Comparative Study on Toxicokinetics of Bisphenol A in F344 Rats, Monkeys (Macaca fascicularis), and Chimpanzees (Pan troglodytes)

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Abstract: We compared the toxicokinetics of bisphenol A (BPA) among three animal species: rats, cynomolgus monkeys and chimpanzees. Rats and monkeys were administered BPA orally or subcutaneously at 10 or 100 mg/kg body weight, while chimpanzees were administered only 10 mg/kg of BPA. BPA in serum was measured by ELISA. In oral administration of BPA at 10 mg/kg, both Cmax and AUC were rats < chimpanzee < monkeys. In oral administration of BPA at 100 mg/kg, both Cmax and AUC were rats < monkeys. Subcutaneous BPA administrations also revealed similar results, although the values of toxicokinetic parameters in subcutaneous administration were higher than those in oral administration. These results suggest that orally or subcutaneously administered BPA in primates is more easily absorbed than that in rats. We conclude that there are considerable differences in distribution, metabolism, and excretion of BPA between rodents and primates.

Key words: bisphenol A, chimpanzee, cynomolgus monkey

Recently, there is an increasing concern about the risk of human exposure to bisphenol A (4,4'-isopropylidene-2-diphenol, BPA) which is a volume chemical used in the manufacture of polycarbonate plastics. BPA is known to have various hormone disrupting effects, such as estrogenic [4], anti-androgenic [14], and anti-thyroid activities [8]. Studies using rodents suggested that perinatal exposure to BPA results in abnormalities in reproductive function [16], hormonal function [11], central nervous system development [5, 12] and behavior [2, 9].

In toxicological studies, rodents, especially rats, are the most popular experimental animals. Considering human risk, however, primates would be more useful...
because of their similarities to humans in their physiological characteristics. Chimpanzees, our closest relative, are considered to be the best models for humans among the non-human primates. Although there are a number of reports on the bioavailability of BPA in rats [6, 10, 15, 17] and monkeys [7], we tried for the first time to compare the toxicokinetics of BPA among three species, i.e., rats, cynomolgus monkeys and chimpanzees, to consider the human risk of BPA exposure. BPA was administered to animals of three species orally or subcutaneously at low (10 mg/kg) or high (100 mg/kg) doses to evaluate and compare dependency on dose as well as route of BPA administration among the three species.

Seventy-eight female F344/N rats (140–150 g body weight) were purchased from SLC (Shizuoka, Japan) and used for administrations of BPA. Collections of whole blood and serum preparations (see below) in rats were performed at the University of Tokyo (Tokyo, Japan). BPA administrations, collections of whole blood, and serum preparations in six female cynomolgus monkeys (Macaca fascicularis) (4.0 to 5.0 kg body weight), and the two female Western chimpanzees (Pan troglodytes verus) (40 to 50 kg body weight), were performed at Shin Nippon Biomedical Laboratory (Kagoshima, Japan) and Sanwa Kagaku Kenkyusho (Kumamoto, Japan), respectively. BPA (Tokyo Kasei Kogyo, Tokyo, Japan) was first dissolved in distilled water with 0.5% CM-cellulose (Wako Pure Chemical, Osaka, Japan) for oral administrations, and in a mixture of dimethylacetamide (Wako) and polyethylene glycol (Wako) (1 : 1) for subcutaneous administrations. BPA at 10 mg/kg or 100 mg/kg was administered to rats and monkeys by oral gavage or dorsal subcutaneous injection, while only 10 mg/kg BPA was administered to chimpanzees by the same ways as rats and monkeys for the ethical reason of avoiding any acute adverse effect at the higher dose of BPA (100 mg/kg). This study was approved by the Animal Care and Use Committee of the Graduate School of Agricultural and Life Sciences, the University of Tokyo. Rats were euthanatized by drawing whole blood under diethylether anesthesia before and 0.5, 1, 2, 4, 6, and 24 h after BPA administration (n=3 at each point). Monkeys (n=3 for 10 mg/kg and n=3 for 100 mg/kg) and chimpanzees (n=2 for 10 mg/kg) were first orally administered BPA at the two dosages and low dose (10 mg/kg), respectively. After one week for entire excretion of BPA [3], the above dosages were repeated subcutaneously. Serial blood samples were taken from each monkey before and 0.5, 1, 2, 4, 6, and 24 h after administration or from each chimpanzee before and 0.25, 0.5, 1, 2, 3, 4, 8, and 24 h after administration. Serum samples were harvested by centrifugation at 3,000 g for 15 min and were kept at −20°C until analysis. BPA in serum was measured by BPA ELISA kit (Japan EnviroChemicals, Japan). Methanol was first added to the serum (1:5, methanol: serum), and then the sample was centrifuged at 10,000 g for 15 min. Supernatant was diluted with the same volume of purified water. Prepared samples were subjected to ELISA according to product manuals. Briefly, the sample and the antigen (BPA)-enzyme complex solution were mixed and added to each microplate well, the inside of which was coated with the BPA-specific antibody. After 60 min competitive assay, unbound or excess reagents were washed out and substrate chromogen was added to each well to develop the color. The optical density at 490 nm was measured to determine the amount of BPA in the sample. In the present method, the detection limit of BPA in serum was 12.5 µg/L. Toxicokinetic parameters were determined from the individual serum BPA concentration-time curves. Peak serum concentrations (C_max) and the time to reach C_max (T_max) were obtained from observed data. The area under the serum BPA concentration-time curves for 4 h (AUC_{0–4 h}) and for 24 h (AUC_{0–24 h}) were calculated by the linear trapezoidal method.

