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Distribution of persistent organic pollutants in serum, omental, and parietal adipose tissue of French women with deep infiltrating endometriosis and circulating *versus* stored ratio as new marker of exposure

Stéphane Ploteau [a,b], Jean-Philippe Antignac [a,c,*], Christelle Volteau [d], Philippe Marchand [a], Anaïs Vénisseau [a], Vincent Vacher [a], Bruno Le Bizec [a]

[a] LUNAM Université, Oniris, USC 1329 Laboratoire d'Etude des Résidus et Contaminants dans les Aliments (LABERCA), Nantes, France
[b] Service de gynécologie-obstétrique, CIC FEA, Hôpital Mère Enfant, CHU Hôtel Dieu, Nantes, France
[c] INRA, Nantes F-44307, France
[d] Plateforme Biometrie, CHU Hôtel Dieu, Nantes, France

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ABSTRACT

Several studies have assessed the potential role of environmental chemicals in the onset, growth, and/or physiopathology of endometriosis. However, their contour in terms of considered exposure markers remains limited. The present study aimed to characterize the internal exposure levels of 78 persistent organic pollutants (POPs, including dioxins, polychlorobiphenyls, brominated flame retardants and organochlorine pesticides) in a set of 113 adult French women (45 controls, 68 cases), and to characterize the distribution of these POPs within three biological compartments (omental adipose tissue, parietal adipose tissue, and serum). For all targeted substances, the correlation between the concentrations measured in omental *versus* parietal adipose tissue was found strongly significant ($p < 0.0001$). An equivalence of the measures performed in parietal and omental adipose tissue was moreover observed with median levels of 6.4 *vs.* 7.4 pg/g l.w. for WHO-TEQ_{2005} PCDD/F, 4.5 *vs.* 4.7 pg/g l.w. for WHO-TEQ_{2005} dl-PCB, 137.1 *vs.* 147.9 ng/g l.w. for sum of 6 ndl-PCB, and 2.1 *vs.* 2.0 ng/g l.w. for sum of 7 i-PBDE, respectively. The same observation was made for individual targeted OCs compounds. Significant correlations were also observed between these concentrations determined in adipose tissue and those measured in serum for dioxins (WHO-TEQ_{2005} PCDD/F = 6.1 pg/g l.w), PCBs (WHO-TEQ_{2005} dl-PCB = 3.6 pg/g l.w., sum of 6 ndl-PCB = 81.1 ng/g l.w.), and brominated flame-retardants (sum of 7 i-PBDE = 0.9 ng/g l.w.). The circulating *versus* stored ratio of some exposure markers (Sum PCDDs, 1,2,3,6,7,8-HxCDF, slightly *versus* highly chlorinated PCBs ratio, PBDE 99 and PBB 153) was found statistically different for control and case individuals. These extended exposure data from deep infiltrating endometriosis patients are the first ones available for France and give a new insight about the equilibrium of chemicals between storage and circulating compartments that should be further considered as new marker of exposure in the context of exposure-health relationship studies.

1. Introduction

Endometriosis is a chronic pelvic disease characterized by the presence of endometrial glands and stroma outside the uterus (Sampson, 1927). Symptoms vary widely, including chronic pelvic pain, dysmenorrhea, dyspareunia, dyschezia and sometimes infertility (Eskenazi et al., 2002). In industrialized countries, endometriosis is one of the main gynecologic causes of hospitalization and its prevalence is increasing (Koninckx, 1999; Bulun, 2009). The prevalence is estimated to 5 to 10% of reproductive age women among which at least one-third are infertile (de Ziegler et al., 2010; Parazzini et al., 2016; Prescott et al., 2016). Endometriosis appears as a multicausal estrogen-dependent disease. Indeed, suppression of ovarian hormonal production reduces the painful symptoms (Giudice, 2010). Several risk factors were evoked, *e.g.* genetic susceptibility, but which cannot explain the majority of endometriosis cases (Nisolle and Donnez, 1997). Among other factors appeared the hypothesis that exposure to environmental chemicals, in particular those persistent with endocrine disrupting properties, may plausibly play a role in the endometriosis etiology (Bulun, 2009; Upson et al., 2013). These persistent organic pollutants (POPs) include historical substances such as dioxins (PCDD/F), polychlorinated biphenyls (PCB) or organochlorine pesticides (OC), and also brominated flame

