

The analysis on the expression of gasotransmitters in early trauma patients

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SUMMARY

Objective: Nitric oxide (NO), carbon monoxide (CO) and hydrogen sulfide (H₂S) were endogenously-generated molecules gas. They owned important biological activity and participated in many pathophysiological processes. This study aimed to examine the levels of three gasotransmitters in the early phase of trauma patients. **Method:** Blood samples were collected from 60 trauma patients and ten healthy volunteers. Concentration of serum iNOS and HO-1 were analyzed by enzyme linked immunosorbent assay and plasma H₂S was determined by colorimetric method. Meanwhile, the occurrence of multiple organ dysfunction syndrome (MODS) was also monitored.

Results: The levels of iNOS, HO-1 and endogenous H₂S in the patients group were significantly different from the healthy control group, and the difference was more obvious with the increase of ISS score. iNOS levels were positively correlated with ISS scores and blood lactic acid values, and HO-1 and endogenous H₂S were negatively correlated with ISS scores and blood lactic acid values. Of 60 trauma patients, eight (13.33%) developed MODS. The level of iNOS in the MODS group was higher than that in non-MODS group, while HO-1 and H₂S were significant lower in the MODS group.

Conclusion: The three gasotransmitters participated in systemic inflammatory responses during early trauma and could be used as important indicators for trauma severity. Their measurements were meaningful for evaluating the severity and prognosis of trauma.

Keywords: wounds and injuries, nitric oxide synthase type II, heme oxygenase-1, hydrogen sulfide, multiple organ failure.

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INTRODUCTION

Recently, severe trauma involving multiple locations and multiple organs has become one of the major causes of deaths. There are three peak incidences of death for the patients with multiple trauma: (i) within several seconds to several minutes after trauma, immediate death is always caused by damage in the brain, heart and large blood vessels, as well as high spinal cord injury; (ii) within several minutes to several hours after trauma, early death is mainly caused by asphyxia, circulatory insufficiency and uncontrolled massive hemorrhage; (iii) within several days to several weeks after trauma, late death is caused by organ failure and infection. In all of the three situations, systemic inflammatory response syndrome (SIRS) exists in different degrees. The excessive release of inflammatory

mediator and the over-activation of inflammatory cells would lead to an excessive immune response in severe trauma.¹ In addition to the direct injury, inflammatory mediators also cause a “second injury” to human body. Thus, control of inflammatory mediators is critically important in the inhibition of SIRS in multiple-organ dysfunction syndrome (MODS).

Endogenous gasotransmitters not only play an important role in the regulation of immunological reactions, but also function in the regulation of vascular activity. It was found that gasotransmitters are the important signaling pathways in inflammatory reactions. Among them, nitric oxide (NO), carbon monoxide (CO) and hydrogen sulfide (H₂S), the three gases and their corresponding synthetases compose an interactional system. NO is an endothelium-

-derived relaxing factor, generated from L-arginine under the catalysis of inducible nitric oxide synthase (iNOS). Endogenous NO is activated in the conditions of severe trauma, infection and shock, and abundant NO is produced. Furthermore, endogenous NO also has a negative inotropic effect; it can inhibit cardiac function, and even cause low blood pressure and shock. Meanwhile, it also interacts with the accumulated superoxide anion (O_2^-) and forms peroxy nitrite anion ($ONOO^-$), thus more poly polymerase is over-activated and causes cellular injury.

CO has anti-inflammatory, anti-oxidation and cell-protection properties. HO-1 is considered one of the major rate-limiting enzymes for synthesis of endogenous CO. Under the influence of hypoxia, endotoxins and cytokines, it can catalyze the degradation of heme to CO. Studies found that endogenous CO activates guanylate cyclase, and thus leads to increase of cGMP and induces the relaxation of vascular smooth muscle.² In addition, it also inhibits the expression of iNOS and decreases the cytotoxicity caused by excessive NO.³

Endogenous H_2S was mainly produced from L-cysteine-substrate by cystathionine- β -synthetase (CBS) and cystathionine-lyase (CSE). The former mainly exists in liver, brain and other parenchymal organs, while the latter mainly exists in the cardiovascular system. The distribution of endogenous H_2S in the human body has tissue specificity. However, there is no consensus regarding the anti-inflammatory ability or pro-inflammatory ability of H_2S .^{4,5}

The participation of the above three gasotransmitters in inflammatory reactions has been widely investigated. However, their roles in different diseases and during different disease courses still calls for further study. So far, knowledge about the three gasotransmitters in early trauma is inadequate. Our research aimed to examine the changes in NO, CO and H_2S in the early phase of severe trauma, and explore the relationship between incidence of MODS and blood lactic acid.

