Analysis of Changes in Bile Acids Concentration in Bile in Response to the Degree of Liver Ischemia and the Method of Organ Preservation

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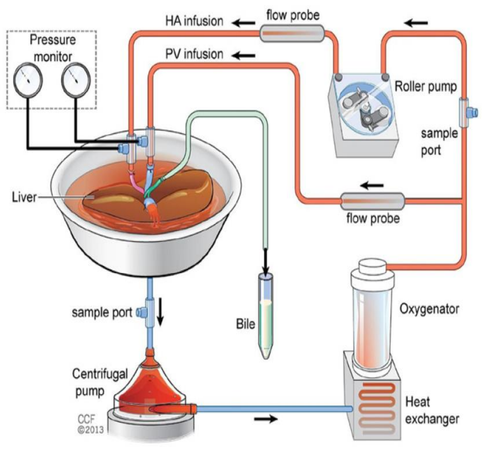
Introduction

Liver transplant surgery is currently the standard of treatment in patients with end-stage organ failure. Nowadays, the dominant method of organ preservation used by most transplantation centers is static cold storage (SCS). However, a better method of organ preservation is sought, which would allow extending the storage time of the graft while maintaining its proper quality The proposed method is normothermic ex-vivo liver perfusion (NEVLP), based on maintaining normal metabolic activity, which gives the opportunity of better assessment of liver viability before implantation. One of the possibilities is to assess the production of bile by the liver perfused in these conditions. It is considered that the production of bile alone is not sufficient evidence for the proper functioning of the liver and directs the research to assess the composition of bile. Therefore, it is assumed that changes in the concentration of bile acids, which are the main component of bile, may correlate with changes occurring in the transplanted organ.

Methods

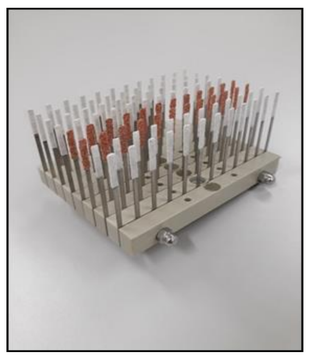
1. Collection of bile during SCS or NEVLP [1]



The study was performed on bile samples obtained from two types of porcine model donors: heart beating donor (HBD) and donor after cardiac death (DCD). Samples were collected during SCS and NEVLP at specific time points: before organ harvest, during perfusion (for NEVLP), reperfusion and the first few days after transplantation. The DCD group was divided due to the time of organ ischemia: 30’ for SCS and 30’, 60’, 90’ for NEVLP (n=3 in each group).

2. Sample preparation

Sample preparation was performed according to the thin-film solid phase microextraction (TF-SPME), using C18 sorbent as the extraction phase.



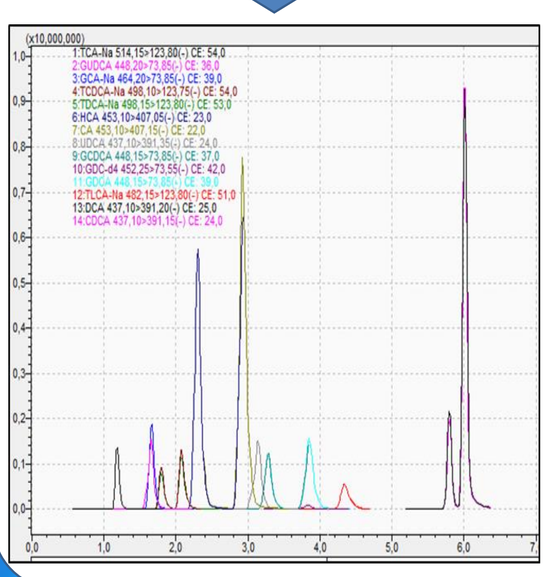
A. Extraction:
- 10 µL bile:990 µL PBS + 10 µL IS
- TF-SPME (5 mm C18 coating)
- 60 min, 25°C, 1000 rpm agitation

