Medical Hypotheses 85 (2015) 612–617

Contents lists available at ScienceDirect

Medical Hypotheses

journal homepage: www.elsevier.com/locate/mehy

An evolutionary perspective on the immunomodulatory role of hydrogen sulphide

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ARTICLE INFO

Article history:
Received 1 September 2014
Accepted 29 July 2015

ABSTRACT

Most preclinical studies on endogenous hydrogen sulphide signalling have given little consideration to the fact that the human body contains more bacterial cells than human cells, and that evolution provides the context for all biology. Whether hydrogen sulphide is pro or anti-inflammatory is heavily debated within the literature, yet researchers have not fully considered that invasive bacteria produce hydrogen sulphide, often at levels far above the endogenous levels of the host. Here I argue that if hydrogen sulphide is an endogenous signalling molecule with immunomodulatory functions, then it must have evolved in the presence of virulent bacteria which produce hydrogen sulphide. This context leads to two competing theories about the evolution of endogenous hydrogen sulphide signalling. The *detectable emission theory* proposes that bacteria produce hydrogen sulphide as part of normal metabolism and hosts which evolved to detect and respond to this hydrogen sulphide would gain a selective survival advantage. This predicts that the endogenous production of hydrogen sulphide is a mechanism which amplifies the bacterial hydrogen sulphide signal. The opposing *protective agent theory* predicts that bacterial hydrogen sulphide is an effective defence against the bactericidal mechanisms of the host's immune response. In this case, endogenous hydrogen sulphide production is either at inconsequential levels to alter the immune response, or is involved in the inflammation resolution process. Evidence suggests that the direct interactions of hydrogen sulphide with the bactericidal mechanisms of the innate immune system are most congruent with the *protective agent theory*. Therefore, I argue that if hydrogen sulphide is an immunomodulatory endogenous signalling molecule its effects are most likely anti-inflammatory.

Introduction

Hydrogen sulphide has tentatively been labelled the third endogenous gaseous signalling molecule along with nitric oxide and carbon monoxide [1]. Hydrogen sulphide has been reported to have a plethora of effects elicited through an equally broad range of molecular interactions. Recently, these direct interactions have become better described and include sulphhydration of proteins [2], breaking of disulphide bridges [3], metal ion reduction [4] and chelating [5], acting as an electron donor to the electron transport change [6], competitive inhibition of complex IV of the electron transport chain [7], and changes to redox status [8]. However, the importance of each of these molecular interactions has not been established due to a lack of consensus on what constitutes normal endogenous levels of hydrogen sulphide in plasma or tissues. The lack of agreement on endogenous levels and the importance of each mechanism of action has made the hydrogen sulphide research field a very controversial area. A particularly controversial area of hydrogen sulphide research is whether it is a pro or anti-inflammatory endogenous signalling molecule. This controversy is partly caused by the many effects hydrogen sulphide may have on the complex systems of tissue damage and the inflammatory response [9]. For example the anti-oxidant and vasodilatory effects of hydrogen sulphide may provide tissue protection in some models of disease, which in turn reduces the inflammatory response [9]. Conversely, the toxic and respiration effects of hydrogen sulphide may cause increased tissue damage and more severe inflammation [9]. In each case, the immune response would appear to be modulated by hydrogen sulphide without a direct interaction between hydrogen sulphide and the immune system. However, less complicated experimental systems, such as those which employ cell culture, have reported equally conflicting accounts of the pro/anti-inflammatory effects of hydrogen sulphide [10–13]. Recent articles have highlighted the potential short comings of preclinical research and each has emphasised the need for greater theoretical enquiry, exploring

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http://dx.doi.org/10.1016/j.mehy.2015.07.029
0306-9877/

the probability of a hypothesis using a broader level of biological thinking [14]. For this reason, this article aims to evaluate which hypothesis on the immunomodulatory effects of hydrogen sulphide is most congruent with the theory of evolution through natural selection.

Is there a selective advantage in hydrogen sulphide detection or production?

