Review

Is ^{1}H NMR metabolomics becoming the promising early biomarker for neonatal sepsis and for monitoring the antibiotic toxicity?

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Metabolomics, the latest of omics disciplines, has been successfully used in various fields of basic research such as pharmacology and toxicology. Recently, this new science has gained an important role in the translational research of diagnostics. In this regard, the challenge for neonatologists and medical laboratories is to diagnose neonatal sepsis, a disease with high mortality and morbidity due to the difficulty in diagnosing it. Metabolomics, through its ability to identify perturbations caused by this condition, aims at recognizing metabolites that characterize neonatal sepsis with high specificity and sensitivity. The purpose of this review is to highlight the ability of metabolomics to find early biomarkers for this condition, as well as to predict the toxic effects caused by antibiotics.

Keywords: Metabolomics, ^{1}H NMR, Neonatal sepsis, Renal toxicity, Antibiotics, Aminoglycosides

Introduction

Neonatal sepsis is defined as a complex clinical syndrome usually associated with several agents of disease, including bacteria, virus, and fungi. The global sepsis alliance (GSA), an association of physicians, has recognized neonatal sepsis as a major public health problem and one of the most significant causes of preterm infant morbidity and mortality (http://www.globalsepsisalliance.org/). Recent studies have shown that more than 21% of very low birth weight (VLBW) infants surviving beyond 72 hours have at least one episode of blood culture-confirmed sepsis. Its incidence ranges between 0.5 and 1% in low income countries and the timing-identification is a major diagnostic problem due to the nonspecific clinical signs.[1–3]

Currently, the diagnostic procedures can be direct or indirect. Direct methods include isolation of microorganisms from body fluid, whereas indirect methods include a variety of tests such as the white blood cell (WBC) count and differential count. However, for most of biochemical and haematological tests, the range of false negative and false positive is still high.[1,3] Since neonatal sepsis is a complex multiorgan dysfunction resulting in large changes in the organism's metabolites (the metabolome), the analysis of the whole metabolome may be an attractive methodology for the determination of metabolites perturbations. Metabolomics is a holistic approach that studies the complete set of low molecular weight metabolites contained in human bio-fluids. By providing access to a portion of bio molecular space not covered by other profiling approaches (e.g., proteomics and genomics), metabolomics offers unique insights into small molecule regulation and signalling in biology.[4,5] Proton nuclear magnetic resonance spectroscopy (^{1}H NMR) is an analytical tool that allows detection of the low molecular weight metabolites present in the samples, which are not usually subjected to any sample extraction or modification. The ^{1}H NMR spectrum is characterized by a number of peaks resulting from different functional groups of molecular components. Identification of hundreds of molecules in a single spectrum of a complex mixture can be accomplished thanks to the changes in the electronic situations of the nuclei.[6]

The use of ^{1}H NMR metabolomics for medical purposes is well documented by several studies covering multiple medical specialities, including oncology, pharmacology, and toxicology.[7–9] However, very few research have been developed and published using the metabolomics approach in the field of sepsis and its treatment, including the effects caused by antibiotics.[10] So far, only three recent papers have been published on this subject.[11–13] Here, we will focus on the most significant findings reported by these papers with the intent to discuss their clinical relevance and implications.

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Metabolomics and Sepsis

Since severe burns are one of the most frequent causes of death from septicaemia occurring in intensive care units, early diagnosis of sepsis is essential to avoid major complications. In this regard, Liu *et al.*[11], using plasma samples obtained from an experimental model of thermally injured rats, has investigated the metabolic modification related to the septic condition. In this study, an ultra performance liquid chromatography-quadrupole time of flight mass spectrometry (UPLC-Q-TOF-MS) was used and the metabolomics analysis underlined the most important differences between the group of rats suffering from septicaemia and the group without sepsis (control group). The representative metabolites related to the septic condition were nine: hypoxanthine, indoxylsufate, glucuronic acid, gluconic acid, proline, uracil, nitrotyrosine, uric acid, and trihydroxy cholanoic acid. A deeper analysis of these metabolites suggested their involvement in oxidative stress and tissue damage.

