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Letter to the Editor

The recognition of LpxC inhibitors as potential antibiotics could revolutionise the management of sepsis in veterinary patients if their unknown biological properties are widely evaluated in suitable animal models

KEYWORDS

LpxC-2;
Sepsis;
Large animal models;
Multi-drug resistant Gram-negative bacteria;
Antibiotic;
Lipopolysaccharide;
Infection

Abstract Current studies continue to show that UDP-3-O-((R)-3-hydroxymyristoyl)-N-acetyl glucosamine deacetylase (LpxC) inhibitors such as pyridone methylsulfone hydroxymate 1 and 2a (LpxC-2) downregulate the lethal effects of sepsis initiated by multi-drug resistant Gram-negative bacteria (MDRGNB) by curtailing lipopolysaccharide (LPS) synthesis in murine models. Sepsis initiated by MDRGNB is a leading cause of shock and systemic inflammatory response syndrome (SIRS) in intensive care unit (ICU) patients. To date however, the biological effects of LpxC-2 and related molecules in companion and production animals remain largely unexplored *in vivo* and are therefore unknown. Such studies would be greatly informative in the expectation of LpxC-2 progressing to human clinical trials. Mechanistic studies to interrogate this novel antibiotic candidate in realistic and clinically applicable large animal models of veterinary importance are sorely lacking. To be relevant, the physiology of the chosen animal models should closely match that of humans such as ovine or porcine, or even better, non-human primate based studies, as they are more genetically similar to humans than murine models. If discovered to have subtle or negligible side effects, LpxC-2 could have a future role in the treatment and management of MDRGNB-induced infections that lead to sepsis in both animals and humans. More research is indicated on LpxC-2 use in many veterinary species, as data remains scarce.

1. Introduction

Advances in scientific research have identified a novel antibiotic target with the potential to combat multi-drug resistant Gram-negative bacteria (MDRGNB) infections via enzymatic inhibition of endotoxin biosynthesis [1,2]. The target is LpxC – a metalloenzyme that catalyses the deacetylation of UDP-3-O-(R-3-hydroxymyristoyl)-N-acetyl glucosamine, the first committed step in the biosynthesis of lipid A which is an essential component of the outer membrane of Gram-negative bacteria [1,3]. Lipid A also protects the bacterium from attack by many antibiotics and detergents. Due to its ability to block lipopolysaccharide (LPS) synthesis, pyridone methylsulfone hydroxymate 1 and 2a (LpxC-2), are members of a class of antibiotics that do not kill bacteria, but avert their ability to activate the sepsis cascade by promoting phagocytic killing [4,5].

Abbreviations: LpxC, UDP-3-O-((R)-3-hydroxymyristoyl)-N-acetyl glucosamine deacetylase; LpxC-2, pyridone methylsulfone hydroxymate 1 or 2a; MDRGNB, multi-drug resistant Gram-negative bacteria (MDRGNB); SIRS, systemic inflammatory response syndrome; ICU, intensive care unit; LPS, lipopolysaccharide

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2. What are the currently known risk factors for contracting MDRGNB?

Thus far, MDRGNB are the commonest and most important sepsis-causing pathogens [4]. The selective pressure generated by antibiotic use has led to the development of antibiotic-resistant hospital microorganisms that are resistant to commonly used antibiotics. This, in some cases has caused hospital epidemic infections [6]. These organisms have acquired multiple mechanisms of antimicrobial drug resistance. They have the potential to spread to the community and continue to exert enormous public health and economic burdens [7,8].

Invasive intensive care procedures, including artificial ventilation, transfusion, artificial organs and long hospital stays augment the risk of infection with MDRGNB [9]. For example, MDRGNB-induced sepsis is the most common contributor to the death of patients with ventricular assist devices [10,11]. Until now, pathogenic MDRGNB have continued to pose the greatest and most serious health threat of hospital acquired infections, especially for patients with chronic indwelling lines or cannulae as they can lead to catheter-related blood stream infections [8,12]. This cohort of patients includes those undergoing extra corporeal membranous oxygenation and blood transfusion [9].

There is need for the development of novel treatment options to alleviate resistance to antibiotics, for instance those targeting LPS and fatty acid biosynthesis and shifting from traditional antibiotic targets [13].

