

Basic Study

H₂S mitigates severe acute pancreatitis through the PI₃K/AKT-NF- κ B pathway *in vivo*

Chun-Yan Rao, Lan-Ying Fu, Chang-Lun Hu, Dai-Xing Chen, Tian Gan, Yi-Cheng Wang, Xiao-Yan Zhao

Chun-Yan Rao, Lan-Ying Fu, Dai-Xing Chen, Tian Gan, Xiao-Yan Zhao, Department of Gastroenterology, Xinqiao Hospital, Third Military Medical University, Chongqing 400037, China
Chang-Lun Hu, Yi-Cheng Wang, Department of Endocrinology, Chongqing Corps Hospital of Chinese People's Armed Police Forces, Chongqing 40061, China

Author contributions: Rao CY and Zhao XY designed the study; Rao CY, Fu LY and Hu CL performed the study; Chen DX and Tian G performed statistical analysis; Rao CY and Fu LY wrote the paper; all authors contributed to the writing of the manuscript and interpretation of the data.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Correspondence to: Xiao-Yan Zhao, Professor, Department of Gastroenterology, Xinqiao Hospital, Third Military Medical University, No. 30 Gaotanyan Street, Shapingba District, Chongqing 400037, China. zhaoxx@medmail.com.cn

Telephone: +86-23-68755604

Received: July 29, 2014

Peer-review started: July 31, 2014

First decision: August 27, 2014

Revised: September 20, 2014

Accepted: December 5, 2014

Article in press: December 8, 2014

Published online: April 21, 2015

Abstract

AIM: To study the effect of hydrogen sulfide (H₂S) on severe acute pancreatitis (SAP) in a rat model.

METHODS: Sprague-Dawley (SD) rats were administered an intraperitoneal injection of saline containing 20% L-Arg (250 mg/100 g) hourly for over 2 h to induce

SAP. The rats were treated with DL-propargylglycine (PAG, 50 mg/kg) or different dosages of NaHS (5 mg/kg, 10 mg/kg, 20 mg/kg or 100 mg/kg). PAG or NaHS was administered 1 h before induction of pancreatitis. Rats were sacrificed 24 h after the last L-Arg injection. Blood and pancreas tissues were collected.

RESULTS: The H₂S and cystathionine- γ -lyase mRNA levels in SAP rats were significantly lower than those in the control group, and treatment with PAG further reduced the H₂S level. Nevertheless, H₂S was significantly increased after NaHS administration compared with the SAP group, and the degree of upregulation was associated with the NaHS dosage. NaHS reduced the levels of plasma amylase, interleukin-6 and myeloperoxidase in pancreatic tissue. NaHS suppressed the degradation of I κ B α and the activity of nuclear factor- κ B, as well as the phosphorylation of PI₃K/AKT.

CONCLUSION: H₂S plays an anti-inflammatory role in SAP *in vivo*.

Key words: Severe acute pancreatitis; Hydrogen sulfide; Cystathionine- γ -lyase; Cytokine; Signaling pathway

© The Author(s) 2015. Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: In this research, we provide wide-ranging dosages of exogenous H₂S (NaHS), from 5 mg/kg to 100 mg/kg, to study the role of H₂S in severe acute pancreatitis (SAP) *in vivo*, and finally found that the level of H₂S was reduced in rat SAP models, suggesting that H₂S donor NaHS reduced the inflammatory indices of SAP, by inhibiting PI₃K/AKT phosphorylation and down-regulating I κ B α degradation and nuclear factor- κ B activity.

Rao CY, Fu LY, Hu CL, Chen DX, Gan T, Wang YC, Zhao

XY. H₂S mitigates severe acute pancreatitis through the PI3K/AKT-NF-κB pathway *in vivo*. *World J Gastroenterol* 2015; 21(15): 4555-4563 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v21/i15/4555.htm> DOI: <http://dx.doi.org/10.3748/wjg.v21.i15.4555>

INTRODUCTION

Severe acute pancreatitis (SAP) is an inflammatory condition of the pancreas that is characterized by elevated pancreatic enzymes in the blood, systemic inflammatory response syndrome (SIRS), and multiple organ dysfunctions syndrome (MODS), which is ultimately responsible for most pancreatitis-associated mortality and morbidity^[1]. Since the study by Rindernecht in 1988^[2] implicated cytokines and stress-activated pathways in SAP, cytokines have received much attention in SAP. These inflammatory mediators interact with microcirculation, thereby increasing vascular permeability and inducing thrombosis and hemorrhage and ultimately leading to tissue necrosis^[1]. Thus, blocking inflammatory cytokine expression at the time of initial injury might obstruct the development of inflammation. However, various cytokines participate in the inflammatory response in SAP, and the efficient regulation of the production and interaction of cytokines is important for preventing and treating SAP. Therefore, it is reasonable to speculate that regulating the key cytokines and signal transduction pathways in inflammatory responses will help develop an effective anti-inflammatory therapy for SAP.

Nuclear factor kappa B (NF-κB) comprises a family of transcriptional activators, which include homodimers or heterodimers of RelA (p65), p50, p52, c-Rel, and RelB^[3]. In resting cells, NF-κB is sequestered in the cytoplasm *via* interaction with the inhibitor of κBα (IκBα) proteins^[4]. The phosphorylation of IκBα induces ubiquitination and proteasomal degradation upon stimulation, and the subsequent release of NF-κB transcription factors, which translocate to the nucleus and bind to cognate DNA binding sites to initiate the transcription of a wide array of genes, including cytokines^[4]. IκBα phosphorylation is triggered by activating the IκB kinase (IKK) complex, through serine/threonine kinase (AKT). AKT is a major downstream target of phosphatidylinositol-3-kinase (PI3K) and is rigorously modulated by PI3K. PI3K inhibition reduces the LPS-induced expression of interleukin (IL)-1β in mouse macrophages^[5].

Hydrogen sulfide (H₂S) is a small molecular weight liposoluble gas, which permeates through the cell membrane freely to exert important physiological functions. H₂S is predominantly produced through the action of three enzymes: cystathionine-β-synthase (CBS) and cystathionine-γ-lyase (CSE), which generate H₂S using L-cysteine as a substrate and pyridoxal 5'-phosphate (PLP) as a cofactor^[6],

and 3-mercaptopyruvate sulfurtransferase (3-MST), which produces H₂S using 3-mercaptopyruvate, a metabolite of L-cysteine, as a substrate^[7]. CSE is the major H₂S-producing enzyme in the peripheral tissue. Numerous studies have shown the beneficial effects of H₂S, particularly in cardiovascular and neurological disorders^[8]. However, the role of H₂S in inflammation is only recently beginning to emerge, and the exact role of this gas is still not clearly understood.

