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Environmental toxicants impair liver and kidney function and sperm quality of captive pandas

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ABSTRACT

Captive pandas are exposed to higher concentrations of environmental toxins in their food source and from atmospheric pollution than wild pandas. Moreover, the Qinling panda subspecies had significantly higher concentrations of toxic chemicals in its feces. To determine whether these toxicants also accumulate in panda's blood and impair its health, concentrations of persistent organic pollutants (POPs) and heavy metals were measured in blood samples. Four heavy metals (As, Cd, Cr and Pb), PCDD/Fs and PCBs were detected in blood drawn from captive Qinling pandas. Time spent in captivity was a better predictor of toxicant concentration accumulation than was panda age. More than 50% of the studied pandas were outside the normal levels for 11 health parameters, and five (ALT, LDH, Ca, Cl, TB) of the 11 parameters classified as abnormal were correlated with blood pollutant concentrations. The proportion of live sperm was significantly lower and the aberrance ratio of sperm was significantly greater for captive pandas than for wild ones. A short-term solution to reduce the health impacts of pollution and toxicant exposure of Qinling pandas is to relocate breeding centers to less contaminated areas and to strictly control the quality of their food provided. A longer term solution depends on improving air quality by reducing toxic emissions.

1. Introduction

The giant panda (*Ailuropoda melanoleuca*) is one of the most endangered animals in the world, and it is recognized worldwide as a symbol for conservation. Conservation zones and captive breeding centers have been established to protect giant pandas, but our previous work has shown that conservation efforts are being compromised by increasing levels of pollution associated with China's rapid industrialization and urbanization (Chen et al., 2016, 2017). Both the Sichuan and Qinling subspecies of giant panda are exposed to high concentrations of polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs), polychlorinated biphenyls (PCBs), chromium (Cr), cadmium (Cd), arsenic (As), and lead (Pb), and captive pandas are exposed to higher concentrations of pollutants than wild pandas (Chen et al., 2016). Moreover, droppings from the Qinling subspecies contain significantly higher concentrations of As, Cd, and Pb than those from the Sichuan subspecies (Chen et al., 2016).

Recently issued data from State Forestry Administration (SFA) showed that there are only 345 remaining individuals of the Qinling subspecies of the giant panda (SFA, 2015), and it is a higher priority for conservation than the Sichuan subspecies. The Shaanxi Wild Animal Research Center (SWARC) (Louguantai, Zhouzhi County, Xi'an city, 34°04′N, 108°19′E) is the only center in China focused on conservation of the Qinling subspecies. There, environmental pollutants (e.g., PCDD/Fs, PCBs, Cd, Cr, As, and Pb) are present in high concentrations in the food fed to the captive pandas (Chen et al., 2016) and atmospheric deposition was the most likely origin of heavy metals and persistent organic pollutants (POPs) in the diets of captive and wild Qinling pandas (Chen et al., 2016).

Persistent organic pollutants and heavy metals are persistent hazardous toxicants that may be transported over long distances in air and water. Both humans and wild animals are vulnerable to these toxicants. For example, PCDD/Fs are associated with developmental toxicity, immunotoxicity, and reproductive toxicity in humans and other animals (Lohmann et al., 2007; Sfriso et al., 2014; Fernandez-Rodriguez et al., 2015). Similarly, PCBs and their breakdown products are known endocrine disrupters, cause the loss of renal cell viability, and are associated with increased risk of chloracne, goiter, anemia, and cancer (Qiu,

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2013; Sfriso et al., 2014; Eqani et al., 2015; Fernandez-Rodriguez et al., 2015; Gustavson et al., 2015). Heavy-metal exposure has been linked with increased incidence of cancer (Cr and As), nephrotoxicity and bone damage (Cd), and reduced reproductive function (Pb) (Neal and Guilarte, 2013; Brahmia et al., 2013; Uddh-Söderberg et al., 2015).

