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Viable skin efficiently absorbs and metabolizes bisphenol A ☆,☆☆

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ABSTRACT

Skin contact has been hypothesized to contribute to human exposure to bisphenol A (BPA). We examined the diffusion and metabolism of BPA using viable skin models: human skin explants and short-term cultures of pig ear skin, an alternative model for the study of the fate of xenobiotics following contact exposure. ^{14}C-BPA [50–800 nmol] was applied on the surface of skin models. Radioactivity distribution was measured in all skin compartments and in the diffusion cells of static cells diffusion systems. BPA and metabolites were further quantified by radio-HPLC. BPA was efficiently absorbed in short-term cultures, with no major difference between the models used in the study [viable pig ear skin: 65%; viable human explants: 46%; non-viable (previously frozen) pig skin: 58%]. BPA was extensively metabolized in viable systems only. Major BPA metabolites produced by the skin were BPA mono-glucuronide and BPA mono-sulfate, accounting together for 73% and 27% of the dose, in pig and human, respectively. In conclusion, experiments with viable skin models unequivocally demonstrate that BPA is readily absorbed and metabolized by the skin. The trans-dermal route is expected to contribute substantially to BPA exposure in human, when direct contact with BPA (free monomer) occurs.

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1. Introduction

Bisphenol A (BPA) is widely used to produce polycarbonate plastics (bottles and food containers) as well as epoxy resins used as internal lacquer in cans. Residual (unreacted) monomers of BPA, but also free BPA released by these polymers under certain pH conditions or during microwave heating (Lim et al., 2009) can migrate into beverages and foods (Brotons et al., 1995; Kubwabo et al., 2009), partly explaining human exposure. Food contamination can also occur along the food chain, due to the very large amounts of BPA-based products manufactured, which result in its presence in the environment. In the last decade, BPA has attracted considerable attention because it is a model xenoestrogen able to trigger reproductive disorders in laboratory animals (Richter et al., 2007). Recently, it was also hypothesized that early exposure to BPA could play a role in the onset of obesity and other metabolic syndromes (Rubin and Soto, 2009), and impact cognitive functions (Palanza et al., 2008). Although a large controversy regarding BPA is still underway (vom Saal and Hughes, 2005; Vandenberg et al., 2009), the amount of evidence supporting the effects of low doses of BPA in animal models is increasing each year. Human exposure to BPA has been clearly demonstrated (Vandenberg et al., 2010) and its possible consequences have become a critical issue in the field of endocrine disruption.

According to recently published reports on risk assessment (EFSA, 2006; Chapin et al., 2008) in the general population, human exposure mostly occurs via residues contained in food or beverages. However, there is ongoing controversy on whether external exposure resulting from food contamination is really a good estimate for internal exposure, because of the discrepancy between the concentrations of BPA in the blood reported by several authors and exposure calculations. Based on PBPK (physiologically-based pharmacokinetic) models, Mielke and Gundert-Remy (2009) noted that the blood concentration calculated for a dose of 0.9 g kg^{-1} d^{-1}, which is the highest daily intake estimated from 3509 biomonitoring samples, is several orders of magnitude lower than the values reported in the literature. Furthermore, Völkel et al. (2002) were unable to measure plasma concentrations above the level of detection of 2.3 ng mL^{-1} after a single oral dose of 5 mg BPA, which is 5000 times higher than the external exposure estimated from biomonitoring data. Several hypotheses can be put forward to explain this inconsistency, among which underestimation of exposure levels.

Additional routes (inhalation and contact) could contribute to the overall human exposure to BPA. Data is still lacking on these issues (Vandenberg et al., 2007), but the contribution of the

Abbreviations: BPA, bisphenol A; HPLC, high-performance-liquid-chromatography.

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inhalation route is likely limited in the case of BPA (Wilson et al., 2007; Geens et al., 2009; von Goetz et al., 2010), with the exception of specific occupational exposure, suggested by studies carried out in BPA manufacturing workplaces (He et al., 2009). Large amounts of BPA are used in thermal paper made for printers relying on the thermal transfer technology, in which BPA is often used as a color developer. This printing technique, mainly used in lightweight printing devices, begun to be extensively used in the 1970s. Over the years, the use of thermal printers has increased (cash registers, credit card terminals). Nowadays, most people come in contact with thermal paper on a daily basis. Not all thermal paper (but a large part) contains BPA. When this is the case, a powdery layer of BPA is used to coat one side of the paper. Under heat or pressure, BPA reacts with the thermal paper dye to produce a color-developing complex based on hydrogen bond interactions (Takahashi et al., 2002). Not only can this result in oral contamination (direct contact of unwashed hands with the mouth), but thermal paper is also a major source for the contamination of recycled paper (Gehring et al., 2004).

