Review

Pharmacology

Hydrogen sulphide, systemic inflammatory response syndrome and compensatory anti-inflammatory response syndrome following sepsis

J Rivers-Auty, M Bhatia*

Abstract

Introduction

Polymicrobial sepsis (PMS) is a systemic infection, which results in a highly evolved inflammatory response by the body; this response is ideally controlled by a signalled anti-inflammatory response. However, tissue damage and infection can cause these responses to become unregulated and pathological. Initially, a hyperimmune response can cause clotting and result in high levels of reactive oxygen species and inflammatory cytokines; when unregulated, this can result in multiple organ failure and death. Conversely, the anti-inflammatory response can become unregulated and result in immune paralysis, uncontrolled infection and pathological hypothermia. The novel signalling molecule hydrogen sulphide appears to be involved in immune function following sepsis. Several studies show that inhibiting the endogenous production of hydrogen sulphide is beneficial following sepsis, and administration of hydrogen sulphide donors appears to exacerbate sepsis. Some studies appear to show the opposite effect. In this review, we argue that these discrepancies could be due to the animal model used that resulted in a hyper- or hypoimmune response in conjunction with differing effects of hydrogen sulphide depending on its concentration and source.

*Corresponding author
Email: madhav.bhatia@otago.ac.nz

Department of Pathology, University of Otago, Christchurch, PO Box 4345, Christchurch 8140, New Zealand

Conclusion

PMS results in two responses by the body, systemic inflammatory response syndrome (SIRS) and compensatory anti-inflammatory response syndrome (CARS). The body produces hydrogen sulphide, and manipulation of it can be therapeutic to SIRS and CARS. Further studies on animal models need to be done to get a greater understanding of the role of hydrogen sulphide in SIRS and CARS, so that such knowledge can then be translated to the clinic.

Introduction

Polymicrobial sepsis (PMS) is a severe infection that infiltrates into the circulatory system, becoming a whole-body infection[1]. PMS typically results in a whole-body immune response, which can build to a pathological level; this is referred to as systemic inflammatory response syndrome (SIRS)[2]. SIRS is characterized by activated circulating leukocytes that produce pathological concentrations of cytokines and reactive oxygen species (ROS). This in turn leads to further tissue damage that causes sensitive organs to fail and eventually death[2]. Patients that survive the initial inflammatory response can later succumb to an infection or tissue damage that the body does not appear to resist or repair[3]. This is because following SIRS the body can respond by producing anti-inflammatory mediators, and like SIRS, the body can over-respond causing paralysis of the immune system; this is referred to as the compensatory anti-inflammatory response syndrome (CARS)[3].

Infections that result in sepsis are most commonly made up of Gram-positive and/or Gram-negative bacteria; however, sepsis can be caused by fungi, yeast or virus infections[1]. Sepsis is a widespread and growing problem, with developed nations reporting the incidence of sepsis at 1 in 10 people admitted to the intensive care unit and 0.5–1.5 cases per 1,000 of the general population[4]. Over 800,000 cases of sepsis are reported annually in the USA, and of these approximately 200,000 are fatal[1]. The incidence and severity of sepsis is greatly affected by the ability to control infection. Currently, antibacterial resistance is a growing problem and the World Health Organisation stated that 25,000 people died in 2011 solely because of antibacterial resistance, and this number will increase dramatically as antibacterial resistant infections continue to grow in numbers[5]. This puts greater importance on sepsis research, as the understanding of the pathophysiology of sepsis is vital to the development of effective treatments. Hydrogen sulphide has recently been discovered to play an important role in many biological functions. It is now considered the third gaseous signalling molecule alongside nitric oxide and carbon monoxide. Hydrogen sulphide's role in immune function is still highly controversial; however, it does appear to play a significant role in the pathology of sepsis. This review addresses the recent developments into the roles of hydrogen sulphide and how intervention in the production or reception of this signalling molecule affects the pathophysiology of sepsis.