Bacterial sources of hydrogen sulphide are often overlooked in studies of hydrogen sulphide in mammalian systems. However, bacterial production of hydrogen sulphide can be substantial from both non-virulent intestinal flora and invasive pathogenic bacteria [15–17]. From an evolutionary perspective it seems unlikely that it is a pure coincidence that many pathogenic bacteria produce relatively high amounts of hydrogen sulphide and that hydrogen sulphide is thought to modulate the mammalian response to infection [18]. From this perspective the competing theories on the immunomodulatory effects of hydrogen sulphide can be rephrased in terms of each organisms of the parasite–host relationship attempting to gain an adaptive advantage. One theory may propose that as hydrogen sulphide is emitted by bacteria, and an immune system which adapted to detect and respond to hydrogen sulphide would gain a selective advantage through an increased ability to sense pathogenic bacteria (Fig. 1A and B). This theory will be hence forth referred to as the *detectable emission theory* and it provides an evolutionary explanation for the proposed pro-inflammatory effects of hydrogen sulphide. An opposing theory may state that the highly reactive molecule of hydrogen sulphide may, by its very nature, inhibit the bactericidal mechanisms of the mammalian immune system, therefore, bacteria which produce more hydrogen sulphide would have a selective advantage (Fig. 1D and E). This theory will be hence forth referred to as the *protective agent theory* and it provides an evolutionary explanation for the proposed anti-inflammatory effects of hydrogen sulphide. Each of these theories makes several predictions about the nature of hydrogen sulphide including how and why it is produced, its specific effects on the bactericidal mechanisms of the immune system and the role of hydrogen sulphide as an endogenous signalling molecule. To avoid the complicated interactions of tissue protection, toxicity and inflammation, this article compared these predictions with research which focused on direct molecular relationships of hydrogen sulphide and the bactericidal mechanisms of the immune systems as well as the mechanisms and regulation of hydrogen sulphide production in bacteria. From this it was concluded that the evidence is most congruent with the *protective agent theory* which predicts that hydrogen sulphide is primarily an anti-inflammatory molecule.

Bacterial sources of hydrogen sulphide

The *protective agent theory* predicts that although bacterial hydrogen sulphide production may have originally evolved as a by-product of normal metabolism, invasive bacteria which generated higher levels of hydrogen sulphide would have a selective advantage and therefore mutations which lead to greater hydrogen sulphide production would be favoured (Fig. 1D and E). In this scenario, hydrogen sulphide would gradually shift from by-product to a metabolite that is synthesized specifically to inhibit the actions of the mammalian immune system (Fig. 1D and E). The *detectable emission theory* predicts that hydrogen sulphide is produced during normal bacterial metabolism and the mammalian immune system evolved cellular responses to hydrogen sulphide (Fig. 1A and B). As such, invasive bacteria which produced lower levels of hydrogen sulphide would have a selective advantage through evading detection by immune cells (Fig. 1C).