B. Desorption:
- 1 mL MeOH
- 60 min, 25°C, 1000 rpm agitation

**For glycochenodeoxycholic, glycoursodeoxycholic and taurochenodeoxycholic acid, the extracts were diluted 200x.*

3. LC-MS/MS analysis

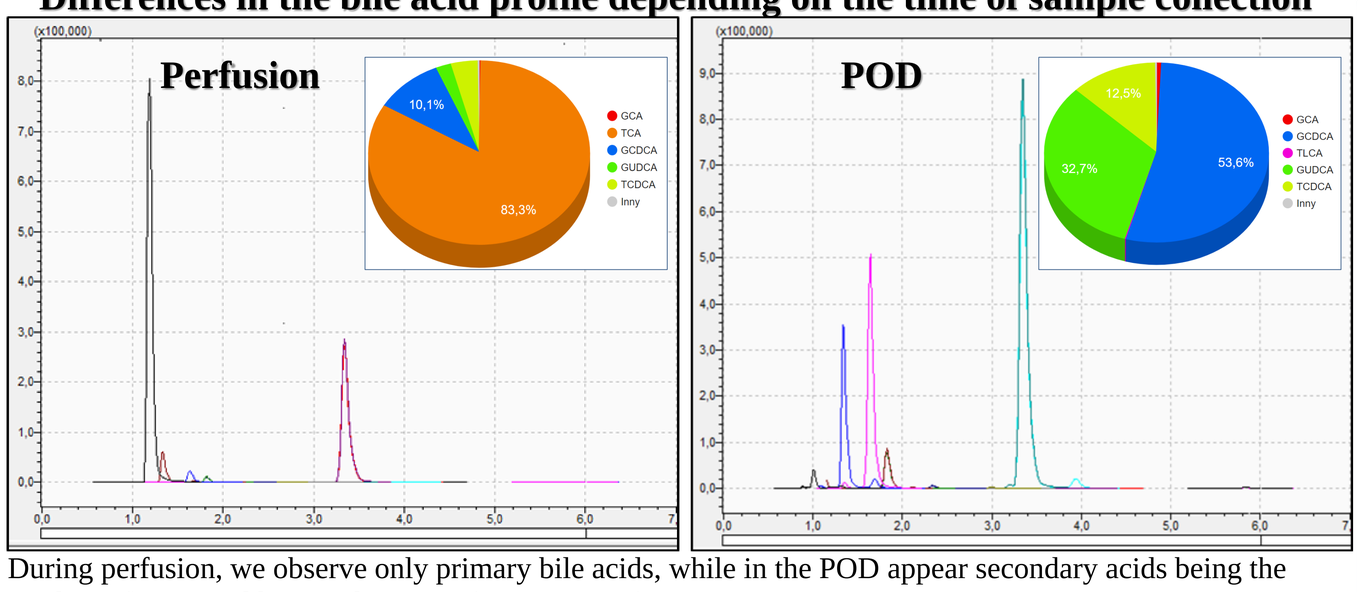
Analyte	Rt [min]	M [g/mol]	MRM transition (m/z)	CE
GCA (Glycocholic acid)	1,66	465.62	464,2→73,85 464,2→402,15 464,2→410,1	39 36 46
TCA (Taurocholic acid)	1,17	515.70	514,15→123,8 514,15→106,8 514,15→280,9	54 55 30
GCDCA (Glycochenodeoxycholic acid)	3,29	449.62	448,15→73,85 448,15→386,4 448,15→330,05	37 35 47
TLCA (Taurolithocholic acid)	4,22	483.71	482,15→123,8 482,15→106,8 482,15→80,05	51 54 55
GUDCA (Glycoursodeoxycolic acid)	1,67	449.62	448,2→73,85 448,2→386,25 448,2→384,10	36 36 40
TDCA (Tauroursodeoxycholic acid)	2,04	499.70	498,15→123,8 498,15→106,8 498,15→80,05	53 54 55
GDCA (Glycodeoxycholic acid)	3,85	449.62	448,15→73,85 448,15→404,15 448,15→402,25	39 33 38
CA (Cholic acid)	2,93	408.57	453,10→407,15 453,10→343,2 453,10→353,35	22 42 41
CDCA (Chenodeoxycholic acid)	5,82	392.57	437,1→391,15 437,1→44,95 437,1→373,2	24 45 43
DCA (Deoxycholic acid)	6,03	392.57	437,1→391,2 437,1→345,15 437,1→343,2	25 41 48
HCA (Hyocholic acid)	2,30	408.57	453,1→407,05 453,1→389,15 453,1→44,75	23 42 55
TCDCA (Taurochenodeoxycholic acid)	1,77	499.70	498,1→123,75 498,1→106,8 498,1→80,05	54 54 55
UDCA (Ursodeoxycholic acid)	3,13	392.57	437,1→391,35 437,1→44,8	24 41