However, little is known about whether POPs (PCDD/Fs and PCBs) and heavy metals (As, Cd, Cr, and Pb) accumulate in the blood of captive pandas, and if they do, whether these pollutants present a health risk to these animals. To answer these questions, blood samples of Qinling pandas at SWARC were taken and analyzed for toxicants. We then examined relationships between toxicant concentrations and time spent in captivity, and panda health as assessed by hematological and biochemical parameters, and by analysis of sperm quality.

2. Materials and methods

All blood samples were collected from captive Qinling pandas housed in the Shaanxi Wild Animal Research Center ("SWARC": 34°06′N, 108°32′E). SWARC is located in Louguantai, Zhouzhi County, Xi'an city. It was established in 1987 and is the only center in the world for conservation of the Qinling subspecies of the giant panda.

Pandas were anesthetized with 25% ketamine at a dose of 8 mg/kg of panda body mass. Blood samples, which were residuals from physical examinations of the individual pandas at SWARC, were collected from the jugular vein of each of fifteen pandas ranging from 4 to 21 years of age that had been in captivity for 3–20 years. Blood samples were placed in EDTA tubes for hematological analysis of heavy metals, PCDD/Fs, and PCBs, and into serum tubes for biochemical analysis. Fresh blood samples were digested and analyzed using standard methods, usually within 1 h of collection.

2.1. Heavy metal analysis

500-mL blood samples were placed into Teflon bombs to which were added 5 mL of HNO_3 for digestion with a microwave system (CEM, Mars 6, CEM, USA). After digestion, samples were diluted to 50 mL with deionized water. Concentration of Cr was measured using the air–-acetylene flame method (AAS; ZEEnit 700P, Analytik, Jena, Germany) with electrically modulated deuterium–HCl background correction. The hydride-forming element As was measured using the HS55 Hydride System (AAS; ZEEnit 700P, Analytik, Jena, Germany). Concentrations of Cd and Pb were measured using a graphite furnace AAS coupled to a MPE 60 (ZEEnit 700 P, Analytik, Jena, Germany) graphite autosampler with two-field mode Zeeman effect background correction. Heavy metal concentrations are expressed as ng/g blood (Chen et, 2017).

2.2. Analysis of PCDDs, PCDFs, and PCBs

Blood samples (volume = 10 mL) were freeze-dried before being spiked with ^{13}C-labeled surrogate standards (Environmental Protection Agency [EPA] method 1613B and 1668A) and undergoing accelerated solvent extraction (ASE350; Thermo, MA, USA) with dichloromethane: hexane (1:1). After determining the lipid content of each sample, the extract was adjusted to 50 mL with hexane; 15 g of acid silica (30% w/w) (44 g H_2SO_4 (98%, GR; Sinopharm, China) + 100 g Silica gel) was added to remove lipids. The acid silica was stirred for 2 h and the extract was poured through 5 g of anhydrous sodium sulfate (Sigma-Aldrich; St. Louis, MO, USA). All the extracts were concentrated to 2 mL by rotary evaporation.

All solvents were purchased from Fisher Scientific (Fairlawn, NJ, USA). Silica gel was obtained from Merck (silica gel 60; Darmstadt, Germany). Basic alumina was obtained from Aldrich (Brockmann I, standard grade; Milwaukee, USA). Florisil was obtained from Riedel-de Haën (60–100 mesh ASTM; Seelze, Germany). Calibration standard solutions, ^{13}C-12-labeled surrogate standards, and ^{13}C-12-labeled injection standards were purchased from Wellington Laboratories (Guelph, Canada).