METHOD

Subjects

A total of 70 participants were included in the study: 60 patients who were treated at the Department of Emergency of the Third Hospital of Hebei Medical University between October 2012 and November 2013, and ten healthy volunteers. In all, there were 61 males and nine females.

The enrollment of these patients met the following conditions: 1. severe trauma; 2. received treatment within 3 hours after severe trauma; 3. aged ranged between 18 and 60 years. The exclusion criteria of the subjects were

as follows: 1. the interval of severe trauma and blood drawing was more than 3 hours; 2. received blood transfusion; 3. received IV transfusion more than 2,000 mL; 4. surgical history; 5. coagulation dysfunction or anti-coagulation medication history; 6. common diseases and simultaneous liver and kidney function damages; 7. severe cardio-cerebral vessel diseases; 8. pregnant and lactating women. Informed consent was obtained from all participants. All the procedures were approved by the ethics committee of the Third Hospital of Hebei Medical University.

Measurement

Severity of the patients' injuries was evaluated using the Abbreviated Injury Scale (AIS) and the Injury Severity Score (ISS). According to the results, the patients were classified into three groups: mild group (score ≤ 16), medium group (score in 16-25) and severe group (score ≥ 25). And based on their prognosis, they were divided into MODS group and non-MODS group.

Cardiograph, blood pressure and finger pulse oxygen saturation were monitored continuously to evaluate the clinical condition of the patients. Routine blood test, liver and kidney function evaluation, blood gas analysis and blood lactic acid monitoring were examined. The diagnosis of MODS was performed according to the modified Fry-MODS diagnosis criteria.⁶

Blood samples were collected within 3 hours of the injury for a routine blood test, measurement of kidney function and procalcitonin. 3 mL of venous blood was collected and centrifuged, the supernatant was stored at $-80^\circ C$ for the analysis of inducible nitric oxide synthase (iNOS), heme oxygenase-1 (HO-1) and plasma H_2S . Concentration of serum iNOS and HO-1 were analyzed by enzyme-linked immunosorbent assay (ELISA) using commercially available ELISA kits (Jiancheng, Nanjing, China) and following the manufacturer's instructions. Plasma H_2S was determined by colorimetric method.

Statistical analysis

SPSS 19.0 was used to perform statistical analyses. The data were presented as mean \pm standard deviation (SD). Normally distributed continuous data were assessed by independent sample t-test. ANOVA test was performed to compare the differences among the groups. Non-normally distributed parameters were analyzed with nonparametric sum rank test. Correlation analysis was performed by Pearson correlation analysis for normally distributed data and Kendall correlation analysis for non-normally distributed data. A p-value of less than 0.05 is indicated as statistically significant differences.

RESULTS

Table 1 listed the general characteristics and the serum gasotransmitter levels of the study subjects. There were no statistical differences between the groups in terms of age, gender ratio and the time from trauma to treatment ($p < 0.05$). The levels of iNOS, HO-1 and endogenous H₂S in the patient group were significantly different from the healthy control group, and the difference was more obvious with the increase of ISS score. The expression of iNOS in the patient group was higher than that found in the healthy control group, and the expression found an upward trend depending on injury severity ($p < 0.05$). Meanwhile, the HO-1 and endogenous H₂S contents were significant lower compared with the control group ($p < 0.05$) (Figure 1).

TABLE 1 The clinical characteristics and serum gasotransmitter levels of the study subjects.

| | Healthy control group | Mild group (ISS ≤ 16) | Medium group (ISS in 16-25) | Severe group (ISS ≥ 25) |
|---------------------------|-----------------------|-----------------------|-----------------------------|-------------------------|
| n | 10 | 23 | 27 | 10 |
| Age (years) | 34±7 | 38±9 | 35±8 | 32±7 |
| Gender (male/female) | 9/1 | 20/3 | 24/3 | 8/2 |
| Time (h) | - | 1.6±0.4 | 1.5±0.5 | 1.5±0.5 |
| iNOS (U/mL) | 474.5±35.9 | 547.1±52.9* | 688.48±74.8* | 753.4±92.33* |
| HO-1 (ng/mL) | 3.54±0.42 | 2.92±0.65* | 2.44±0.45* | 1.77±0.655* |
| H ₂ S (μmol/L) | 50.77±2.00 | 48.64±1.72* | 46.40±1.82* | 44.15±1.60* |

Compared with the healthy control group, * $p < 0.05$.