- LCMS-8060 Triple Quadrupole Mass Spectrometer (Shimadzu)
- ESI source in the negative MRM mode:
- Mobile phase – A: H2O+ 0,1% FA; B: ACN+ 0,1%F A
- Column: ACQUITY UPLC BEH C18, 130Å, 1.7 µm, 2.1 mm X 50 mm

Results

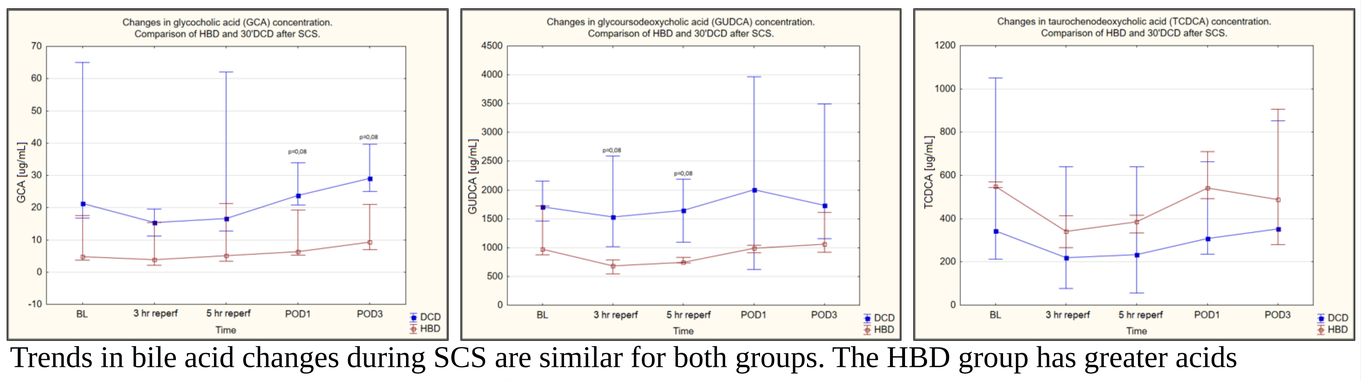
Differences in the bile acid profile depending on the time of sample collection



During perfusion, we observe only primary bile acids, while in the POD appear secondary acids being the product of intestinal bacterial activity (e.g. GUDCA).

