partially support hypothesis

If I obtain this result it means that Pb activates the NF-kB pathway and reactive oxygen species however it doesn't affect the cell permeability. In other words, the exposure of lead in cells won't affect cellular permeability. Specifically, this result means that lead has no relationship with cellular permeability.

3.3. Possible result 3: partially support hypothesis

If I obtain this result it means that pb activates the NF-kB pathway however not through the emission of reactive oxygen species and this increase the cell permeability. The reactive oxygen species wouldn't emit however the NF-kB pathway is activate through other stimuli and therefore affecting the cell permeability.

3.4. Possible results 4: partially support hypothesis

If I obtain this result, and it means that there are positive results in reactive oxygen species and cell permeability but not in the activation of NF-kB pathway. In other words, lead exposure affects the reactive oxygen species but not activating the NF-kB pathway through it. However, this also affect the cell permeability but the activation of NF-kB pathway won't involve.

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3.5. Possible results 5: partially support hypothesis

If I obtain this result, it means that there is negative result in reactive oxygen species and activation of NF-kB pathway. However, the exposure of lead somehow affects the cell permeability. The reactive oxygen species doesn't emit, and therefore not activating the NF-kB pathway.

3.6. Possible result 6: partially support hypothesis

If I obtain this result it means that there are negative results in cell permeability and NF-kB pathways. Furthermore, the exposure of leads in cell would emit reactive oxygen species but there is no relations with the activation of NF-kB pathways and cellular permeability.

3.7. Possible result 7: partially support hypothesis

If I obtain this result it means that there is activation of NF-kB pathways and won't increase the cellular permeability and wouldn't emit reactive oxygen species. The activation of NF-kB pathway won't have any effect on the reactive oxygen species and cell permeability.

3.8. Possible result 8: fully contradicts hypothesis

If I obtain this result it means that all three results are negative and doesn't support my hypothesis. In specific, the exposure of lead in cells won't have any effect in activation of the NF-kB pathway, emission of Reactive oxygen species, and increasing in cell permeability. The appearance of antibodies is unchanged or less than the control group. The relative fluorescence units are lower in comparison with the control group and there is no effect when the cells deal with propidium iodide.

Table 1. Combination of possible results (CR)

Combination Result # (CR#)	Pb increases phospho-IKB by western blot	Pb increases ROS by ROS assay	Pb increases cell permeability by FACS for propidium iodide	support of hypothesis
1	+	+	+	Full
2	+	+	-	Partial
3	+	-	+	Partial
4	-	+	+	Partial
5	-	-	+	Partial
6	-	+	-	Partial
7	+	-	-	Partial
8	-	-	-	Fully contradicts

"+" means that the results obtain has better/similar effect compared as Fenretinide, "-" means that the result obtain has similar effect as PBS

3.9. Possible results for the variables of concentration and treatment duration

When concentration of lead decrease there might be a negative correlation

- when the concentration of lead decreases the result might change and cause a negative correlation since a lower concentration can made the Beas 2b cells to get less exposure of lead

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which would then affect the detection of the reactive oxygen species, cellular permeability, and nf-kb pathway.

When concentration of lead increase there might be a positive correlation

• The higher the concentration the result obtain might get a higher exposure of lead. This also means that the nf-kb might get better results in the activations. However, a surplus of concentration might damage the cell and cause harm, and therefore get inadequate or invalid results.

When concentration stays constant the result might stay the same

• When the concentration stays the same there might be a higher reliable result since the different trials have small difference; However, the same concentration might not get a result that is best since if the concentration set is too low or high it influences the result.

When treatment duration decreases there might be a different result.

When treatment duration increases there might be a different result.

When treatment duration is constant the result might be similar.

4. Discussion

4.1. Possible result 1

When this result is obtained it perfectly support my hypothesis since pb increases phospho-IKB by western blot, pb also increases reactive oxygen species, and increase cell permeability. When I obtain this result it might mean that lead exposure in cells activates NF-kB is possibly caused by the emission of reactive oxygen species. Theoretically it is possible that lead activates the NF-kB pathways through oxidative stress in other words reactive oxygen species. If lead exposure in cells activates the NF-kB pathway and also increase reactive oxygen species it proves that lead activates NF-kB pathways through ROS. The reason it's possible to obtain this result is that when cells are exposed in lead that cause reactive oxygen species. This activate IκB kinase (IKK), which phosphorylates the NF-kappaB (IκB) proteins inhibitors [15]. This also cause inflammatory response like increasing of cellular permeability.

We can see this when there is PI-positive cells in Pb-treated samples; Higher ROS levels in Pb-treated cells; Increased levels of phospho-IKB in treated cells.