* Corresponding author at: Laboratoire d'Etude des Résidus et Contaminants dans les Aliments (LABERCA), Oniris, Atlanpole - La Chantrerie, CS50707, Nantes F-44307, France.
E-mail address: laberca@oniris-nantes.fr (J.-P. Antignac).

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retardants (BFR) which were very poorly investigated with regard to their association with endometriosis.

These contaminants were widely used in the latter half of the 20th century and despite restrictions and interdiction dispositions for some of them, are still present in environmental, food and human matrices (Patterson et al., 2009). Regarding dioxins and dioxin-like PCB, the main exposure sources include incineration processes and isolating components in electric devices, and the main human contamination route is food exposure (Van den Berg et al., 2006). It is now demonstrated that lactation is an excretion route for dioxins and PCB, resulting in decreased maternal levels (Nelson et al., 2006; Sasamoto et al., 2006). Moreover, the body burden of dioxins and PCBs generally increases with age (Uemura et al., 2008). Polybrominated flame retardants are used in many manufactured products (textiles, electronic devices…) to diminish their inflammability, and the main human contamination route is also food exposure for adult, while the contribution of inhalation (indoor air) and direct contact (textiles, toys…) may be an exposure route for infants (Antignac et al., 2009; Frederiksen et al., 2009; Vorkamp et al., 2011). However, the major exposure route for infants is breastfeeding (Adgent et al., 2014). Organochlorine pesticides are a main concern for occupational exposure but are also present in the global exposome of the general population (Upson et al., 2013). Most of these POPs present the capability to interact with different nuclear receptors (ER, AR, AhR, …) so that the exposure to these POPs can lead to endocrine disruption (Safe, 1990; Arisawa et al., 2005; Van den Berg et al., 2006) in particular regarding the reproductive function (Alvarez et al., 2000; Andersen et al., 2002; Chao et al., 2007; Harley et al., 2010).

While brominated flame-retardant were investigated in only two studies related to endometriosis (Hoffman et al., 2007; Buck Louis et al., 2012), several studies have assessed the association between the others POPs internal exposure levels (dioxins, polychlorinated biphenyls, or organochlorine pesticides) and the onset or growth of endometriosis (Heilier et al., 2005; Louis et al., 2005; Porpora et al., 2009; Buck Louis et al., 2012; Upson et al., 2013; Martinez-Zamora et al., 2015). There are some arguments in favor of a causal relationship between endometriosis and organochlorine's pesticides in an experimental study of female primates (Crain et al., 2008). Indeed, two large cohort studies found a positive association for hexachlorocyclohexane HCH (Buck Louis et al., 2012; Upson et al., 2013). Now these studies investigating the potential role of POPs in the physiopathology of endometriosis remain globally non convergent and finally fairly conclusive. Their heterogeneity in terms of methodology, size and nature of the populations studied, as well as the nature and number of monitored markers of exposure and possible confounders contribute to explain this situation. Notably, the simultaneous determination of major historical and more emerging POPs including dioxins (PCDD/F), polychlorobiphenyls (PCBs), organochlorine pesticides (OCs), and polybromodiphenylethers (PBDE), has not yet been reported in a single study. The biological compartment in which contaminants are determined also appears as a non-consensual issue with regard to its relevancy for revealing the exposure-effect relationships. While concentrations in adipose tissue better reflect the long-term exposition, serum samples are less invasive, that may contribute to explain the lower number of studies having used adipose tissue.