In this study, we aimed to investigate the hypothesis that the exogenous H₂S vehicle (NaHS) could be beneficial against SAP induced by intraperitoneal administration of L-Arg in Sprague-Dawley (SD) rats *in vivo*. We observed that H₂S donor NaHS protects against experimental SAP *in vivo* by suppressing NF-κB activation *via* the down-regulation of PI3K/AKT phosphorylation.

MATERIALS AND METHODS

Induction of SAP in rats

Animal care and experimental protocols were performed in compliance with the animal management guidelines of the Government of the People's Republic of China. Male SD rats (223-265 g) were obtained from the Animal Center, Daping Hospital, Third Military Medical University. The rats were randomly assigned to either a control (*n* = 7) or an experimental (*n* = 10) group.

The control group animals were given hourly intraperitoneal injections of normal saline.

The SAP group animals were given hourly intraperitoneal injections of saline containing 20% L-Arg (250 mg/100 g) for over 2 h^[9].

The PAG + SAP group animals were treated as previously described for the SAP group above, except that PAG (50 mg/kg, Sigma), a CSE inhibitor, was dissolved in saline and administered intraperitoneally 1 h before the first L-Arg injection.

In the 5, 10, 20 and 100 mg/kg NaHS + SAP groups, different dosages of NaHS (Sigma) were diluted in saline and administered 1 h before the first L-Arg injection.

In the wortmannin (W) + SAP, 5 mg/kg NaHS + W + SAP, and 100 mg/kg NaHS + W + SAP groups, the animals were treated as described for the SAP group, with 5 mg/kg NaHS + SAP and 100 mg/kg NaHS + SAP, except that wortmannin (1.4 mg/kg, Sigma)^[10], a PI3K inhibitor, was dissolved in dimethyl sulfoxide (DMSO) and administered 30 min before the first L-Arg injection.

Twenty-four hours after the first L-Arg injection, the animals were sacrificed using an intraperitoneal injection of a lethal dose of pentobarbital (50 mg/kg). Blood and pancreas samples were collected.

Real-time PCR to quantitate CSE mRNA expression

Total RNA was collected from pancreatic tissues

Table 1 Optimal annealing temperature, optimal cycles, and desired product sizes for detecting cystathionine-γ-lyase and GAPDH

Gene	Primer sequence	Size
CSE	Forward: 5'GCGCTGCTCCAACGTGTGTATAA 3' Reverse: 5'GGGTCCGGTTTCAGCATGTTT 3'	253 bp
GAPDH	Forward: 5'CTCATGACCACAGTCCATGC3' Reverse: 5'TTCAGCTCTGGGATGACCTT3'	155 bp

using TRIzol Reagent (Sigma) according to the manufacturer's instructions. Total RNA (2 µg) was reverse transcribed using a ReverTra Ace-α-cDNA Synthesis Kit (Toyobo) at 42 °C for 10 min, 30 °C for 20 min, and 99 °C for 5 min, followed by 4 °C for 5 min. The cDNA was used as a template for detecting CSE and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression. The optimal annealing temperature, optimal cycles, and desired product sizes are shown in Table 1. Real-time PCR was performed on an iCycler iQ apparatus (Fermentas) using the iCycler optical system software (version 3.1) and the SYBR Green PCR Master Mix (ABI). The ΔCT value was determined, and the formula $2^{-\Delta\Delta CT}$ was used to determine the relative quantity of the amplified fragments.

Measurement of plasma H₂S and amylase in rats

Aliquots (120 µL) of plasma were mixed with distilled water (100 µL), trichloroacetic acid (10% w/v, 120 µL), zinc acetate (1% w/v, 60 µL), N,N-dimethyl-p-phenylenediamine sulfate (NNDPP) (20 µmol/L; 40 µL) in 7.2 mol/L HCl, and FeCl₃ (30 µmol/L; 40 µL) in 1.2 mol/L HCl in 96-well plates. The absorbance of the resulting solution was measured after 10 min at 670 nm^[11]. All samples were assayed in duplicate, and the H₂S was calculated against a calibration curve of NaHS (3.125-100 µmol/L). The results are expressed as the plasma H₂S concentration in micromoles per liter. Plasma amylase was assessed using auto-biochemistry equipment.

ELISA analysis of IL-6

To measure the plasma IL-6, ELISA kits from R&D Systems were used according to the manufacturer's instructions. The ELISA results were reproducible with an interassay variability of < 9.5% and an intraassay variability of < 6.5%. The results were corrected for the DNA content of the tissue samples and expressed as picograms per microgram of DNA.

Myeloperoxidase detection

Inflammatory cell sequestration in the pancreas was quantified as a measurement of the tissue myeloperoxidase (MPO) activity^[12]. The tissue samples were thawed, homogenized in 20 mmol/L phosphate buffer (pH 7.4) and centrifuged at 13000 rpm for 10 min at 4 °C, and the resulting pellet was resuspended

in 50 mmol/L phosphate buffer (pH 6.0) (Sigma). The suspension was subjected to four cycles of freezing and thawing and further disruption through sonication (40 s). The sample was subsequently centrifuged at 13000 rpm for 5 min at 4 °C, and the supernatant was used for the MPO assay. This mixture was incubated at 37 °C for 110 s; the reaction was terminated after adding 50 µL of 0.18 mol/L H₂SO₄, and the absorbance was measured at 405 nm.

Western blot analysis of p-AKT and IκBα

The frozen pancreatic tissue samples were cut into small pieces, homogenized in 0.5 mL of RIPA buffer, and incubated at 4 °C overnight. The dissolved proteins were collected after centrifugation at 10000 g for 30 min, and the supernatant was collected. The pancreatic tissue proteins were subsequently separated through SDS-polyacrylamide gel electrophoresis and transferred to a PVDF membrane (Millipore). The membrane was incubated with primary antibodies against p-AKT (1:300, Santa), IκBα (1:500, Abcam), and GAPDH (1:8000, ProMab), followed by incubation with the appropriate secondary horseradish peroxidase conjugated anti-rabbit IgG antibodies (1:3000, Santa; 1:3000, Abcam; and 1:8000, ProMab, respectively).