Further experiments will be required after obtaining these results, since it not enough to certify that this is what happened in other cell type and in real life. So, the experiment needs to be held in Vivo studies in other word in real organisms, and in a variety of cell types for confirmation. On top of that time course studies that researches on how different time duration affect the result also need to be held for the data's validity.

4.2. Possible result 2

When this results is obtained, it partially support my hypothesis that lead exposure activates the NF-κB pathway, through the emission of reactive oxygen species (ROS). While the activation of NF-κB does not have relationship with cellular permeability. We can measure this by the absence of propidium iodide (PI)-positive cells in Pb-treated samples, where this might also suggest a different cellular response. The elevated ROS levels in Pb-treated cells, combined with increased phospho-IκB levels, indicate that lead exposure may trigger NF-κB activation through oxidative stress. This implies that Nf-kB was activate through the induce of oxidative stress, however, different from possible result 1 this result explains there might be other impact nf-kb cause but not permeability. Therefore, further experiments on the other negative impacts of Nf-kB and ROS may be conduct

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such as other inflammatory response. Furthermore, the result obtain might also because of the specific cell type, so an experiment in other cell type may confirm the results.

4.3. Possible result 3/4

When these result are obtained, it partially supports my hypothesis. These two results all indicates that lead exposure have an effect on cellular permeability, yet it either wouldn't affect the ros or the nf-kb pathway. In other words, it indicates that there is a confusion between the relationship between Reactive oxygen species and Nf-kB pathway. The Nf-kB pathway is not triggered by the reactive oxygen species and oxidative stress but other stimuli causing cellular permeability. This result is quite unrealistic since there are already theories that suggest that Nf-kB is activated through oxidative stress. However, obtaining this result can also mean that lead doesn't activate the nf-kb through ros but maybe directly or in other stimuli like heat and ph levels etc. Therefore, for further experiments we need to find out and conduct researches on what really cause the increasing cellular permeability: is it still the nf-kb pathway or reactive oxygen species or its other stimulus that activities it? In simple words we need to investigate in its mechanism.

4.4. Possible result 5/6/7

When I obtain these three results, it partially supports my hypothesis and might means that lead either activates the Reactive oxygen species, Nf-kB pathways, or increases cellular permeability but only one of them. This suggests that lead have potential effects on all of them however, there is no relationship between each other. Since, either it a activates the pathway but not affecting th permeability and ros etc. The reason this result might be obtain is through unsuccessful experiments and the treatment duration or concentration that affected the lead exposure since theoretically we can predict that there is somehow relationships between the three of them. Therefore, for further experiments we need to investigate in the activation of ROS, nf-kb pathway, and cell permeability specifically how lead cause it to activates and relationship between each other. In other words, conduct experiments that researches the potential reason that led activate one but not the other.

4.5. Possible result 8

When I obtain this result it fully contradicts my hypothesis that exposure won't activates the NF-kB pathway, ROS, and wouldn't increase in cellular permeability. Known of the three experiments would get a positive result and therefore lead wouldn't have any relationship between the nf-kb pathway and cellular permeability. The reason we might obtain this result is possibly due to low exposure level, inaccurate measurements, invalidity etc. So, when we obtain this result, we should investigate the correlation of lead and the three other aspects Is this result due to invalid experiments or duration etc. On top of that, we can also investigate in other impacts of lead that we might have observe like poisonous etc.

5. Conclusion

The purpose of this research is to determine how exposure to lead affects cellular permeability and how material permeability is affected by heavy metals. Additionally, the study will explore the function of NF-kB, a crucial transcription factor that controls inflammation and immunological responses. Furthermore, it will evaluate the impact of reactive oxygen species (ROS) in relation to lead exposure, researching their possible function in oxidative stress and cell damage. In order to

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provide further understanding of the possible effects of lead on the cells, this study tries to find the relation between lead exposure and cellular damage.

With the success of this study, it allows us to:

1) understand mechanism: It allow us to know more about the knowledge about lead exposure affecting cellular functions and the activation of inflammatory pathway.

2) Pathway research: Understand the relationship between reactive oxygen species and nf-kb pathway.

3) Public health: Help medical surgery and determination on lead exposure treatments.

4) Therapeutic Targets: Finds potential treatment targets to reduce the impact of lead intoxication.

References

[1] Denchak, M. (2024, April 16). Flint water crisis: Everything you need to know. Be a Force for the Future. https: //www.nrdc.org/stories/flint-water-crisis-everything-you-need-know#summary

[2] World Health Organization. (n.d.). Chemicals of public concerns I lead. World Health Organization. https: //www.who.int/teams/environment-climate-change-and-health/chemical-safety-and-health/health-impacts/chemicals/lead

[3] World Health Organization. (n.d.-a). Almost 1 million people die every year due to lead poisoning, with more children suffering long-term health effects. World Health Organization. https: //www.who.int/news/item/23-10-2022-almost-1-million-people-die-every-year-due-to-lead-poisoning--with-more-children-suffering-long-term-health-effects#: ~: text=Lead%20exposure%20is%20estimated%20to%20account%20for%2021.7, diseases%20can%20be%20attributed%20to%20exposure%20to%20lead.

