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Pharmacelsus News

Dear Customer,

With this e-mail you receive the third issue of the Pharmacelsus newsletter 2013. It is intended to inform you with the latest news regarding drug development and regulatory issues as well as keep you informed about additions to the growing service portfolio of Pharmacelsus.

Pharmacelsus has recently expanded its portfolio of *in vivo* models and conducted a pilot study for the assessment of metabolomic biomarkers in a rat model of liver fibrosis in collaboration with BIOCRATES Life Sciences, Innsbruck, Austria. Today, we present to you the results of these studies.

Further information can be found on our website at www.pharmacelsus.com.

If you are interested in more information on our services or to visit Pharmacelsus, don't hesitate to contact us any time.

Sincerely yours,

Dr. Christine Batzl-Hartmann	Dr. Klaus Biemel
CEO	COO

Induction of liver fibrosis by bile-duct ligation (BDL) in rats:
Model setup at Pharmacelsus and evaluation of non-invasive metabolomic biomarkers in collaboration with BIOCRATES Life Sciences

Bettina Husen
(Pharmacelsus GmbH)

Guido Krebiehl, Denise Sonntag
(Biocrates Life Sciences)

Hepatic fibrosis represents a consequence of alcoholic liver disease (long-term excessive alcohol consumption), non-alcoholic fatty liver disease (NALFD) / non-alcoholic steatohepatitis (NASH, metabolic syndrome), cholestatic liver disease, autoimmune liver disease, chronic viral hepatitis and exposure to hepatotoxic drugs. This means that it affects a large number of patients. Antifibrotic therapies, as well as non-invasive biomarkers as a promising alternative to classical methods for the assessment (staging and differentiation) of liver disease are urgently needed.

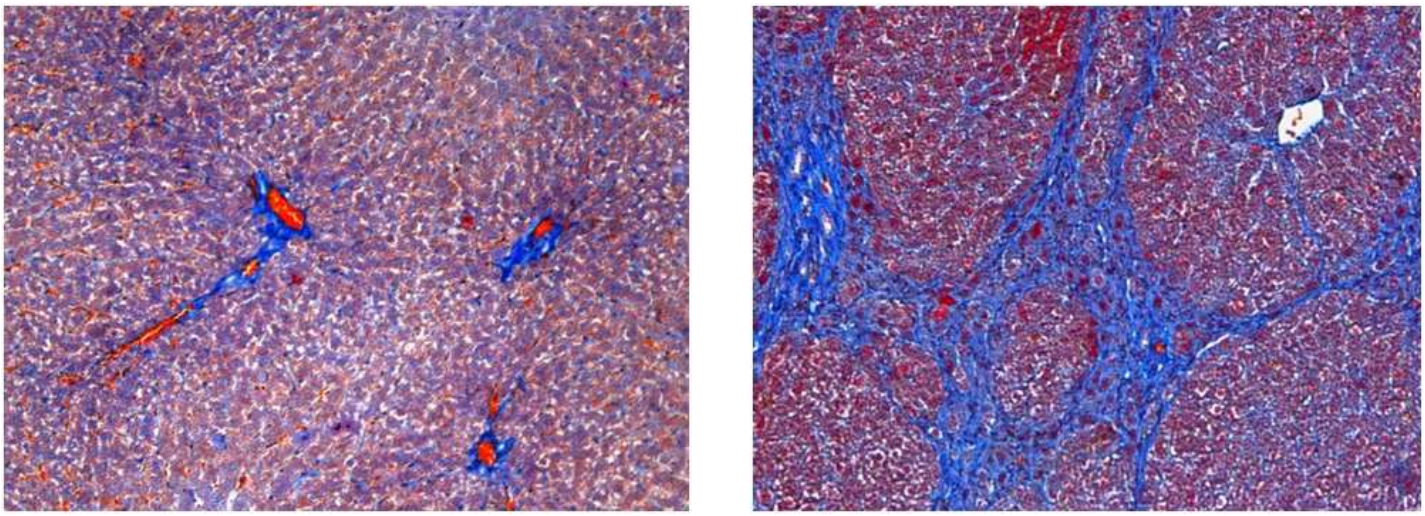
We evaluated the benefits of endogenous plasma metabolites (metabolite classes as specified in the table below) as predictive biomarkers in a well-known rat model of liver fibrosis and cholestatic liver disease.

At Pharmacelsus, adult male Sprague Dawley rats were subjected to bile duct ligation (BDL) or sham surgery (n=8 BDL, n=8 sham). General condition and liver enzymes (ALT, AST) were followed up for 6 weeks. Qualitative assessment of liver histology was performed in week 6.

