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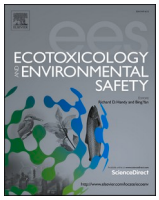

Effects of arsenic exposure on the PI3K/Akt/NF-κB signaling pathway in the hippocampus of offspring mice at different developmental stages

Yingying Qi [a,c,1], Jiaqi Sun [a,1], Huan Wang [a,b], Haiyang Yu [b,d], Xiaoxia Jin [a,b], Xu Feng [e], Yan Wang [a,b,*]

[a] Department of Occupational and Environmental Health, School of Public Health, Shenyang Medical College, Shenyang, Liaoning, People's Republic of China
[b] Key Laboratory of Environment and Population Health of the Educational Department of Liaoning Province, Shenyang, People's Republic of China
[c] Zhuhai Center for Chronic Disease Control(the Third Hospital of Zhuhai), People's Republic of China
[d] Department of Toxicology, School of Public Health, Shenyang Medical College, Shenyang, Liaoning, People's Republic of China
[e] Department of Health Statistics, School of Public Health, Shenyang Medical College, Shenyang, Liaoning, People's Republic of China

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ABSTRACT

The 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 at different developmental stages. Sodium arsenite ($NaAsO_2$) at doses of 0, 15, 30 or 60 mg/L administered to female mice and their pups. The nuclear translocation levels of NF-κB were assessed by EMSA. Real-time RT-PCR was used to measure Akt, NF-κB and PI3K mRNA levels. Protein expressions of PI3K, p-Akt, inhibitor kappa B kinase (IKK), p-NF-κB, protein kinase A (PKA), inhibitor kappa B (IκB), and cAMP response element-binding protein (CREB) were measured by Western blot. Results disclosed that exposure to 60 mg/L $NaAsO_2$ could suppress NF-κB levels of nuclear translocation of postnatal day (PND) 20 and PND 40 mice. Arsenic downregulated the transcriptional and translational levels of PI3K, Akt and NF-κB. Additionally, protein expressions of p-IKK, p-IκB, PKA and p-CREB also reduced. Taken together, results of present study indicated that arsenic could downregulate the PI3K/Akt/NF-κB signaling pathway, particularly on PND 40, which might be involved in the cognitive impairments.

1. Introduction

Researches showed that chronic exposure to arsenic through contaminated water is related to vascular diseases, skin lesion and neurological disorders (Hall et al., 2017; Zeng and Zhang, 2020; Zhang et al., 2023). Epidemiological studies disclosed that drinking of arsenic-contaminated water can lead to cognitive impairments of children (Wasserman et al., 2018; Vahter et al., 2020). Researches demonstrated that arsenic could cause brain damage, impair neural structure and development (Zhao et al., 2017; Niño et al., 2022; Lu et al., 2023). These results concerned neurotoxicity induced by arsenic. Nevertheless, the neurotoxic mechanisms caused by arsenic are still not well understood.

LTP is considered an ideal model for neuronal synaptic plasticity related to learning and memory (Nicoll et al., 2017). Research suggested that the creation of new proteins is necessary for late stages of LTP, occurring quickly through the controlled translation of mRNA located at synapses, and persisting over time through the transcription of new gene (Abraham et al., 2008). Nuclear transcription factor-κB (NF-κB) exerts a critical role in LTP and synaptic signaling (Freudenthal et al., 2004; Dresselhaus et al., 2018). NF-κB exists predominantly in hippocampus, cortex and cerebellum within the central nervous system (CNS) (O`Sullivan et al., 2010). Inactive NF-κB protein is normally bound to an inhibitory inhibitor kappa B (IκB) protein and sequestered in cytoplasm (Shim et al., 2011). NF-κB is activated through the phosphorylation and degradation of IκB by the inhibitor kappa B kinase (IKK), which results

Abbreviations: $NaAsO_2$, sodium arsenite; PI3K, phosphatidylinositol 3-kinase; Akt, protein kinase B; NF-κB, nuclear transcription factor-κB; IκB, inhibitor kappa B; IKK, inhibitor kappa B kinase; PND, postnatal day; LTP, long-term potentiation; EMSA, electrophoretic mobility shift assays; WB, western bolt analysis.

* Correspondence to: Department of Occupational and Environmental Health, School of Public Health, Shenyang Medical College, No. 146 Huanghe North Street, Yuhong District, Shenyang 110034, People's Republic of China.
E-mail address: yanwang@symc.edu.cn (Y. Wang).

[1] Yingying Qi and Jiaqi Sun contributed equally to this study.

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in NF-κB dissociating from the complex and translocating into the nucleus, in which NF-κB binds to κB sequence on DNA, so as to play a part in transcriptional regulation (Karin et al., 2000). Study revealed that the targeted overexpression of the super-repressor in forebrain neurons and lack of NF-kB in neuron-targeted NF-kB-deficient animals, resulted in slight impairment of hippocampal basal synaptic transmission and inhibition of LTP , which proposed that NF-kB regulated synaptic plasticity via protein kinase A (PKA)/CAMP response element-binding protein (CREB) (Kaltschmidt et al., 2006). Zhao et al. (2023) demonstrated that reconstruction of normal gut microbiota treatment alleviated neurobehavioral impairments by affecting toll-like receptor 4 (TLR4)/myeloid differentiation factor 88 (Myd88)/NF-κB signaling pathway in the striatal tissues of offspring rats prenatally exposed to arsenic through microbiota-gut-brain axis. This suggested that NF-κB might be involved in developmental arsenic neurotoxicity. Moreover, Zhu et al. (2018) found that phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) signal pathway could activate translocation of NF-κB P65 into nucleus.