The concentration-time profiles of oral BPA administration at 10 mg/kg in rats, monkeys and chimpanzees, and those at 100 mg/kg in rats and monkeys are shown in Figs. 1A and B, respectively. These profiles indicate lower BPA bioavailability in rats compared to that in both monkeys and chimpanzees. Indeed, no sample of rats contained detectable levels of BPA except for one (33 µg/L) of three samples at 2 h after oral BPA administration at 10 mg/kg, thus C_max, AUC_{0–4 h} and AUC_{0–24 h} could not be calculated (Table 1), and T_max was not defined. In oral administration of BPA at 10 mg/kg, C_max, AUC_{0–4 h}, and AUC_{0–24 h} showed the same tendency, that is, rats < chimpanzees < monkeys (Table 1). In oral administration of BPA at 100 mg/kg, C_max, AUC_{0–4 h}, and AUC_{0–24 h} were all rats < monkeys (Table 1). Subcutaneous BPA administrations at 10 or 100
mg/kg showed similar results, although the values of toxicokinetic parameters were higher than those in oral administrations at each dose (Fig. 2 and Table 1).

We demonstrated the direct comparison of toxicokinetics of oral or subcutaneous administration of BPA among F344 rats, cynomolgus monkeys, and Western chimpanzees. Oral administration resulted in lower availability of BPA when compared to subcutaneous administration in all species examined in this study, suggesting a route dependency common to mammals, which is consistent with a previous report [10]. Fast-pass metabolism by the intestine and/or liver as well as intestinal secretion probably contributed to the lower bioavailability of orally administered BPA. We also observed clear dose dependency in toxicokinetic parameters between 10 and 100 mg/kg administrations in rats and monkeys as expected. Irrespective of route of administration, bioavailabilities of BPA at 10 or 100 mg/kg were rats < chimpanzees < monkeys, or rats < monkeys, respectively, in this study. These results suggest that either orally or subcutaneously administered BPA in primates is more easily absorbed than in rats. This might be due to the differences in the abilities of metabolism and/or excretion of BPA between rats and monkeys. Further researches about the species differences in alteration of hepatic and intestinal enzyme expression and function accompanying BPA disposi-

Fig. 1. Serum levels of bisphenol A after oral administration at 10 mg/kg in female rats, monkeys and chimpanzees (A), and at 100 mg/kg in rats and monkeys (B). Data represent the mean ± SEM of three rats and three monkeys. Data of chimpanzees are indicated as plots from each subject (chimpanzee #1 and #2). No error bar in rats or monkeys indicates that the error is included within the symbol. Plots in gray area mean that serum BPA concentration is under the detection limit in this study.

Fig. 2. Serum levels of bisphenol A after subcutaneous administration at 10 mg/kg in female rats, monkeys and chimpanzees (A), and at 100 mg/kg in rats and monkeys (B). Data represent the mean ± SEM of three rats and three monkeys. See also the figure legend of Fig. 1.
tion, such as CYP450 and UDP-glucuronosyl transferase (UDPGT), among rodents and primates are important, since BPA is metabolized principally to its monoglucuronide conjugate and is excreted via feces or urine [13]. It is possible that humans might also absorb BPA more easily than experimental rats. Both behavioral and neurological alterations in rodents by perinatal low-dose BPA exposure per os even at < 100 µg/kg/day [1] would strongly suggest potential adverse effects of chronic environmental exposure to BPA in humans (< 10 µg/kg/day), if the differences in bioavailability between rodents and primates including humans also applies to much lower dose exposure to BPA. We conclude that there are considerable differences in distribution, metabolism, and excretion of BPA between rodents and primates.

### Table 1. Toxicokinetic parameters in rats, monkeys, and chimpanzees after oral or subcutaneous injection of BPA at 10 or 100 mg/kg

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<tr>
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<th>10 mg/kg BPA</th>
<th>100 mg/kg BPA</th>
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<tr>
<td></td>
<td>Rats (n=3)a) (mean±SD)</td>
<td>Monkeys (n=3) (mean±SD)</td>
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<tr>
<td>Oral injection</td>
<td></td>
<td></td>
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<tr>
<td>Cmax (µg/L)</td>
<td>N.C. b)</td>
<td>2,793 ± 920</td>
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<tr>
<td>Tmax (h)</td>
<td>N.D. c)</td>
<td>0.7 ± 0.2</td>
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<tr>
<td>AUC₀–₄h (µg·L⁻¹·h)</td>
<td>N.C.</td>
<td>3,209 ± 536</td>
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<tr>
<td>AUC₀–2₄h (µg·L⁻¹·h)</td>
<td>N.C.</td>
<td>3,247 ± 587</td>
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<td>Subcutaneous injection</td>
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<tr>
<td>Cmax (µg/L)</td>
<td>872 ± 164</td>
<td>57,934 ± 1,902</td>
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<tr>
<td>Tmax (h)</td>
<td>1.0</td>
<td>2.0 ± 0.0</td>
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<tr>
<td>AUC₀–₄h (µg·L⁻¹·h)</td>
<td>1,912 ± 262</td>
<td>15,316 ± 5,856</td>
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<tr>
<td>AUC₀–2₄h (µg·L⁻¹·h)</td>
<td>3,377 ± 334</td>
<td>39,040 ± 10,738</td>
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a) Note that T max in rats was determined by using the mean of each time point. C max in rats represents mean ± SD calculated by the serum BPA concentrations of three animals at T max. b) N.C.: Not calculated. c) N.D.: Not defined.

### References