Metabolomics analysis of rats subjected to hemorrhagic/traumatic shock and resuscitation with/without stem cell supplementation

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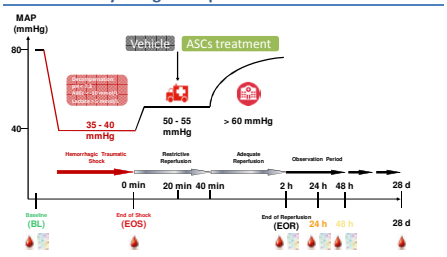
Background & Aim

Hemorrhagic traumatic shock (HTS) and reperfusion are often associated with general inflammatory response and organ dysfunction, resulting in death of some patients. Most recently, we have shown that rat adipose-derived stem cells (ASCs) supplemented resuscitation improves long term outcome after HTS in rats[1]. Using metabolomics we aimed to a) characterize HTS-related metabolic alterations and b) evaluate potential effects of ASCs treatment on those alterations in a HTS rat model.

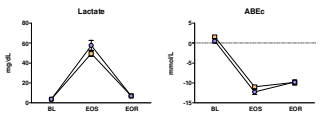
Materials & Methods

Rats were subjected to HTS (mean arterial pressure (MAP) 30-35 mmHg until decompensation) and a resuscitation protocol, including pre-hospital restrictive reperfusion (30 ml/kg/h, MAP maintained at 50-55 mmHg for 40 min), followed by an adequate reperfusion phase (75ml/kg/h for 60 min, MAP to baseline). 20 min after onset of reperfusion animals either received 2x10^6 ASCs, ASC secretome or vehicle intravenously. Blood samples were obtained at baseline (BL), end of reperfusion (EOR), 24h and 48h after shock.

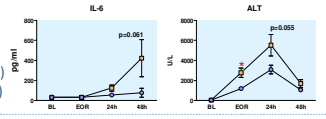
Study Design & Experimental Procedure



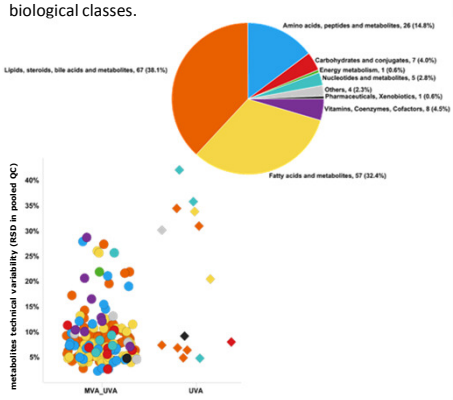
Equal shock severity in both groups prior to treatment



ASC treatment ameliorated [1] inflammation (IL-6) & liver injury (ALT)



With HILIC-HRMS metabolomics 190 known metabolite levels were investigated, 176 thereof were suitable for multi- and univariate statistical analysis, 14 for univariate analysis only. Median technical variability was very low (7.5%) and detected metabolites covered all important biological classes.

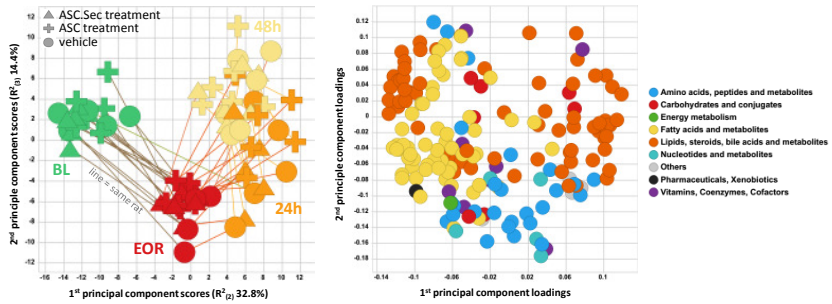


Statistical analysis:
To achieve sufficient normal distribution and homo-scedasticity data was $\log_{10}$ transformed (Kolmogorov-Smirnov test, Brown-Forsythe Levene type test).
PCA analysis was performed centered and scaled to unit variance. Missing values were imputed by a regularized expectation-maximization.
ANOVA model was selected according to logLik, AIC, log-likelihood ratio test, distribution of residuals and q-q plots.

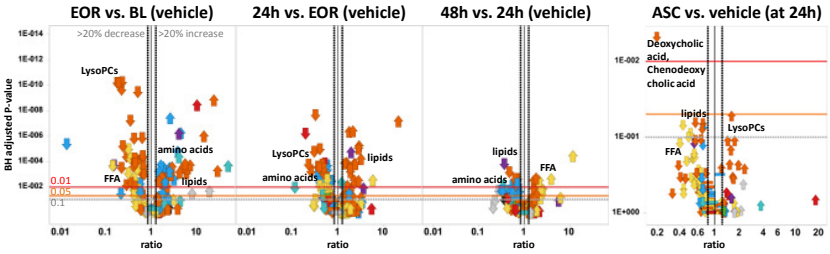
Results

Metabolomics revealed a profound and significant impact of HTS on plasma metabolite levels at EOR, 24h and 48h after shock induction. Especially lipids were increased, while fatty acids were decreased, indicating trauma induced hypermetabolism and elevated energy expenditure. Metabolic alterations after shock continued over time, without returning to baseline state within 48h.

PCA reveals strong metabolic impact of HTS



More detailed analysis by ANOVA revealed additional trauma induced increase in amino acids as substitutional energy source. With ongoing time after trauma, lipids and fatty acids neared baseline levels while amino acids decreased at 24h and 48h. ASC treatment reduced overall mortality to 0% (28% vehicle). Determination of metabolic impact was challenging due to strong trauma induced changes and the exploratory design of the study. Nevertheless, important trends of ASC-supplemented resuscitation are visible, e.g. lower levels of lipids, fatty acids with increased lysolipids, indicating more efficient lipolysis and energy generation.



Conclusions

- **Metabolomics proved to be useful to characterize HTS-induced metabolic alterations**
- **HTS & resuscitation induces profound metabolic alterations up to 48 hours**
- **Metabolic changes in rats are in line with reports in human[2] & porcine[3] models**
- **Rats appear to be a suitable pre-clinical model for metabolomics studies**
- **ASCs mode of action includes among others metabolic effects**
- **ASCs treatment seems to improve energy provision**

Outlook

- Further studies to better characterize metabolic changes after trauma
- Evaluating metabolic alterations for predicting complications after trauma
- Using metabolomics to better understand ASC's efficacy in emergency setting
- Evaluating rats as a preclinical model for metabolomics studies

References

1) Ashmwe et al. (submitted); 2) Parent et al. JAMA 2016; 3) Lexcen et al. J Trauma Acute Care Surg 2012

Funded by:

Work done in "CBmed" was funded by the Austrian Federal Government within the COMET K1 Centre Program, Land Steiermark and Land Wien.

The project was in part supported by the Lorenz Böhler Fond 05/3