金属化合物の新規化学形態分析法の構築と毒性発現機構解明における応用

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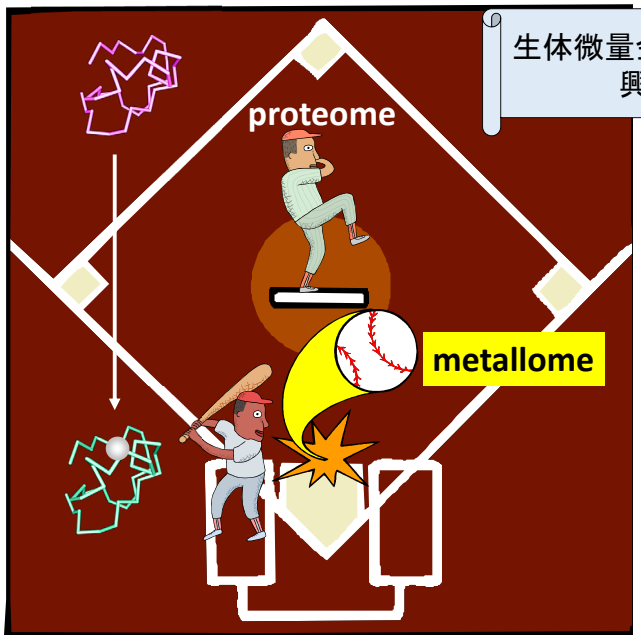
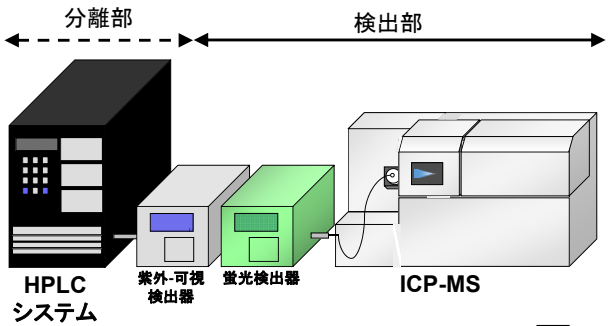
生体微量金属研究における興味と手法



生体微量元素の化学形態分析（スペシエーション、speciation）



LC-ICP-MS

分離部には、LCの他、GC、CE、AF4等も利用可能であるが、生体試料との相性から、LCが多用される。

ICP-MS以前は、原子吸光光度計やICP-発光分光計も使われていた。

selenium

Σελήνη, Selene

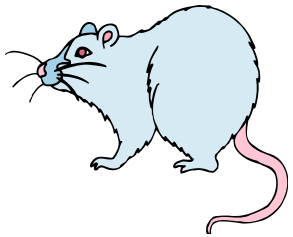
- essential for animals but not plants

- 25 genes encoding selenoproteins in human (ex. glutathione peroxidase, thioredoxin reductase, etc.)

- ambivalent effects (essential but poisonous)

Se is transformed on its metabolic pathway with forming organic Se compounds.

"Identification of Se metabolite" is synonymous with "elucidation of Se metabolism."



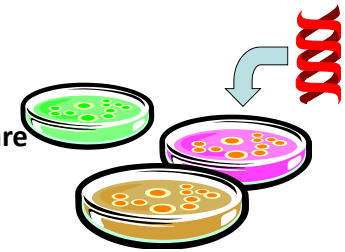
some advantages of cultured cells

- **animal welfare**
- **cost**
- **easier gene modification**

etc....

From experimental animals to cultured cells

However, limited information about the Se metabolism in cultured cells are available.....



