（人間環境医工学専攻生体環境学コース）　　（別記様式第８号）
（課程・論文博士共通）

論　文　内　容　要　旨

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論文題目	Pharmacological levels of hydrogen sulfide inhibit oxidative cell injury through regulating the redox state of thioredoxin（硫化水素によるチオレドキシン酸化還元状態の調節及び酸化的細胞傷害の抑制）		

論文内容要旨

[Purpose]

Hydrogen sulfide (H_2S) is a gaseous mediator with multifaceted biological activities. It protects cells against oxidative stress, but the mechanisms involved are not fully understood. Given that Trx/ASK1/P38 signaling pathway mediates many oxidative cell responses and that its activation is determined by the redox state of Trx, we speculated that H_2S might regulate Trx and its downstream signaling pathway. The purpose of this study was to address this hypothesis.

[Methods]

Murine podocytes and human hepatoma HepG2 cells were cultured and exposed to various chemicals. The cell death was detected by Calcein-AM/Propidium Iodide staining, Hoechst staining and WST assay. Oxidative stress was evaluated by protein carbonylation. TXNIP/Trx/ASK1/P38 signaling pathway was analyzed using immunoprecipitation and western blot analysis. Trx redox state was detected by AMS-shift assay. Trx sulfhydration was determined with maleimide labeling. H_2S level was measured using the lead sulfide method.

[Results]

(1) Exposure of glomerular podocytes to adriamycin, an anti-tumor drug, induced P38-mediated oxidative cell injury, as evidenced by the increased reactive oxygen species (ROS) generation, oxidative activation of ASK1 and P38, as well as the prevention of cell death by an antioxidant, NADPH oxidase inhibitor, and P38 inhibitor.

(2) Supplement of podocytes with H_2S donor NaHS blunted the activation of the ASK1/P38 signaling pathway and protected cells from adriamycin-induced oxidative cell injury. It also alleviated cell damage elicited by H_2O_2, superoxide, thioredoxin (Trx) inhibitor PX12, and Trx reductase inhibitor auranofin.

(3) Mechanistic analysis revealed that H_2S did not affect the protein level of Trx and TXNIP (a negative regulator of Trx), but it promoted the dissociation of TXNIP from Trx. Furthermore, it prevented the H_2O_2-induced formation of Trx dimers in a way similar to DTT. Moreover, it increased the conversion of

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4　論文内容要旨は，（研究の目的），（方法），（結果），（考察），（結論）の順に日本語（２，０００字程度）もしくは英語（半角５，０００字程度）でまとめ，タイプ等で印字すること。（文字数を記載してください。）

論文内容要旨（続紙）

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(4) In HepG2 cells, inhibition of the H_2S-producing enzyme CSE increased Trx oxidation and exaggerated Trx reductase inhibitor-induced cell death. Consistently, the promotion of endogenous H_2S production through the supplement of cells with L-cysteine exerted the opposite effects.

[Discussion]

In this study, we demonstrated that H_2S protected cells from oxidative cell injury through mechanisms involving its regulation on the redox state of thioredoxin and P38 signaling pathway. Given the importance of Trx and the pathway in the control of multifaceted cell responses, our findings could have significant implications.

In this study, we found that H_2S alleviated oxidative stress, inhibited oxidative sensitive Trx/ASK1/P38 signaling pathway, and attenuated oxidative cell injury induced by several different stimuli, indicating a protective effect of H_2S on oxidative stress. This effect of H_2S was associated with dissociation of Trx with its inhibitor TXNIP, elevated thiol activity of Trx and disruption of the disulfide bonds among Trx and/or other molecules (PX12 or Trx itself), indicating that H_2S promoted Trx in its reduced form. Given that Trx is located at the upstream of the ASK1/P38 signaling pathway and that it is the major cellular defense mechanism against oxidative stress, it is conceivable that this effect of H_2S contributed to its protective effect on oxidative cell injury.

Our findings could have significant implications. First, Trx/ASK1/P38 signaling pathway mediates many oxidative cell responses, including inflammation, senescence, and survival. Intriguingly, all these cell responses are also regulated by H_2S. It is likely that H_2S exerts its biological actions through interference with Trx/ASK1/P38 signaling pathway. Second, our results indicate that targeting H_2S could be used to modulate cell responses to ADR. Depending on the situations, H_2S could be manipulated to potentiate the tumor-killing efficacy of ADR or protect normal cells from the toxicity of ADR. Third, Trx is reported to be required for H_2S production by 3MST. Our finding of regulation of Trx activity by H_2S suggests the existence of a positive reciprocal regulation between Trx and H_2S.

[Conclusion]

Collectively, our study indicates that H_2S inhibits oxidative activation of the ASK1/P38 signaling pathway and mitigates oxidative cell injury through regulation of Trx redox status. Our study thus provides novel mechanistic insight into the anti-oxidative actions of H_2S and suggests that it could be used to prevent oxidative cell injury.

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