EMSA analysis of NF-κB activity

Nuclear extracts from pancreas (100 mg) tissues were prepared using a nuclear extraction kit, according to the manufacturer's instructions (Kaiji Biotech). Briefly, the NF-κB oligonucleotide probe (5'-TCAACTCCCCTGAAA GGGTCCG-3') was labeled with biotin and purified on a NICK column (Yuantai Biotech). The binding reaction was performed in a 20 µL reaction mixture containing 6 µg of incubation buffer, 8 µg of nuclear extract, and 20 fm of biotech labeled oligonucleotides. After incubating for 20 min at room temperature, 2 µL of 0.1% bromophenol blue was added, and the samples were electrophoresed on a 6% non-denaturing polyacrylamide gel at 100 V for 30 min.

Survival analysis

To observe the therapeutic effect of NaHS, the rats were randomly assigned to normal saline + SAP (*n* = 10), SAP (*n* = 10), 5 mg/kg NaHS + SAP (*n* = 10), and 100 mg/kg NaHS + SAP (*n* = 10) groups. NaHS and L-Arg were administered as previously described, but the rats were allowed to drink water freely and fast after the procedure.

Statistical analysis

The data are expressed as mean ± SE. Differences between the mean values of multiple groups were analyzed using one-way analysis of variance (ANOVA) followed by the LSD test and survival analysis through Kaplan-Meier using SPSS 13.0 software, and *P* < 0.05 was considered statistically significant.

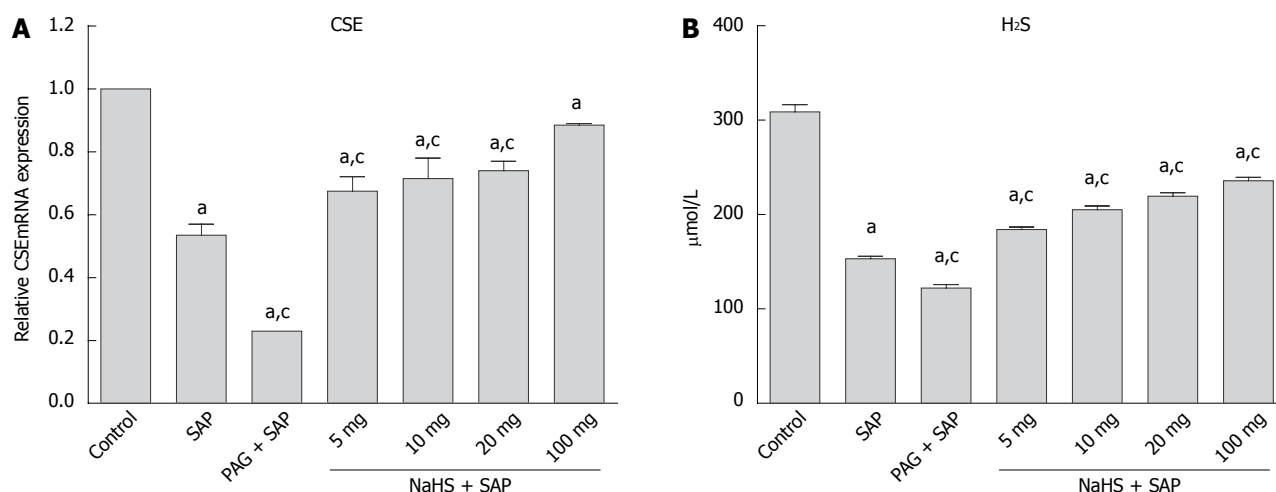


Figure 1 Levels of cystathionine-γ-lyase mRNA (A) and plasma H₂S (B) in pancreatic tissue. ^a $P < 0.05$ vs the control; ^c $P < 0.05$ vs the SAP group.

RESULTS

Plasma H₂S levels and mRNA expression of CSE in vivo

As shown in Figure 1A, CSE mRNA expression in the pancreatic tissue was significantly suppressed in the SAP group compared with the control group ($P < 0.05$), and the CSE mRNA expression was further down-regulated after the PAG ($P < 0.05$) treatment. Compared with the control group, plasma H₂S levels were significantly lower ($P < 0.05$) in the SAP rats, and administering PAG further reduced the H₂S level. However, after treatment with different dosages of NaHS, plasma H₂S levels in these groups were all significantly increased compared with the SAP group ($P < 0.05$), and within the NaHS groups, the H₂S levels were gradually upgraded with increasing NaHS dosage (Figure 1B).

Levels of plasma amylase, IL-6, and MPO in pancreatic tissue

As shown in Figure 2A, plasma amylase was significantly increased in the SAP group compared with the control group ($P < 0.05$), and the level in the PAG + SAP group was further increased ($P < 0.05$). However, this increase could be abolished after administering different doses of NaHS, and no significant differences between the SAP group and 5 mg/kg NaHS + SAP group was observed; however, a significant reduction in the plasma amylase activity was observed in the rats receiving 10, 20, or 100 mg/kg NaHS ($P < 0.05$). Within these NaHS groups, the levels of plasma amylase were reduced with decreasing NaHS dosage. The histological examination of the pancreas samples obtained from the L-Arg, PAG and NaHS treated rats was also performed (Figure 2B). As shown in Figure 2C, a significant increase in interleukin (IL)-6 expression was observed in the SAP group. The pretreatment of SAP rats with PAG further increased the IL-6 levels, while treatment with different dosages

of NaHS induced a significant reduction in IL-6 compared with SAP rats. There was a significant MPO increase in SAP rats compared with the controls ($P < 0.05$), and the rats that were administered PAG showed an even higher MPO increase than the SAP rats ($P < 0.05$). Nevertheless, treatment with different doses of NaHS significantly reduced the MPO activity compared with SAP rats ($P < 0.05$, Figure 2D).

