Tetramethylpyrazine Ameliorate High-Glucose Enhanced Hypoxia by Suppresing BNIP3 Expression to Inhibit H9C2 Cardiomyoblast Apoptosis

Shao-Yu Chen (陳劭瑀), Shih-Ping Liu (劉詩平), Chih-Yang Huang (黃志揚)
Graduate Institute of Basic Medical Science, China Medical University.

Abstract

Cardiovascular diseases have become more and more serious in all over the world, and it costs a lot in health care and medicine treatment. Cardiovascular diseases, including coronary heart disease, cardiac infarction and heart failure, are related with many risk factors, ex: diabetes high blood pressure, smoking, and obesity. However, current medicine treatment is not the effective therapeutic approach. To develop new and more effective medicine to protect cardiomyocyte is a very important object to every country.

One of important event in acute myocardial infarction is hypoxia, and it caused myocyte death. On the other hand, diabetes caused high-glucose concentration in blood, and it will cause diastolic dysfunction and cardiac hypertrophy. According to previous data in our lab, hypoxia combined with high-glucose will enhance BNIP3 expression which will cause cell apoptosis. In this study, we tried to find out a medicine to repress the BNIP3 expression level to decrease the cell apoptosis.

After screening 100 kinds of Chinese herbal medicine in cardiac hypoxia SD rats by heavy bleeding, there's only Chuanxiong treatment can increase the survival marker p-Akt expression and decrease inflammation marker p-NFkB expression. For this reason, we used Tetramethylpyrazine (TMP), one of the Chuanxiong component, to study the cardioprotectionn effect.

We found out that hypoxia induced BNIP3 expression, even high-glucose doesn't effect to BNIP3, but when hypoxia combined with hypoxia, would enhance BNIP3 expression in cardiomyoblast cell line H9C2. In time dependent experiment, hypoxia only and hypoxia combined with high-glucose up-regulated BNIP3 and Bak while down-regulated, apoptosis-related protein p-Akt and BcL-xL expression levels. We silenced BNIP3 to prove that hypoxia only or combine with high-glucose induced cell apoptosis via regulating BNIP3 and down stream caspase-8 expression.

By using TMP to treat H9C2 in hypoxia only and hypoxia combine with high-glucose experiment, we found out that TMP treatment can inhibited hypoxia only or hypoxia combine with high-glucose induced cell apoptosis by regulating BNIP3 expression and down stream cleavage caspase-3 protein levels.

In summary, we demonstrated that hypoxia and hypoxia combine with high-glucose induced cell apoptosis via elevation of BNIP3 expression and TMP can inhibit hypoxia and hypoxia combine with high-glucose induced cell apoptosis via down regulation of BNIP3 expression.

TMP may become a cardioprotection drug in the future.

Introduction

In many countries, heart disease patients are increasing in recent years. Statistics show that it cost a lot of money in health care and medical treatment. It is a very important issue to try to find out how to prevent heart disease or improve the related medicine and try to reduce the disease incidence.

Heart disease including aorta disease, cardiomyopathy, heart value disease, pericarditis, arrhythmia, coronary heart disease, cardiac infarction and heart failure and they are related with many reasons like lifestyle, smoking, physical inactivity, high cholesterol, high blood pressure, diabetes and obesity.

cardiomyocyte apoptosis is an important event after acute myocardial infarction (AMI) and may be responsible for a significant portion of myocyte death during the acute ischemic stage. And our lab's previous paper had shown that there is one possible apoptotic mechanism for the development of heart failure in obesity with nocturnal sustained hypoxia.

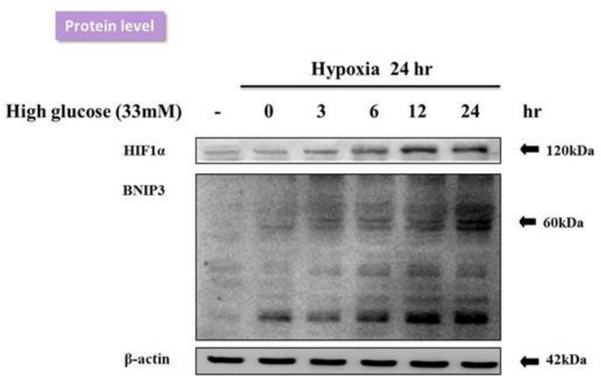
During hypoxia situation, it would keep HIF-1α stable, and induce BNIP3 expression, which leads cells to apoptosis.

