NMR-based metabolomics of bacterial infections studied in a mouse-model

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Introduction: The clinical diagnosis of bacterial infections traditionally requires the recovery of microorganisms in patients’ blood, urine or specimen. The conventional culture methods are time-consuming, labour-intensive and show a high rate of failure, but are still routinely employed. In recent years, metabolomic studies applied magnetic resonance spectroscopy to define metabolic profiles of biological fluids. Combining metabolomic profiling and multivariate data analysis, different bacterial groups could be automatically identified in growing cultures [1] and allowed for the distinction between bacterial and viral meningitis by analyzing cerebrospinal fluid samples [2]. Here we demonstrate in mouse-models of Streptococcus pneumoniae and Staphylococcus aureus infection that NMR-based metabolomics can be a powerful tool to distinguish between two bacterial strains which are both anaerobic and gram-positive, regarding metabolic information of bacteria and host and using statistical pattern recognition techniques.

Experiments: NMR-based metabolic profiling: For NMR profiling, one-dimensional nuclear overhauser effect spectroscopy (NOESY) spectra were acquired on a 600 MHz Bruker Avance NMR spectrometer with a 5 mm TXI probe head equipped with z-gradient. The metabolic profiles were created using targeted profiling in Chenomx NMR Suite 4.6. After normalizing the data to minimize the diurnal variation, the profiles were used as input in the software package SIMCA-P for statistical modelling. Mouse-models: The experiments were performed using C57BL/6 wild-type mice. 9 male mice were infected with Staphylococcus aureus: 7 male mice were infected with Staphylococcus aureus strain Xen 29 possessing a stable copy of the modified Photobacteriulx ABCDE operon at a single integration site on the bacterial chromosome. 100 microlits of the bacterial suspension (1 x 10^6 CFU) were injected subcutaneously [3]. After 4 h and 24 h of infection, lesion development was monitored by bioluminescence and 300 µl of serum were collected. Streptococcus pneumoniae: 6 male mice were infected with S. pneumoniae strain SPN 15814 by transorally injecting 50 microlits of bacteria suspended in PBS (2 x 10^6 CFU) into the lungs [4]. After 24 h of infection serum samples were collected, pooled and included in the NMR study.

Results: Metabolites: 45 metabolites were identified and quantified in the mouse serum samples by NMR-based metabolomic profiling. The 1H NMR spectra for infected mice were distinctly different form those for control subjects. In Staphylococcus aureus infected mice resonances attributed to malonate, ethanol, 2-hydroxybutyrate and the amino acids (phenylalanine, tryptophan, ornithine, lysine threonine, proline, histidine, alanine, creatine, leucine and isoleucine) were elevated while the significant energy metabolites (2-oxoglutarate, glucose, citrate, succinate) were depressed.

Conclusion: Metabolic technologies allow for the investigation of a large number of metabolites in small volumes of body fluids. In this pilot study we have shown by investigating serum samples of mice, that NMR-based metabolomic analysis can be a powerful diagnostic tool for distinguishing between different bacterial infections.

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References: