様式３

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共同利用・共同研究拠点「化学汚染・沿岸環境研究拠点」
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下記の共同研究について、別紙の通り報告します。

１　研究課題

Screening and Quantification of Target and Non-Target Compounds in Human Samples from Zambia and Ghana

２　研究組織

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拠点対応教員 Kei Nomiyama	愛媛大学	准教授	

３　研究内容　（別紙）

別紙

研究課題名

Screening and Quantification of Target and Non-Target Compounds in Human Samples from Zambia and Ghana

研究目的

Over the last decades an increasing number of chemicals (xenobiotics) has been used in various applications and consequently been released to the environment. By an uptake through skin, food, water and air either through direct usage of consumer products or through the environment these chemicals can reach human tissues, and can be stored in tissues such as fat or blood before they can be metabolized and excreted via the urine. Hence human biomonitoring programs in developed countries monitor human body fluids such as urine and blood for a number of compounds and biomarkers. However, such screening analysis has never been done from developing countries, especially from Africa.

Pesticides are one of the important pollutants in Africa. Neonicotinoids (NNs) which were designed to specifically target insects` nicotinic Acetylcholine receptors (nAChRs) have been widely used in various pests management systems since the early 1990`s. However, recent reports have extensively highlighted on their potential binding affinity for the α4β2 subtype of the nAChRs which is predominantly expressed in the thalamus of the mammalian brain (Chen et al. 2014, Li et al. 2011). In mammals, the thalamus has been cited for its essential role in relaying sensory signals to the cerebral cortex and regulation of consciousness, sleep, and alertness. As such, alterations in the density of the α4β2 neuroreceptor subtype in the thalamus may lead to many central nervous system disorders in humans. In a recent report, Wang et al. (2017) extensively elaborated on the potency of NN and NN metabolites in damaging protein, DNA and lipids of vertebrates through oxidative stress mechanisms. Developing foetus have also been found to be highly susceptible to NN exposure through the blood-brain-barrier. The European Food Safety Authority (EFSA) even predicted a possible neurodevelopmental toxicity of some NNs (especially, acetamiprid and Imidacloprid) in infants. These reports trigger great concern about the budding toxicological impacts of NNs in humans and highlights the need to mount effective monitoring systems for NN exposure levels among various age groups within the society.

In Ghana, most farmers use synthetic pesticides in crop production, with the aim of reducing pre-harvest and post-harvest losses (Clarke et al., 1997). Currently, the Ghana

Cocoa Research Institute has regularised the use NNs (thiamethoxam and Imidacloprid) in cocoa farming (Owusu-Manu, 2001; Antwi-Agyakwa, 2015). The general population of Ghana may be exposed to various NNs as a result of pesticide mishandling, misapplications, and/or consumption of NN treated produce. The current study therefore, sought to evaluate the exposure levels of NNs among the residents of Kumasi, the second largest city Ghana.

研究内容

In this study, five native NN compounds (Imidacloprid, Clothianidin, Thiamethoxam, Nitenpyram and Dinotefuran) and one NN metabolite (*N*-desmethyl-acetamiprid) were detected among the subjects. The mean concentrations, detection frequencies and percentiles of the detected NNs have been presented in Table 1.

From the results, *N*-dm-acetamiprid, a major metabolite of acetamiprid was found with the highest detection frequency (92%) among the subjects (Table 1). This suggests that most residents of the city are often exposed to acetamiprid treated products. Meanwhile, imidacloprid was also detected in most (70.7%) of the subjects (Table 1). The mean and the maximum concentrations of imidacloprid obtained among the subjects of this study (3.41±24.07 µg/l and 211.62 µg/l respectively) far exceeded the levels of all the other detected NNs (Table 1). These suggest that the residents of the study area might be regularly exposed to high doses of imidacloprid than other NNs. Meanwhile, the mean concentration of imidacloprid recorded in this study (3.41±24.07 µg/l) was found to exceed its mean concentration reported among Japanese adults (1.54±2.7 µg/l) by two folds *(Ueyama et al., 2014*). Moreover, the peak concentration of imidacloprid recorded among the subjects from Kumasi (211.62 µg/l) was found to be more than 25 times higher than its maximum levels reported among Japanese adults (8.20 µg/l) by *Ueyama et al. (2014*).

Table 1: Detection frequencies and statistical summary of urinary NNs (µg/l) in residents of Kumasi (n = 76).

			Percentiles			
Neonicotinoids	**%DF**	**GM±STDEV**	**25th**	**50th**	**75th**	**100th**
Imidacloprid	70.67	3.41±24.07	BDL	0.14	0.97	211.62
Clothianidin	40.00	0.03±0.08	BDL	BDL	0.03	0.45
N-dm-Acetamiprid	92.00	0.82±1.46	0.16	0.42	0.81	10.65
Thiamethoxam	18.60	0.02±0.04	BDL	BDL	0.00	0.21
Nitenpyram	23.00	0.01±0.02	BDL	BDL	0.00	0.14
Dinotefuran	28.00	0.03±0.09	DBL	BDL	0.02	0.60

*%DF means percentage of detection frequency, GM±STDEV means geometric mean ±standard deviation, BDL means below detection limit.