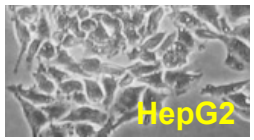
Experimental design

intact cells

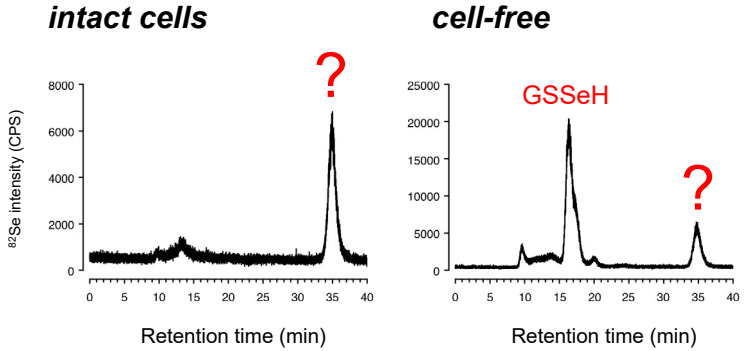
- Human hepatocellular carcinoma cells, HepG2, were used.
- HepG2 cells were exposed with 10 μM selenite for 24 h.
- The supernatant was obtained by ultracentrifugation, and subjected to an LC-ICP-MS.

cell-free

- The homogenate of HepG2 was prepared.
- The homogenate was mixed with 100 μM selenite and 10 mM GSH for 1 h at 37˚C.
- The supernatant was obtained by ultracentrifugation, and subjected to an LC-ICP-MS and LC-ESI-MS-MS.

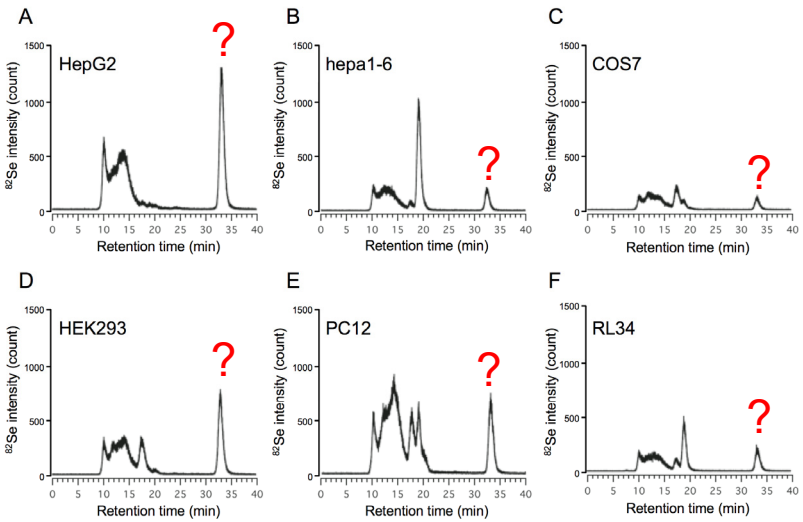


Elution profile of Se in the supernatant of HepG2 cells obtained by LC-ICP-MS

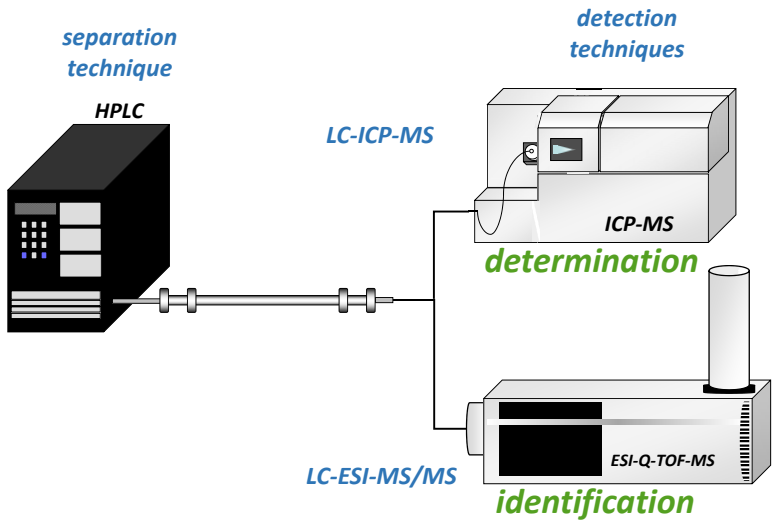


The unknown Se metabolite was also detected in the supernatant of human embryonic kidney cells (HEK293), pheochromocytoma cells of the rat adrenal medulla (PC12), mouse hepatoma cells (hepa1-6), rat liver cell (RL34) and fibroblast-like cells from a monkey kidney (COS7) although an amount of the unknown Se metabolite varied among these cell lines.

