P-46

四塩化炭素を単回投与したマウス肝臓におけるheat shock protein及びその遺伝子発現の肝小葉内分布/Immunohistochemistry and in situ hybridization for Heat shock protein from mice treated with single administration of carbon tetrachloride

○神鳥仁志/Hitoshi Kandori, 青木正美/Masami Aoki, 宮本由美子/Yumiko Miyamoto, 中村小百合/Sayuri Nakamura, 小林亮介/Ryosuke Kobayashi, 松本光晴/Mitsuharu Matsumoto, 横山孝太朗/Kotaro Yokoyama Axcelead Drug Discovery Partners, Inc.

Materials & Methods

Animals: BALB/c mice (male, 8-week-old). CCl 4 (0.1 mL/kg, PO), single administration. Plasma (EDTA treated) and liver sampling at 6 hours or 1day after administration (n=4).

Blood chemistry (BC): AST, ALT and GLDH by LABOSPECT008 (Hitachi High-Technologies). miR-122 by qPCR using ID3EAL system (MiRXES) and ABI7900 (Thermofisher sciences).

Histopathology (Liver): Left lateral lobule immersed in 10 vol% neutral buffered formalin for 5 days. Paraffin embedded. About 3 μm thick section. HE, in situ hybridization (ISH) for *Hspa1a* and *Hspa5*, and immunohistochemistry (IHC) for HSP70 (*HSPA1a*) and GRP78 (*HSPA5*).

ISH (Advanced Cell Diagnostics Inc.)
- probe: RNAscope® 2.5 LS Probe -Hspa1a
  RNAscope® 2.5 LS Probe -Hspa5
- kit: RNAscope® 2.5 LS Reagent Kit-BROWN
  BOND Polymer Refine Detection kit

IHC
- antibody: Anti-Hsp70 (Abcam/ab181606)
  Anti-GRP78 BiP (Abcam/ab21685)
- kit: Bond Polymer Refine Detection kit
  BOND DAB Enhancer
- Automated staining equipment
  (Leica BOND RX)

Results

BC

Group	Time	AST (U/L)		ALT (U/L)	
Corn oil	6hours	59 ±	9	75 ±	11
	1 day	62 ±	11	66 ±	12
CCl4	6 hours	126 ±	13 **	349 ±	46 &&
	1 day	12539 ±	6802 &	25500 ±	8087 &&

Group	Time	GLDH (U/L)		miR-122 (2^-Δ Δ Ct)	
Corn oil	6 hours	80.3 ±	20.6	1.08 ±	0.51
	1 day	95.7 ±	29.8	1.10 ±	0.55
CCl4	6 hours	73.1 ±	15	15.25 ±	5.31 &
	1 day	5365 ±	864.5 &&	29.91 ±	21.55

**:p≤0.01 (t-test), &:p≤0.05, &&:p≤0.01 (Welch test)

- Increased AST, ALT and miR-122 at 6 hours, GLDH at 1 day.

HE

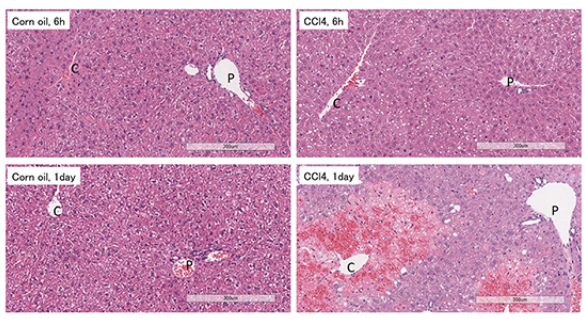
6hours

Group	Corn oil	CCl4
Liver (Centrilobular)		
Vacuolization, Hepatocyte	-	++

1 day

Group	Corn oil	CCl4
Liver (Centrilobular)		
Zonal necrosis, Hepatocyte	-	+++
Infiltrate, Inflammatory cell	-	+
Hemorrhage	-	++

-: Not remarkable, +: Minimal, ++: Mild, +++: Moderate, ++++: Marked



C: centrilobular, P: periportal

- Centrilobular hepatocellular vacuolization at 6 hours.
- Centrilobular zonal hepatocellular necrosis, inflammatory cell infiltration, and hemorrhage at 1 day.