PI3K belongs to the family of lipid kinases, as stated by Fruman et al. (1998). In the classical PI3K/Akt activation pathway, PH domain binding to PtdIns(3−5)P3 in PI3K recruits Akt and its upstream factor into the cell membrane, thereafter Akt is phosphorylated (Alessi et al., 1997). Several substrates crucial in cell survival, metabolism, proliferation and protein synthesis are regulated by catalytically active Akt (Manning et al., 2007). Research revealed that caspase-3 could hinder LTP by cleaving Akt1 (Jo et al., 2011). Data showed that the ability to learn and memorize of rats decreased significantly because of the microinjection of PI3K inhibitor, LY294002, into medial prefrontal cortex (Slouzkey et al., 2016). Furthermore, study also indicated that arsenic may reduce PI3K and Akt levels in rats` hippocampus (Srivastava et al., 2018). According to Li et al. (2019), taurine reduced the impact of arsenite on primary cortical neurons, maybe through the involvement of Akt pathway.

Taken together, PI3K/Akt/NF-κB signaling pathway contributes significantly to learning and memory. To date, there is limited research on the effects of arsenic exposure on the PI3K/Akt/NF-κB signaling pathway in the developing brain. The purpose of this study was to explore the effects of arsenic exposure on the PI3K/Akt/NF-κB signaling pathway in the hippocampus of offspring mice during early life.

2. Materials and methods

2.1. Animal model establishment

Animal model establishment and experimental protocols described in previous report (Wang et al., 2020). This study procedure was approved by Scientific Research Committee of Shenyang Medical College and followed the Chinese National Guidelines for the protection of laboratory animals.

2.2. Morris water maze

The detailed protocols were complied with previous report (Wang et al., 2020).

2.3. Synaptic ultrastructure observation

The detailed protocols described by Yu et al. (2016) were followed.

2.4. Electrophoretic mobility shift assays (EMSA)

The detailed protocols reported by Zheng et al. (2013) were followed.

2.5. Quantitative real-time RT-PCR

The detailed procedures reported in previous paper (Wang et al., 2020). To amplify a fragment of PI3K, Akt, NF-κB P65 and GAPDH. In Table 1, primer sequences were provided.

2.6. Western bolt analysis (WB)

After homogenizing the mice hippocampus, the Minute™ cytoplasmic and nuclear fractionation kit (Invent, USA) was used to isolate the cytoplasmic and nuclear proteins. Fifty micrograms of nuclear or cytoplasmic protein were resolved. Antibodies were PI3K (1:1000), p-IκB (1:1000), PKA (1:500), p-NF-kB P65 (1:800), p-CREB (1:800) (Abcam, UK), p-Akt (1:1000) and p-IKK (1:1000) (Cell Signaling, USA), β-actin (1:2000), Histone (1:1000) (Santa Cruz, USA). An image analyzing software was used to assess the intensity of each band (Gel-Pro analyzer v4.0), and which were adjusted to β-actin or Histone intensity.

2.7. Statistical analysis

IBM SPSS version 22.0 was utilized for the statistical analysis. The mean ± SD was used to describe the results. Group mean differences were evaluated using one-way ANOVA, with multiple comparisons conducted using SNK method. A significance level of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Influence of arsenic on mice's body and brain weights

Body weights of PND 20 mice treated with 60 mg/L $NaAsO_2$ and PND 40 mice administered 30 and 60 mg/L $NaAsO_2$ respectively experienced a substantial drop in comparison to the control. Additionally, in comparison to the control, brain weights of PND 40 mice treated with 60 mg/L $NaAsO_2$ reduced obviously (Table S1).

3.2. Influence of arsenic on PND 40 mice's capacity for learning and memory

This results showed that escape latency and distance travelled of mice treated with arsenic were obviously longer in the last two days in place trial of Morris water maze. Moreover, duration of stay, number of crossing in target area of mice given 30 and 60 mg/L $NaAsO_2$ reduced notably compared to the control in probe trial (Fig. 1).

3.3. Influence of arsenic on hippocampal synaptic ultrastructure

As shown in Fig. 2, the synaptic ultrastructure of CA1 area in mice's hippocampus in the control was normal with more round synaptic vesicles, abundant postsynaptic density. However, the synapses of mice exposed to arsenic showed fewer synaptic vesicles and thinner postsynaptic density at various developmental stages.

Table 1
The primer sequence for PCR.

Gene	Primer sequences (5'→-3')	Length (bp)
PI3K	Sense: TGTGGCACAGACTTGGTGTT Antisense: TTCTTCCCTTGAGATGTCTCCC	153
Akt	Sense: CCGCCTGATCAAGTTCTCCT Antisense: TTCAGATGATCCATGCGGGG	118
NF-κB P65	Sense: GCGTACACATTCTGGGGAGT Antisense: GTTAATGCTCCTGCGAAAGC	118
GAPDH	Sense: CAATGTGTCCGTCGTGGATCT Antisense: GTCCTCAGTGTAGCCCAAGATG	124