Because of the extensive use of thermal paper and because large amounts of free BPA can be found in it, skin contact may contribute to the overall exposure of humans to BPA. It is therefore critical to examine if skin can absorb and/or metabolize BPA. The best way to do this is to use radio-labeled BPA, but this approach cannot be used for *in vivo* studies in humans. It is therefore necessary to use the most accurate skin models to address this issue, in accordance with international guidelines. We have developed an *ex vivo* alternative skin model, based on pig ear skins (which can easily be obtained from local slaughterhouses) to examine the fate and penetration of model toxicants. Short-term culture of pig ear skin incubated in proper conditions using static diffusion cells, has been shown to be an efficient skin model that expresses both phase I and phase II functional enzymatic activities and can be used for a period of 72 h with good conservation of the skin barrier function (Jacques et al., 2010a,b,c). In this study, we used this model to examine the percutaneous absorption and biotransformation of BPA, using ^{14}C-BPA. We carried out the experiments using a range of BPA doses (50–800 nmol) applied on the surface of skin explants, and quantified the residues in all compartments (the surface of the skin, the skin itself, and diffusion cells). Similar experiments were conducted using frozen pig skin (control) and viable human dermal explants on which 50 nmol of BPA were applied. Parent BPA and its metabolites were quantified by radio-HPLC. This is the first report of the absorption and metabolism of BPA in viable skin models, with full description of the repartition and metabolic fate of BPA.

2. Materials and methods

2.1. Chemicals

[U-^{14}C]-BPA, with a specific activity of 2.074 GBq mmol^{-1} was purchased from Amersham Biosciences (UK). Its radio-purity was >98.5% based on radio-HPLC analysis. Unlabeled BPA (>99% purity) was purchased from Sigma–Aldrich (St.-Quentin-Fallavier, France). Other chemicals and solvents (analytical grade) were purchased from the following sources: bovine liver β-glucuronidase (type B1 1000 units g^{-1} solid), sulfatase from *Aerobacter aerogenes* (3.9 units mg^{-1} protein), ammonium acetate, sodium acetate, phosphate buffer and sodium hydroxide: Sigma–Aldrich; acetonitrile and ethyl acetate: Scharlau Chemie S.A. (Barcelona, Spain); ethanol and acetic acid: Merck (Briare-Le-Canal, France). Ultrapure water from Milli-Q system (Millipore, Saint-Quentin-en-Yvelines, France) was used for *ex vivo* preparations and for the preparation of HPLC mobile phases.

2.2. Short-term culture of pig ear skin

Skin organ culture used ears of domestic pigs (Pietrain breed, 6-month old females) obtained from a local slaughterhouse. Ears were taken from the animals within 5 min after slaughtering and were kept at 4 °C during the transport to the laboratory, which lasted a maximum of 2 h. After cleaning and shaving, skins were immediately excised with a scalpel, then sectioned at a thickness of 500 μm using a Padgett dermatome (Michael's France, Neuilly-sur-Seine, France) and punched into 28 mm diameter discs. The punch areas were free of structural changes such as scratches, erosions and scars, which could affect the diffusion and metabolism of BPA.

For incubations, skin punches were seeded dermal side down in Transwell® inserts (23 mm diameter, 8 μm pore size; Corning Life Sciences, Avon, France) in 6-well plates prefilled with 1.5 mL culture medium at 37 °C in a 5% CO_2 air incubator. In this *ex vivo* organ culture system, explants are maintained at the air/liquid interface and dermal/epidermal feeding is achieved by the diffusion of nutriments across the insert. The culture medium was Dulbecco's Eagle Modified Medium supplemented with L-Glutamine (0.584 g L^{-1}), streptomycin/penicillin (100 μg mL^{-1}), fungizone (2.5 μg mL^{-1}), gentamycin (50 μg mL^{-1}), all from Sigma–Aldrich.

^{14}C-BPA (8333 Bq sample^{-1}) adjusted with unlabeled BPA to reach the required dose (50, 100, 200, 400 and 800 nmol, corresponding to 2.75, 5.5, 11, 22 and 44 μg cm^{-2}, respectively) was applied in 60 μL ethanol/phosphate buffer 0.1 M pH 7.4 (1:2, vol/vol) on the surface of the skin. Culture media collected at 24, 48 and 72 h (end of the experiment) were kept at −20 °C until analysis. All incubations were carried out in triplicate.

2.3. Control incubations

The influence of skin viability on percutaneous absorption was investigated by comparing BPA diffusion in fresh explants with the corresponding skin preparations which had previously been frozen, following the method of Kao et al. (1985). Skin punches were stored at −20 °C for 1 month. After this enzyme inactivation period, they were incubated in the same conditions as fresh skin punches, with ^{14}C-BPA (8333 Bq sample^{-1}) adjusted with unlabeled BPA to reach the required dose (50, 200 and 800 nmol), in triplicate. Culture media collected at 24, 48 and 72 h were kept at −20 °C until analysis.

2.4. Human skin explants

Human skin from the abdominal region of Caucasian female donors (36–44 years old) with no skin diseases was obtained from surgery and supplied by Biopredic (Rennes, France). The samples were sectioned at a thickness of 500 μm using a dermatome and punched into 28 mm diameter discs. Human skin punches were incubated in the same conditions as pig skin punches with 50 nmol ^{14}C-BPA (8333 Bq), in triplicate. Culture media collected at 24, 48 and 72 h were kept at −20 °C until analysis.

2.5. Radioactivity measurements

At the end of the 72 h experiment, media and skin were removed. Skin surfaces were washed twice with cotton swabs impregnated with ethanol/water (3:1, vol/vol). Cotton swabs were plunged in acetonitrile (2 mL) and placed in a sonicator for 20 min.

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