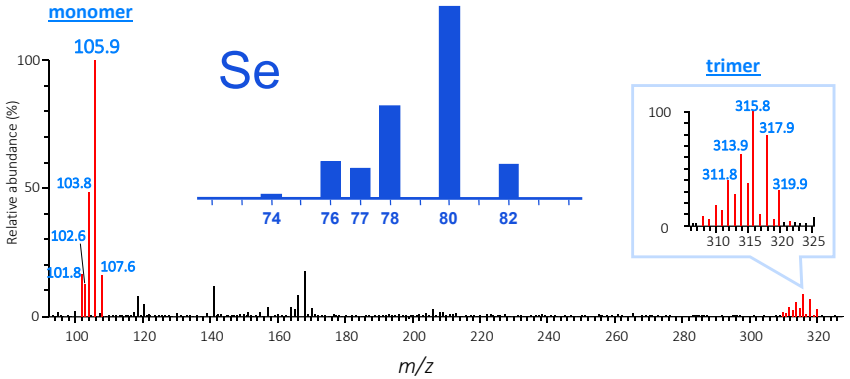
Elution profile of Se in the several supernatants of cells obtained by LC-ICP-MS



Hyphenated techniques for the identification of unknown metallometabolome



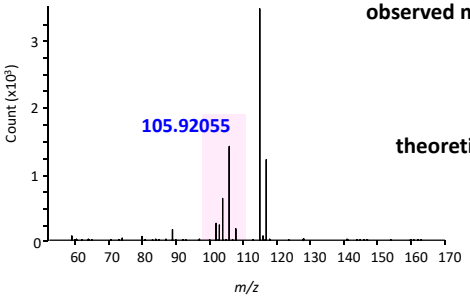
ESI-QMS spectrum of the supernatant of HepG2 cells incubated with sodium selenite and glutathione



LC-ESI-MS-MS conditions (Waters Quattro Micro)

Column	GS-320A-2E (multi-mode size exclusion)
Buffer	10 mM ammonium acetate pH 6.5
Flow rate	0.04 mL/min
Injection volume	5 µL
Detection mode	negative ion mode

ESI-Q-TOF-MS spectrum of the supernatant of HepG2 cells incubated with sodium selenite and glutathione



observed mass containing ^{80}Se 105.92055

Δ9.06 ppm

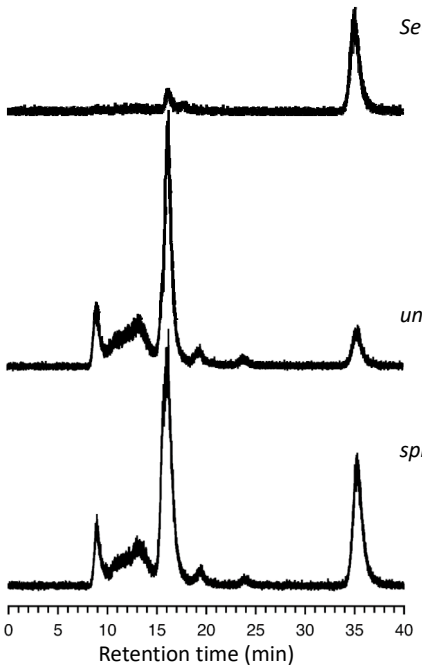
theoretical mass of $^{80}SeCN^-$ 105.91959