3. Is LpxC-2 the answer to the management of MDRGNB induced sepsis?

Studies are ongoing on LpxC-2 as a representative of new class of antibiotics that is reportedly in the final stages of testing in small animals before being trialled clinically [1,3,5]. Inhibition of LpxC has recently been shown to protect mice from the lethal effects of sepsis caused by *Acinetobacter baumannii*, a MDRGNB, by modulating inflammation, enhancing phagocytosis and thereby reducing serum LPS levels [4].

Virulent MDRGNB characteristically shed LPS during growth, leading to the activation of the sepsis cascade and culminating in systemic inflammatory response syndrome (SIRS) [4]. Sepsis is an important cause of shock, which affects millions of people and animals around the world. In people, for instance, the global incidence of sepsis continues to escalate. It remains a major cause of death in intensive care units (ICUs) and the community, and causes a large burden of disease and has a negative economic impact [14]. An Australian and New Zealand study calculated the incidence of severe sepsis in adults treated in ICUs at 0.77 (0.76–0.79) per 1000 of population [15]. The same study reported that 26.5% of patients with severe sepsis died in the ICU, 32.4% perished within 28 days of the diagnosis of severe sepsis and 37.5% died in hospital [15].

Irrespective of the species, organ failure is one of the most severe manifestations of SIRS. Mortality and morbidity are directly related to the number of failing organs, therefore preventing the activation of the sepsis cascade is a priority of LpxC-2 enzymatic activity. The prevention and the treatment of LPS-initiated sepsis represent the only prospect for improved survival and quality of life for patients in intensive care who are at risk of acquiring infections from MDRGNB [16].

4. Does LpxC-2 have a future use in veterinary medicine and food animal production?

Resistant strains of bacteria found in animals have mainly resulted from the use of antimicrobials in veterinary medicine; and similarly resistant strains of bacteria found in humans are due the use of antimicrobials in human medicine [17]. The recognition of companion pet animals as a source of MDRGNB [18] raises serious concerns due to their close interaction with humans.

The vast majority of currently used antibiotics were developed to either inactivate or damage bacteria or both. The promising aspect of LpxC-2 is that it prevents Gram-negative bacteria from synthesising LPS, therefore making the bacteria both unviable and curtailing their production of endotoxin, allowing the immune system to fight infection more effectively. This is an attractive attribute of LpxC-2, however, the toxicity and safety profiles, and withdrawal periods in food animals are still unknown. LpxC-2 could potentially be added to antibiotic cocktails in bull semen meant for artificial insemination to inhibit MDRGNB such as *Pseudomonas*, *Stenotrophomonas* and *Sphingomonas spp*. that have been identified as pathogens affecting animal reproduction [19]. LpxC-2 also has the potential of being used to mitigate the effects of Salmonella. Salmonella has been identified as a major cause of economic losses in domestic livestock, and is also a serious zoonotic pathogen worldwide [20]. LpxC-2 could potentially be used in controlling a wide spectrum of economically important livestock diseases such as those causing mastitis [21–23], endometritis, abscesses and general malaise by pathogens such as *Pasteurella multocida* [24], and infections caused by several members of the *Enterobacteriaceae* family such as *Escherichia coli*.

5. What opinions are out there regarding testing LpxC inhibitors in large animals?

It is true that LpxC inhibitors could be tested in large animals and their effect in reducing sepsis remains an interesting possibility that has yet to be demonstrated. However, it should be realised that LpxC inhibitors are lethal to many Gram-negative pathogens, including many outlined in this paper. Acinetobacter is an exception in that certain strains of Acinetobacter apparently can survive *in vitro* without lipid A biosynthesis. However, for the vast majority of Gram-negative bacteria, this is thought not to be the case, but where is the evidence? It is known that LpxC inhibitors are not anti-virulence factors, so antibiotic resistance could rise quickly. Therefore, the suggestion about using LpxC inhibitors in livestock ought to be carefully considered for its potential impact on humans.