Concomitantly, Stringer *et al.*[12] performed a pilot study using an ^{1}H NMR approach on plasma samples collected from two groups of subjects: (1) patients with acute lung injury (ALI) and (2) healthy volunteers as control. The aim of this study was to investigate the ALI condition, which is characterized by a high rate of morbidity and a 30–50% of mortality. Using ^{1}H NMR coupled by a mathematical analysis on the water soluble component of the plasma samples, it was possible to generate a specific metabolites table, which seemed to point out a promising means to help in the early diagnosis of this severe disorder. The metabolites responsible for the discrimination between the groups were: total glutathione, adenosine, phosphatidylserine, and sphingomyelin.

In 2011 Izquierdo-García *et al.*[13] used the ^{1}H NMR metabolomics approach for the diagnosis of sepsis. They collected and analyzed bronchoalveolar lavage fluid (BALF), lung tissue, and serum samples from 28 male Sprague-Dawley rats, which were divided into two groups (14 per group). One group underwent sepsis infection due the caecal ligation and puncture while the second group did not receive the puncture (control). Using the metabolomics approach, the authors were able to build a predictive model discriminating between the two groups; the model showed significant changes in metabolites amount between groups, reaching up to 100% of both sensitivity and specificity. The discriminant metabolites in the septic rat model were: alanine, creatine, phosphoethanolamine, and myoinositol increase in lung tissue; creatine increase and myoinositol decrease in BAL fluid; and alanine, creatine, phosphoethanolamine, and acetoacetate increase in serum.

Taking into consideration the studies carried out so far, it is reasonable to argue that the metabolomics technique can be considered an effective tool for the diagnosis of sepsis. Nonetheless, further studies are required to confirm these promising preliminary results. Very interesting data on metabolomics in bacterial pneumonia and viral bronchiolitis are also available in paediatric literature.[14] In particular, it is now possible to achieve a metabolic fingerprint of the pneumococcal pneumonia, which may help to understand the metabolic alteration due to this aetiological agent.[15]

Metabolomics as Tool for Monitoring Antibiotic Toxicity

One of the capabilities of the metabolomic technique is to detect the metabolic perturbations caused namely by the nephrotoxicity and epatotoxicity effects of drugs. This can be a new effective tool to measure the toxicity.[16,17] Indeed, these properties play an important role in featuring tailored drug therapies and reducing the risk of drug toxicity, which may cause remarkable damage to organs mainly involved in the catabolism of drugs, such as the kidneys.[18,19] Unfortunately, current available biochemical markers for assessing toxicity and kidney injury are characterized by unsatisfactory sensitivity and specificity.[19–21]

Despite the great potential of metabolomics as a tool for the identification and classification of metabolic patterns associated with kidney injury or dysfunction, only few articles exploring the metabolomics approach in perinatal pharmacology have been published.[21]

Currently, only specific data are available on aminoglycosides[10] as they are the most widely used and studied drugs. Aminoglycosides are oligosaccharides derived from amino sugars. These antibiotics have a broad spectrum of activity against Gram+, Gram− mycobacteria, spirochaetes, anaerobes, mycoplasma, chlamydia, etc. The drug distribution is usually variable because of the strong water solubility, exhibiting the most effective action at alkaline pH. The aminoglycosides are catabolized in the kidney, filtered by glomerulus and then reabsorbed by tubular cells; the direct toxic effect therefore takes place within renal tubule cells.[22]