PCBs, PCDDs, and PCDFs were analyzed at the POP laboratory of the Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences (RCEES-CAS, Beijing, China); all concentrations were corrected for lipid weight. Sample extraction, cleanup, and chemical analysis followed established methods with some modifications (Liu et al., 2006, 2008). 12 dioxin-like PCB congeners were quantified by an isotope dilution method using high-resolution gas chromatography coupled with high-resolution mass spectrometry (HRGC/HRMS) (Liu et al., 2006, 2008). Total organic carbon (TOC) concentration was analyzed on a TOC Analyzer (OI Analyzer; College Station, TX, USA). A 0.1-g sample was weighed and loaded into the combustion cup, which was packed with quartz wool. Prior to combustion, the samples were wetted with 5% phosphoric acid and heated to 250 °C for 1 min to purge inorganic carbon. The signal was detected by non-dispersed infrared (NDIR) detection when flashed at 900 °C for 6 min in the combustion chamber.

The quantification of 17 PCDD/Fs homologues was done using HRGC/HRMS on an Agilent 6890 gas chromatograph coupled with an Autospec Ultima mass spectrometer (Waters Micromass, Manchester, UK) operating in the EI (Electron Impact Ionization) mode at 35 eV; the trap current was 600 IA. The GC was equipped with a CTC PAL autosampler. One- or two-mL samples were injected in splitless mode (splitless time = 2 min for PCDD/Fs) in a DB-5MS fused silica capillary column (60 m for PCDD/Fs and PCBs) with helium as carrier gas at a constant flow rate of 1.2 mL/min. The oven temperature programs were as follows: for PCDD/Fs, start 150 °C held for 3 min, 150–230 °C at 20 °C min^{-1} held for 18 min, 230–235 °C at 5 °C min^{-1} held for 10 min, 235–320 °C at 4 °C min^{-1} held for 3 min; for PCBs, start 120 °C held for 1 min, 120–150 °C at 30 °C min^{-1}, 150–300 °C at 2.5 °C min^{-1} held for 1 min.

2.3. Quality control and quality assurance

All data were subject to quality control and quality assurance. All glassware was washed two times with distilled water, and then with dichloromethane after use. After washing, glassware was dried for 6 h at 400 °C in a muffle furnace. All performance criteria required for the analysis of PCBs and PCDD/Fs followed US EPA methods (1668A and 1613B).^{13}C-labeled surrogated standards (1668A-LCS and 1613-LCS) were spiked in the sample for qualification and quantification, and ^{13}C-labeled injection standards (EPA 68A-IS and 1613-IS) were added for recovery calculation. The recoveries of the surrogate standards ranged from 76.7 ± 25.2% and 49.2 ± 13.6% for PCB sand PCDD/Fs, respectively, which met the requirements of US EPA methods 1668A and1613B. Limit of detection (LOD) in the sample was defined as a signal to noise (S/N) ratio = 3. The LOD values were in the range of 0.01–0.82 pg/g for PCBs and 0.04–8.40 pg/g for PCDD/Fs. Laboratory blanks were analyzed with samples quality control at set intervals, and there was no detection of target compounds in the blanks.

2.4. Hematological and biochemical analysis

Blood samples for serum were centrifuged at 2000 rpm, usually within 1 h of collection, and the serum was removed and stored at −20 °C. The hematology and serum biochemistry of samples were analyzed at the Shaanxi Sengong Hospital, Xi'an City. Counts or concentration of white blood cells (WBC: ± 0.1 × 10^9 /L), red blood cells (RBC: ± 0.5 × 10^{12} /L), hemoglobin (HGB: ± 2 g/L), platelets (PLT: ± 10 × 10^3 /μL) and lymphocytes (± 0.5% LYM) were determined using a Micro 60 Hematology Analyzer (Horiba ABX, Montpellier, France; guaranteed cv of measurements 0.5–5%).

Total protein (TP: ± 1 g/L), globulin (GLB: ± 0.5 g/L), albumin (ALB: ± 0.5 g/L), sodium (Na: ± 2.5 mmol/L), potassium (K: ± 0.025 mmol/L), chlorine (Cl: ± 1 mmol/L), calcium (Ca: ± 0.01 mmol/L), glucose (GLU: ± 0.025 mmol/L), alanine