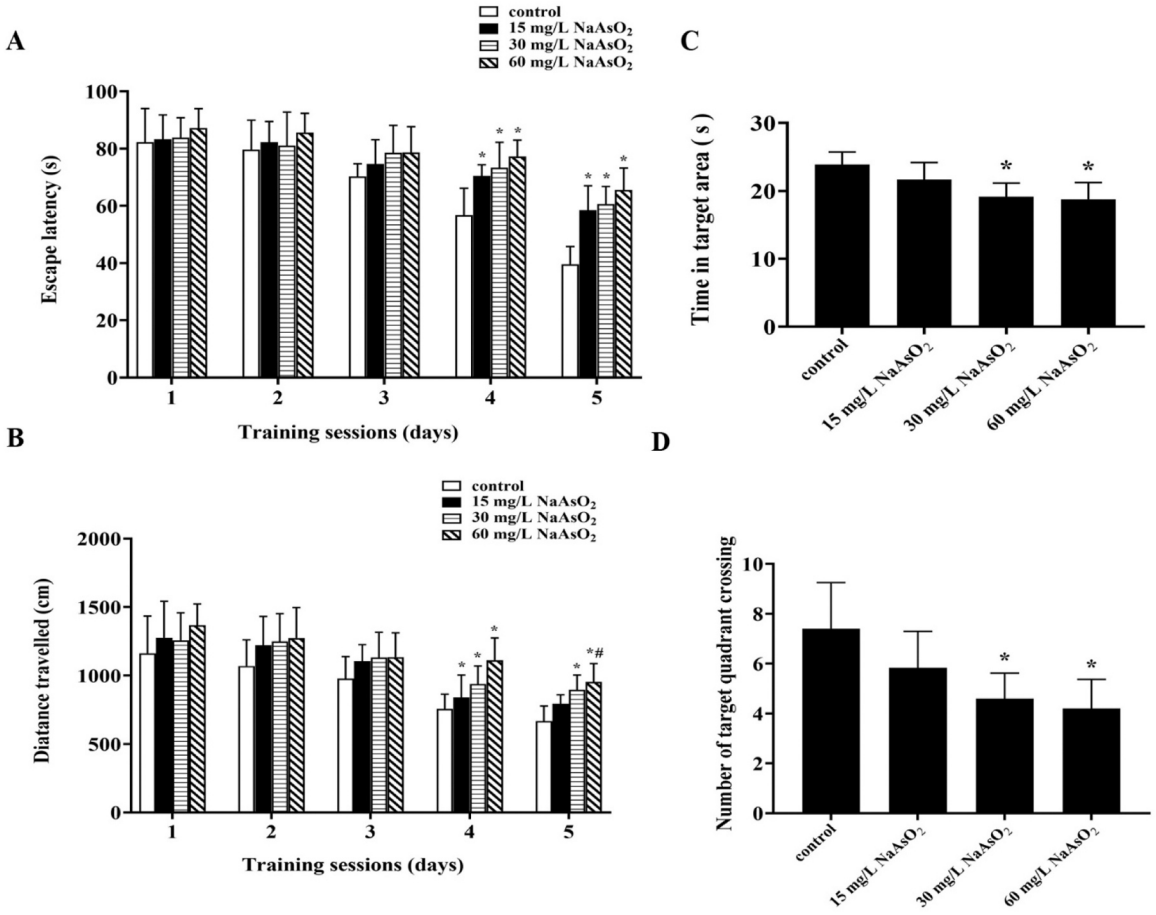

Fig. 1. Effects of arsenic on learning and memory of PND 40 mice. (A) The place trail in Morris water maze was used to detect the spatial learning ability of PND 40 mice. The escape latency for each mouse was automatically recorded. (B) Changes of distance that mice travelled in place trial. (C) Alterations of time the offspring mice spent in target area in probe trial of Morris water maze. (D) Changes of number crossing the target area in probe trial. Results were represented as mean ± SD, n=6. $P < 0.05$, * vs. the control, # vs. 15 mg/L $NaAsO_2$ group.