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Discussion

The authors have referenced some of their own studies in this review. The protocols of these studies have been approved by the relevant ethics committees related to the institution in which they were performed. Animal care was in accordance with the institution guidelines.

Following a systemic infection, the body responds with an inflammatory response; this is a highly evolved process that is regulated by the body's own anti-inflammatory response, as represented by Patient B (Figure 1). However, the process can become uncontrolled in individuals whose physiologic function has been significantly compromised by infection and tissue damage. These cases are common in PMS and can result in two physiologically different but equally lethal syndromes. SIRS is the hyperimmune response syndrome represented by Patient A (Figure 1) and CARS is the hypoimmune response syndrome represented by Patient C (Figure 1). Each of these syndromes has similar symptoms but very different physiologies that require very different interventions.

SIRS

Severe sepsis follows a chain of events that end with multiple organ failure and death. The exotoxin from the pathogen leaves the initial site of infection and enters the bloodstream. Leukocytes detect these exotoxins and become activated. The activated leukocytes release proinflammatory cytokines such as tumour necrosis factor alpha (TNFα), interleukin (IL)-1 and IL-6 as well as ROS such as nitric oxide. The exotoxins also initiate the coagulation cascade. These events lead to SIRS, which is commonly characterized by hyperthermia, increased capillary permeability, tachycardia, endothelial injury, decreased blood pressure, impaired haemodynamics, vasodilation, vascular thrombosis, microischemia, free radical damage, hypermetabolism and ultimately multiple organ failure

(Figure 2)[6]. SIRS can also be caused by other pathologies such as pancreatitis, as well as significant injuries such as burns and severe trauma. Initially, SIRS was believed to be the primary cause of sepsis-related deaths; so animal models were designed to replicate sepsis with SIRS. Due to this, preclinical animal research found that treatment of sepsis animals with immune suppressants was highly therapeutic. However, this failed to translate into the clinic, with large-scale studies showing no effect or even a negative

effect of the immune suppressants[3]. This led to the hypothesis that the body was already initiating its own anti-inflammatory response and that the immune-suppressing pharmacological intervention led to further immune system shutdown[3]. From this, it was clear that the role of the immune system in sepsis is complicated and not completely unwanted.

CARS

CARS was first described by Bone[2]; it was defined as a systemic
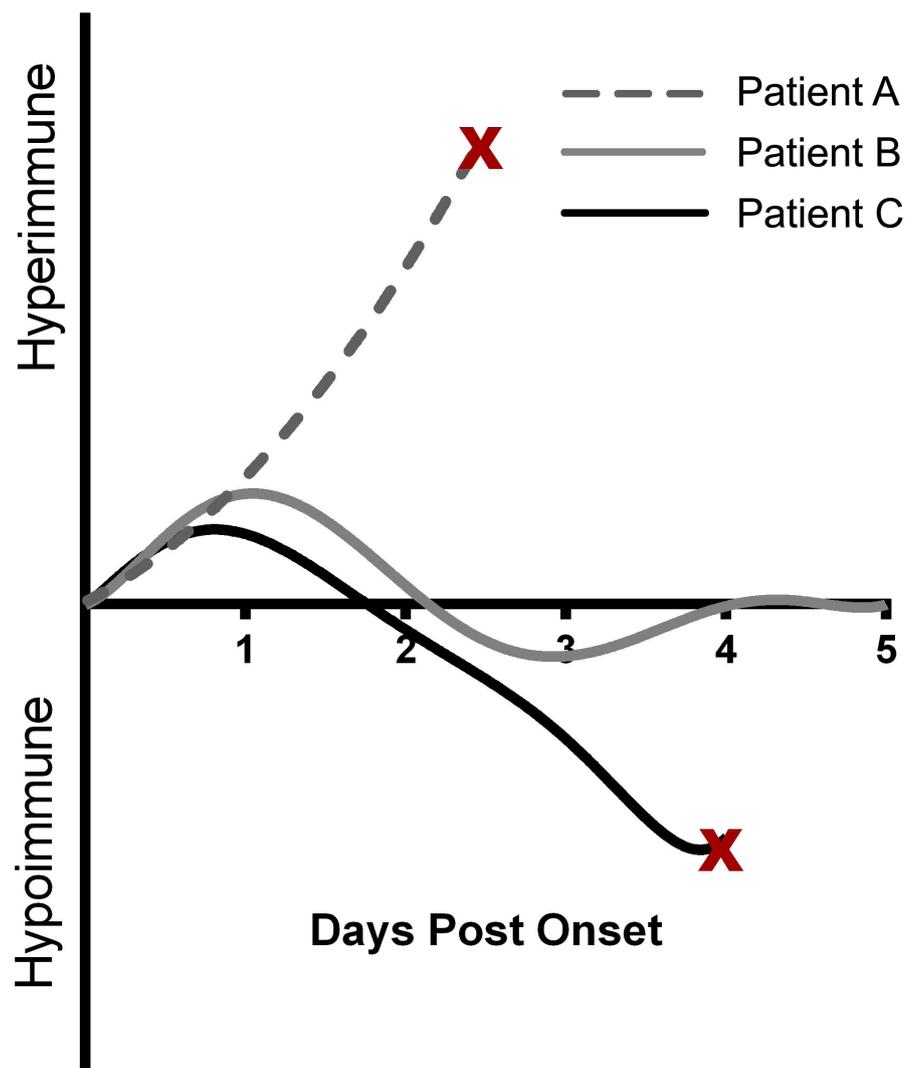