To clarify whether the changes in serum gasotransmitter levels played a role in the trauma, the relationship between gasotransmitter levels and ISS scores as well as blood lactic acid values were further investigated (Table 2). The results of the analysis demonstrated that iNOS levels were positively correlated with ISS scores ($r^2=0.586$, $p < 0.001$) and blood lactic acid ($r^2=0.440$, $p < 0.001$) and HO-1 and endogenous H₂S values were negatively correlated with ISS scores ($r^2=0.473$, $p < 0.001$; $r^2=0.590$, $p < 0.001$) and blood lactic acid values ($r^2=0.525$, $p < 0.001$; $r^2=0.515$, $p < 0.001$).

TABLE 2 The relation between gasotransmitter levels with ISS scores and blood lactic acid values.

| | Association with ISS scores | | Association with blood lactic acid values | |
|------------------|-----------------------------|----------|---|----------|
| | r^2 | p | r^2 | P |
| iNOS | 0.586 | <0.001 | 0.440 | <0.001 |
| HO-1 | 0.473 | <0.001 | 0.525 | <0.001 |
| H ₂ S | 0.590 | <0.001 | 0.515 | <0.001 |

Among the 60 trauma patients, eight (13.33%) developed MODS. There were no statistical differences between the non-MODS and MODS in age, gender ratios and the time from trauma to treatment ($p < 0.05$) (Table 3). The level of iNOS in the MODS group was higher than that in the non-MODS group, while HO-1 and H₂S were significant lower in the MODS group ($p < 0.05$) (Figure 2).

TABLE 3 The rate of MODS and serum gasotransmitter levels.

| | Control group | Non-MODS | MODS |
|---------------------------|---------------|--------------|---------------|
| n | 10 | 52 | 8 |
| Age (years) | 34±7 | 33±9 | 34±7 |
| Gender (female/male) | 9/1 | 45/7 | 7/1 |
| Time (h) | - | 1.6±0.5 | 1.5±0.4 |
| iNOS (U/mL) | 474.5±3.9 | 628.9±102.3* | 736.5±86.93*# |
| HO-1 (ng/mL) | 3.54±0.42 | 2.61±0.61* | 1.98±0.85*# |
| H ₂ S (μmol/L) | 50.77±2.00 | 47.30±2.18* | 44.53±1.92* |

Compared with the healthy control group, * $p < 0.05$. Compared with the non-MODS group, # $p < 0.05$.

DISCUSSION

NO, CO and H₂S were endogenously-generated gas molecules. They present important biological activity and participated in many pathophysiological processes. Gasotransmitters were freely permeable across cell membranes and served as regulators of a multitude of biochemical pathways and physiological processes. Recently, the roles of gasotransmitters in trauma were recognized, which led to a new broad perspective on inflammatory mediators.

The release of inflammatory factors into the blood causes a "second strike" to the tissues and cells in trauma sites, and is the main reason for the systemic inflammatory response at the early stage of injury. Lactic acid is an important intermediate product in the glycometabolism of the human body in the presence of hypoxia. Thus, it is an important and sensitive indicator of cellular hypoxia. In acute trauma, the effective blood capacity is insufficient and accumulates large amounts of lactic acid. In the process of fluid resuscitation, dynamic observation of the lactic acid values could help understand characteristics of blood perfusion.⁷ A decline in lactic acid after fluid resuscitation usually means a better prognosis, while an increase in lactic acid might indicate ischemic reperfusion. In the present study, the level of blood lactic acid was increased, and it was positively correlated with NO content and negatively correlated with CO and H₂S contents. The synthesis of iNOS and endogenous NO was high after being damaged.