More than 1000 genera of bacteria produce hydrogen sulphide as a product of normal metabolism. These include: *Desulfovibrio*, *Helicobacter*, *Streptococcus*, *Staphylococcus*, *Escherichia*, *Salmonella* and *Mycobacterium* [19]. Respiration and protein degradation are the two major metabolic activities in which hydrogen sulphide is a product. The electron transport chain of sulphate reducing bacteria utilises electrons from the oxidisation of organic molecules or diatomic hydrogen to generate a hydrogen ion gradient between the cytosol and intermembrane space [20]. The electrons are then donated onto an activated sulphate in a reaction facilitated by ATP-sulphurylase producing the reduced product of hydrogen sulphide [20]. This form of anaerobic respiration is estimated to be over 3.5 billion years old [20]. Although, respiration as a source of hydrogen sulphide in a contributor to intestinal lumen hydrogen sulphide levels, protein degradation is responsible for most of the luminal sulphide [17]. Furthermore, it is likely that hydrogen sulphide from protein degradation is more relevant to the evolutionary background to the immune response to hydrogen sulphide due to this pathway being very common in virulent pathological bacteria [19]. Protein degradation involves the digestion of proteins down to amino acids to be used for bacterial proteins. However, these amino acids can also be further metabolized into compounds that are essential for many processes within the cell. The sulphur containing amino acids, such as L-cysteine and methionine, can be converted into other amino acids or metabolites involved in respiration including pyruvate and acetyl-CoA [19]. During these reactions the sulphur containing side chain is cleaved to produce hydrogen sulphide. These pathways are also thought to have evolved well before multi-cellular life forms [19]. The time which these pathways evolved and the essential nature of these pathways suggest that hydrogen sulphide is a by-product of metabolism and this supports the *detectable emission theory*. However, several studies provide evidence that suggests that bacterial hydrogen sulphide is integral to virulence. Chen et al. [21] found that the more advance stages of gingivitis correlated with bacterial strains which produced higher levels of hydrogen sulphide. Furthermore, Shatalin et al. [19] found that the enzymes involved in the digestion of L-cysteine to produce hydrogen sulphide were integral to bacterial resistance to antibacterials and hydrogen peroxide. They proposed that hydrogen sulphide is protective through an antioxidant mechanism which is synergistic with bacterial nitric oxide. Notably, gene deletion of the enzymes involved in hydrogen sulphide production did not affect the normal growth rates of the bacteria, furthermore, treatment of the bacteria with antibiotics or hydrogen peroxide elevated the levels of hydrogen sulphide production. Collectively, these studies strongly indicate that bacterial hydrogen sulphide is not just a passive by-product and has crucial cytoprotective functions in stressful conditions. As hydrogen peroxide is utilised by neutrophils for its bactericidal properties, it is likely that hydrogen sulphide production aids some strains of bacteria to resist the cytotoxic activity of the mammalian immune system (Fig. 1E).

Bacterial production of hydrogen sulphide in many cases is a normal by-product of protein metabolism. This is evident in the many hydrogen sulphide producing bacteria that exist in the many niches that are not exposed to the mammalian immune system. However, it is also clear that hydrogen sulphide is a protective metabolite that may aid bacterial survival in hostile environments through an antioxidant mechanism (Fig. 1E). Given that the immune system produces oxidants as a weapon against bacterial infection it seems likely that bacterial production of hydrogen sulphide may aid in virulence (Fig. 1D and E). This is supported by evidence that demonstrates up-regulation of hydrogen sulphide