In vivo expression of p-AKT, IκBα, and NF-κB

The Western blot analysis showed that the *in vivo* expression of p-AKT was notably increased in the SAP rats compared with the controls ($P < 0.05$, Figure 3A), and p-AKT expression was further augmented after the PAG administration. NaHS limited the increase in SAP-induced p-AKT levels, and the role of NaHS was enhanced gradually as the NaHS dosage increased. To determine the contribution of AKT in H₂S-mediated inflammation during SAP, we treated rats with the PI3K inhibitor wortmannin for 30 min after the NaHS administration. We observed that the inactivation of PI3K with wortmannin treatment further reduced p-AKT expression. NF-κB is a downstream target of the PI3K/AKT pathway, and the release of NF-κB from the inhibitory protein IκBα is essential for activation of NF-κB. The phosphorylation of IκBα is a prerequisite for the degradation of this protein, which is primarily dependent on AKT activation. The induction of SAP resulted in significant IκBα degradation (Figure 3B) and NF-κB activation (Figure 3C), which was enhanced through PAG treatment, while the effect of H₂S was suppressed through the NaHS application. We also observed that the role of NaHS was increased through the robust inhibition of PI3K activity using wortmannin.

Levels of plasma IL-6 and MPO in vivo

Administering the PI3K inhibitor wortmannin for 30 min after the NaHS treatment further reduced the plasma IL-6 levels and MPO activity in pancreatic tissue (Figure 4).

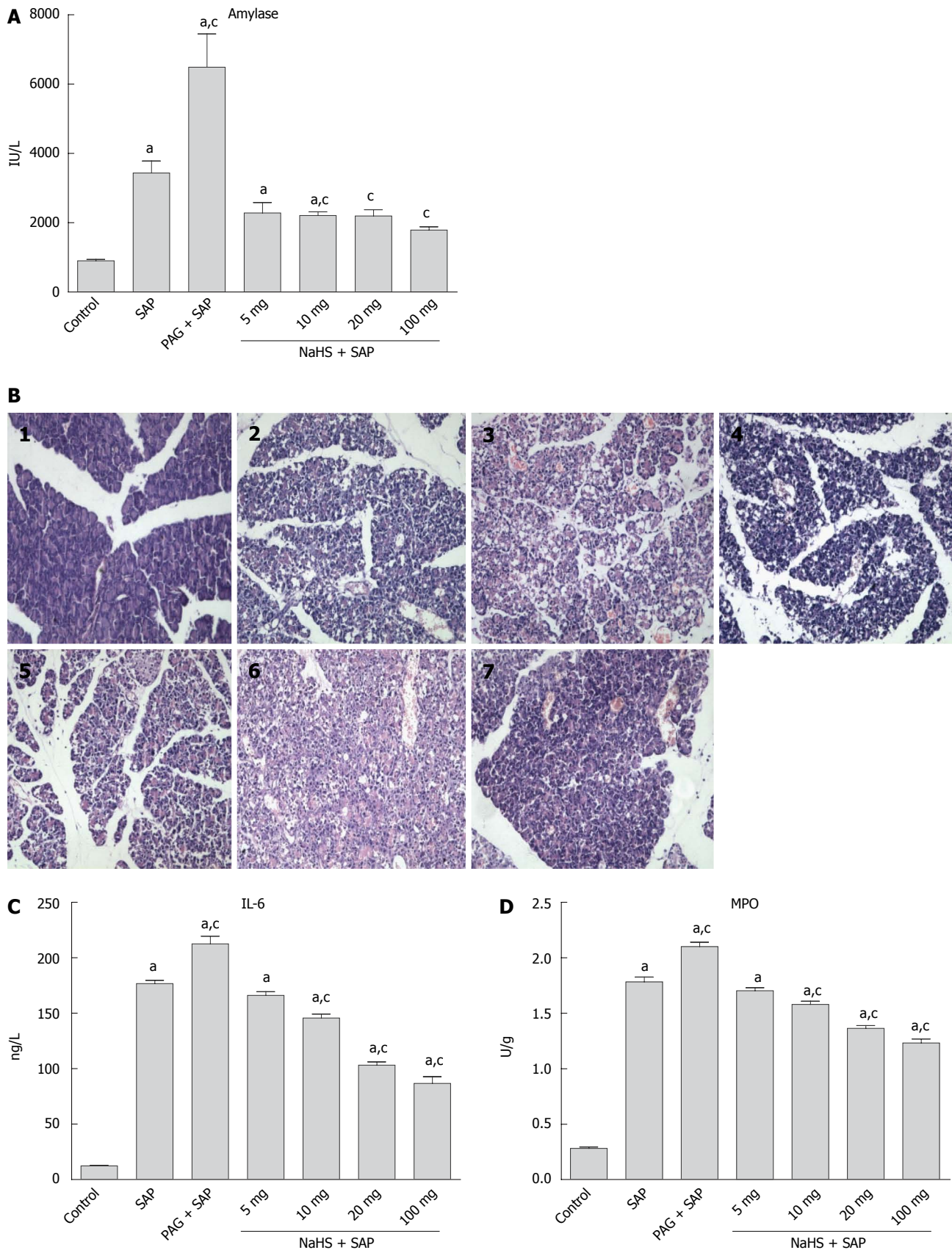


Figure 2 Levels of amylase, interleukin-6, and myeloperoxidase in pancreatic tissue. A: Plasma amylase level; B: Pancreatic histology (HE × 200) [1: Control (saline-injected) pancreas; 2: L-Arg-induced severe acute pancreatitis (the arrow indicates edema, destruction, histoarchitecture bleeding of the acini and the inflammatory cell infiltration); 3: Rats pre-administered PAG 50 mg/kg show more severe destruction and histoarchitecture bleeding of the acini and inflammatory cell infiltration; 4: Pretreatment with NaHS at 5 mg/kg; 5: Pretreatment with NaHS at 10 mg/kg; 6: Pretreatment with NaHS at 15 mg/kg; 7: Pretreatment with NaHS at 100 mg/kg]; C: Plasma IL-6 level; D: MPO activity in the pancreatic tissue. ^a*P* < 0.05 vs the control; ^c*P* < 0.05 vs the SAP group. PAG: Propargylglycine; MPO: Myeloperoxidase; SAP: Severe acute pancreatitis.