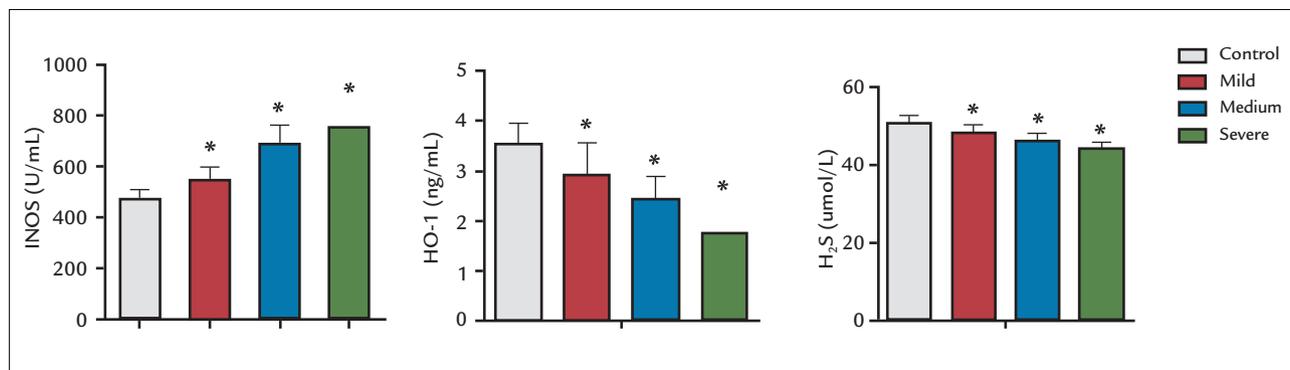


FIGURE 1 Serum gasotransmitters levels of the study subjects in traumatic patients group and healthy control group. *indicates $p < 0.05$ compared with control.

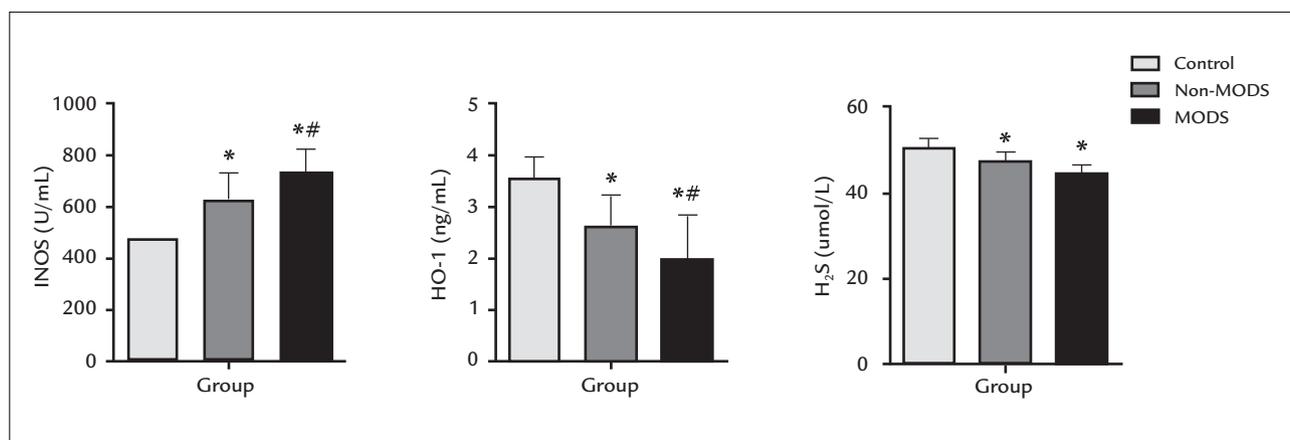


FIGURE 2 Serum gasotransmitters levels of the study subjects in non-MODS and MODS group.

*indicates $p < 0.05$ compared with healthy control, # indicates $p < 0.05$ compared with non-MODS.