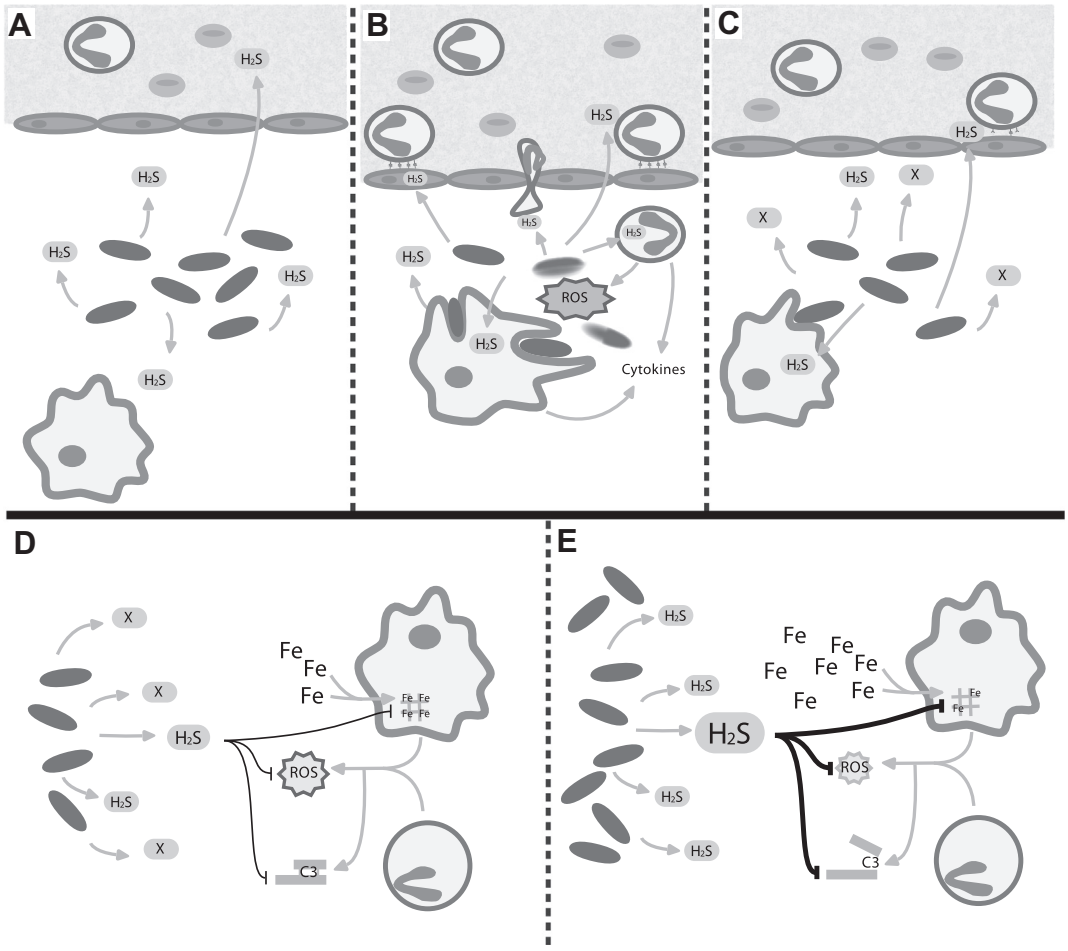

Fig. 1. (A)–(C) represent a simplified evolutionary process of bacterial hydrogen sulphide production predicted by the *detectable emission theory*. Bacteria produce hydrogen sulphide during normal metabolism and individuals with immune systems that can detect hydrogen sulphide (B) gain a selective advantage over those which cannot (A). (B) Depicts the expected responses of the immune system to bacterial hydrogen sulphide including the respiratory burst of reactive oxygen species (ROS), cytokine release, leukocyte infiltration, phagocytosis and vasodilation. (C) Depicts the expected evolution of bacteria to a phenotype which produces less hydrogen sulphide possibly by converting it into other metabolites (X), thus lowering the activation of the immune response. (D) and (E) represent the evolutionary process of bacterial hydrogen sulphide production predicted by the *protective agent theory*. Bacteria in figure (E) will have a selective advantage of those in figure (D) as they produce more hydrogen sulphide which inhibits the immune system's responses by preventing iron (Fe) storage, inhibiting ROS production, acting as an antioxidant, and breaking the disulphide bonds of the complement protein C3b disabling its role in opsonisation.

production in bacteria exposed to bactericidal agents produced by the mammalian immune system. Together, these arguments support the *protective agent theory* of hydrogen sulphide production.

Properties of hydrogen sulphide

The physical properties of hydrogen sulphide largely support the *protective agent theory*. The innate immune systems response to a bacterial infection has three main lines of attacks: the production of oxidants, activation of complement proteins and nutrient deprivation. How hydrogen sulphide interacts with each of the bactericidal approaches indicates that bacteria which produce hydrogen sulphide would have a strong selective advantage through an ability to resist host defence mechanisms (Fig. 1E).

Oxidants are produced by leukocytes in response to infection. Nicotinamide adenine dinucleotide phosphate-oxidase facilitates the electron donation onto oxygen to produce the free radical superoxide, this then spontaneous combines with other molecules within the cell to produce a range of free radicals which are cytotoxic [22]. The enzyme superoxide dismutase converts superoxide into hydrogen peroxide, a cytotoxic free radical with membrane soluble properties that allow it to have a large area of action [22]. Leukocytes also produce the enzyme myeloperoxidase which facilitates the reaction of hydrogen peroxide with a chloride ion to generate hypochloric acid; this is the active ingredient of household bleach. As discussed above, hydrogen sulphide is an antioxidant which directly deactivates the bactericidal activity of these free radicals. Furthermore, research by Palinkas et al. [23] found that hydrogen sulphide directly reacts with the ferrous complex of the myeloperoxidase enzyme inhibiting its action. Palinkas et al. found that myeloperoxidase inhibition would occur even with relatively low concentrations of hydrogen sulphide. From this it appears that hydrogen sulphide cannot only act as an anti-oxidant but also reduce the immune system's ability to produce bactericidal oxidants. Therefore, hydrogen sulphide production would provide a selective advantage for bacteria through protection from the oxidative burst produced by leukocytes (Fig. 1E).