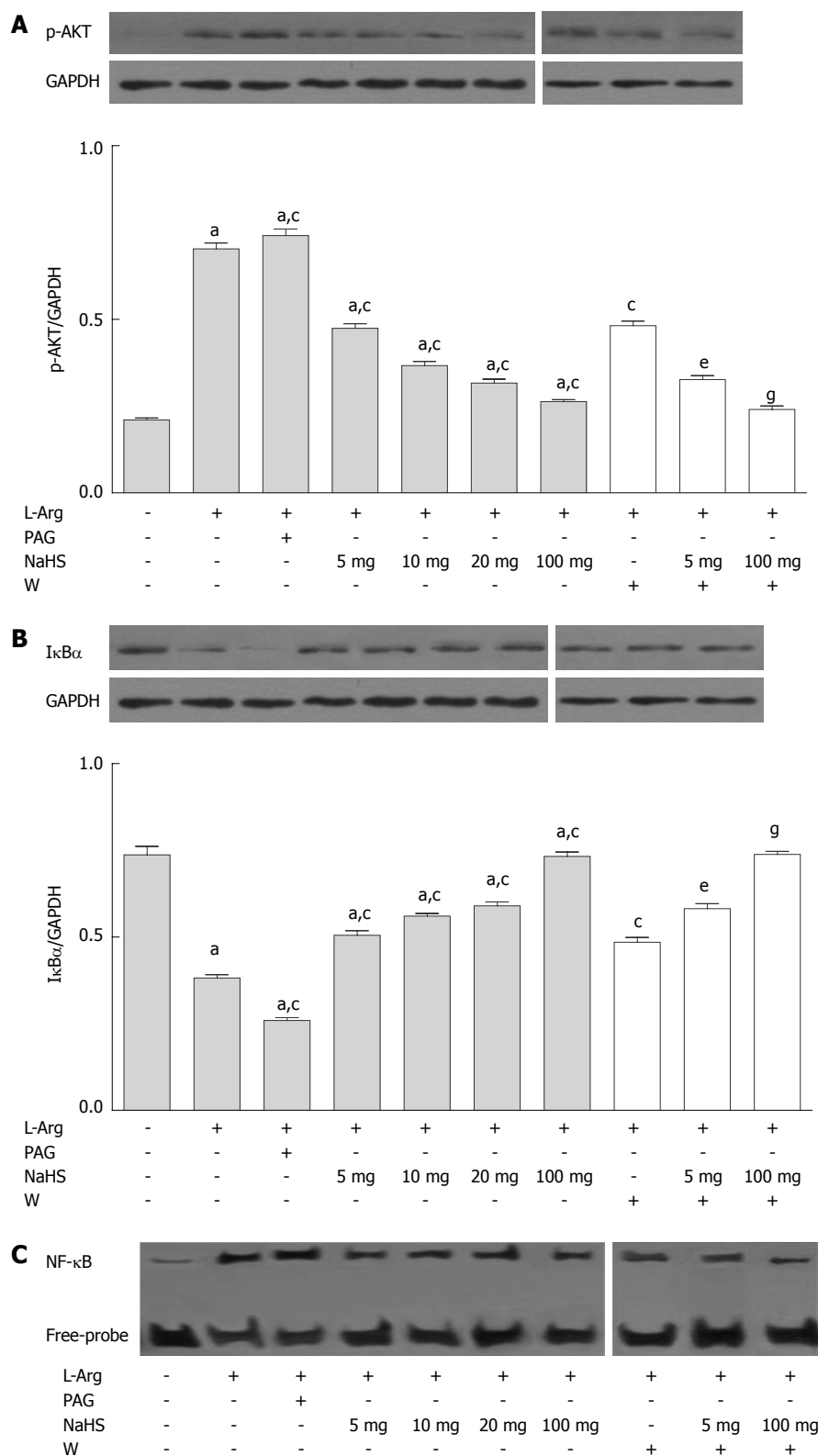


Figure 3 Levels of p-AKT (A), IκBα protein expression (B) and nuclear factor-κB activity *in vivo* (C). Western blot and EMSA analyses of the protein expression and NF-κB activity after treatment with L-Arg and NaHS or PAG and the PI3K inhibitor wortmannin. ^a*P* < 0.05 vs the control; ^b*P* < 0.05 vs the severe acute pancreatitis (SAP) group; ^c*P* < 0.05 vs the 5 mg/kg NaHS + SAP group; ^d*P* < 0.05 vs the 100 mg/kg NaHS + SAP group.

Survival analysis

The Kaplan-Meier survival curves are shown in Figure 5 for the 7-d study period. The results showed that the

normal saline + SAP and SAP rats all died within 6 d without administering the H₂S donor NaHS; however, the survival rate in the groups treated with NaHS was 70%.

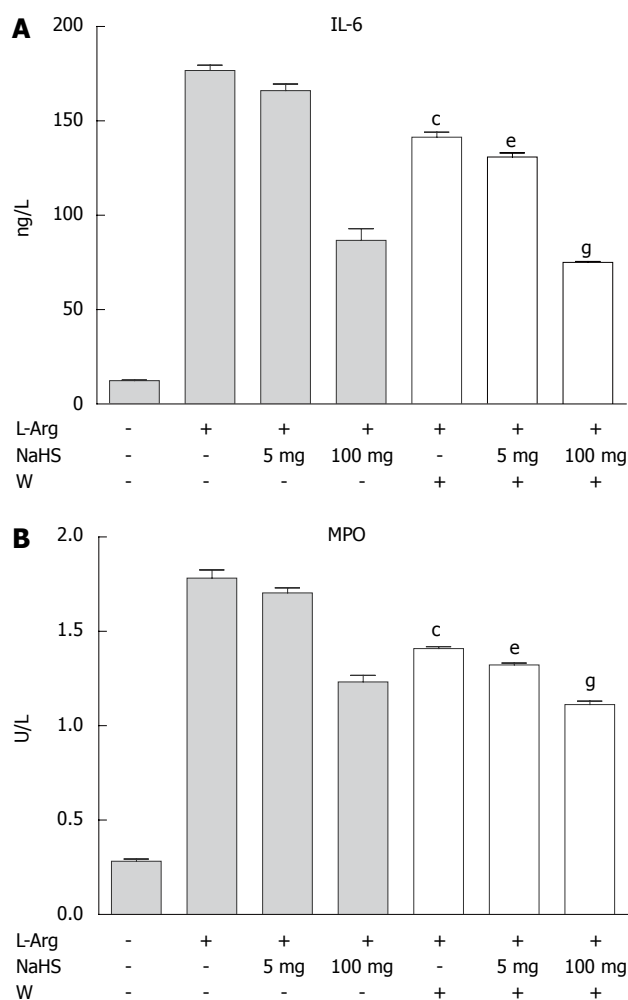


Figure 4 Level of plasma interleukin-6 and myeloperoxidase activity in the pancreas. A: Plasma interleukin (IL)-6 level after treatment with wortmannin; B: Myeloperoxidase (MPO) activity in pancreatic tissue after treatment with wortmannin. ^c $P < 0.05$ vs the severe acute pancreatitis (SAP) group; ^e $P < 0.05$ vs the 5 mg/kg NaHS + SAP group; ^g $P < 0.05$ vs the 100 mg/kg NaHS + SAP group.

