Accumulation of persistent organochlorine pollutants and polybrominated diphenyl ether in wild rats, and toxicogenomic analyses of their effects

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Introduction

Norway or Brown rats (Rattus norvegicus) inhabit large areas of the world, especially close to human populations. They are known to eat birds, small fish, insects, plant and garbage from dumps. Especially, Brown rats feed on human-generated waste, thus exposure scenarios are sometimes similar to those of humans. Therefore, analysis of environmental chemicals in Brown rats may be considered as indirect measure of toxic contaminants in humans. The Brown rat is also a useful indicator for the effects of environmental contamination on terrestrial wildlife due to their position in the food-chain.

Chlorinated/brominated persistent toxic substances (PTS) are ubiquitous contaminants in the environment. Due to the high lipophilicity/resistance to biological degradation, wildlife animals and humans accumulate notable levels of them through food chain. In a previous study, we analyzed several chlorinated and brominated organic contaminants such as PCDDs, PCDFs, PCBs, DDTs, HCHs, chlordane compounds, HCB, cyclodienes and PBDEs, in wild Brown rats collected from urban, rural, waste dumping or land fill sites as well as isolated remote island in Japan. In that study we found greater accumulation of PCB in liver extracts of Brown rats from urban areas in Japan. In the present study, additional analysis of PCDDs, PCDFs and co-planar PCBs in individual Brown rat were performed by isotope dilution technique using HRGC-HRMS. Furthermore, we examined the effects of these contaminants on testis and liver mRNA expression profiles using GeneChip technology.

Materials and Methods

Sampling and Analysis

Brown rat (Rattus norvegicus) are considered to be a bio-indicator species because they are one of the most common animals that encountered around the world. They live both as a commensal in close association with man. Consequently, in this study, Brown rat (several individuals in each location) were collected and determined their age and gender. In this study 8-49 week-old male rats were used for chemical analysis. For each location, a maximum of 3 extracts was combined for organochlorine pesticides, PCBs and PBDEs analyses. For the analyses of PCDF, PCDD and co-planar PCBs, we used individual samples (n=1-3). The laboratory Wistar rats (age of 7 weeks) were used as non-contaminated control animals. Sampling locations selected in this study were, two highly urbanized localities of Tokyo (Shinjuku "Tokyo-Snj" and Ikebukuro "Tokyo-Ikb"), Osaka (Umeda "Osaka-Umd" and Nanba "Osaka-Nnb"), waste dumping or landfill site of Hokkaido, "Landfill" and remote Teuri Island, Hokkaido "Teuri-Is-Hk" in 2003. In addition, 3 laboratory raised Wistar rat (WR) used as control animals that analyzed individually. All the animals were dissected, liver was removed and stored frozen until analysis. The homogenized liver was spiked with internal standards such as $^{13}C_{6}$-$_{12}$-labeled OCPs, PCDDs, PCDFs, PCBs and PBDEs, homogenated with Na_2SO_4, Soxhlet extracted with dichloromethane (DCM) for 16-h, cleaned up. The identification and quantification was performed using high resolution gas chromatography -high resolution mass spectrometry (HRGC-HRMS). Two methods blank sample analyzed contain less than detection limit of any target analytes.

For DNA microarray analysis, total RNA was isolated from liver or testis of laboratory and wild rats, and was purified to mRNA. cRNA probes were generated from total RNA samples using the Message-AmpTM cRNA kit (Ambion Inc., Austin, TX). Biotinylated cRNA probes were hybridized to Rat Expression 230 Array and Rat Genome 230 Array chips using the Affymetrix Fluidics Station 400 according to the manufacturer's standard protocol. The image data on each individual microarray chip was scaled to 250 target intensity, using the Microarray Suite software (Affymetrix, Santa Clara, CA).

Results and Discussion

The average of pg/g lipid based WHO-TEQ concentrations were in the decreasing order of 7,800 (Tokyo-Snj), 1,900 (Osaka-Nnb), 1,300 (Tokyo-Ikb and landfill), 500 (Osaka-Umd), 310 (Teuri-Is-Hk) and 21 (Wistar rat). Except for Tokyo-Snj rats, PCDD/DFs were greater TEQ contributors in other places as well as in control Wistar rats (Table 1). One of the Tokyo-Snj Brown male rats showed elevated concentration of 21,000 pg-TEQ/g lipid due to high PCB levels. Exception with the highest TEQ value in Tokyo-Snj rat, the hepatic TEQ concentrations in wild Brown rats showed a tendency to depend on their age (R=0.52, p=0.05).

Table1. TEQ concentrations (pg / g lipid) of PCDDs, PCDFs and co-planar PCBs in liver of wild and laboratory Brown rats

	Lab. Contol	Teuri-Is-Hk	Landfill	Tokyo-Snj	Tokyo-Ikb	Osaka-Umd	Osaka-Nnb
TEQ pg/g lipid	N=3	N=1	N=3	N=3	N=3	N=3	N=3
PCDF	7	130	790	1300	680	300	1300
	(5.9- 7.9)	(130)	(320- 1300)	(150- 3200)	(380- 1000)	(260- 280)	(430- 2300)
PCDD	8.1	66	270	880	230	86	310
	(7.2- 8.6)	(66)	(130- 350)	(55- 120)	(150- 270)	(71- 83)	(160- 560)
PCDD/F	15	200	1100	2100	910	390	1600
Non-ortho PCB	4.7	110	210	5500	380	110	310
	(3.0- 6.4)	(110)	(190- 210)	(53- 16000	(270- 350)	(82-110)	(200-420)
Mono-ortho PCB	0.13	1.4	11	130)	6.6	2.3	6.5
	(0.15-0.22)	(1.4)	(4.7- 14)	(2.1- 362)	(4.6-7.7)	(1.7-2.7)	(5.2-6.6)
Total TEQ	21	310	1300	7800	1300	500	1900
	(16- 23)	(310)	(640- 1900)	(260- 21000)	(890- 1600)	(440- 470)	(880- 3300)

From the results of GeneChip experiments and the pathway analyses, the mRNA expression levels of cytokine, vitamin or steroid hormone metabolizing enzymes altered in wild Brown rat livers compared with those of control rats. The expression levels of stress protein, such as HO-1 (heme oxigenase 1), metallothionein, HSP 70 (heat shock protein 70), were raised in wild rat livers. The expression of phase I enzymes, e.g., cytochrome (CYP) 1A1, CYP2A1, NADPH: Quinone Oxidoreductase-1 were also induced in wild rats. However, we did not find any marked inductions of phase II enzyme expressions. The combined mRNA mixture of three testes from Tokyo-Snj rats showed the reduced expression of testosterone synthesis enzymes and relating binding protein genes, e.g., StAR, P450scc, CYP17, HSD (hydroxysteroid dehydrogenase) 3beta, HSD17beta, CYP19. The Tokyo rat, which had the highest TEQ values, showed drastic suppression of testosterone concentration in blood.