Complement proteins are a range of molecules produced by leukocytes that are either directly bactericidal or aid the immune

system in identifying, killing and phagocytising the bacteria. Complement 3bi is made up of two fragments that are bound through a disulphide bridge. Complement 3bi acts by covalently binding to the surface of the bacteria, it then interacts with the complement receptor 1 on the surface of leukocytes and this induces phagocytosis. Granlund-Edstedt et al. [3] found that hydrogen sulphide splits the disulphide bridge between the two fragments of Complement 3bi and this prevented the bactericidal activity of human leukocytes (Fig. 1D and E). Bacteria have evolved a number of mechanisms to resist the activity of the complement pathway including proteases which digest the complement proteins and surface coatings which prevent complement protein binding to the bacteria; the work of Granlund-Edstedt et al. [3] suggests that hydrogen sulphide production may be another simple and effective adaptation for repelling the actions of the complement pathway.

In response to an infection the immune system produces a number of proteins which bind nutrients that are essential for bacterial growth. This lowers the bioavailable levels of these nutrients which in turn stops the expansion of the infection [4]. Ferritin is an iron storing protein that is over expressed during an infection to limit the bioavailability of iron [4]. Several studies have reported that hydrogen sulphide can reach the ferritin core and reduce the ferric ions solubilising them into a bioavailable form [4] (Fig. 1D and E). Again this suggests that hydrogen sulphide production provides and adaptive advantage to bacteria. It appears that the chemical nature of hydrogen sulphide inhibits many of the mechanisms the immune system utilises to fight bacterial infection. This strongly supports the *protective agent theory* of hydrogen sulphide production by bacteria. Yet, from what has been discussed the *protective agent theory* and the *detectable emission theory* are not mutually exclusive theories. It is entirely possible and perhaps expected that the immune system has mechanisms to detect an agent which is produced by bacteria to resist the host's response to infection. However, these theories are at odds due to the fact that the host's immune cells have enzymes which metabolise cysteine to produce hydrogen sulphide (Fig. 2). This endogenous production of hydrogen sulphide must either function as a pro-inflammatory signalling molecule or a molecule which facilitates the resolution of the inflammatory response; it seems impractical for hydrogen sulphide to perform both roles.

The *detectable emission theory* and endogenous production of hydrogen sulphide

The enzymes which facilitate reactions which produce hydrogen sulphide from cysteine metabolism have largely conserved sequences between a range of life forms including bacteria and mammals [19]. This suggests that these reactions are universally beneficial to normal metabolism. However, hydrogen sulphide production is far more substantial in many virulent strains of bacteria, extra cellular concentrations can reach several orders of magnitude higher than those reached during normal mammalian metabolism [24]. Therefore the detection of hydrogen sulphide production by immune cells would provide a selective advantage to the host. Interestingly, research has shown that the immune cells elevate expression of hydrogen sulphide producing enzymes in the presence of bacterial toxins as well as sterile tissue damage [25]. Assuming the primary function of these enzymes is to elevate the levels of hydrogen sulphide and not the other products of the reactions; the *detectable emission theory* predicts that the purpose of these enzymes is to utilise the existing bacterial hydrogen sulphide detection pathways to activate and recruit immune cells to the site of infection or injury (Fig. 2A). As more immune cells arrive at the site of injury or infection they too will produce hydrogen sulphide amplifying the existing pro-inflammatory signal. This positive feed-back mechanism is also seen with pro-inflammatory cytokines which cause the synthesis and secretion of more pro-inflammatory cytokines in leukocytes. However, the flaw in this line of thinking is that, as discussed above, hydrogen sulphide directly reduces the efficacy of many of host's approaches to fight the infection. This would make hydrogen sulphide a poor signalling molecule to elicit a positive feed-back activation of the immune system, as the increasing concentrations of hydrogen sulphide would only act to inhibit the host's protective responses to the vir-