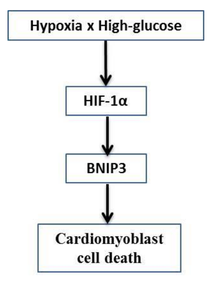
Ligusticum chuanxiong (LCX) is a commonly used traditional Chinese medicine (TCM) for empiric treatment of cerebrovascular and cardiovascular diseases for many centuries. LCX's single ingredient extracts Tetramethylpyrazin (TMP) also has significant activity in animal stroke models and preserves the structural and functional integrity of mitochondria.

So, by using TMP to treat cardiomyoblast cell line H9C2 in hypoxia only and hypoxia combine with high-glucose, we hypothesis that it could have protective effect to cardioblast cells.

Results

Fig 1. A.





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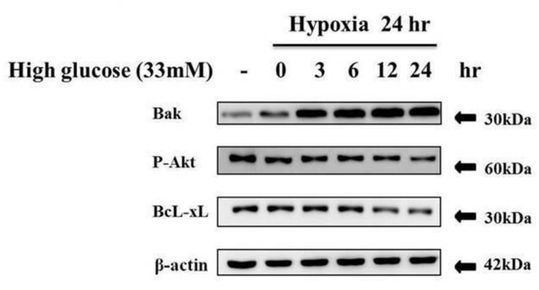
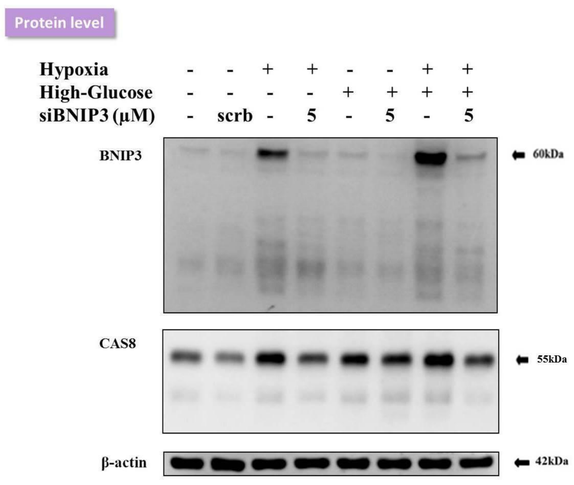


Fig.1. **Effects of HG plus hypoxia induce BNIP3 expression in cell.** Cells were cultured in HG medium (33mM) and incubated in hypoxia(5%) for different time periods as indicated. (A) HIF1α and BNIP3 levels detected by western blot show that apoptosis were increased by HG plus hypoxia.

Fig 2. A.



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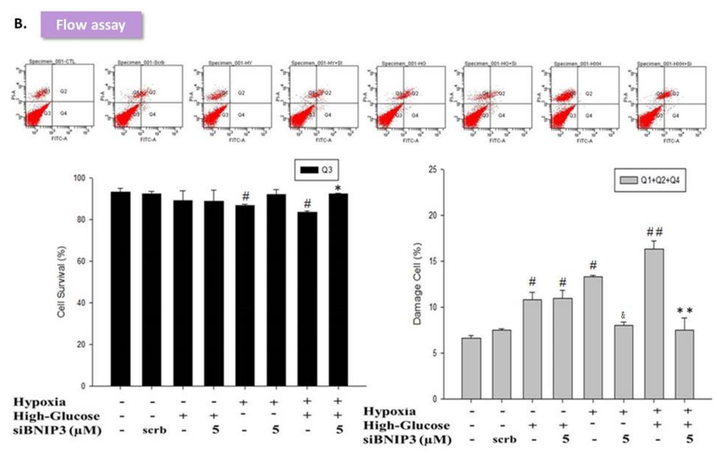


Fig.2. **Hypoxia only or combine with high-glucose induced cell apoptosis via regulating BNIP3 expression.** BNIP3 and down stream cas8 levels are increase by hypoxia and hypoxia plus high-glucose, and expression levels both silence siBNIP3(5μM).

Fig 3. A.