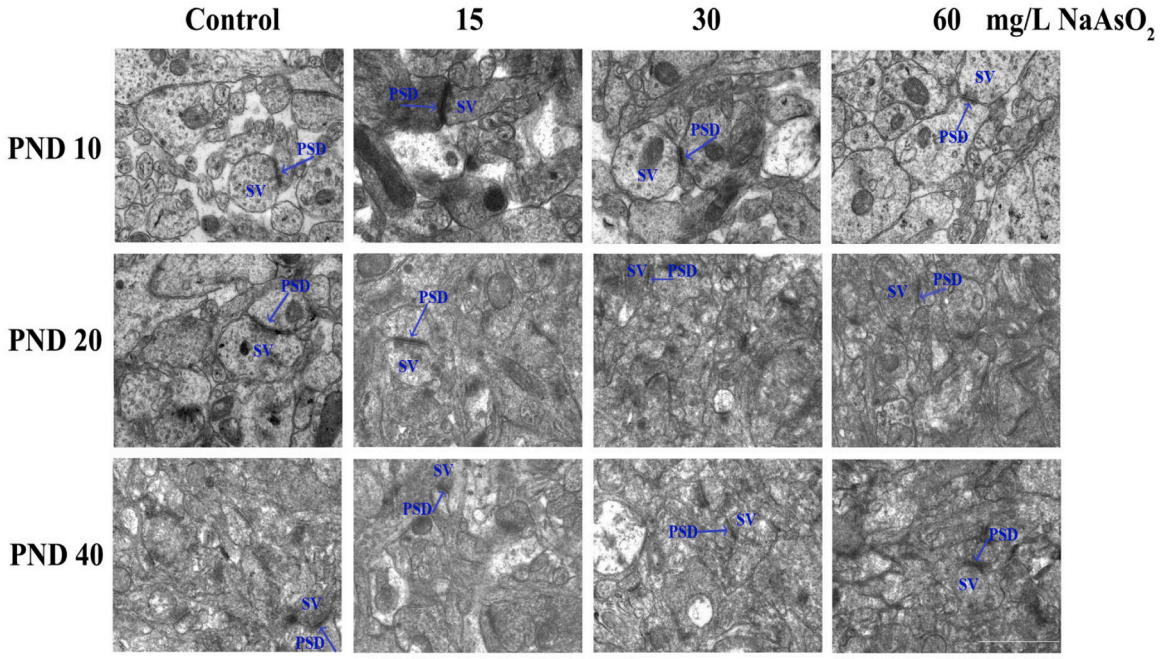

Fig. 2. Effects of arsenic on hippocampal synaptic ultrastructure. PSD, postsynaptic density; SV, synaptic vesicle; Slice thickness=70 nm. Micrographs were captured by 30 000 × , scale bar=1 μm.

3.4. Effects of arsenic on PI3K and Akt levels at early developmental stages

Considerably lower PI3K protein levels in the hippocampus of PND 20 mice treated with 30 and 60 mg/L $NaAsO_2$ showed in present study. PI3K protein expressions of PND 40 mice exposed to arsenic reduced remarkably than the control. Otherwise, exposure to 60 mg/L $NaAsO_2$ evidently suppressed PI3K mRNA levels of PND 20 mice. PND 40 mice treated with arsenic showed a substantial decrease in PI3K mRNA levels. Conversely, protein and mRNA levels of PI3K in mice did not differ markedly on PND 10 (Fig. 3A-C).

p-Akt protein levels dramatically declined in PND 20 mice treated with 60 mg/L $NaAsO_2$. Otherwise, protein and mRNA levels of Akt in PND 40 mice treated with arsenic reduced remarkably. However, the changes in Akt protein and mRNA levels of PND 10 mice did not reveal any significance (Fig. 3D-F).

3.5. Effects of arsenic on NF-κB levels at early developmental stages

Compared to the control, significantly lower levels of NF-κB activation showed in PND 10 and PND 20 mice given 60 mg/L $NaAsO_2$ and PND 40 mice administered 30 and 60 mg/L $NaAsO_2$. In contrast to the control, PND 20 mice given 60 mg/L $NaAsO_2$ showed a significant decline in NF-κB P65 protein levels, while obvious decrease also was found in PND 40 mice exposed to arsenic. Furthermore, exposure to 30 and 60 mg/L $NaAsO_2$ could downregulate NF-κB mRNA levels in PND 40 mice (Fig. 4).

3.6. Influence of arsenic on p-IKK and p-IκB protein expressions

p-IKK protein levels in PND 20 mice treated with 60 mg/L $NaAsO_2$ were notably lower. Furthermore, arsenic significantly reduced p-IKK protein levels in PND 40 mice. Conversely, the significant differences of p-IKK protein levels in PND 10 mice were not found (Fig. 5A-B).

Compared to the control, significantly less p-IκB protein levels observed in PND 40 mice treated with 60 mg/L $NaAsO_2$. However, the differences in p-IκB protein expressions of PND 10 and PND 20 mice did not indicate any significance (Fig. 5C-D).

3.7. Effects of arsenic on PKA and p-CREB protein levels

Compared to the control, PKA protein levels evidently reduced in mice given 30 and 60 mg/L $NaAsO_2$ at various developmental stages. Additionally, in contrast to 30 mg/L $NaAsO_2$ exposed group, PKA protein levels in PND 40 mice treated with 60 mg/L $NaAsO_2$ were much lower (Fig. 6A-B).

Exposure to 30 and 60 mg/L $NaAsO_2$ could obviously decrease the p-CREB protein levels in PND 20 and PND 40 mice. But there was no evident differences of p-CREB protein levels in PND 10 mice (Fig. 6C-D).

4. Discussion

Morris water test indicated that the escape latency and distance travelled of mice exposed to arsenic were obviously longer, time spent in target area and the numbers crossing the target area of mice exposed to