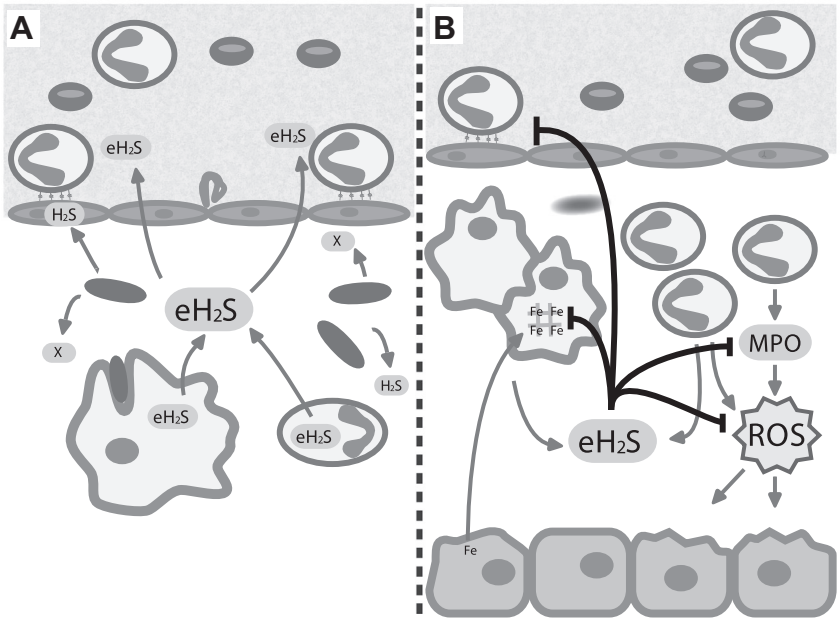

Fig. 2. The role of endogenous hydrogen sulphide (eH_2S) predicted by the *detectable emission theory* (A) and the *protective agent theory* (B). (A) Bacteria produce hydrogen sulphide as a by-product of their metabolism, immune cells detect this emission and up-regulate their production of hydrogen sulphide to amplify the signal to recruit and activate more leukocytes. (B) As the immune system enters the resolution phase of inflammation, hydrogen sulphide production is up-regulated to prevent collateral damage caused by nutrient sequestering and ROS production.

ulent infection. Therefore, it seems likely that the elevation of hydrogen sulphide production by the immune cells is part of a resolution process.

The *protective agent theory* and endogenous production of hydrogen sulphide

The immune response appears to exacerbate many pathologies, including sterile pathologies such as stroke and pancreatitis, as well as diseases involving infections such as sepsis [26]. This is thought to be due to collateral damage from the non-specific responses by leukocytes, particularly the respiratory burst which results in high levels of the cytotoxic compounds of hydrogen peroxide and hypochlorite [27] (Fig. 2B). Also, storing essential nutrients such as iron comes at a cost to the host (Fig. 2B). However, there is a selective advantage to initially over-responding to infection as the cost of the collateral damage of this response is minor compared to the potential costs of a persistent infection [28]. However, once initiated there must be appropriate mechanisms to resolve the inflammatory response. This is done through a number of mechanisms with the most obvious example being the anti-inflammatory cytokine IL10 which is elevated during infections to manage the response and avoid collateral damage [29]. Having multiple systems which regulate the immune response, particularly those which have the most collateral effects, would provide a survival advantage. The enzymes which facilitate hydrogen sulphide producing reactions are up-regulated during inflammation and given the direct effects on mechanism which the immune system utilises to fight the infection, it seems likely that the hydrogen sulphide produced by immune cells is a regulatory response intended to inhibit inflammation (Fig. 2B). This supports the *protective agent theory*.

Is endogenous production of hydrogen sulphide irrelevant to inflammation?