^{80}Se : 79.91652
^{12}C : 12.00000
^{14}N : 14.00307

$SeCN^-$

ESI-Q-TOF MS conditions (Agilent 6450)

Buffer	0.3 % ammonia
Flow rate	0.1 mL/min
Injection volume	5 µL
Detection mode	negative ion mode

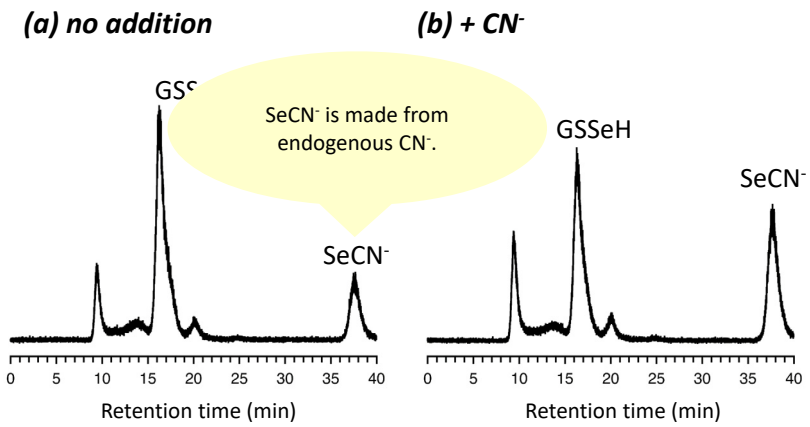


LC-ICP-MS conditions (Agilent 7700)

Column	GS-520 HQ (multi-mode size exclusion)
Buffer	50 mM Tris-HCl, pH 7.4
Flow rate	0.6 mL/min
Injection volume	20 µL

Elution profiles of Se in the supernatant of HepG2 cells incubated with sodium selenite and glutathione

Elution profiles of Se in the supernatant of HepG2 cells incubated with sodium selenite, glutathione and cyanide



The addition of cyanide (CN^-) increased the amount of selenocyanate ($SeCN^-$).

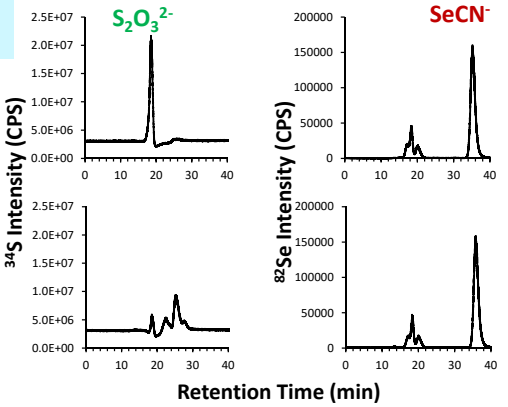
Effect of rhodanese on the production of selenocyanate

reaction mixture
- thosulfate / GSSeH /(selenosulfate)
- cyanide
- cofactors
- buffer
- rodanese (-) / (+)

$SeCN^-$ is non-enzymatically synthesized.

rhodanese (-)

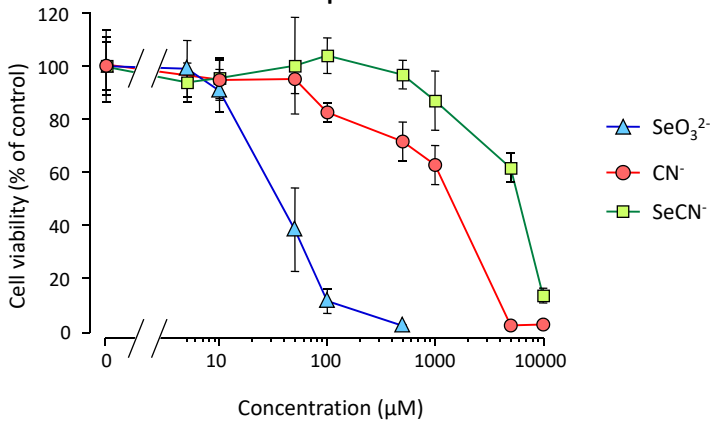
rhodanese (+)



Why is endogenous cyanide generated?