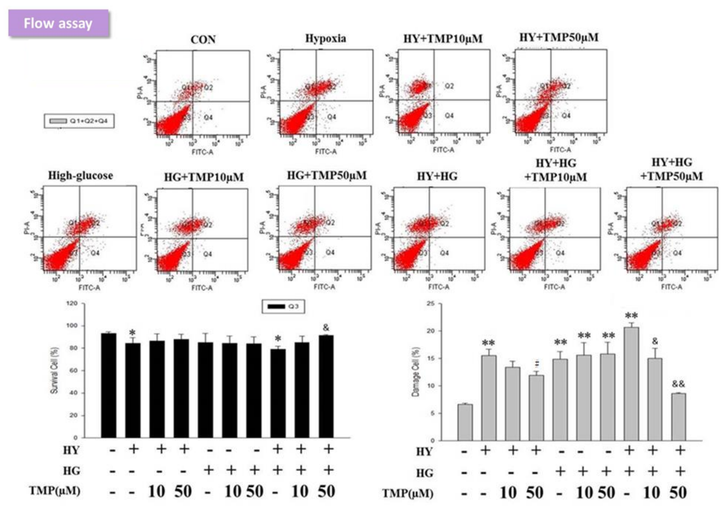
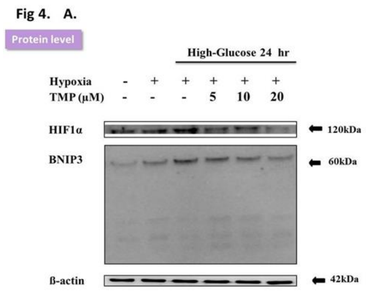


Fig.3. **HG enhances hypoxia-induced BNIP3 expression lead to cell apoptosis and inhibited by TMP.** The damage cell percentage increase by hypoxia and enhance when hypoxia combine with high-glucose. And the damage cell percentage decrease by TMP 10 and 50 μM.

Fig 4. A.



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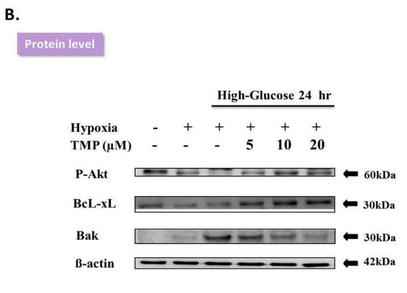

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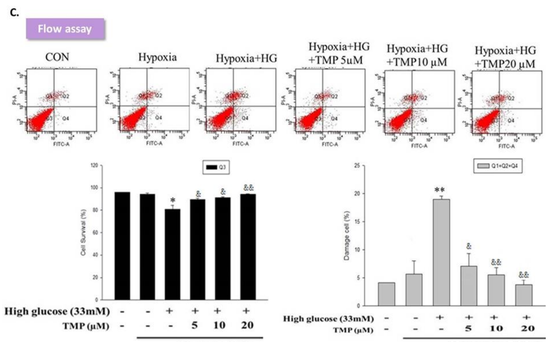
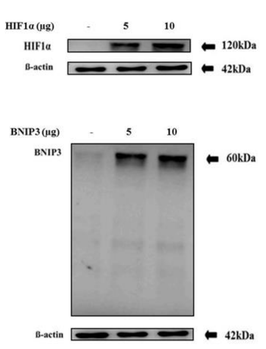


Fig.4. **Tetramethylpyrazine down regulated of HIF-1α and downstream BNIP3 and other related proteins in H9c2 cells which exposed to hypoxia combine with high-glucose.** (A) Both HIF1α and BNIP3 levels are increase by hypoxia and hypoxia plus high-glucose, and both of HIF1α and BNIP3 expression inhibited by TMP 5, 10, 20(μM).

Fig 5. A.



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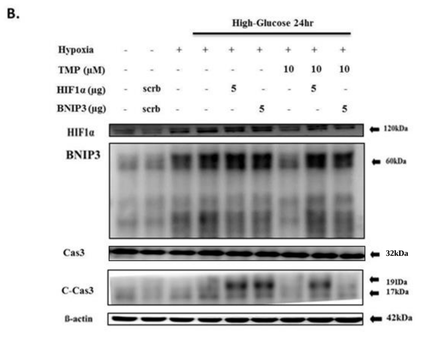

Fig.5. **HG enhances hypoxia-induced BNIP3 expression lead to cell apoptosis after over expression and both inhibited by TMP.** Both HIF1α and BNIP3 levels are increase by hypoxia and hypoxia plus high-glucose, and enhance when we use plasmid to overexpression. Both of HIF1α and BNIP3 expression inhibited by TMP 10(μM), and the same effect shows after overexpression, TMP was more effect to BNIP3.

Conclusion

Hypoxia or high-glucose enhanced hypoxia induce cell apoptosis via elevation of BNIP3 expression.

Tetramethylpyrazine (TMP) inhibits hypoxia or high-glucose enhanced hypoxia induce apoptosis via down regulation of HIF-1α-BNIP3 expression.