[4] Juan, C. A., Pérez de la Lastra, J. M., Plou, F. J., & Pérez-Lebeña, E. (2021, April 28). The chemistry of reactive oxygen species (ROS) revisited: Outlining their role in biological macromolecules (DNA, lipids and proteins) and induced pathologies. International journal of molecular sciences. https: //www.ncbi.nlm.nih.gov/pmc/articles/PMC8125527/#: ~: text=In%20mitochondria%2C%20physiological%20levels%20of%20%E2%80%A2%20O%202%E2%88%92, and%20the%20action%20of%20the%20antioxidant%20defence%20system.

[5] Jakubczyk K; Dec K; Kałduńska J; Kawczuga D; Kochman J; Janda K; (n.d.). Reactive oxygen species - sources, functions, oxidative damage. Polski merkuriusz lekarski : organ Polskiego Towarzystwa Lekarskiego. https: //pubmed.ncbi.nlm.nih.gov/32352946/#: ~: text=Reactive%20oxygen%20species%20%28ROS%29%20are%20molecules%20capable%20of, singlet%20oxygen%2C%20as%20well%20as%20free%20nitrogen%20radicals.

[6] Wortzel, I., & Seger, R. (2011, March). The ERK cascade: Distinct functions within various subcellular organelles. Genes & cancer. https: //www.ncbi.nlm.nih.gov/pmc/articles/PMC3128630/#: ~: text=The%20extracellular%20signal-regulated%20kinase%201%2F2%20%28ERK1%2F2%29%20cascade%20is, survival%2C%20but%20apoptosis%20and%20stress%20response%20as%20well.

[7] Wu Y; Huang H; Wu J; Qin Y; Zhao N; Chen B; Nong Q; Huang Y; Hu L; (n.d.-a). Lead activates neutrophil degranulation to induce early myocardial injury in mice. Ecotoxicology and environmental safety. https: //pubmed.ncbi.nlm.nih.gov/37984289/

[8] Encyclopædia Britannica, inc. (2024, August 23). Encyclopædia Britannica. https: //www.britannica.com/science/cell-biology/Transport-across-the-membrane

[9] Osmosis - selective permeability of the cell membrane: Video | osmosis. (n.d.). https: //www.osmosis.org/learn/Selective_permeability_of_the_cell_membrane

[10] Author links open overlay panelYingying Qi a c 1, a, c, 1, b, d, e, & AbstractThe primary purpose of present study was to explore the effects of arsenic exposure on the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt)/nuclear transcription factor-κB (NF-κB) signaling pathway in the hippocampus of offspring mice a. (2024, August 6). Effects of arsenic exposure on the PI3K/AKT/NF-KB signaling pathway in the hippocampus of offspring mice at different developmental stages. Ecotoxicology and Environmental Safety. https: //www.sciencedirect.com/science/article/pii/S0147651324009060?ref=pdf_download& fr=RR-2& rr=8bddf3a28bd2735c

[11] Ng, N. S., & Ooi, L. (2021, January 5). A simple microplate assay for reactive oxygen species generation and rapid cellular protein normalization. Bio-protocol. https: //www.ncbi.nlm.nih.gov/pmc/articles/PMC7952951/#: ~: text=2', 7'-dichlorofluorescein%20(DCF)%20and

DOI: 10.54254/2753-8818/2026.34023

[12] Reactive oxygen species (ROS) fluorometric assay kit. CN. (n.d.). https: //www.thermofisher.cn/order/catalog/product/EEA019#: ~: text=The%20assay%20works%20by%20utilizing%20the%20fluorescent%20probe, oxidized%20by%20ROS%20to%20the%20strong%20fluorescent%20DCF.
[13] A AmiyaRanjanRout, A AmiyaRanjanRout, A AmiyaRanjanRout, A, AmiyaRanjanRout, & Follow. (2024, August 6). Student's t-distribution in statistics. GeeksforGeeks. https: //www.geeksforgeeks.org/students-t-distribution-in-statistics/
[14] Admin. (2021, May 28). Comparison of population means with ANOVA table. ESTAMÁTICA. https: //estamatica.net/anova-table-with-spss/
[15] Israël, A. (2010, March). The IKK complex, a central regulator of NF-kappab activation. Cold Spring Harbor perspectives in biology. https: //www.ncbi.nlm.nih.gov/pmc/articles/PMC2829958/