HBD + SCS vs 30’DCD + SCS

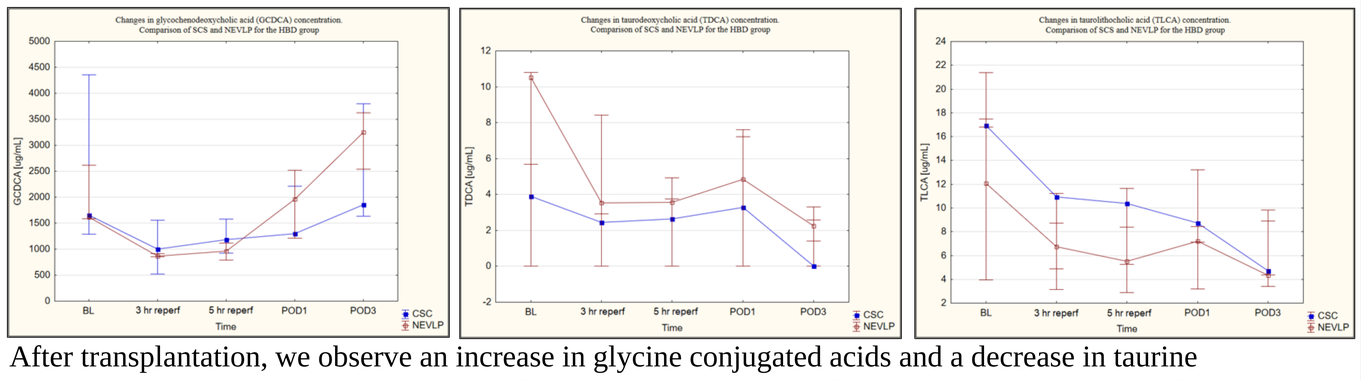
GCA | GUDCA | TCDCA



Trends in bile acid changes during SCS are similar for both groups. The HBD group has greater acids conjugation with taurine, while the 30'DCD group is dominated by glycine conjugates.

HBD + SCS vs HBD + NEVLP

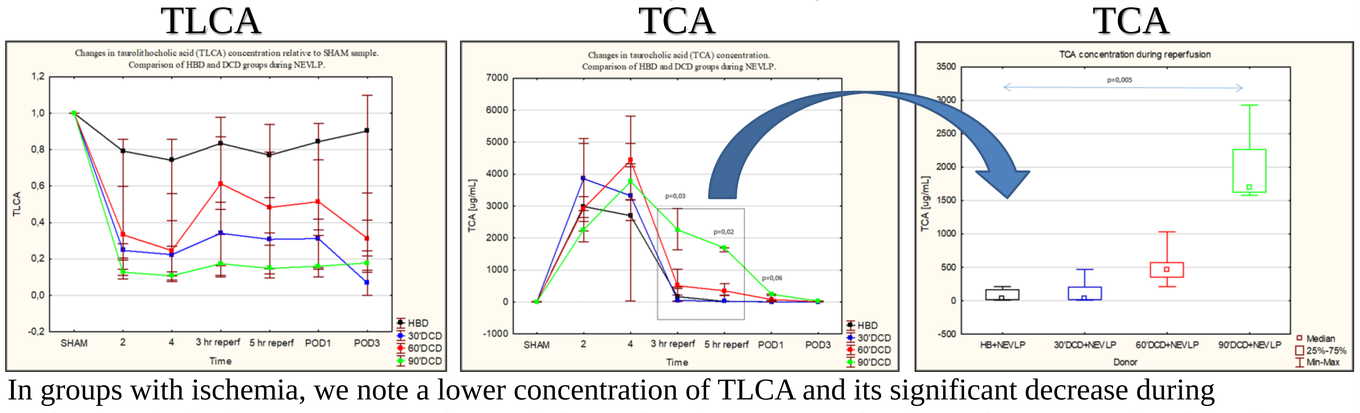
GCDCA | TDCA | TLCA



After transplantation, we observe an increase in glycine conjugated acids and a decrease in taurine conjugated acids in both groups. GCDCA levels increase more rapidly in the first postoperative days after NEVLP compared to SCS.

HBD + NEVLP vs 30’DCD,60’DCD,90’DCD + NEVLP

TLCA | TCA | TCA



In groups with ischemia, we note a lower concentration of TLCA and its significant decrease during perfusion. The high concentration of taurocholic acid is characteristic for the perfusion period and is still present in the reperfusion of the 90’DCD group.

**The results are presented as Median and Min-Max*

Conclusions

- TF-SPME is a high-throughput sample preparation method that can be effectively used for profiling bile samples.
- Only trace amounts of free bile acids were found in bile. There is a change in the concentration of conjugated bile acids during transplantation.
- Changes in bile acid concentrations in bile samples may correlate with the metabolic processes occurring in the transplanted organ.
- Further research of bile composition extended to other bile acids and their metabolites may allow to find biomarkers of liver function.

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[1] A. Nassar et al., “Surgical Innovation,” no. April, 2014