DISCUSSION

The role of H₂S in inflammation is ambiguous. Here, we show an anti-inflammatory role for this compound in *in vivo* experiments involving SAP. Similarly, we observed that CSE mRNA expression was significantly reduced in the L-Arg-induced rat models, and treatment with the CSE inhibitor (PAG) further reduced the CSE mRNA levels, accompanied by increases in plasma amylase, IL-6, and MPO activity *in vivo*. Thus, we initially proposed that the down-regulation of the endogenous H₂S/CSE system might be involved in the pathogenesis of inflammatory infiltration in SAP and that H₂S/CSE alterations were associated with the activity and severity of this disease. We pre-treated the rats with different dosages of NaHS before inducing SAP *in vivo*. NaHS successfully reduced the levels of plasma amylase, IL-6 and leukocyte infiltration (shown by MPO activity) in the pancreas of SAP rats, accompanied by the up-regulation of CSE mRNA expression. Intriguingly, the effect of NaHS

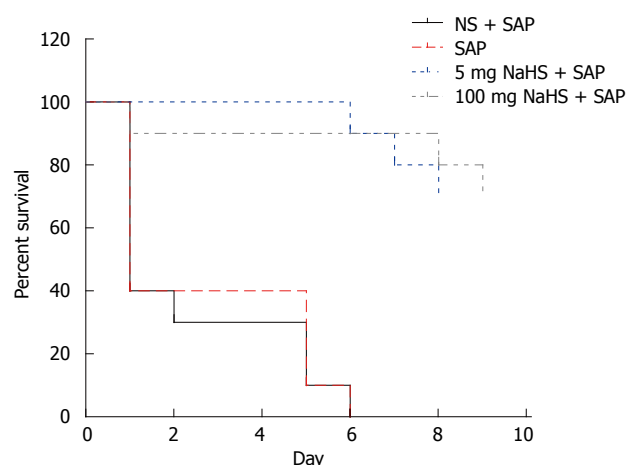


Figure 5 Survival analyses between different groups of rats. The survival rate was monitored for 10 d [$P < 0.05$, when severe acute pancreatitis (SAP) rats with NaHS injections were compared with SAP rats without NaHS injection, $n = 10$ in these groups].

was gradually increased with increasing NaHS dosage *in vivo*. These results suggested that H₂S could be a negative regulator in SAP.

IL-6 is produced by many cell types, including monocytes/macrophages, endothelial cells, fibroblasts, and smooth muscle cells^[1,13], which indicates the profile of leukocyte recruitment during the inflammatory response *via* the selective regulation of inflammatory chemokines and apoptotic events^[14,15]. Circulating levels of IL-6 are a sufficient indicator of the severity of acute pancreatitis^[16]. We observed that the levels of plasma IL-6 were significantly up-regulated in SAP rats. The concentrations of IL-6 in the plasma were obviously attenuated after pre-administration with different dosages of NaHS. The present study suggests that the anti-inflammatory action of H₂S might be mediated through inhibition of IL-6. This result is consistent with previous data, showing that the H₂S donor in OA-treated rats significantly reduced IL-6 and IL-8 levels in the plasma and in the lungs^[17], and the administration of NaHS attenuated the increase in plasma TNF- α and ICAM-1 levels in hepatic ischemia-reperfusion (HIR) rats^[18].

There is much evidence confirming that the development and progression of SAP depend on NF- κ B transcription, which plays an important role in the regulation of inflammatory mediators. Furthermore, evidence suggests that NF- κ B transcription plays a role in exogenous H₂S-induced cytokine expression in human leukocytes^[19], confirming the close relationship between NF- κ B and H₂S. We observed that the role of H₂S in SAP involved the suppression of I κ B α degradation, NF- κ B transcription, and pro-inflammatory gene expression. Moreover, the PI3K inhibitor wortmannin further decreased NF- κ B transcription, plasma IL-6 levels, and MPO activity in pancreatic tissue.

AKT is a major PI3K effector and the full activation

of AKT requires phosphorylation at Thr308 and Ser473, and Thr308 phosphorylation by 3'-PDK is also a PI3K effector^[20]. Although AKT is not directly phosphorylated through PI3K, these post-translational modifications are strictly dependent on PI3K activity^[20]. Tarassishin *et al.*^[21] suggested that activation of the PI3K/AKT pathway could play an anti-inflammatory role. Therefore, we determined whether H₂S inhibits AKT phosphorylation *in vivo* and examined the effect of the PI3K/AKT pathway in the H₂S-mediated inflammatory response. For this purpose, we administered the PI3K inhibitor wortmannin before SAP induction and observed that the effect of NaHS was enhanced by wortmannin. Therefore, we supposed that H₂S regulates AKT through a node downstream of PI3K. Previous studies have demonstrated that H₂S significantly improves cardiodynamic functions and attenuates the size of myocardial infarction *via* AKT stimulation^[22]. Furthermore, Tamizhselvi *et al.*^[23] reported that the H₂S-mediated down-regulation of TNF- α and IL-1 β in cerulein-treated acinar cells was associated with the activation of PI3K/AKT.

H₂S protects gastric mucosal epithelial cells against oxidative stress through the activation of p38 mitogen-activated protein kinases (MAPK)^[24]. It has been reported that H₂S significantly attenuated the phosphorylation of MAPK and activated NF- κ B and expression of the anti-apoptotic protein Bcl-2^[25]. The treatment of C28/I-2 cells with H₂S deactivated extracellular-regulated kinase (ERK1/2), inhibited the phosphorylation of NF- κ B p65 at Ser536 and reduced IL-6 and IL-8 expression^[26]. Taken together, these results demonstrated that H₂S possesses an extensive pathophysiological action and exerts a protective role *via* intra- and extra-cellular signal transduction pathways or genetic transcription in various animal models and cell culture.