Whilst this is an interesting area for additional research, LpxC inhibitors have the potential to be very useful in treatment of infections due to multi-resistant Gram-negative bacteria. Veterinary or human drug development use of large animal species in particular is usually called for to validate drug development. Some authorities however believe that the emphasis on the use of large animal models, based on

assumptions that these would be more relevant also in the human perspective is questionable. The relevance of species used (in the context of human and veterinary drug development) should be based on pharmacokinetic, as well as pharmacodynamic particulars. Further to this, there is no species, not even humans, that is 100% relevant for humans. From the pharmacological and mechanistic point of view currently used standard animal models such as murine models of systemic and skin and soft tissue infection models have proven useful in the early phases of the drug development process, prior to initiation of clinical trials, to establish proof of concept. In subsequent studies where the toxicological profile of the test article is investigated, two species, one rodent and one non-rodent are commonly used. The non-rodent species is usually dog and occasionally monkey, the availability of historical control data as well as group sizes limit choice of species such as sheep and bovine in these safety studies. So is there room to explore non-model species for drug development or this paradigm shift is contra-indicated?

6. Perspectives

The possibility of investigating the *in vivo* effects of LpxC-2 in clinically relevant large animal models needs a serious consideration. Reports on large animal models with the ultimate goal of characterising the biological effects of LpxC-2 *in vivo* are scarce. Well-developed relevant large animal studies such as those in sheep and swine [25] could provide insights into using models based on LPS-induced sepsis as a starting point. Established large animal studies such as these on LPS could set a foundation for testing MDRGNB challenge through incremental and validation experiments. Work in this area could embrace objectives such as the determination of physiological, haematological, biochemical, immunological, histopathological, and inflammation effects, and of proteogenomic studies [26] of LpxC-2 *in vivo*. The studies should able to determine the kinetics of LpxC-2 and related molecules in blood, tissues and urine. Swine may offer many advantages compared to other experimental large animals for having been extensively studied, the ease of management and that their physiological attributes are comparable to those of man [27,28]. Animals such as baboons offer all the advantages of a large animal and are comparable with humans in nearly all physiological and immunological aspects [29]. The downside in the use of baboons in whole animal experiments is that studies that enrol non-human primates can provoke intense scrutiny from regulatory and oversight groups [30], potentially making it practically impossible for studies to get initiated.

The use of LPS in initial studies would exclude the effects of confounders from other live bacterial products that may independently propagate inflammation. It would be interesting to know if the presence of LpxC-2 has any influence on LPS-induced pathology despite the fact that LpxC-2 is designed to "merely" prevent the synthesis of LPS in live bacteria. After LPS challenge, further studies could involve using live pathogenic Gram-negative bacteria. It is also possible that LpxC-2 may have properties that may influence inflammation in its own right, rather than through the known LpxC enzymatic inhibition.

It has so far been documented that LpxC does not share homology with any known mammalian protein, making it an excellent target for the design of novel antibiotics [31]. Most studies however, have largely been *in vitro* or in mouse models and so studies involving large animals should be conducted in anticipation of the advancement of LpxC inhibitors into human clinical trials [1,3,5]. While there is dissenting debate that animal-based research has been unable to predict human response to drugs because animals and humans have different evolutionary trajectories, the alternative view is that animal models should be genetically closer to that of humans to be relevant [32]. Observations from small animal models may not be relevant in humans, for instance, current evidence points out that mouse models are remote from human conditions [32,33]. The body size similarities, repeated sampling and the opportunity for continuous invasive monitoring are some of the major benefits of large animal experimental models [34]. Moreover, the translational benefit of animal studies has been shown to help to identify and minimise the chances of iatrogenic harm during critical care situations. This underscores the need to design experiments to understand the pharmacokinetic, pharmacological and side effects of LpxC-2 in the presence of simulated infection, which are currently unknown in large animals *in vivo*.

The benefit of trialling LpxC in relevant large animal models might help in understanding the fight against sepsis in man. The community benefits could include optimum health outcomes because of decreased morbidity and mortality, all of which translate to improved economic productivity. Similar benefits are expected in animal patients as well. For now, more data is needed regarding the effects of LpxC-2 use in well-designed large animal studies. In summary, very little is known about the effects of LpxC-2 in large animals and any new information would make a useful contribution to science and medicine.

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