Environment International 119 (2018) 20–25

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Isolated hypospadias: The impact of prenatal exposure to pesticides, as determined by meconium analysis

Elodie Haraux[a,b,*], Pierre Tourneux[b,c], Christelle Kouakam[b], Erwan Stephan-Blanchard[b], Bernard Boudailliez[d], Andre Leke[b,e], Celine Klein[a], Karen Chardon[b]

[a] Paediatric Surgery Unit, Amiens University Hospital, F-80054 Amiens, France
[b] PeriTox - UMI 01, UFR de Médecine, Jules Verne University of Picardy, F-80054 Amiens, France
[c] Paediatric Intensive Care Unit, Amiens University Hospital, F-80054 Amiens, France
[d] Department of Paediatrics, Amiens University Hospital, F-80054 Amiens, France
[e] Neonatal Intensive Care Unit, Amiens University Hospital, F-80054 Amiens, France

ARTICLE INFO

Handling Editor: Lesa Aylward

Keywords:
Hypospadias
Meconium
Pesticides
Environmental exposure

ABSTRACT

Although endocrine-disrupting chemicals (EDCs, including pesticides) are thought to increase the risk of hypospadias, no compounds have been formally identified in this context. Human studies may now be possible via the assessment of meconium as a marker of chronic prenatal exposure. The objective of the present study was to determine whether or not prenatal exposure to pesticides (as detected in meconium) constitutes a risk factor for isolated hypospadias. In a case-control study performed between 2011 and 2014 in northern France, male newborns with isolated hypospadias (n = 25) were matched at birth with controls (n = 58). Newborns with obvious genetic or hormonal anomalies, undescended testis, micropenis, a congenital syndrome or a family history of hypospadias were not included. Neonatal and parental data were collected. Foetal exposure was assessed by determining the meconium concentrations of the pesticides or metabolites (organophosphates, carbamates, phenylurea, and phenoxyherbicides) most commonly used in the region. Risk factors were assessed in a multivariate analysis. The pesticides most commonly detected in meconium were organophosphates (in up to 98.6% of samples, depending on the substance) and phenylurea (> 85.5%). A multivariate analysis revealed an association between isolated hypospadias and the presence in meconium of the phenylurea herbicide isoproturon and of the phenoxyherbicide 2-methyl-4-chlorophenoxyacetic acid (odds ratio [95% confidence interval]: 5.94 [1.03–34.11] and 4.75 [1.20–18.76]) respectively). We conclude that prenatal exposure to these two herbicides (as assessed by meconium analysis) was correlated with the occurrence of isolated hypospadias. The results of our case-control study (i) suggest that prenatal exposure to pesticides interferes with the development of the male genitalia, and (ii) emphasize the importance of preventing pregnant women from being exposed to EDCs in general and pesticides in particular.

1. Introduction

Hypospadias (the most common congenital malformation of the penis) results from the impaired development of ventral penile tissues. Most cases of hypospadias require surgical correction to prevent subsequent urinary and sexual disorders. The pathophysiology of hypospadias appears to be linked to a number of endocrine, genetic, vascular and environmental factors (Haraux et al., 2016). The increase in the incidence of hypospadias (Botta et al., 2014), and other components of testicular dysgenesis syndrome (cryptorchidism, low sperm counts, testicular germ cell cancer, etc.) (Skakkebaeck et al., 2015) during the 20th century might be linked to environmental exposure to toxics. Endocrine-disrupting chemicals (EDCs) may interfere with the normal development of the male external genitalia during early pregnancy (Wang and Baskin, 2008) by altering the endocrine system's normal regulatory functions. Among the many suspected EDCs certain pesticides are known to disrupt the endocrine system in in vitro (Lin and Garry, 2000) and in animal studies (Marouani et al., 2017) including the induction of hypospadias in rats (Vilela et al., 2007). However, no one substance has been formally identified as a risk factor for hypospadias in humans. Indeed, foetal exposure during early pregnancy is currently difficult to assess. In clinical studies, foetal exposure during

* Corresponding author at: Service de Chirurgie de l'enfant, Pôle Femme, Couple, Enfants, CHU Amiens, Bd Laennec, 80054 Amiens, France.
E-mail addresses: haraux.elodie@chu-amiens.fr (E. Haraux), tourneux.pierre@chu-amiens.fr (P. Tourneux), erwan.stephan-blanchard@u-picardie.fr (E. Stephan-Blanchard), boudailliez.bernard@chu-amiens.fr (B. Boudailliez), leke.andre@chu-amiens.fr (A. Leke), klein.celine@chu-amiens.fr (C. Klein), karen.chardon@u-picardie.fr (K. Chardon).

https://doi.org/10.1016/j.envint.2018.06.002
Received 7 February 2018; Received in revised form 4 June 2018; Accepted 4 June 2018

early pregnancy has been evaluated by means of indirect markers (such as the annual estimated amount of pesticides applied (Carbone et al., 2006)) or direct markers of exposure (such as maternal blood levels (Giordano et al., 2010)).

Meconium - the first stools excreted by newborns after birth - appears to be the most sensitive matrix (Ostrea et al., 2009) for detecting chronic foetal exposure. The excreted substance is derived from bile secretions and amniotic fluid swallowed from the 12th week of gestation onwards (Esteban and Castaño, 2009) and therefore constitutes a very good biomarker of chronic exposure. Although very few studies have used this matrix to analyse foetal exposure to pesticides, promising results have been obtained (Berton et al., 2014; Jeong et al., 2016; Ostrea et al., 2009; Whyatt et al., 2009). To the best of our knowledge, the putative association between foetal exposure and isolated hypospadias (IH) has not previously been investigated.