Figure 1: Three theoretical patients that had PMS[2,3,26]. Patient A shows an uncontrolled hyperimmune response typical of SIRS (dark grey dashed line). Patient B shows a successful immune response (light grey line). Patient C shows an uncontrolled hypoimmune response typical of CARS (black line).
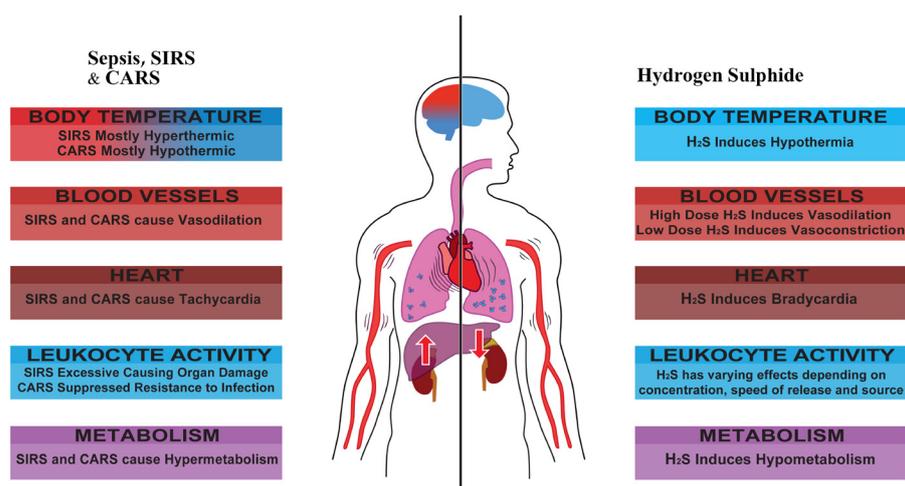
Figure 2: A diagram demonstrating the overlapping effects of SIRS, CARS and hydrogen sulphide on the body[2,3,6,7,18,25,27–29].

deactivation of the immune response in a pathological manner, that is, it was not the body merely recovering from a hyperimmune state such as SIRS, but a pathology where anti-inflammatory cytokines and hormones resulted in the near complete shutdown of the immune system in such a way that not only could the immune system not respond to the infection but the hormones and cytokines produced directly caused pathological symptoms such as hypothermia (Figure 2)[3,6–8]. From clinical research, it became clear that CARS has its own clinical ramifications and patients appeared to die either during the peak of the immune response of SIRS such as Patient A (Figure 1) or they would survive the initial hyperimmune response and die later when their immune response was suppressed (CARS) such as Patient C (Figure 1). These patients showed symptoms of hypothermia and low leukocyte numbers. Clinical samples have shown that during CARS, there is a dramatic increase in the anti-inflammatory cytokine IL-10 and transforming growth factor-β. This gives two separate and opposite potential causes of death following sepsis, and each would require completely different treatments (Figure 1).