Sidhapuriwala *et al.*^[27] considered that exogenous H₂S (NaHS) at 10 mg/kg significantly down-regulated amylase levels in the plasma and MPO activity in acute pancreatitis; however, the role of NaHS was not enhanced at 15 mg/kg. Faller *et al.*^[28] recently showed that only inhalation of H₂S at 80 ppm prevents lung injury in ventilated mice. In myocardial ischemia-reperfusion injury, treatment with different doses of H₂S donor (NaHS) generated a U-shaped dose response curve^[29,30]. However, we showed that the protective role of H₂S in SAP was gradually increased from 5 to 100 mg/kg *in vivo*; thus, further studies are needed to confirm these findings.

In conclusion, in the present study, we showed that the down-regulation of the CSE/H₂S system plays an important role in SAP development and progression and in preconditioning the H₂S donor with NaHS-induced anti-inflammatory activity in SAP. This protective effect was associated with PI3K/AKT phosphorylation inhibition, the repressed degradation of I κ B α , and NF- κ B activation. We also observed that the prophylactic administration of NaHS significantly

reduced the mortality of SAP rats. These data indicated that H₂S/CSE plays a crucial role in SAP development, which suggests that NaHS might provide new valuable therapeutic strategies for improving the clinical course of this disease.

COMMENTS

Background

Severe acute pancreatitis (SAP) is an inflammatory disease with high morbidity and mortality, but the pathogenesis of SAP remains unknown. Recent studies have implicated H₂S in different inflammatory reactions. However, the exact role of this gas is still not clearly understood, especially in SAP.

Research frontiers

Cystathionine- γ -lyase (CSE) is the major H₂S-producing enzyme in the peripheral tissue. Numerous studies have shown the beneficial effects of H₂S, particularly in cardiovascular and neurological disorders.

Innovations and breakthroughs

The authors provide wide-ranging dosages of exogenous H₂S (NaHS), from 5 mg/kg to 100 mg/kg, to study the role of H₂S in SAP *in vivo*, and finally certified the role of H₂S in SAP rats through survival analysis.

Applications

These data indicated that H₂S/CSE plays a crucial role in SAP development, which suggests that NaHS might provide new valuable therapeutic strategies for improving the clinical course of this disease.

Terminology

SAP is an inflammatory condition of the pancreas that is characterized by elevated pancreatic enzymes in the blood, SIRS and MODS, which is ultimately responsible for most pancreatitis-associated mortality and morbidity. H₂S is predominantly produced through the action of three enzymes: CBS and CSE, and 3-mercaptopyruvate sulfurtransferase (3-MST), however, CSE is the major H₂S-producing enzyme in the peripheral tissue.

Peer-review

The paper is important because the effects of H₂S on pancreatitis have not been investigated thoroughly in the Arginine model. The conclusions are supported by the well-presented and executed experiments.

REFERENCES

- 1 Bhatia M. Novel therapeutic targets for acute pancreatitis and associated multiple organ dysfunction syndrome. *Curr Drug Targets Inflamm Allergy* 2002; **1**: 343-351 [PMID: 14561181]
- 2 Rinderknecht H. Fatal pancreatitis, a consequence of excessive leukocyte stimulation? *Int J Pancreatol* 1988; **3**: 105-112 [PMID: 2834471]
- 3 Vallabhapurapu S, Karin M. Regulation and function of NF-kappaB transcription factors in the immune system. *Annu Rev Immunol* 2009; **27**: 693-733 [PMID: 19302050 DOI: 10.1146/annurev.immunol.021908.132641]
- 4 Ojaniemi M, Glumoff V, Harju K, Liljeroos M, Vuori K, Hallman M. Phosphatidylinositol 3-kinase is involved in Toll-like receptor 4-mediated cytokine expression in mouse macrophages. *Eur J Immunol* 2003; **33**: 597-605 [PMID: 12616480]
- 5 Rommel C, Camps M, Ji H. PI3K delta and PI3K gamma: partners in crime in inflammation in rheumatoid arthritis and beyond? *Nat Rev Immunol* 2007; **7**: 191-201 [PMID: 17290298]
- 6 Moore PK, Bhatia M, Mochhala S. Hydrogen sulfide: from the smell of the past to the mediator of the future? *Trends Pharmacol Sci* 2003; **24**: 609-611 [PMID: 14654297]
- 7 Shibuya N, Tanaka M, Yoshida M, Ogasawara Y, Togawa T, Ishii K, Kimura H. 3-Mercaptopyruvate sulfurtransferase produces hydrogen sulfide and bound sulfane sulfur in the brain. *Antioxid Redox Signal* 2009; **11**: 703-714 [PMID: 18855522 DOI: 10.1089/ARS.2008.2253]
- 8 Szabó C. Hydrogen sulphide and its therapeutic potential. *Nat Rev Drug Discov* 2007; **6**: 917-935 [PMID: 17948022]
- 9 Tani S, Itoh H, Okabayashi Y, Nakamura T, Fujii M, Fujisawa T,