- To reduce the toxicity of selenite

Cytotoxicity of selenite (SeO_3^{2-}), cyanide (CN^-) and selenocyanate ($SeCN^-$) on HepG2 cells

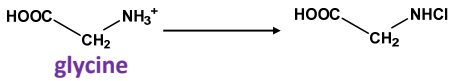


Cell viability was determined by MTT assay. Values are represented as means ± SD, $n = 4$

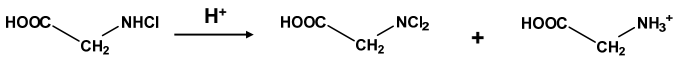
How is endogenous cyanide generated?

- Proposed mechanisms underlying the cyanide generation

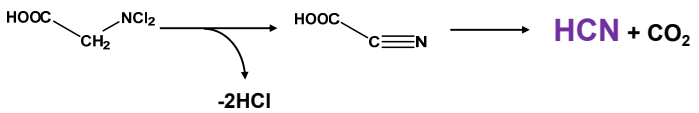
(A) *N*-chlorination by myeloperoxidase



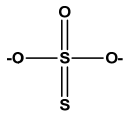
(B) acidic dismutation of *N*-chloroglycine



(C) Decomposition to nitrile and cyanide



Cipollone and Visca, *IUBMB Life* **59**, 187-189, 2007



Exogenous CN^- + thiosulfate —rhodanese→ thiocyanate (SCN^-)

Excess amount of Se (-II) ($GS\text{-}SeH$, HSe^-) + Endogenous CN^-

Reactive Cyanogen Species (RCNS)

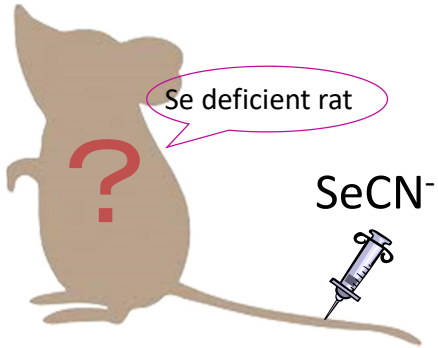
enzymatically → *utilization for selenoprotein synthesis, excretion into urine*

non-enzymatically → selenocyanate ($SeCN^-$) → ***detoxification of excess Se***

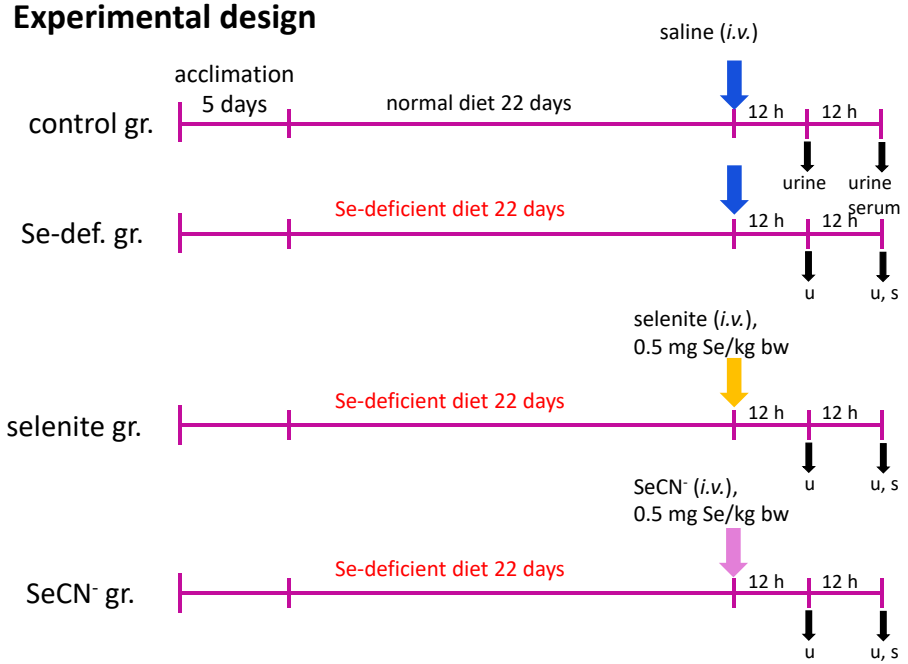
Y. Anan, M. Kimura, M. Hayashi, R. Koike and Y. Ogra: *Chem. Res. Toxicol*. (2015) **28**, 1803–1814
10.1021/acs.chemrestox.5b00254