Hydrogen sulphide
Hydrogen sulphide is a foul-smelling toxic gas that is now recognized as a gaseous signalling molecule produced by the body through enzymatic metabolism of L-cysteine to elicit a number of varying effects[6]. Many of these effects are related to the symptoms of both SIRS and CARS (Figure 2). For example, high levels of hydrogen sulphide induce vasodilation, decreased blood pressure, hypothermia, decreased metabolism, smooth muscle relaxation and immune modulation (Figure 2)[9]. Therefore, it seems clear that depending on the stage of sepsis and whether the patient is undergoing SIRS or CARS, intervening in hydrogen sulphide signalling could have therapeutic effects. Hui et al.[10] reported the first indication that hydrogen sulphide is involved in eliciting some of the symptoms of sepsis. They reported that plasma levels of hydrogen sulphide increased significantly in both endotoxin-induced and cecal ligation and puncture (CLP)-induced models of sepsis in Sprague–Dawley rats. These plasma level increases coincided with vasodilation and a subsequent drop in blood pressure. The therapeutic potential of intervening in the endogenous production of hydrogen sulphide during

an endotoxemia model was then explored in a groundbreaking study by Li et al.[11]. This involved injecting mice with *Escherichia coli* lipopolysaccharide to produce a systemic inflammatory response; this model of endotoxemia results in symptoms similar to those of sepsis, although it does not represent a true model of PMS. In this study, DL-propargylglycine (PAG) was used to inhibit cystathionine-γ-lyase (CSE), a key enzyme involved in hydrogen sulphide production; this inhibition provided significant protection from organ failure during the endotoxemia. This therapeutic approach was then translated to the gold standard of animal PMS models, the CLP model, where Zhang et al.[12] found that prophylactic and therapeutic administrations of PAG provided significant tissue protection and attenuated lethality. They also found that administering the hydrogen sulphide donor NaHS at the time of the CLP procedure increased tissue damage and lethality. This study was the first to directly show that elevated hydrogen sulphide levels contributed to the pathology of PMS. More recently, hydrogen sulphide has been implicated in the vascular injury that occurs during endotoxemia; this vascular injury is crucial to organ failure during PMC. Furthermore, PAG inhibition of the endogenous production of hydrogen sulphide was found to be protective of this vascular injury[13]. Together, these studies provided strong evidence in the pathological role of hydrogen sulphide production during sepsis and indicate that pharmacological inhibition of this production may be a therapeutic avenue that needs further exploration in the clinical setting.

The mechanisms of the pathological role of hydrogen sulphide have been explored thoroughly with a number of potential pathways implicated. The most commonly proposed mechanism of action of therapeutic inhibition of hydrogen sulphide

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production is the reduction in the inflammatory response. This is a contentious issue, with different studies showing differing effects of hydrogen sulphide depending on the model of inflammation used, the cell type studied and the source and concentration of hydrogen sulphide. However, the bulk of evidence suggests that in sepsis models, endogenous hydrogen sulphide is a proinflammatory signalling molecule, and by inhibiting its production the pathological inflammatory response is reduced. These inflammatory effects appear to involve increasing leukocyte migration and activation, and this most likely signalled through the direct sulphhydration of signalling molecules such as the cysteine-38 residue on the P65 subunit of NF-κB[14]. These changes then result in alterations in gene transcription and activation of SP-vanilloid, COX-eicosanoid and cytokine pathways[14–21]. This suggests that CSE inhibitors as well as other pharmacological interventions that target hydrogen sulphide inflammation signalling could be a potential therapy for SIRS following sepsis; however, this should be avoided if the patient has declined and is showing CARS-like symptoms.