Even though Clothiandin, Thiamethoxam, Nitenpyram and Dinotefuran were detected in less than 50% of the subjects, their cumulative levels were significant (Table 1). Most of the subjects (77.1%) were found to be exposed to two or more NNs at the same time (Fig. 1). This suggests that most residents of the study area might be exposed to multiple NN compounds, and as such may be susceptible to the synergistic impacts of NNs.

成果発表リスト

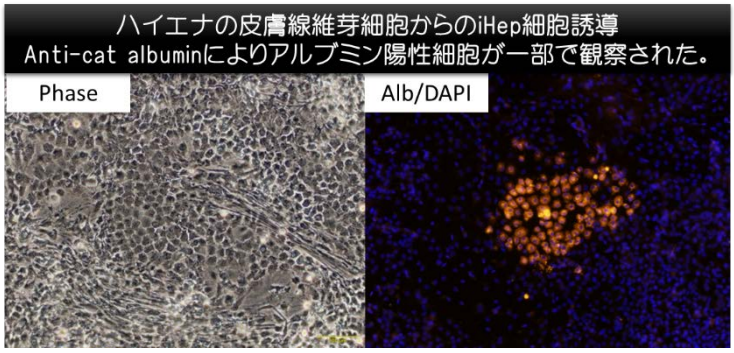
1：Yared B. Yohannes, Shouta M.M. Nakayama, Haruya Toyomaki, John Yabe, Kaampwe Muzandu, Andrew Kataba,Yoshinori Ikenaka, Hazuki Mizukawa, Russell Dowling, Jack Caravanos, Mayumi Ishizuka. A Pilot Study on Exposure of Lead (Pb) and Its Blood Level Burden in Children from Kabwe, Zambia: Implication to the General Population. 第26回環境化学討論会、2017年6月7日～9日、静岡県コンベンションアーツセンター「グランシップ」、静岡市、静岡県（口頭発表）

2：Collins Nimako, Yoshinori Ikenaka, Osei Akoto, Mayumi Ishizuka, Nester Bortey-Sam、Quantification of Urinary Neonicotinoids in Patients of three Hospitals in Kumasi, Ghana、9th International Toxicology Symposium in Nigeria、2017年9月7日～8日、University of Benin, Benin, Nigeria（口頭発表、受賞)

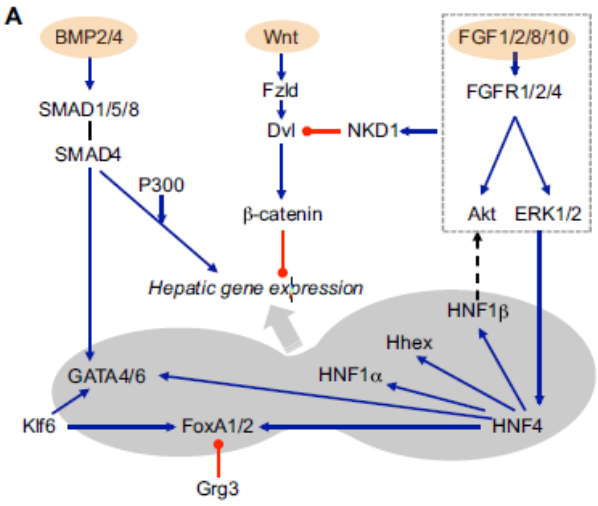
3：Yared Beyene Yohannes, John Yabe, Shouta M.M. Nakayama, Haruya Totomaki, Kaampwe Muzandu, Yoshinori Ikenaka, Hazuki Mizukawa, Russell Dowling, Jack Caravanos, Hokuto Nakata, Mayumi Ishizuka, Elevated Blood Lead Levels among Children, Infants and Mothers in Kabwe, Zambia, SETAC North America 38th Annual Meeting, 11-16 November 2017, Minneapolis Convention Center, Minneapolis, Minnesota, USA (Oral)

研究内容

① iHep 細胞への誘導の試み



当該研究では、食肉目として、ハイエナに注目した。ハイエナの皮膚線維芽細胞に鈴木因子である Hnf4α と Foxa (Foxa1, Foxa2, Foxa3 のいずれか一つ)を導入した。その結果、肝臓マーカーであるアルブミンや E-cadherin の顕著な増加を確認できた(上図)。しかし、誘導効率が低い事や cytochrome P450 や UGT 等、異物代謝に関与する酵素群の発現量が低い事などが改めて浮き彫りとなった。