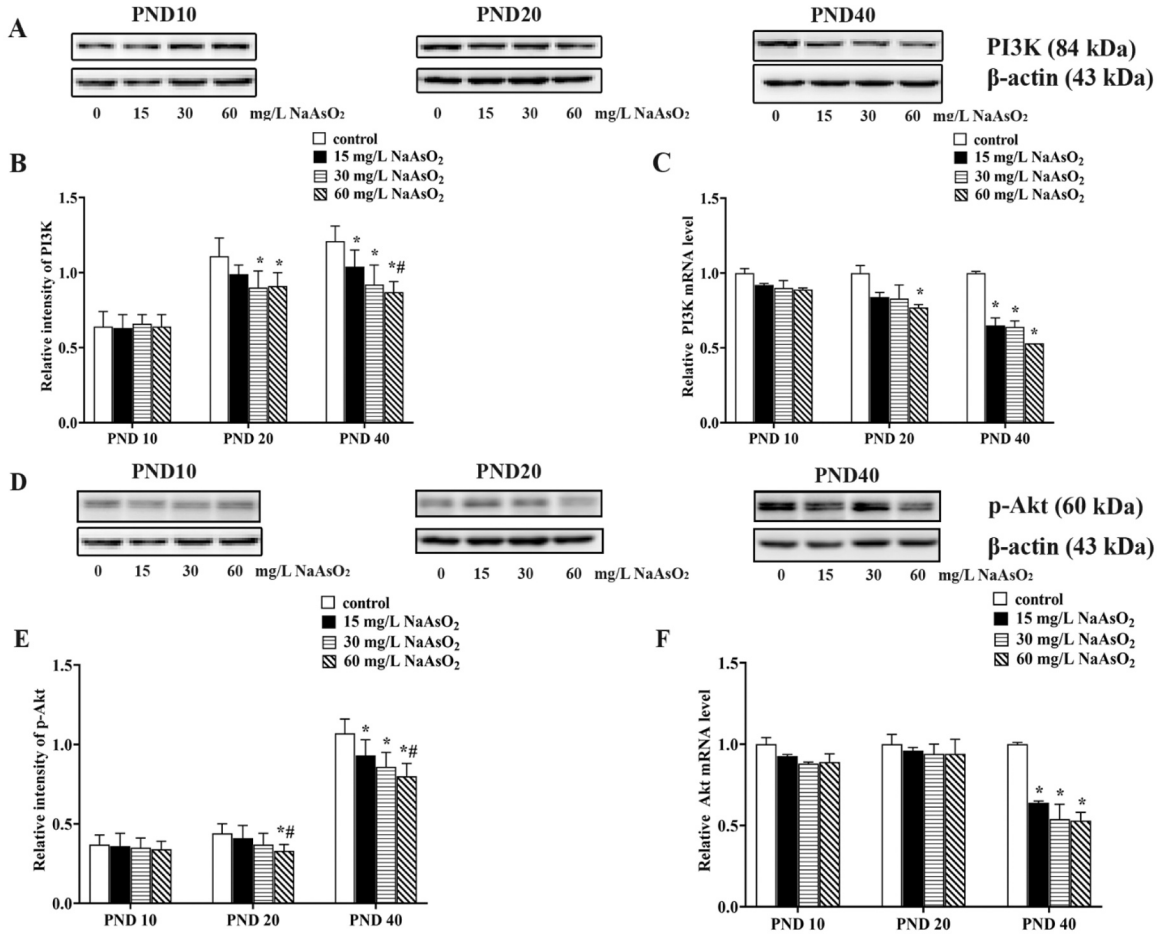

Fig. 3. Effects of arsenic on PI3K and Akt levels during the early developmental stages. (A, D) The levels of PI3K and p-Akt protein in the hippocampus of offspring mice were detected by WB. Images were the representative blots. (B, E) Statistical analysis of PI3K and p-Akt protein levels. (C, F) Real-time RT-PCR was used to detect PI3K and Akt mRNA levels in the hippocampus. Results were represented as mean ± SD, n=6. $P < 0.05$, * vs. the control, # vs. 15 mg/L $NaAsO_2$ group.