However, there is also research that suggests that increasing plasma levels of hydrogen sulphide can be protective during sepsis and/or SIRS. Recently, Sidhapuriwala et al.[22] found that a slow-releasing hydrogen sulphide donor was therapeutic in a pancreatitis model that induces SIRS. In this study, they proposed an anti-inflammatory mechanism of action of the hydrogen sulphide. This suggests that the speed of hydrogen sulphide release may have significant effects on the way it affects the immune response. However, a study by Spiller et al.[23] was very similar to the Zhang et al.[12] with very different results. In the Spiller et al.[23] study, two variations of CLP were used to model mild and severe sepsis in mice; they administered PAG as an endogenous

hydrogen sulphide production inhibitor or one of two hydrogen sulphide donors including NaHS, a fast-releasing donor and Lawesson's reagent, a slow-releasing donor, just prior to the CLP procedure. In this study, they reported that both hydrogen sulphide donors were protective and reduced mortality; PAG made outcomes worse by increasing mortality. They specifically investigated leukocyte migration and adhesion, proposing that hydrogen sulphide was protective through preventing systemic activation of the immune response and promoting a focal immune response via ATP-K+ channels[23]. Looking closely at the methods, nothing was substantially different to explain the contrasting results between the Spiller et al.[23] and the Zhang et al.[12] studies. One explanation is that both sepsis and hydrogen sulphide have differing effects on the body depending on a number of factors (Figure 2). Interestingly, a study by Faller et al.[24] also reported hydrogen sulphide to be protective in an LPS-induced lung inflammation model; however, the study by Spiller et al.[23] showed the opposite effect. They reported that inhaled hydrogen sulphide prevented leukocyte infiltration and prevented a focal immune response to the LPS. Therefore, the two papers reported hydrogen sulphide to be protective in two models that reproduce symptoms of SIRS, yet they proposed opposite modes of action of hydrogen sulphide. It is clear that the diverse symptoms of sepsis, SIRS and CARS, and how they interact with the diverse physiological effects of elevated hydrogen sulphide levels, require further investigation (Figure 2).

Another potential mechanism of hydrogen sulphide as a protective signalling molecule in sepsis was proposed by Wagner et al.[25]. They found that inhaled hydrogen sulphide induced hypothermia and protected mice from CLP sepsis, and that conduction-induced hypothermia was also protective. However,

combining the two treatments was not more protective than each separately, suggesting that hydrogen sulphide was mostly providing protection through a hypothermic mechanism. However, hypothermia would most likely be protective during SIRS such as Patient A (Figure 1). If hydrogen sulphide was used to treat Patient C (Figure 1), it is likely that the induced hypothermia would compound the hypothermic symptoms of CARS, and this would cause the patients' condition to deteriorate (Figure 2).

Conclusion
PMS can result in two extreme responses by the body. SIRS and CARS each has separate and highly pathological symptoms that overlap with the effects of hydrogen sulphide on the body. It is now clear that the body produces hydrogen sulphide as a signalling molecule and that manipulation of this production may be therapeutic in a number of inflammatory conditions such as SIRS and CARS. To truly understand the roles of hydrogen sulphide in PMS, animal models must establish whether SIRS or CARS has been achieved, and the therapeutic potential of hydrogen sulphide inhibition or administration must be tested in each syndrome. Otherwise, similar to other classical anti-inflammatories, hydrogen sulphide may also fail to be translated from preclinical animal research to the clinic as a therapeutic target for PMC.

Abbreviations list
CARS, compensatory anti-inflammatory response syndrome; CLP, cecal ligation and puncture; SE, cystathionine-γ-lyase; DL-PAG,

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propargylglycine; IL, interleukin; PMS, polymicrobial sepsis; ROS, reactive oxygen species; SIRS, systemic inflammatory response syndrome; TNFα, tumour necrosis factor alpha

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