In 2009 Boudonck *et al.*[23] investigated how metabolomics could be used as a tool for the early detection of renal toxicity. The authors pointed out how the current markers of renal toxicity such as serum creatinine and the blood urea nitrogen (BUN) had several limitations. In fact, these markers become sensitive when about two-thirds of nephrons lose the ability to filtrate. The authors designed a metabolomics experiment using Sprague-Dawley rats treated with three different drugs: gentamicin, tobramycin (aminoglycosides), and cisplatinum. The rats were 2
treated at 1, 5, and 28 days respectively, and at each of these time points, urine samples were collected;

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subsequently, rats were sacrificed for further histological investigations. Histological findings were then compared with the metabolomics analysis performed using liquid chromatography (LC) and gas chromatography (GC) coupled to mass spectrometry (MS). The analysis of urine revealed a set of metabolites associated with the nephrotoxic damage caused by drugs. In particular, a mathematical model based on the level of amino acids (leucine, hippurate, isoleucine, glucose, and valine) identified the group of rats with nephrotoxic damage with 100% accuracy after 28 days, 93% after 5 days, and surprisingly with 70% after just 1 day. The latter was absolutely undetectable by histology. In conclusion, by the metabolomic approach, it was possible to highlight a number of biomarkers capable of predicting early kidney damage before its demonstration by histology.

In another recent work, Tzovaras *et al.*,[24] studied the nephrotoxicity caused by aminoglycosides using ^{1}H NMR. The aim of that study was to characterize the early stage of aminoglycoside induced nephrotoxicity identifying a cluster of metabolites by ^{1}H NMR. The study was performed on nineteen healthy patients; for each patient a sample of urine and one of plasma were taken before the treatment with aminoglycosides and after 5 days of treatment. The ^{1}H NMR analysis showed a significant positive increase of alanine and lactic acid metabolites, whereas a reduction of hippurate in the samples collected after 5 days of treatment was observed. In addition, a correlation with an increased escretion fraction of sodium, magnesium, and calcium clearly indicated that aminoglycosides can induce both proximal and distal renal tubular disfunction. 3

Conclusion

In summary, published studies suggest that metabolomics may become a promising tool for diagnosing neonatal sepsis and monitoring therapy in the near future. Even if no evidence supporting this hypothesis can be stated. This approach may indeed be a powerful instrument providing knowledge about the factors responsible of the metabolic modifications, which can help in identifying metabolic patterns of function, disease, and injury, as well as pharmacodynamic and toxicodynamic monitoring.

Conflict of Interests

The authors declare that they have no conflict of interests.

References

1 Mussap M, Fanos V. Neonatal nephrology and laboratory medicine: an effective interdisciplinary model to improve the outcome in neonatal intensive care unit. J Matern Fetal Neonatal Med. 2011;24(Suppl 2):1–2.

2 Mussap M, Degrandi R, Cataldi L, Fanos V, Plebani M. Biochemical markers for the early assessment of neonatal sepsis: the role of procalcitonin. J Chemother. 2007;19(Suppl 2):35–8.

3 Mussap M, Cataldi L, Plebani M, Fanos V. Laboratory investigation for the early diagnosis and monitoring of neonatal urinary tract infections: the present situation and future perspectives. J Chemother. 2006;18(Spec no 3):9–13.

4 Fanos V, Barberini L, Antonucci R, Atzori L. Metabolomics in neonatology and pediatrics. Clin Biochem. 2011;44:452–4.

5 Nicholson JK, Lindon JC. Systems biology: metabonomics. Nature. 2008;455:1054–6.

6 Lindon JC, Nicholson JK, Holmes E, Everett JR. Metabonomics: metabolic processes studied by NMR spectroscopy of biofluids. Concepts Magn Reson. 2000;12:289–20.

7 Fiehn O. Metabolomics–the link between genotypes and phenotypes. Plant Mol Biol. 2002;48(1–2):155–71.

8 Dunn WB, Broadhurst DI, Atherton HJ, Goodacre R, Griffin JL. Systems level studies of mammalian metabolomes: the roles of mass spectrometry and nuclear magnetic resonance spectroscopy. Chem Soc Rev. 2011;40(1):387–426.