Hence, this present study was designed to evaluate the association between IH in newborns and foetal exposure to pesticides during pregnancy, as assessed by meconium assays.

2. Patients and methods

2.1. The study population

We conducted a prospective case-control study between March 2011 and March 2014 in the Picardy region of northern France, within 70% of the land is farmed. The study was approved by the independent ethics committee at Amiens University Hospital (reference: RCB 2010-A01491-38). Parents aged under 18 years or having been deprived of their parental rights were excluded from the study. Newborns with a life-threatening condition were also excluded.

After the provision of written, informed consent by parents, newborns with suspected hypospadias were prospectively included at birth by the 11 maternity units involved in the study. A paediatric endocrinologist and a paediatric urologist confirmed the diagnosis and classified the malformation according to the position of the urethral meatus (distal, middle or proximal). Associated abnormalities of the genitalia were noted - particularly the position of the testicles and the appearance of the scrotum. After their inclusion of each case, the next three male infant born at a similar gestational age (< or > to 35 weeks of amenorrhea (WA)), in the same month and in the same maternity unit were then recruited as controls. All newborns underwent a comprehensive clinical examination. Newborns with hypospadias and normal hormonal profile (Table 1) but no other genital malformations (e.g. undescended testis or micropenis), congenital syndromes or a known family history of hypospadias were included the IH group (n = 32) (Fig. 1). Matched controls with no genital anomalies, no associated syndromes and no known family history of hypospadias were included in a control group (n = 90).

With regard to neonatal data, low birth weight (LBW) and low birth length (LBL) were defined as below the 5th percentile for normative data in France and after adjustment for the mother's age, parity and the baby's gender (http://audipog.net/module_ligne.php).

Medical records and the study questionnaire were used to collect a number of parental data: age, height, weight, the mother's educational level, employment status, smoking status, medication and folate intakes during pregnancy, gynaecological, obstetric and endocrine histories (age at menarche, the use of oral contraceptives during the trimester preceding pregnancy, any miscarriages, etc.), and any family history of hypospadias, undescended testis or testicular cancer.

2.2. Evaluation of prenatal exposure

Foetal exposure to pesticides was evaluated directly by assaying the meconium for pesticides. Meconium samples were collected daily by a nurse directly from the newborn's diapers, up until the time when the first stools were expelled. The samples were initially stored at 4 °C for transport and then store at −80 °C until analysis. Using an assay method developed in our laboratory, the concentrations of the six pesticides most commonly used in the Picardy region (and concentrations of their metabolites, for some compounds) were analysed using an ultra-high-pressure liquid chromatography-tandem mass spectrometry method (Table 2) (Berton et al., 2014).

2.3. Statistical analysis

We performed a descriptive analysis of the study population. We excluded newborns for whom meconium assays had not been performed (7 with IH and 32 controls), and then compared the IH group (n = 25) with the control group (n = 58).

The levels of pesticide exposure were classified with regard to the detection rate. When a substance was detected in < 50% of samples, the individuals were classified into two classes ("low" when < LOD or a "high" when ≥LOD). When a substance was detected in between 50 and 70% of the samples, the individuals were classified into three

Table 1
Target compounds: concentrations (ng/g) and detection rates for parent pesticides and metabolites (Berton et al., 2014).

Class	LOD	LOQ	Concentration (median; interquartile range)	% > LOD Overall (IH/controls)
		Organophosphates		
Chlorpyrifos	0.05	0.20	6.6; 6.5, 7.2	92.7 (96.0/91.4)
DETP	0.50	2.00	82.4; 37.9, 166.2	98.6 (100.0/98.3)
Diazinon	0.01	0.05	1.6; 1.4, 3.0	90.4 (92.0/89.6)
DEP	0.05	0.70	0.1; 0.0, 0.4	62.6 (64.0/62.1)
Malathion	0.05	0.30	9.1; 4.4, 11.6	74.7 (80.0/72.4)
DMP	0.50	2.00	0.1; 0.0, 0.4	26.5 (32.0/22.4)
		Carbamates		
Propoxur	0.10	0.50	12.1; 9.3, 22.1	89.2 (100.0/84.5)
ETU	1.00	4.00	1.0; 0.3, 2.4	34.9 (32.0/36.2)
		Phenylurea		
Isoproturon	0.05	0.10	7.0; 6.9, 7.8	90.4 (96.0/87.9)
Desmethylisoproturon	0.05	0.20	7.9; 7.8, 8.7	85.5 (92.0/82.8)
		Phenoxyherbicides		
MCPA	0.40	1.00	0.0; 0.0, 284.4	15.6 (28.0/10.3)

Pesticides and metabolites: chlorpyrifos and its metabolite diethyl thiophosphonate (DETP), diazinon and its metabolite diethyl phosphate (DEP), malathion and its metabolite dimethyl phosphate (DMP), propoxur, ethyl thiourea: 2-imidazolidinethione (ETU, a metabolite of mancozeb (not measured)), isoproturon and its metabolite desmethylisoproturon (1-(4-isopropylphenyl)-3-methylurea), 2-methyl-4-chlorophenoxyacetic acid (MCPA); LOD: limit of detection; LOQ: limit of quantification; IH: isolated hypospadias.