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ORIGINAL ARTICLE

Role of sodium-hydrogen exchanger isoform 1 in regulating hepatocyte apoptosis induced by hyperammonaemia

Peng Wang[a,1], **Xin Wang**[a,*,1], **Ling Li**[b], **Quancheng Kan**[c], **Zujiang Yu**[c], **Rongfang Feng**[a], **ZiXiao Chen**[a], **Yan Shi**[a], **Jinling Gao**[a]

[a] *Department of Basic Medicine, Nursing College, Zhengzhou University, Zhengzhou, Henan, PR China*
[b] *Department of Palliative and Hospice Care, The Ninth People's Hospital of Zhengzhou, Henan, PR China*
[c] *Department of Infectious Disease, First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan, PR China*

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KEYWORDS
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Abstract
Background: The ''secondary injury'' theory of liver failure indicated that hyperammonaemia due to liver failure causes further deterioration of hepatocytes. Our previous studies have demonstrated that high blood ammonia levels may lead to hepatocyte apoptosis, as NH_4Cl loading caused metabolic acidosis and an increase in sodium-hydrogen exchanger isoform 1 (NHE1). In this study, we established a hyperammonia hepatocyte model to determine the role of NHE1 in the regulation of hepatocyte apoptosis induced by NH_4Cl.
Materials and methods: In current studies, intracellular pH (pHi) and NHE1 activity were analyzed using the pHi-sensitive dye BCECF-AM. The results showed that intracellular pH dropped and NHE1 activity increased in hepatocytes under NH_4Cl treatment. As expected, decreased pHi induced by NH_4Cl was associated with increased apoptosis, low cell proliferation and ATP depletion, which was exacerbated by exposure to the NHE1 inhibitor cariporide. We also found that NH_4Cl treatment stimulated PI3K and Akt phosphorylation and this effect was considerably reduced by NHE1 inhibition.
Conclusion: This study highlighted the significant role of NHE1 in the regulation of cell apoptosis induced by hyperammonaemia.

* Corresponding author.
E-mail address: wang_x@aliyun.com (X. Wang).
[1] Contributed equally to this work and are joint first authors.

PALABRAS CLAVE
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Papel de la isoforma 1 del intercambiador iónico de sodio/hidrógeno en la regulación de la apoptosis de hepatocitos inducida por hiperamoniaquemia

Resumen
Antecedentes: La teoría de la «lesión secundaria» de la insuficiencia hepática mostró que la hiperamoniaquemia provocada por la insuficiencia hepática causa mayor deterioro de los hepatocitos. Nuestros anteriores estudios previos han demostrado que los niveles altos de amoníaco en sangre pueden conducir a la apoptosis de los hepatocitos. Como la carga de NH_4Cl provocó acidosis metabólica y un aumento de la isoforma 1 del intercambiador de sodio/hidrógeno (NHE1). En este estudio, establecimos un modelo de hepatocitos de hiperamonia para establecer el papel de NHE1 en la regulación de la apoptosis de hepatocitos inducida por NH_4Cl.
Materiales y métodos: En los estudios actuales, el pH intracelular (pHi) y la actividad del NHE1 se analizaron con el colorante BCECF-AM, sensible al pHi. Los resultados mostraron que el pH intracelular disminuyó y la actividad del NHE1 aumentó en hepatocitos con tratamiento del NH_4Cl. Como se esperaba, la disminución del pHi inducido por NH_4Cl se relacionó con un aumento de la apoptosis, baja proliferación celular y reducción del ATP, que se exacerbó por la exposición a cariporide, inhibidor del NHE1. También encontramos que el tratamiento del NH_4Cl estimuló la fosforilación de PI3K y Akt, y este efecto se redujo considerablemente por la inhibición del NHE1.
Conclusión: Este trabajo ha destacado el importante papel del NHE1 en la regulación de la apoptosis celular inducida por hiperamoniaquemia.

Introduction

Hepatic failure (HF) is a life threatening disease and is caused by a variety of factors, which induce liver cell damage and liver dysfunction. The mortality rate of HF is very high[1] and the complications associated with HF include hepatic encephalopathy, hepatorenal syndrome and hemorrhaging.[2] It has been proved that hyperammonaemia was involved in the pathogenesis of hepatic encephalopathy and it is commonly studied as the mechanism of encephalopathy.[3]

Recent studies, including the results obtained from our laboratory, have demonstrated that hyperammonemia has a direct adverse effect on hepatocytes and it is therefore both a cause and an effect of hepatic failure.[4–6] But the effect of high blood ammonia on liver cell injury and its underlying mechanism remain unclear.

Proper cell function depends on the maintenance of intracellular and extracellular physicochemical parameters within certain physiological limits. Such an important parameter is pH.[7] In fact, the activity of intracellular enzymes, the interaction of cytoskeletal elements, the rate at which cells grow and differentiate all depend on pHi.[8,9] Cells can minimize significant pHi fluctuations through several H^+ transport systems; the best known system is represented by the sodium/hydrogen exchanger (NHE) family. Among all the NHE isoforms, NHE isoform 1 (NHE1) is the dominant subtype in hepatic tissue and displays a widespread tissue distribution by maintaining pHi and cell volume levels, in contrast, other subtypes have more limited tissue distribution and are thought to be involved in NaCl absorption.[11,12]

Most studies have demonstrated that apoptosis is ultimately accompanied by cytosol acidification.[10,13,14] More recently, it was reported that rats subjected to 280 mmol/L NH_4Cl loading for five days developed metabolic acidosis.[15] Flow cytometric analysis from our previous research work revealed that the cell apoptosis ratio increased with increasing concentrations of NH_4Cl. This demonstrated that high blood ammonia levels may lead to liver cell damage and apoptosis.[4–6,16] A cytoprotective role of NHE1 is also supported by experiments in which NHE1-null proximal tubule cells demonstrated enhanced sensitivity to multiple apoptotic stimuli compared with wild-type cells.[14] Manucha[17] demonstrated that tubular epithelial cell apoptosis was associated with decreased NHE1 expression in a neonatal rat model of ureteral obstruction.

In our present study, we investigated whether hyperammonemia has any effects on the pHi and activity of NHE1 in hepatic cells. A hyperammonia hepatic cell model was established to determined the role of NHE1 in the regulation of hepatocyte apoptosis induced by hyperammonaemia and its mechanism underlying liver failure.

Materials and methods

Materials

All cell culture reagents, including antibiotics, fetal bovine serum (FBS), phosphate-buffered saline (PBS) and RIMP1640 were purchased from Thermo Fisher Scientific, Inc. (Waltham, MA, USA). 2′,7′-bis(2-carboxyethyl)-5(6)-carboxyfluo rescein acetoxymethyl ester (BCECF-AM), cariporide and antibodies were purchased from Santa Cruz