- Koide M, Otsuki M. New model of acute necrotizing pancreatitis induced by excessive doses of arginine in rats. *Dig Dis Sci* 1990; **35**: 367-374 [PMID: 2307082]
- 10 Singh VP, Saluja AK, Bhagat L, van Acker GJ, Song AM, Soltoff SP, Cantley LC, Steer ML. Phosphatidylinositol 3-kinase-dependent activation of trypsinogen modulates the severity of acute pancreatitis. *J Clin Invest* 2001; **108**: 1387-1395 [PMID: 11696584]
- 11 Chunyu Z, Junbao D, Dingfang B, Hui Y, Xiuying T, Chaoshu T. The regulatory effect of hydrogen sulfide on hypoxic pulmonary hypertension in rats. *Biochem Biophys Res Commun* 2003; **302**: 810-816 [PMID: 12646242]
- 12 Yang G, Cao K, Wu L, Wang R. Cystathionine gamma-lyase overexpression inhibits cell proliferation via a H₂S-dependent modulation of ERK1/2 phosphorylation and p21Cip/WAK-1. *J Biol Chem* 2004; **279**: 49199-49205 [PMID: 15347670]
- 13 Bhatia M, Sidhapuriwala JN, Sparatore A, Moore PK. Treatment with H₂S-releasing diclofenac protects mice against acute pancreatitis-associated lung injury. *Shock* 2008; **29**: 84-88 [PMID: 17621252]
- 14 Jones SA. Directing transition from innate to acquired immunity: defining a role for IL-6. *J Immunol* 2005; **175**: 3463-3468 [PMID: 16148087]
- 15 McLoughlin RM, Witowski J, Robson RL, Wilkinson TS, Hurst SM, Williams AS, Williams JD, Rose-John S, Jones SA, Topley N. Interplay between IFN-gamma and IL-6 signaling governs neutrophil trafficking and apoptosis during acute inflammation. *J Clin Invest* 2003; **112**: 598-607 [PMID: 12925700]
- 16 Panek J, Karcz D, Pieton R, Zasada J, Tusinski M, Dolecki M, Winiarski M. Blood serum levels of proinflammatory cytokines in patients with different degrees of biliary pancreatitis. *Can J Gastroenterol* 2006; **20**: 645-648 [PMID: 17066155]
- 17 Li T, Zhao B, Wang C, Wang H, Liu Z, Li W, Jin H, Tang C, Du J. Regulatory effects of hydrogen sulfide on IL-6, IL-8 and IL-10 levels in the plasma and pulmonary tissue of rats with acute lung injury. *Exp Biol Med* (Maywood) 2008; **233**: 1081-1087 [PMID: 18535161 DOI: 10.3181/0712-RM-354]
- 18 Kang K, Zhao M, Jiang H, Tan G, Pan S, Sun X. Role of hydrogen sulfide in hepatic ischemia-reperfusion-induced injury in rats. *Liver Transpl* 2009; **15**: 1306-1314 [PMID: 19790158 DOI: 10.1002/lt.21810]
- 19 Ganster F, Burban M, de la Bourdonnaye M, Fizanne L, Douay O, Loufrani L, Mercat A, Calès P, Radermacher P, Henrion D, Asfar P, Meziani F. Effects of hydrogen sulfide on hemodynamics, inflammatory response and oxidative stress during resuscitated hemorrhagic shock in rats. *Crit Care* 2010; **14**: R165 [PMID: 20836847 DOI: 10.1186/cc9257]
- 20 Ehrhardt C, Ludwig S. A new player in a deadly game: influenza viruses and the PI3K/Akt signalling pathway. *Cell Microbiol* 2009; **11**: 863-871 [PMID: 19290913 DOI: 10.1111/j.1462-5822.2009.01309.x]
- 21 Tarassishin L, Suh HS, Lee SC. Interferon regulatory factor 3 plays an anti-inflammatory role in microglia by activating the PI3K/Akt pathway. *J Neuroinflammation* 2011; **8**: 187 [PMID: 22208359 DOI: 10.1186/1742-2094-8-187]
- 22 Yong QC, Lee SW, Foo CS, Neo KL, Chen X, Bian JS. Endogenous hydrogen sulphide mediates the cardioprotection induced by ischemic postconditioning. *Am J Physiol Heart Circ Physiol* 2008; **295**: H1330-H1340 [PMID: 18660450 DOI: 10.1152/ajpheart.00244.2008]
- 23 Tamizhselvi R, Sun J, Koh YH, Bhatia M. Effect of hydrogen sulfide on the phosphatidylinositol 3-kinase-protein kinase B pathway and on caerulein-induced cytokine production in isolated mouse pancreatic acinar cells. *J Pharmacol Exp Ther* 2009; **329**: 1166-1177 [PMID: 19258518 DOI: 10.1124/jpet.109.150532]
- 24 Yonezawa D, Sekiguchi F, Miyamoto M, Taniguchi E, Honjo M, Masuko T, Nishikawa H, Kawabata A. A protective role of hydrogen sulfide against oxidative stress in rat gastric mucosal epithelium. *Toxicology* 2007; **241**: 11-18 [PMID: 17825973]
- 25 Tripatara P, Patel NS, Collino M, Gallicchio M, Kieswich J, Castiglia S, Benetti E, Stewart KN, Brown PA, Yaqoob MM, Fantozzi R, Thiemermann C. Generation of endogenous hydrogen sulfide by cystathionine gamma-lyase limits renal ischemia/reperfusion injury and dysfunction. *Lab Invest* 2008; **88**: 1038-1048 [PMID: 18679378 DOI: 10.1038/labinvest.2008.73]
- 26 Kloesch B, Liszt M, Steiner G, Bröll J. Inhibitors of p38 and ERK1/2 MAPkinase and hydrogen sulphide block constitutive and IL-1 β -induced IL-6 and IL-8 expression in the human chondrocyte cell line C-28/12. *Rheumatol Int* 2012; **32**: 729-736 [PMID: 21161531 DOI: 10.1007/s00296-010-1682-0]
- 27 Sidhapuriwala JN, Ng SW, Bhatia M. Effects of hydrogen sulfide on inflammation in caerulein-induced acute pancreatitis. *J Inflamm (Lond)* 2009; **6**: 35 [PMID: 20040116 DOI: 10.1186/1476-9255-6-35]
- 28 Faller S, Ryter SW, Choi AM, Loop T, Schmidt R, Hoetzel A. Inhaled hydrogen sulfide protects against ventilator-induced lung injury. *Anesthesiology* 2010; **113**: 104-115 [PMID: 20574227 DOI: 10.1097/ALN.0b013e3181de7107]
- 29 Elrod JW, Calvert JW, Morrison J, Doeller JE, Kraus DW, Tao L, Jiao X, Scalia R, Kiss L, Szabo C, Kimura H, Chow CW, Lefer DJ. Hydrogen sulfide attenuates myocardial ischemia-reperfusion injury by preservation of mitochondrial function. *Proc Natl Acad Sci USA* 2007; **104**: 15560-15565 [PMID: 17878306]
- 30 Ji Y, Pang QF, Xu G, Wang L, Wang JK, Zeng YM. Exogenous hydrogen sulfide postconditioning protects isolated rat hearts against ischemia-reperfusion injury. *Eur J Pharmacol* 2008; **587**: 1-7 [PMID: 18468595 DOI: 10.1016/j.ejphar.2008.03.044]

P- Reviewer: Tulassay ZJ S- Editor: Ma YJ L- Editor: Wang TQ
E- Editor: Zhang DN





Published by **Baishideng Publishing Group Inc**

8226 Regency Drive, Pleasanton, CA 94588, USA

Telephone: +1-925-223-8242

Fax: +1-925-223-8243

E-mail: bpgoffice@wjgnet.com

Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>

<http://www.wjgnet.com>



ISSN 1007-9327