ISH

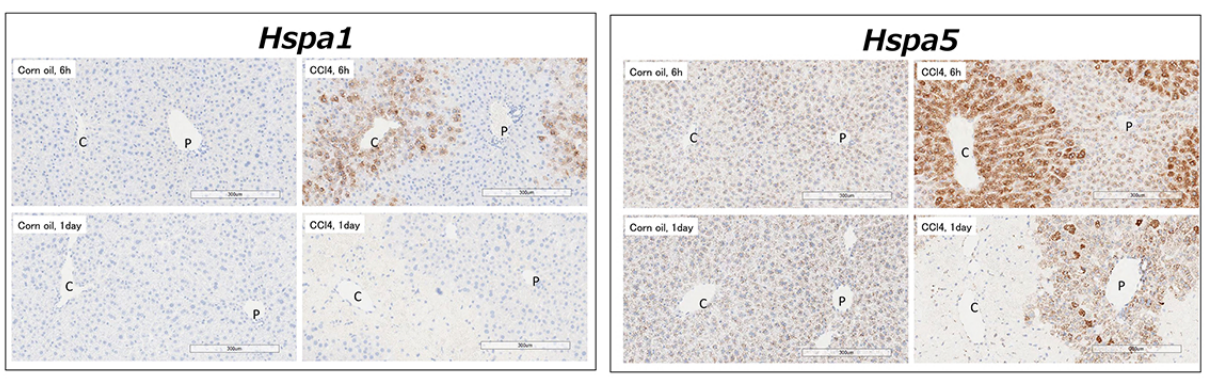
6 hours

Group	Corn oil	CCl4
Centrilobular		
Hspa1a	0	3
Hspa5	2	4
Periportal		
Hspa1a	0	0
Hspa5	2	2

1 day

Group	Corn oil	CCl4
Centrilobular		
Hspa1a	0	NA
Hspa5	2	NA
Periportal		
Hspa1a	0	0
Hspa5	2	3*

Evaluated as 0-4.
NA: Not evaluated due to necrosis.
*: Not judged as significant considering positive control (PPIB).



- Increased *Hspa1a* and *Hspa5* signals in centrilobular area at 6 hours.

IHC

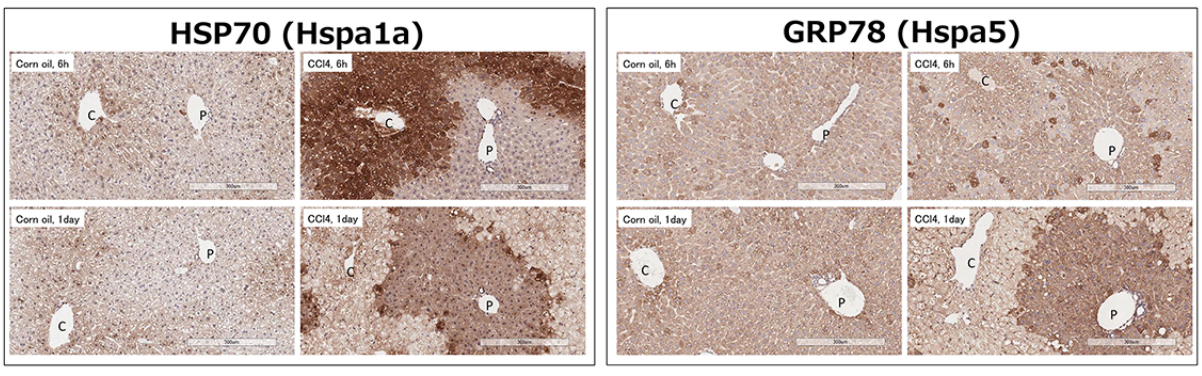
6hours

Group	Corn oil	CCl4
Centrilobular		
HSP70	1	4
GRP78	1	1
Periportal		
HSP70	0	0
GRP78	1	1

1 day

Group	Corn oil	CCl4
Centrilobular		
HSP70	1	NA
GRP78	1	NA
Periportal		
HSP70	0	2
GRP78	1	2

Evaluated as 0-4.
NA: Not evaluated due to necrosis.



- Increased HSP70 signal in centrilobular area at 6 hours.
- Increased HSP70 and GRP78 signals in periportal area at 1 day.

Discussion

- This study was conducted as part of searching for early parameter for hepatotoxicity. Increased gene expression of HSPs, which is recognized as response to cell stress, was observed by ISH at centrilobular area 6 hours after the administration. Since hepatocellular necrosis was not observed at 6 hours, HSPs gene expression detected by ISH might be an early parameter for hepatocellular damage.
- There were some discrepancy between ISH and IHC results. They might be caused partly by the post-transcriptional gene regulation; however, detailed mechanism remained unclear.
- When gene expression, especially gene transcription is the main concerning subject, ISH should be conducted. Because paraffin blocks can be used for ISH by RNAscope®, this method would be applied for pathologic samples routinely prepared in general toxicity studies.

COI Disclosure Information (Hitoshi Kandori)
In connection with this presentation, there is no COI to be disclosed with any companies.