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The role of HIF-1α-BNIP3 pathway in acrylonitrile-induced hippocampal neuronal cell toxicity

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INTRODUCTION & AIM

- **Acrylonitrile (AN)** is an important chemical raw material, and is widely used in the production of fibers, plastics and resins. Exposure to AN occurs in occupational settings.While exposure to AN can cause neurotoxicity in bioorganisms, the exact mechanism remains unclear.
- **Hypoxia-inducible factor-1 (HIF-1)** is an important transcription factor produced under hypoxia. During hypoxia, cytoplasm HIF-1α binds to stably expressed HIF-1β and then is translocated into the nucleus to induce multiple downstream target genes.
- It is essential to explore the impact of AN on the critical transcription factor HIF-1α, aiming to identify new targets for reducing the neurotoxic effects of AN.

METHOD

- CCK assay, LDH release rate, flow cytometry, and Western blotting were used to detect the cell viability, toxicity, apoptosis and expression of related proteins in mouse hippocampal neuronal cell (HT22) after AN exposure.
- Then, cells were pretreated with HIF-1α activator cobalt chloride ($CoCl_2$) and BNIP3 overexpression, followed by detection of relevant indicators.

RESULTS & DISCUSSION

1.1 Acute AN exposure induced apoptosis in HT22 cells though HIF-1α/BNIP3 pathway.

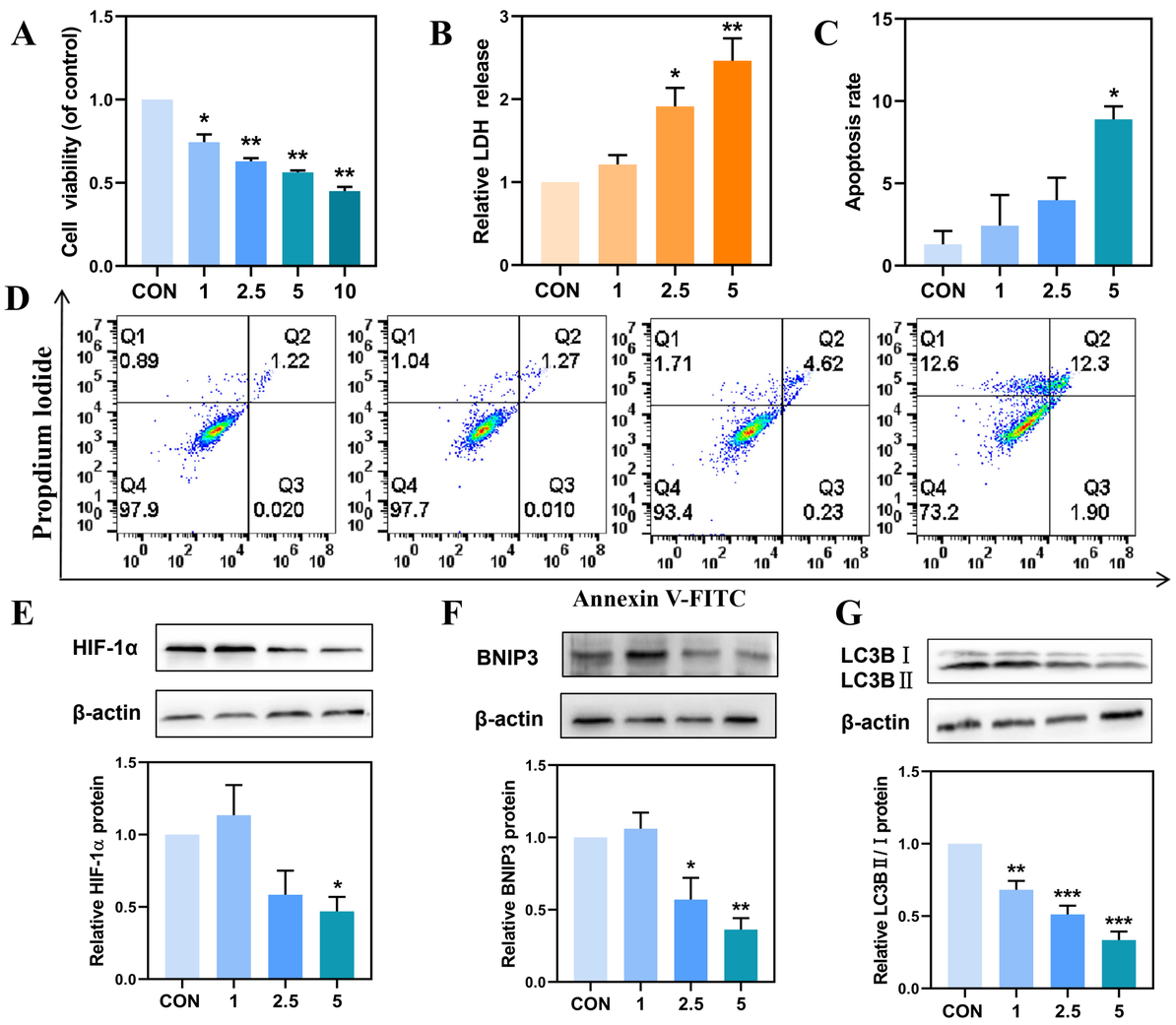

Fig 1. Effects of AN on HT22 cells. (A) Cell viability was detected with CCK8 after treatment with different AN concentration (0, 1, 2.5, 5 mM) for 6 h. (B) HT22 cytotoxicity was detected by LDH release rate. (C, D) Apoptosis levels were detected by flow cytometry. (E, F, G) Representative protein images after AN exposure.

1.2 Activation of HIF-1α pathway can mitigate AN-mediated HT22 cytotoxicity.

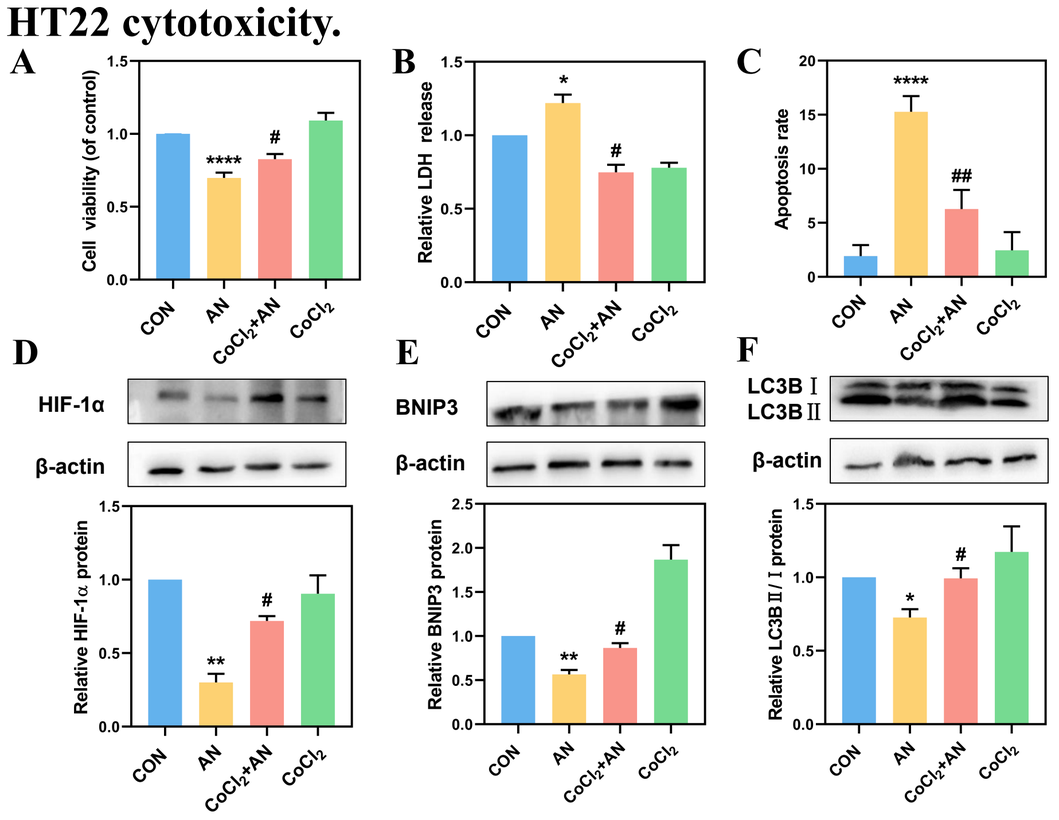

Fig 2. Effects of $CoCl_2$ on AN-induced HT22 cell. (A) Cell proliferation was detected upon pretreatment with $CoCl_2$ (200 μM for 0.5 h) followed by treatment with AN (5 mM, 6 h). (B)LDH. (C)Apoptosis rate. (D, E, F)Protein expression and quantitative analyses.

