Bioanalysis

What role can metabolomics play in the discovery and development of new medicines for infectious diseases?

"...metabolomics provides an important tool to monitor and understand drug activity with applications from early discovery through to preclinical development and clinical usage."

Keywords: anti-infective • antimicrobial • drug mechanism • infectious diseases • metabolomics • pharmacometabolomics

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Infectious diseases represent the greatest cause of morbidity and mortality throughout humanity. Many infectious diseases are fatal if untreated, and the emergence of drug resistance in pathogenic parasites, bacteria and viruses threaten our ability to treat common infections. Despite the successes of antibiotics during the 20th century, the current pipeline of anti-infective drug development is worryingly sparse, and has led to a crisis in the treatment of infectious diseases. Therefore, new systematic approaches to anti-infective drug discovery are urgently required. In recent years, advances in systems biology have provided the opportunity to understand the biochemistry of microbial pathogens, and the host–pathogen interface, at a system-wide level. In particular, an improved knowledge of the metabolism of microorganisms, and the ability to monitor metabolic changes in infected patients, is critical to facilitate the discovery and development of novel anti-infective drugs. In this context, recent advances in metabolomics technologies offer new promise in the quest for new anti-infective medicines.

Metabolomics

Metabolomics involves the comprehensive analysis of many small molecules in a biological system, providing a rapid snapshot of the cellular physiology. This metabolic state is determined by the complex relationships between genetic and environmental factors, providing critical molecular information to link the genotype and phenotype. Therefore, metabolomic approaches have been increasingly used in applications for human health and disease including drug discovery and development [1]. Great progress has been made on various instrumentation for metabolomics over the last decade, with two bioanalytical platforms most widely used for metabolomic studies – mass spectrometry (MS) including LC–MS and GC–MS, and NMR spectroscopy. Based on these technologies, different targeted or untargeted analytical strategies have been employed in pursuit of various experimental aims. Targeted studies involve accurate quantitative analysis of a predefined group of compounds based on existing knowledge of a biological system. Thus, extraction and analysis conditions are optimized for a single metabolite, pathway or class of compounds. On the other hand, untargeted approaches aim to detect a comprehensive metabolite profile, allowing nonbiased analysis of significant metabolites within a system. This unbiased approach offers great potential to investigate metabolic pathways for the discovery of new drug targets [2]. Since many anti-infective drugs act on metabolic targets within infectious pathogens, untargeted metabolomic technology can be used to screen global cellular metabolic changes reflecting the metabolic response to therapeutic intervention. In addition, untargeted metabolomics is suited to cell culture studies of micro-organisms and can improve our knowledge of the metabolism of pathogens and investigate host–pathogen interactions, leading to the discovery of new drug targets [3].

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Drug discovery in the omics age

The discovery of essential enzymes that represent potential novel drug targets requires improved understanding of microbial metabolism at the whole system level. Technical advances in biological sciences have resulted in the generation of a large amount of genomic, transcriptomic and proteomic data, improving our basic understanding of the biochemical networks of many microorganisms. However, the function of metabolic networks can only be fully understood by accurately measuring the actual metabolites. The addition of metabolomics to other established omics approaches offers new promise for the identification and validation of novel microbial drug targets. However, it is still a challenging task to understand how each of these individual levels of 'omics' networks interact with each other, how these interactions lead to complex system responses and ultimately how changes in conditions influence the responses. Therefore, for the successful application of systems biology to drug discovery, computational modeling is necessary to integrate multiple levels of omics data and to develop predictive models leading to the discovery of optimal targets for therapeutic intervention [4,5]. Recently, a computational quantitative model of glycolysis in *Trypanosoma brucei* based on 'omics' data has been constructed and extended to improve our understanding of the metabolism of the system, with the aim of developing optimized antiparasite drugs [6,7].