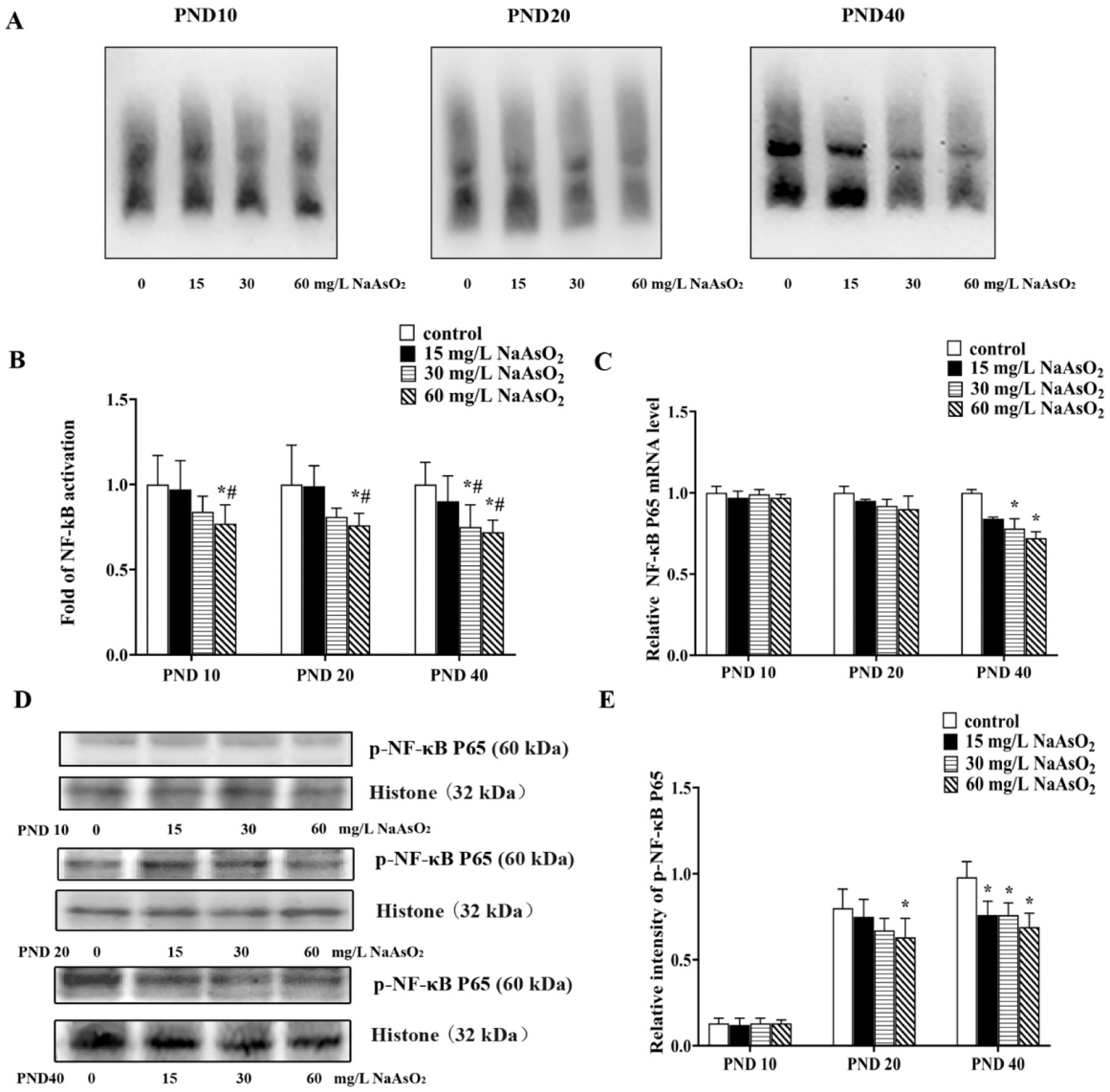

Fig. 4. Effects of arsenic on NF-κB expression during the early developmental stages. (A) The nuclear translocation levels of NF-κB in the hippocampus of offspring mice were assessed by EMSA. Images were the representative blots. (B) Statistical analysis of NF-κB activation levels. (C) Real-time RT-PCR was used to detect NF-κB mRNA levels in the hippocampus. (D) The levels of NF-κB protein in the hippocampus of offspring mice were detected by WB. Images were the representative blots. (E) Statistical analysis of NF-κB protein levels. Results were represented as mean ± SD, n=6. $P < 0.05$, * vs. the control, # vs. 15 mg/L $NaAsO_2$ group.

arsenic were significantly shorter and fewer. These results suggested that arsenic could impair learning and memory capabilities of offspring mice. Moreover, the present study showed that mice exposed to arsenic had fewer synaptic vesicles and thinner postsynaptic density in their synapses, demonstrating a detrimental impact of arsenic on the synaptic ultrastructure.

Studies revealed that PI3K plays a role in the process of learning and memory (Sanna et al., 2002; Jiang et al., 2017; Bai et al., 2023). In response to different extracellular stimuli, Akt, a downstream target of PI3K, controls cellular survival, proliferation and protein synthesis (Li et al., 2019). Qu et al. (2016) revealed that arsenic exposure could affect synaptic plasticity and reduce protein levels of p-Akt in rat brain. PI3K/Akt signaling pathway involves in LTP and synaptic plasticity (Wu et al., 2016). Study disclosed that injecting PI3K inhibitors into the medial prefrontal cortex could suppress phosphorylation of PI3K/Akt and LTP (Sui et al., 2008). Manthari et al. (2018) revealed that arsenic could induced autophagy in the cerebral cortex and hippocampus of developmental mice by inhibiting PI3K/Akt/mammalian target of rapamycin (mTOR) signaling pathway. Findings from this study indicated that PI3K mRNA levels, PI3K and p-Akt protein expressions of PND 20 mice exposed to 60 mg/L $NaAsO_2$ reduced noticeably, PI3K and Akt mRNA and protein expressions of PND 40 mice exposed to arsenic decreased obviously. Results of the present study proposed that arsenic could inhibit the transcriptional and translational levels of PI3K in the hippocampus of PND 20 and PND 40 mice. Furthermore, our findings showed that exposure to arsenic may potentially affect the translational levels of Akt, particularly on PND 40. The transcriptional level of Akt only reduced on PND 40. Findings from the present study disclosed that arsenic could inhibit gene and protein levels of PI3K and Akt with the prolonged arsenic exposure at early developmental stages, which might affect PI3K/Akt signaling pathway, particularly on PND 40. The inhibitory effect of arsenic on the PI3K/Akt signaling pathway in this study was consistent with the results mentioned above.

Research findings increasing indicate that NF-κB family participates in memory and neural plasticity (Freudenthal et al., 2005; Alawdi et al., 2017; Gupta and Guleria, 2022). Genes transcription in neurons can be regulated by NF-κB, which is a crucial regulator of memory formation and synaptic plasticity (Jarome et al., 2015). Study demonstrated that