One possibility is that both the *detectable emission theory* and the *protective agent theory* are true. For reason discussed above it was argued that the existence of endogenous hydrogen sulphide production pathways indicates that only one of theories could be true. However, the bacterial production of hydrogen sulphide can be several orders of magnitude higher than endogenous levels. There is little agreement in the literature on the endogenous levels of hydrogen sulphide due to the difficulties in accurately measuring the highly reactive molecule. However, a recent review by Olson [1] articulately argued that the endogenous concentration must be below 1 μM, this is because of several reasons but most notably humans can detect the odour of hydrogen sulphide at 1 μM and above, and plasma and tissue homogenates do not smell of sulphur. Conversely, bacterial activity within the body can lead to the pungent and toxic concentrations of 2 mM [21,24]. Because of this it could be argued that the endogenous production of hydrogen sulphide is negligible in regards to immune modulation compared to bacterial production and only at concentrations achieved during bacterial infection does the immune system detect and respond. Therefore, the host–parasite relationship could be that the bacteria produce hydrogen sulphide as protection from the host's defences and immune cells evolved to detect this hydrogen sulphide and activate. This theory proposes that the changes in the host's expression levels of the hydrogen sulphide producing enzyme levels during infection or tissue damage merely reflects the changes in metabolic demands of the immunes cells and that the other products of these reactions (including pyruvate and serine) are required in greater concentrations to meet the new metabolism profile; or perhaps these enzymes are involved in safely reducing the levels of a physiologically active substrate. Homocysteine may be an example of this, it is a substrate of two of three major hydrogen sulphide producing enzymes and high levels of homocysteine have been linked to several pathologies [30]. Interestingly, this argument proposes that hydrogen sulphide may be both anti and pro-inflammatory as the net effect of hydrogen sulphide could depend on a number of things as the mechanisms which deactivate the host's response are balanced with the detection and activation of the immune system. Two important factors which may influence the net effect of hydrogen sulphide on the immune response are the concentration of hydrogen sulphide and how inflammation is defined. Low concentration of hydrogen sulphide may activate the immune system without substantially limiting its bactericidal response and at high concentrations the bactericidal actions of the immune systems may be completely inhibited. These complex relationships make it incredibly difficult to elucidate the direct effect of any component of the inflammatory network. What is interesting is that this complexity is what is being reported in the literature, with subtle differences in the dose of hydrogen sulphide or the context in which the dose is administered resulting in completely dissimilar effects on inflammation. This makes the theory that hydrogen sulphide is both a protective agent produced by the bacteria and a bacterial emission that is detected by the immune system quite an attractive theory, however, this theory presumes that endogenous hydrogen sulphide in inconsequential in inflammation signalling.

Conclusion

Elucidating the effects of endogenous hydrogen sulphide production is a very difficult task. The network in which hydrogen sulphide is proposed to operate is incredibly complicated and merely inhibiting its production or elevating its levels with exogenous donors will provide limited information on the direct effects of hydrogen sulphide. Assessing whether hydrogen sulphide production is inflammatory or anti-inflammatory from an evolutionary perspective provides a new insight into the likelihood of each theory. Furthermore, by focusing on direct molecular interaction of hydrogen sulphide with aspects of the immune system, the complicated networks of tissue damage and immune system activation were avoided. From this it was found that there is more evidence to support the *protective agent theory* of hydrogen sulphide. This states that hydrogen sulphide is produced by bacteria to directly inhibit the bactericidal activity of the immune system. It further proposes that endogenous production of hydrogen sulphide is a mechanism adapted by the mammalian immune system to tightly regulate the immune response to reduce collateral damage and eventually aid in the resolution of the inflammatory response. However, using an evolutionary perspective to elucidate the role of endogenous hydrogen sulphide could not rule out a combination of the *protective agent theory* and the *detectable emission theory*. In the context of endogenous production these theories are incompatible, hydrogen sulphide is either a positive feedback mechanism to amplify the immune response or it is part of the resolution of inflammation, however, they are compatible and perhaps expected if you consider the endogenous production of hydrogen sulphide as largely insignificant compared to bacterial production. If the combination theory is true then bacteria produce hydrogen sulphide to inhibit the host's defences, however, the immune system has evolved to detect high levels of hydrogen sulphide and recognise this as a bacterial infection. In either case, this article concludes that endogenous hydrogen sulphide signalling cannot be an endogenous pro-inflammatory signal.

Conflict of interest

The author has no conflict of interests to report.

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