Another exciting role for metabolomics is the *de novo* discovery of drug targets for existing antimicrobial compounds. The mechanisms of many anti-infective drugs in current use are not understood. The lack of known drug target(s) prevents optimization or repurposing of these drugs in order to overcome drug resistance, improve efficacy or minimize toxicity. Furthermore, recent developments in phenotypic high-throughput screening of chemical libraries have discovered thousands of novel chemotypes with antimicrobial activities that provide promising starting points for anti-infective drug discovery [8,9]. However, the mechanisms of action of these compounds are not known, hindering further rational development into drug candidates with promising pharmaceutical properties. Metabolomics can directly interrogate mechanisms of action by profiling the biochemical effects of compounds on microbial cell cultures [10–12]. In some cases, the specific target (or targets) of the test compounds can be observed by the accumulation of substrate and depletion of downstream metabolic products for the target enzyme. This unbiased approach provides a hypothesis-free methodology to discover targets for novel chemotypes. One of the greatest challenges for these pharmacological assays is to confirm the association between the observed metabolic responses and the antimicrobial activity. Fortunately, many microbes can be grown in cell culture conditions, facilitating metabolomics analysis of time- and dose-dependent responses to drug treatment, allowing delineation of specific compound-induced metabolic perturbations from those nonspecific perturbations associated with cell death. In addition, integration of metabolomics into medicinal chemistry programs could allow analysis of several antimicrobial analogs, generating relationships between antimicrobial structure and metabolic perturbations. In all cases, metabolomics offers significant advantages over targeted enzyme inhibition assays, as the system-wide measurement of metabolic changes confirms the biochemical impact of drug action in a live cell system, and the selectivity of drug action at a specific enzyme(s) or pathway(s) can be directly determined. Interestingly, metabolomics also reveals off-target biochemical effects, and can directly highlight cases where potent therapeutic activity is not due to inhibition if the intended target, for example, metabolic profiling of isoprenoid synthesis in *Plasmodium falciparum* revealed that the primary antiparasitic activity of fosmidomycin is due to inhibition of methylerythritol phosphate cytidylyltransferase, rather than deoxyxylulose phosphate reductoisomerase as was previously expected based on *in vitro* enzyme assays [13]. It is only through system-wide analysis of cellular biochemistry (e.g., metabolomics) that mechanisms of drug action can be assessed in this unbiased manner.

Metabolomics in drug development

Pharmacometabolomics involves the analysis of metabolites in an individual's biofluid (e.g., blood, serum or urine), with the goal of predicting the response of an individual to drug treatment [14], since the metabolome closely reflects the phenotypic responses of a host–pathogen system to both genetics and environmental effects. Metabolomic biomarkers offer the potential to monitor and improve the efficacy, resistance, toxicity and pharmacokinetic profiles for anti-infective drugs and can lead to the identification of biomarkers for monitoring disease progression and diagnosis. It may also include the investigation of drug metabolism pathways by detection of drug metabolites that predict the safety and efficacy of a drug. For example, metabolomic strategies have been used to explore the metabolic pathways of ritonavir, which is currently used for the treatment of HIV but causes multiple side effects and the mechanisms are not fully understood [15]. It was reported that four pathways of drug metabolism based on 26 ritonavir metabolites detected by LC–MS were related to the drug toxicity. Pharmacometabolomics can also play a major role in the development of

personalized medicine as it facilitates the selection of the best drug treatment for each individual patient by predicting drug efficacy, toxicity and side effects. Such biomarkers are important in the pharmaceutical industry as the ability to tailor drug treatment to specific individuals or patient groups could optimize the utilization of drugs in the clinic, and minimize the risks associated with full clinical development of new drugs.

Summary & future directions

Metabolomics is a highly efficient approach for determining the mechanisms that underpin the activity, resistance and toxicity of existing and novel anti-infectives. Unlike classical single-target, hypothesis-driven, studies of drug action, metabolomics offers a hypothesis-free approach to measure the impact of drugs across multiple metabolic pathways. The observation of perturbations across the whole metabolic system will allow a more detailed understanding of the global mechanisms involved in activity, toxicity and resistance. Comprehensive metabolomics analysis is heavily dependent on the latest analytical technologies and advanced chemoinformatic software. Interpretation of metabolomics data in the drug discovery context is also dependent on our underlying biochemical knowledge of microbial and human metabolism. It is hoped that continual improvement in annotation and understanding of metabolic pathways, in combination with advanced bioinformatics software, will further improve the biochemical interpretation of metabolomics data, and lead to predictive *in silico* models that will provide significant cost savings for future drug discovery. With existing technologies, metabolomics provides an important tool to monitor and understand drug activity with applications from early discovery through to preclinical development and clinical usage. These system-wide biochemical measurements of drug action will inform rational medicinal chemistry to improve properties of novel drug candidates, and guide clinical usage of new and existing anti-infective drugs or combination therapies to improve efficacy, minimize toxicity and monitor for resistance.

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