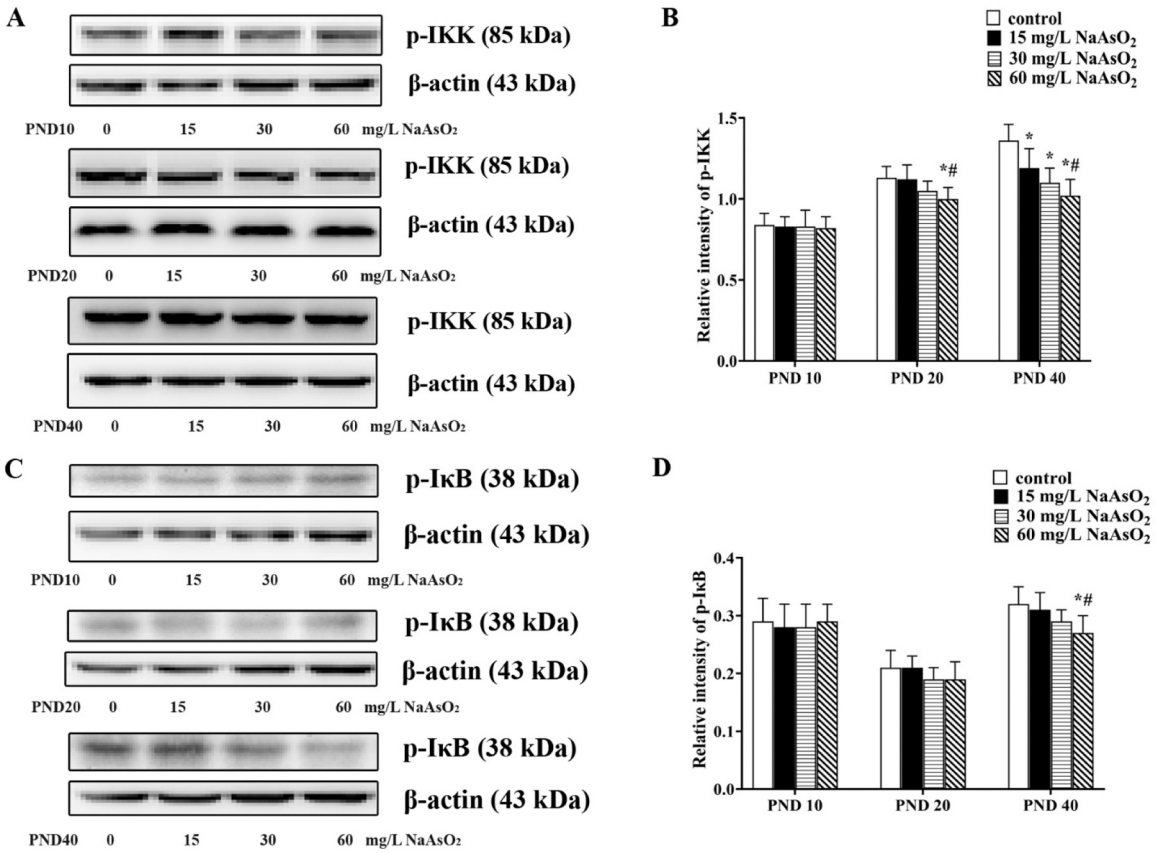

Fig. 5. Effects of arsenic on IKK and IκB levels during the early developmental stages. (A, C) The levels of p-IKK and p-IκB protein in the hippocampus of offspring mice were detected by WB. Images were the representative blots. (B, D) Statistical analysis of p-IKK and p-IκB protein levels. Results were represented as mean ± SD, n=6. $P < 0.05$, * vs. the control, # vs. 15 mg/L $NaAsO_2$ group.

NF-κB P65 subunit-deficient mice had obvious cognitive deficits (Bracchi-Ricard et al., 2008). Results of this study showed that significantly lower NF-κB nuclear translocation levels were found in PND10 and PND 20 mice exposed to 60 mg/L $NaAsO_2$ and PND 40 mice treated with 30 and 60 mg/L $NaAsO_2$. Furthermore, levels of NF-κB mRNA reduced significantly only in PND 40 mice treated with 30 and 60 mg/L $NaAsO_2$. Moreover, PND 20 mice treated with 60 mg/L $NaAsO_2$ had considerably lower p-NF-κB P65 protein expressions, while p-NF-κB P65 protein expressions reduced noticeably in PND 40 mice treated with arsenic. This data indicated that early developmental arsenic exposure could reduce NF-κB levels, particularly on PND 40. Therefore, the alterations of NF-κB levels might participate in the cognitive deficits caused by arsenic exposure. The exact mechanism will be explored in the subsequent research.

The regulation of the NF-κB signaling pathway is intimately linked to IKK and IκB (Shen et al., 2019). Normally, IκB serves to suppress NF-κB activation. However, phosphorylation of IκB by IKK, which is activated by p-Akt, leads to the translocation of NF-κB into the nucleus and the upregulation of its target genes (Xu et al., 2019; Zhao et al., 2021). Yu et al. (2012) indicated that p-IKK protein expression decreased in rats with impaired ability to learn and memorize. Results of present study revealed that p-IKK protein levels were obviously lower in PND 20 mice treated with 60 mg/L $NaAsO_2$, while those in PND 40 mice exposed to arsenic reduced notably. Furthermore, p-IκB protein expressions of PND 40 mice treated with 60 mg/L $NaAsO_2$ reduced significantly. According to this study, continuous exposure to arsenic could inhibit the translational levels of p-IKK and p-IκB, which might further affect NF-κB levels.

PKA, a protein kinase, is a significant signaling component in CNS that regulates cognitive processes (Cho et al., 2015). Jin et al. (2018) revealed that the PKA catalytic subunit could affect the expression level of CREB. Results of the present research indicated that PKA protein levels of mice administered 30 and 60 mg/L $NaAsO_2$ reduced notably at different developmental stages, and protein expressions of p-CREB of PND 20 and PND 40 mice exposed to 30 and 60 mg/L $NaAsO_2$ also reduced obviously. Kaltschmidt et al. (2006) revealed that NF-κB could regulate PKA expression, further affect the function of learning and memory. This data indicated that arsenic exposure could reduce NF-κB levels of offspring mice, in return, might have an effect on PKA and CREB levels. Srivastava et al. (2018) reported that curcumin exerted neuroprotective role involving PI3K/Akt pathway, which might affect N-methyl-D-aspartate (NMDA) receptors and downstream signaling through CREB in arsenic induced cognitive deficits of rats. Zhu et al. (2017) disclosed that p-CREB protein expression in both the hippocampus and cerebral cortex decreased in offspring rats exposed to fluoride and arsenic. In this study, the declined levels of CREB in the hippocampus of offspring mice might be involved in the cognitive impairment induced by arsenic exposure at the early life.