9 Atzori L, Antonucci R, Barberini L, Griffin JL, Fanos V. Metabolomics: a new tool for the neonatologist. J Matern Fetal Neonatal Med. 2009;22(Suppl 3):50–3.

10 Fanos V, Barberini L, Antonucci R, Atzori L. Pharmametabolomics in neonatology: is it a dream or a fact? Curr Pharm Des. 2012;21:2996–3006.

11 Liu X-R, Zheng X-F, Ji S-Z, Lv Y-H, Zheng D-Y, Xia Z-F, *et al.* Metabolomic analysis of thermally injured and/or septic rats. Burns. 2010;36(7):992–8.

12 Stringer KA, Serkova NJ, Karnovsky A, Guire K, Paine R, Standiford TJ. Metabolic consequences of sepsis-induced acute lung injury revealed by plasma ^{1}H-nuclear magnetic resonance quantitative metabolomics and computational analysis. Am J Physiol Lung Cell Mol Physiol. 2011;300(1):L4–11.

13 Izquierdo-García JL, Nin N, Ruíz-Cabello J, Rojas Y, de Paula M, López-Cuenca S, *et al.* A metabolomic approach for diagnosis of experimental sepsis. Intensive Care Med. 2011. 4

14 Atzei A, Atzori L, Moretti C, Barberini L, Noto A, Ottonello G, *et al.* Metabolomics in paediatric respiratory diseases and bronchiolitis. J Matern Fetal Neonatal Med. 2011;(24 (Suppl 2):59–62.

15 Slupsky CM, Rankin KN, Fu H, Chang D, Rowe BH, Charles PGP, *et al.* Pneumococcal pneumonia: potential for diagnosis through a urinary metabolic profile. J Proteome Res. 2009; 8(12):5550–8.

16 Lindon JC, Holmes E, Nicholson JK. Metabonomics techniques and applications to pharmaceutical research & development. Pharm Res. 2006;23(6):1075–88.

17 Atzori L, Griffin JL, Noto A, Fanos V. Metabolomics: a new approach to drug delivery in perinatology. Curr Med Chem. 2012;19(27):4654–61.

18 Antonucci R, Atzori L, Barberini L, Fanos V. Metabolomics: the 'new clinical chemistry' for personalized neonatal medicine. Minerva Pediatr. 2010;62:145–8.

19 Hanna MH, Segar JL, Teesch LM, Kasper DC, Schaefer FS, Brophy PD. Urinary metabolomic markers of aminoglycoside nephrotoxicity in newborn rats. Pediatr Res. 2013;73(5):585–91.

20 Bonventre JV, Vaidya VS, Schmouder R, Feig P, Dieterle F. Next-generation biomarkers for detecting kidney toxicity. Nat Biotechnol. 2010;28(5):436–40.

21 Atzori L, Antonucci R, Barberini L, Locci E, Cesare Marincola F, Scano P, *et al.* 1H NMR-based metabolic profiling of urine from children with nephrouropathies. Front Biosci (Elite Ed). 2010;2:725–32.

22 Lopez-Novoa JM, Quiros Y, Vicente L, Morales AI, Lopez-Hernandez FJ. New insights into the mechanism of aminoglycoside nephrotoxicity: an integrative point of view. Kidney Int. 2011;79(1):33–45.

23 Boudonck KJ, Mitchell MW, Német L, Keresztes L, Nyska A, Shinar D, *et al.* Discovery of metabolomics biomarkers for early detection of nephrotoxicity. Toxicol Pathol. 2009;37(3): 280–92.

24 Tzovaras V, Tsimihodimos V, Kostara C, Mitrogianni Z, Elisaf M. Aminoglycoside-induced nephrotoxicity studied by proton magnetic resonance spectroscopy of urine. Nephrol Dial Transplant. 2011;26(10):3219–24.

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