More globally, the equilibrium between the storage and circulating biological compartments in terms of POP levels appears underconsidered while this may be a potential corner stone of the complex exposure-effect relationship. In particular, the equivalence and representativeness of different adipose tissue depots (*e.g.* deep *versus* superficial) may be an issue if control and case individuals do not authorize the collection of strictly identical samples for practical reasons. Little literature is also available on the serum *versus* adipose tissue relationships with regard to the concentration of POPs, while this may appears as a new integrative marker of relevance. Finally, there is a lack of studies that have characterized this distribution of POPs for same individuals in serum and in adipose tissue considering both deep (omental) and superficial (parietal) adipose tissue.

The primary objective of the present study was to characterize the internal exposure levels of an extended range of 78 POPs (*i.e.* PCDD/F, PCB, PBDE, PBB, HBCD and OCs) in a set of 113 adult French women, as well as the relationships and balance between three biological compartments, namely omental adipose tissue, parietal adipose tissue, and serum, in terms of POP concentrations. From this descriptive point of view, the generated knowledge is expected to contribute to risk assessment and human biomonitoring by providing new and recent internal exposure data for this particular subpopulation. The secondary objective was to assess the concentration ratio between storage (fat) *versus* circulating (serum) compartment as a new marker of exposure, both for control and case individuals. From this more particular and new descriptive point of view, these data are proposed as new insight regarding the exposure - disease relationships.

2. Materials and methods

2.1. Population and samples

Data were collected as part of a population-based case–control study of endometriosis (ENDOTOX) performed in the Region Pays-de-Loire between 2013 and 2015. Case individuals ($n = 68$) were adult women with surgical diagnosis of deep infiltrating endometriosis (DIE) first based on clinical examination. For all cases, magnetic resonance imaging (MRI) and surgery were then performed, as well as anatomo-pathological examination, with the objective of removing the lesions and confirming the diagnosis of DIE. Control individuals ($n = 45$) were adult women consulting for other gynecological problem but without endometriosis at the surgery and without any clinical symptoms of the disease like chronic pelvic pain, dysmenorrhea, dyspareunia or history of infertility. For a subset of this population ($n = 2 \times 38$ individuals), cases and controls were paired matched according to age (± 4 years), geographic origin race (caucasian, African, other), BMI (<25.0 kg/m^2, 25.0–29.9 kg/m^2, ≥ 30.0 kg/m^2) and previous experience of breast feeding (duration in months). This studied population's characteristics are reported in Table 1.

For case and control individuals, samples (2 cm^3) of both omental and parietal adipose tissue were collected during surgery. A blood sample (20 mL) was collected during the day before surgery. This sampling was performed between July 2013 and September 2014. All samples were stored at −80 °C until analysis. The protocol of this study was approved by the Bioethics Committee of the GNEDS (Groupe Nantais d'Ethique dans le Domaine de la Santé) under the clinical trial nb. NCT02651077 and all patients signed an informed consent form. As a very good correlation and equivalence was rapidly demonstrated between internal exposure levels measured in both fat compartments (parietal adipose tissue and omental tissue), the omental tissue sampling and subsequent characterization was not realized for all control individuals. Moreover, the final number of characterized samples for each class of targeted POPs (Tables 2 and 3) was also depending on clinical availability, quality requirements, and other feasibility issues.

2.2. POPs measurements

The methodologies applied to isolate, detect, and quantify the targeted POPs including dioxins ($n = 17$ PCDD/F), polychlorobiphenyls ($n = 12$ dioxin-like $+6$ non-dioxin-like PCB), polybromodiphenylethers ($n = 8$ PBDE), polybromobiphenyls ($n = 3$ PBB) and organochlorine pesticides ($n = 30$ OCs) have been described earlier (Antignac et al., 2006, Costera et al., 2006, Cariou et al., 2005, Antignac et al., 2009, Bichon et al., 2015). Briefly, ^{13}C-labeled congeners were added to each sample for quantification according to the isotopic dilution method. Adipose tissue (400–

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