Bioavailability, metabolism and toxicological effect of $SeCN^-$

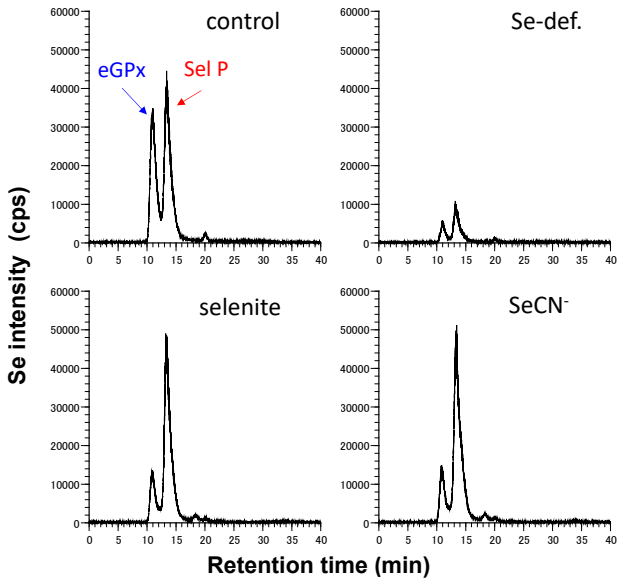
Animal experiment I



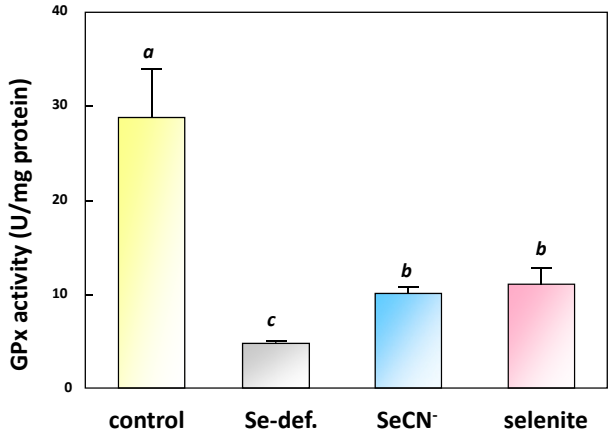
Experimental design



Elution profile of Se in the serum obtained by LC-ICP-MS

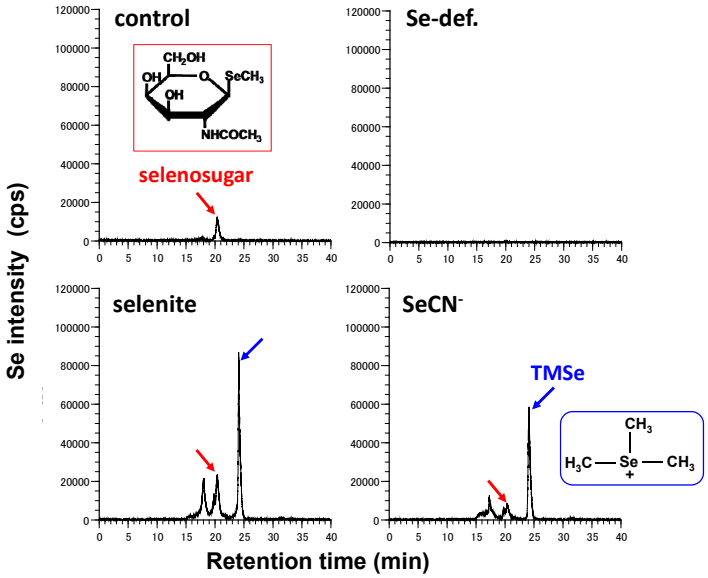


GPx activity in the serum



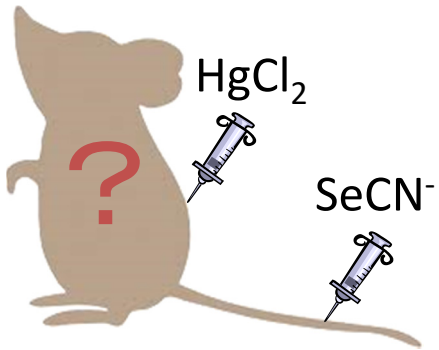
Bars marked by the different letters (***a***, ***b*** or ***c***) are significantly different among groups.