Trauma, infection, hypoxia and excessive stress response could both lead to systemic inflammatory response. In normal physiological conditions, a small amount of NO was catalyzed by iNOS. In our study, the generation of iNOS was increased with the ISS score in the early phase of trauma patients. When a large amount of NO was generated, it could react with superoxide anion and lead to the accumulation of peroxynitrite anion, also causing cell damage.⁸ Besides, excessive NO led blood vessels to expand and produced hypotension shock. Some studies found that different types of inflammation could lead to image enhancement in animal models. In addition, inhibition of iNOS can reduce the production of NO, and subsequently relieve inflammatory response.^{9,10} iNOS is the only rate-limiting enzyme in NO production, decreasing the synthesis of NO could diminish the inflammatory response, septic shock and other symptoms.⁹ Hemoglobin plays a vital role in the synthesis of NOS; it can be degraded by HO-1 and thus

inhibit NOS. HO-1 can influence the activity of iNOS.¹¹ Furthermore, H₂S donors inhibited iNOs production by enhancing the expression of HO-1.¹²

HO-1 is the major rate-limiting enzyme for the synthesis of endogenous CO, it can catalyze the degradation of heme into CO, catalytic iron and bilirubin. Catalytic iron and bilirubin have a strong capacity of anti-oxidation.^{13,14} CO not only plays an important role in anti-inflammation, anti-apoptosis, improving circulation and anti-thrombosis, but also down-regulates the expression of proinflammatory molecules such as IL-8 and TNF- α .¹⁵ Some studies found that a small amount of inhaled CO enhances the ability of anti-oxidation and anti-apoptosis in the experimental model of LPS-induced lung injury; meanwhile, up-regulations with HO-1 could achieve similar effects.¹⁶ In our study, HO-1 level was significantly lower in the early phase of trauma patients than in the healthy control group, and they were negative correlated with ISS score and blood lactic acid level. The results

demonstrated that the HO-1/CO pathway is activated in severe trauma; CO participated in the inflammatory response as an important gasotransmitter.

Similar with CO and NO, H₂S plays important roles in physiological regulation. Due to the specific distribution of its synthetase, the regulatory functions also exhibited apparent tissue specificity. However, there was no consensus regarding the anti-inflammatory ability or pro-inflammatory ability of H₂S. In some researches, pretreatment of mice with H₂S improved leukocyte rolling/adhesion as well as neutrophil migration, which subsequently contributed to reduce bacteria levels and improve survival rate.¹⁷ This suggested that endogenous H₂S played a protective role in systemic inflammatory responses. The study by Li et al. revealed that the damage of ALI/ARDS mouse was aggravated after NaHS was administered, which suggested the proinflammatory role of H₂S.⁴ In the current study, H₂S is significantly lower in patients with ISS ≥ 25 compared with the patients with ISS ≤ 16 .

MODS refers to the sequential failure of more than two organs. Any disease can cause inflammatory immune disorders that may induce MODS. Gasotransmitters, such as NO, CO and H₂S, are the important signaling molecules that participate in the inflammatory response, and at the same time they interact with each other. A study found that the iNOS/NO and the HO-1/CO systems were restricted with each other.¹⁸ A possible explanation is that CO is produced by decomposition of hemoglobin after the oxidation of HO-1; meanwhile, hemoglobin was also the main component of prosthetic group of iNOS. Thus, hemoglobin degradation influenced the production of NO. Furthermore, CO also occupied the coordination center of NOS and reduced its activity. CSE mainly exists in vascular smooth muscle; it could be activated by NO and inhibited by the precursors of NO (hydroxyl ammonium). As for the iNOS/NO and CSE/H₂S system, there were no consensus reached. Both synergetic and inhibitive dynamic interplay between iNOS/NO and CSE/H₂S were reported.¹⁹ CSE/H₂S system showed a synergetic effect on HO-1/CO system.

In the current study, iNOS was found to be significantly higher in the MODS group than in the non-MODS group, which indicated that the iNOS/NO system was activated when severe trauma occurred. A large amount of NO was released into the blood and exacerbated the systemic inflammatory response, caused severe damage to body tissues and organs. HO-1 and H₂S were significantly lower in the MODS group than in the non-MODS group. The results suggested that the three gasotransmitters were closely related with the incidence of MODS and severity of trauma.

CONCLUSION

During the early phase of trauma, it can be suspected that these gasotransmitters not only participate in systemic inflammatory responses, but also facilitate the generation of blood lactic acid. There was a positive correlation between iNOS level and both trauma severity and blood lactic acid value. Meanwhile, a negative correlation was found between HO-1 and H₂S levels, as well as trauma severity and blood lactic acid values. In systemic inflammatory responses, they would not function independently. The interaction between these gas molecules may vary under different conditions. iNOS, HO-1 and endogenous H₂S could be used as important indicators of trauma severity and the onset of post-trauma MODS. Their measurement was meaningful for evaluating the severity and prognosis of trauma.

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