1.3 BNIP3 overexpression increased mitophagy and improved AN-induced cell viability.

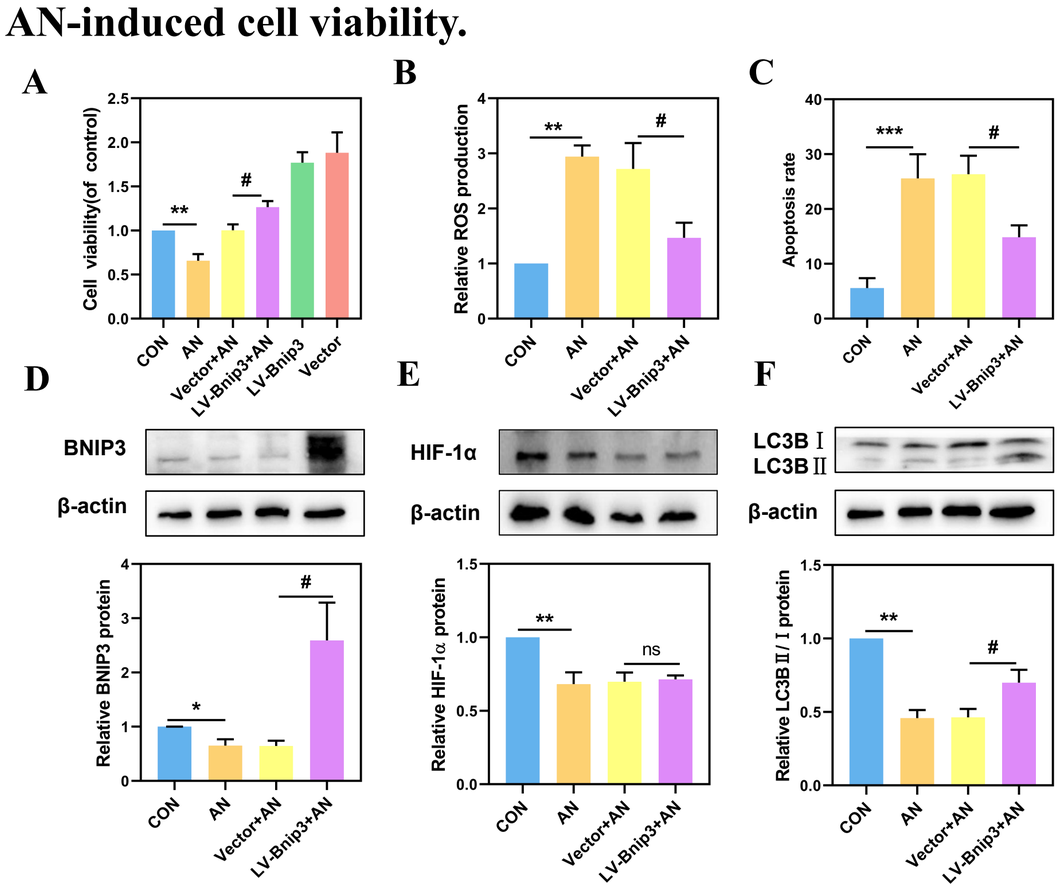

Fig 3. Effects of BNIP3 overexpression on AN-induced HT22 cell . (A) Cell proliferation was detected after BNIP3 stably overexpression followed by AN treatment (5 mM, 6 h). (B) ROS. (C)Apoptosis rate. (D, E, F) Protein expression and quantitative analyses.

CONCLUSION

AN significantly downregulated HIF-1α/BNIP3-mediated mitophagy pathway, and caused damage to HT22 cells. This may be a new mechanism of AN's neurotoxicity, which is expected to provide new targets for precise prevention and treatment of AN poisoning.

FUTURE WORK

Our study will further explore the applicability of this pathway in other nerve cells and whether other mitophagy pathways are involved in AN-mediated mitochondrial function disorder, providing more reliable evidence for AN's clinical treatment.

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