Elution profile of Se in the urine (12-24 h) obtained by LC-ICP-MS

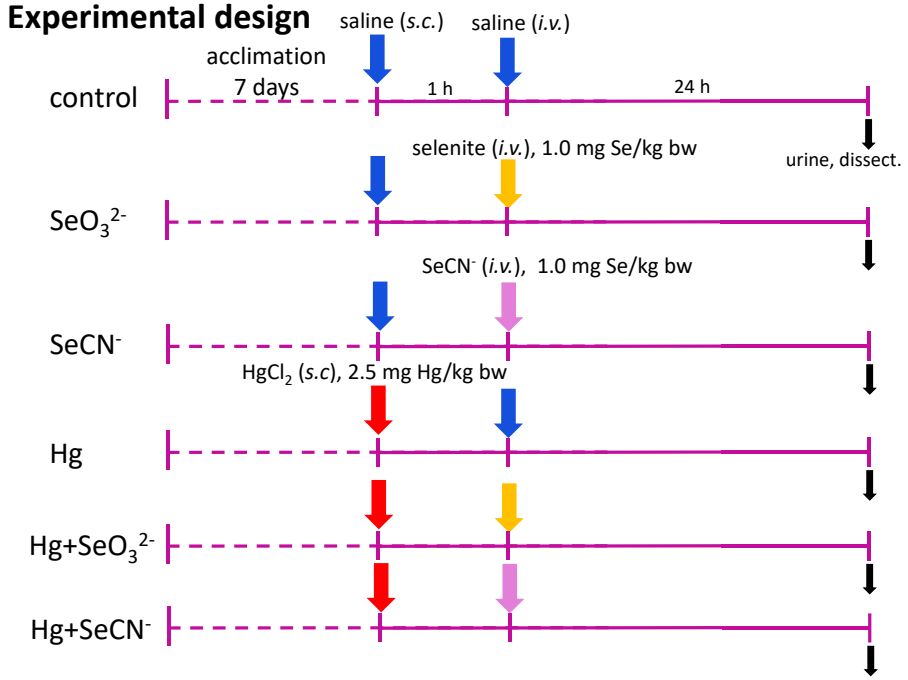


Bioavailability, metabolism and toxicological effect of $SeCN^-$

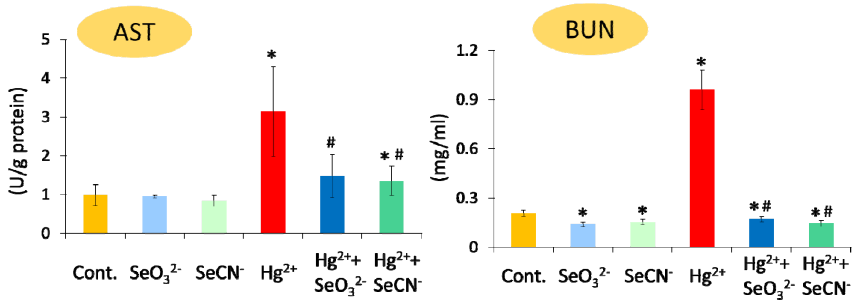
Animal experiment II



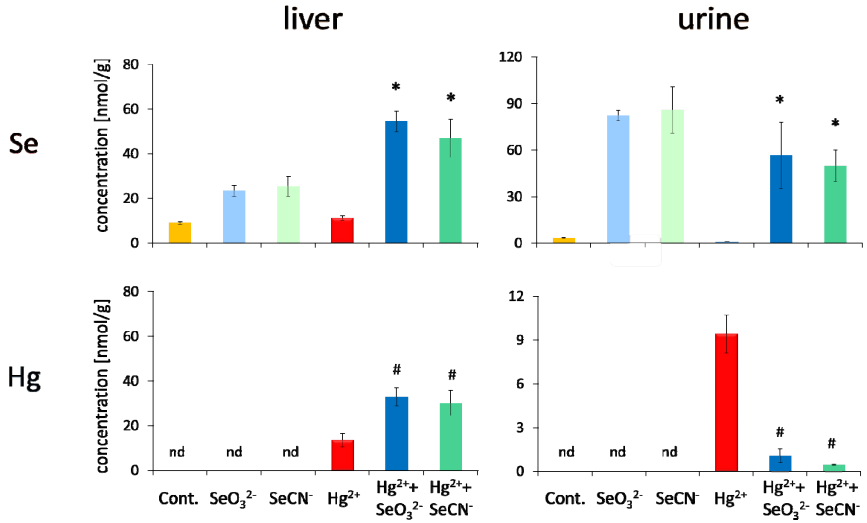
Experimental design



Hepato- and nephrotoxicity

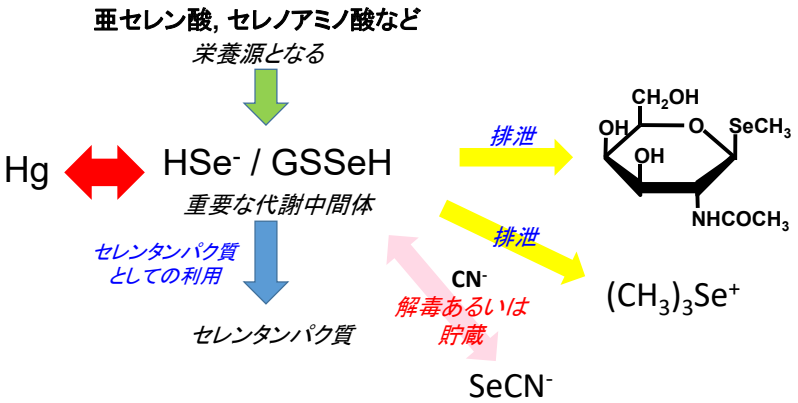


Effects of selenium compounds on AST and BUN.
Data are presented as means ± SD (n=4 - 6) . *p＜0.05 vs. the control group , #p＜0.05 vs. the Hg^{2+} group.
AST: asparate aminotrasferase
BUN: blood urea nitrogen



Concentrations of Se and Hg in the liver and urine.
Data are presented as means ± SD (n=4 - 6) . *p＜0.05 vs. SeO_3^{2-} or SeCN⁻ alone group , #p＜0.05 vs. the Hg^{2+} group. nd:not detected

結論



- 細胞内の過剰なセレンは、一時的に解毒・貯蔵されるが、その際にセレノシアン酸に変換される。
- セレノシアン酸の生合成過程は、非酵素的で“活性シアン種”の存在を想定させるものである。
- 化学形態分析により活性シアン種の想定に辿り着いたが、この毒性学的意義について、今後さらに研究を続ける必要がある。