5. Conclusion

Taken together, arsenic might affect the PI3K/Akt signaling pathway by inhibiting gene and protein levels of PI3K/Akt especially on PND 40, which might further influence the NF-κB levels, PKA and CREB protein levels. The PI3K/Akt/NF-κB signaling pathway might be involved in the learning and memory impairment caused by arsenic exposure. However, the exact underlying mechanisms need further research.

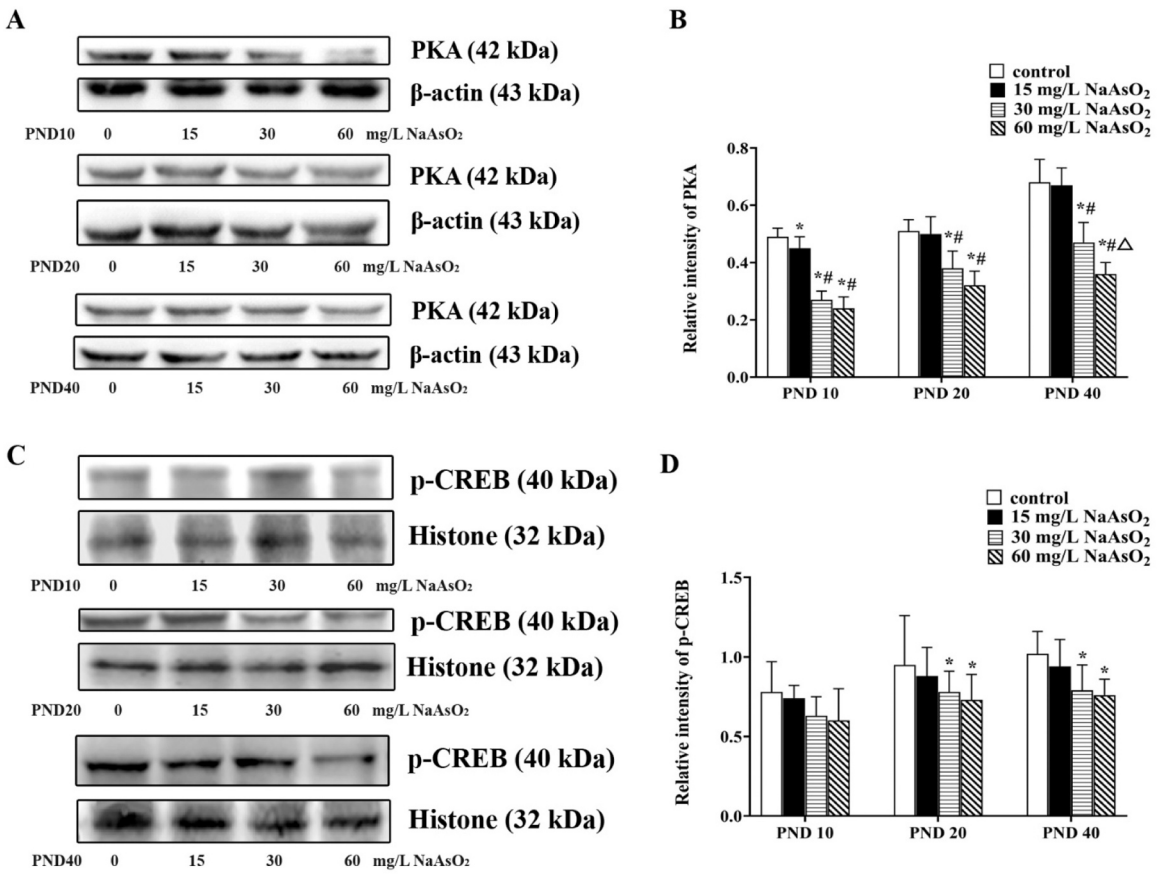

Fig. 6. Effects of arsenic on PKA and CREB levels during the early developmental stages. (A, C) The levels of PKA and p-CREB protein in the hippocampus of offspring mice were detected by WB. Images were the representative blots. (B, D) Statistical analysis of PKA and p-CREB protein levels. Results were represented as mean ± SD, n=6. $P < 0.05$, * vs. the control, # vs. 15 mg/L $NaAsO_2$ group, △ vs. 30 mg/L $NaAsO_2$ group.

CRediT authorship contribution statement

Haiyang Yu: Methodology, Investigation. **Xiaoxia Jin:** Methodology. **Xu Feng:** Methodology. **Yan Wang:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Yingying Qi:** Writing – original draft, Visualization, Investigation, Formal analysis, Data curation. **Jiaqi Sun:** Writing – original draft, Visualization, Investigation, Formal analysis, Data curation. **Huan Wang:** Methodology, Investigation.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Yan Wang reports financial support was provided by the National Natural Science Foundation of China. Yan Wang reports financial support was provided by Liaoning Provincial Natural Science Foundation. Yan Wang reports financial support was provided by Innovative Talents Support Plan of Colleges and Universities in Liaoning Province. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2024.116830.

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