線維芽細胞から肝細胞への誘導に、今回は鈴木因子を用いた。一方、最近の報告では HNF や Foxa 以外にも、肝臓の初期発生にはいくつかの重要な Key factor が報告され始めている(右図)。また、これらの因子には種差があり、例えば、マウスの肝臓への分化誘導には HNF4α が重要であるが、ヒトでは HNF1α が重要であると言った知見である。

今後、種を超えた iHep 細胞への誘導を考慮すると、発生学的に各種でどのような因子が重要であるのか、明らかにしていく必要がある。また、これら遺伝的因子に依存しない誘導法の確立も重要になる。Chemical を用いた誘導法もその一つであり、今後はこれらの組み合わせにより“種共通”の分化因子を探索していく予定である。

② 食肉目の硫酸抱合活性

遺伝子データベースの解析より SULT 分子種の遺伝子は 1 ファミリーに属する分子種として食肉目で 1A1, 1B1, 1C1, 1C2, 1C4, 1D1, 1E1 の存在が明らかとなった。

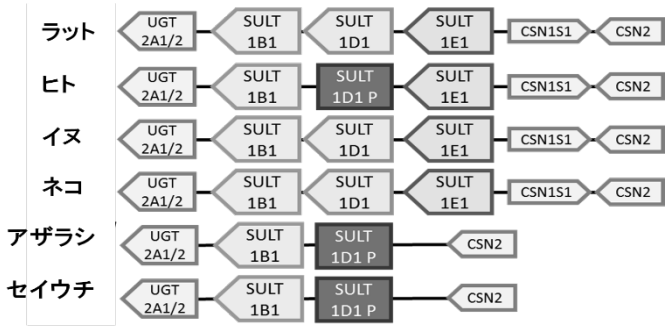


図 1：SULT1 ファミリーの遺伝子コード領域の一部

遺伝子コード領域の比較検討の結果、ヒトとほとんどの食肉目動物において SULT1B1, 1D1, 1E1 の 3 分子種は UGT2A1/2 遺伝子と CSN1S1 遺伝子の間にコードしていることが明らかとなった。しかし、鰭脚類のアザラシとセイウチでは UGT2A1/2 遺伝子と CSN2 遺伝子の間にコードされ、SULT1E1 分子種の欠損が示唆された(図 1)。

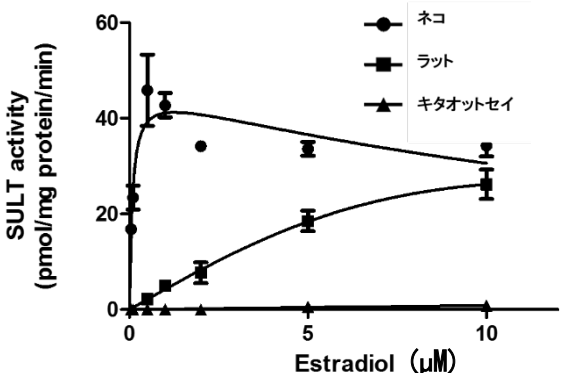


図 2：SULT1E1 活性の Michaelis-Menten 図

更に、*in vitro* にて SULT1E1 特異的な基質を用いた活性試験の結果、ネコ、およびラットと比較して鰭脚類のキタオットセイでは非常に低い *Vmax* および *Vmax/Km*、更には非常に高い *Km* 値が見受けられた(図２)。これらのことから鰭脚類では SULT1E1 分子種の活性が非常に低く、機能的な役割を持っていないことが示唆される。SULT1E1 分子種は Bisphenol A や Hydroxy-polychrorinated biphenyls 等の環境化学物質の代謝に重要な酵素である一方、内因性物質のエストロゲン代謝を担う酵素群である。エストロゲン代謝は UGT と SULT で相補的に担われているが、ネコでは SULT 活性が比較的高く、主に SULT を用いた代謝が行われている可能性が考えられた。一方鰭脚類では UGT と SULT 共にエストロゲンに対する活性が低い。そのため鰭脚類においては代償的な代謝経路を含む、その他のエストロゲン代謝経路に大きな種差がある可能性が示唆された。

成果発表リスト

1：Kondo T, Ikenaka Y, Nakayama SMM, Kawai YK, Mizukawa H, Mitani Y, Nomiyama K, Tanabe S,　Ishizuka M. UDP-glucuronosyltransferase (UGT) 2B subfamily interspecies differences in carnivores. Tox. Sci. 158: 90-100 (2017) DOI: 10.1093/toxsci/kfx072

2：Saengtienchai A, Ikenaka Y, Kawata M, Kawai Y, Takeda K, Kondo T, Bortey-Sam N, Nakayama SMM, Mizukawa H, Ishizuka M. Comparizon of xenobiotic metabolism in phase I oxidation and phase II conjugation between rats and bird species. Comparative Biochemistry and Physiology, part C (accepted)