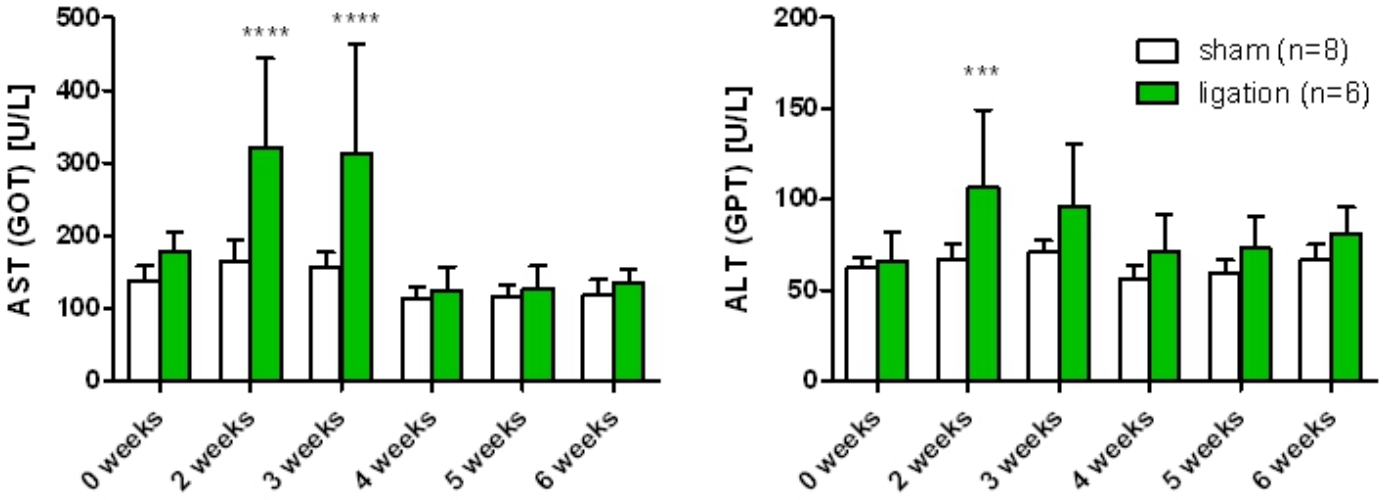
At BIOCRATES Life Sciences AG, an LC-MS-based targeted metabolomic approach was used to quantify various classes of metabolites

(207 individual metabolites in total) in plasma and liver.

Liver histology after 6 weeks of BDL revealed clear signs of fibrosis. Plasma profiles of liver enzymes, 2, 3, 4, 5 and 6 weeks after BDL, as well as relative weights of liver and spleen were consistent with published data.



Histology of rat livers 6 weeks after surgery. Left: normal liver architecture after sham surgery, right: biliary cirrhosis after bile-duct ligation, i.e. accumulation of extracellular matrix (stained blue) and ductular proliferation at portal areas



Serum concentrations of classical markers of liver injury (liver enzymes: AST, ALT: mean ± SD, 1-way ANOVA, Bonferoni post-test for significant differences between sham-treatment and ligation)

At necropsy, the number of metabolites that showed significantly different concentrations in BDL rats compared to sham rats was 44 in liver and 17 in plasma. Nine of these metabolites were changed in the same way in liver and in plasma giving rise to the assumption that they may serve as direct plasma markers of chronic liver damage (e.g. tyrosine and sphingomyelin C16:0).

Metabolite class	Number of metabolites analysed	Number of metabolites > LOD in liver d42	Number of metabolites > LOD in plasma d42	Significant change ($p < 0.1$) in liver d42	Significant change ($p < 0.1$) in plasma d42	Significant change ($p < 0.1$) in liver AND plasma d42
Acylcarnitines	41	25	22	0	0	0
Amino acids	22	21	17	9	3	1
Biogenic amines	21	15	16	6	0	0
Phosphatidylcholines	77	71	70	20	8	4
Lyso-phosphatidylcholines	14	13	12	5	4	3
Sphingomyelins	15	14	15	3	2	1
Bile Acids	17	12	11	1	0	0
Total	207	171	163	44	17	9

Another set of plasma metabolites appeared to indicate acute, rather than chronic liver injury (specific bile acids and phosphatidylcholines). Concentrations of these metabolites returned to sham levels by week 4 of the study like the classical functional parameters ALT and AST.

We conclude that targeted metabolomic analysis bears the potential to identify novel non-invasive biomarkers of liver injury and fibrosis, which might reveal increased sensitivity and specificity compared to classical markers.

Bile-duct ligation in rats in combination with targeted metabolomic analysis can be regarded as a predictive model of acute and chronic liver injury and might be of interest for

- pharmacodynamic profiling of antifibrotic drug candidates,
- pharmacokinetic profiling of drug candidates to be used in patients with impaired liver function,
- identification and profiling of new biomarkers for liver disease progression and/or therapeutic monitoring.

Get more details or request an offer from Dr. Bettina Husen (husen@pharmacelsus.de)

Services available at Pharmacelsus: ADME & in vitro Pharmacology, Target Finding & Biological Profiling, in vivo Pharmacology & PK, Bioanalytics, Target-Based Bioassays

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Pharmacelsus GmbH
Science Park 2
66123 Saarbrücken
GERMANY

Phone: +49 (681) 3946 - 7510
Fax: +49 (681) 3946 - 7511

http://www.pharmacelsus.de

Rechtsform: GmbH, Sitz: Saarbrücken
Amtsgericht Saarbrücken HRB 11950
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Geschäftsführerin: Dr. Christine Batzl-Hartmann (CEO)

Geschäftsführer: Dr. Klaus Biemel (COO)