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Uptake of Pyrene in a Breast-Fed Child of a Mother Treated with Coal Tar

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Abstract: **A woman was treated for atopic dermatitis with coal tar containing ointments. Coal tar containing ointments contain genotoxic polycyclic aromatic hydrocarbons. Over a period of 50 days the accumulated dose of different coal tar containing ointments treatments corresponded to 993 mg of pyrene and 464 mg of benz[a]pyrene. During this treatment she gave breast milk to her 3-month-old daughter. Analysis of urine samples from the breast-fed child showed elevated levels of urinary excretion of a metabolite of pyrene (1-hydroxypyrene, 1-OHP). These levels were in the same range as urinary excretion levels of this metabolite observed in the mother's urine. As no pyrene was observed in breast milk at a limit of determination of 0.0035 μmol/L, transfer of pyrene from mother to child via breast milk is not likely. Also, a low level of 1-hydroxypyrene observed in the mother's milk did not account for the observed urinary excretion levels in the child. It must therefore be assumed that pyrene was transferred from mother to child via another route, presumably direct skin-to-skin or skin-to-mouth contact. Dermatologists should inform their patients who receive treatment with coal tar containing ointments of the risk of transfer of polycyclic aromatic hydrocarbons by skin-to-skin or skin-to-mouth contact.**

Coal tar is used by dermatologists for treatment of skin disorders such as psoriasis and eczema. Coal tar is a complex mixture of organic compounds that is only partly chemically characterized but coal tar is known for its content of polycyclic aromatic hydrocarbons (PAH). Some members of this group have been classified as human carcinogens (1). Although evidence from controlled trials is scarce, dermatologists consider the use of coal tar containing ointments (CTO) as safe and efficacious (2,3).

Polycyclic aromatic hydrocarbons (PAH) are ubiquitous genotoxic substances and all humans are exposed somehow, including during the prenatal and neonatal period of our lives (4). The most important sources of exposure in urban areas are diet, active smoking, indoor air quality (open fire and passive smoking), and outdoor air pollution (domestic heating and traffic) (5,6). In this

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case report we will discuss the unexpected relative high exposure of a child that received breast milk from a mother who was treated with CTO.

CASE REPORT

A 34-year-old woman (nonsmoker) with atopic dermatitis was seen in our dermatologic day care unit twice a week. She received daily applications of CTO. Part of those treatments were self-administered at home. Two types of ointment were used: a 'dark' colored ointment containing 5 w% pix lithantracis in zinc oxide paste and a 'light' colored ointment based on a 10% solutio carbonis detergens (containing 16 w% of coal tar solution) in white petrolatum and lanette wax (50/50 by volume), both according to the Dutch Pharmacopeia. The amount of CTO applied was carefully registered. In Table 1 the amounts of applied CTO are presented, including the contents of pyrene (PYR) and benz[a]pyrene (B[a]P). The entire body of the patient was treated, except for the mammae and the face. The total amount of PYR and B[a]P from both types of ointments corresponded to topically applied dose of 993 mg PYR and of 464 mg B[a]P.

During the entire period of treatment the woman planned to breast feed her 3-month-old daughter. Because of concerns of the mother with respect to possible transfer of PAH from the ointment to her child, we asked her to collect urine samples from herself and her child and also collect some breast milk samples before the start of the treatment and during the entire treatment period. At the end of the treatment period she had collected spot urine samples on the day before the start of the treatment and on 5 days during the period of treatment (50 days). Urine samples were collected from the child before the start of the treatment and on four occasions during the treatment. These samples were collected by the mother using a system designed for collection of urine from small children (Urincoll Fille, Braun Biotrol S.A. Paris, France). Breast milk samples were collected before the start of treatment (one sample) and on 11 occasions during the treatment period. All urine and breast milk samples were stored at −20°C prior to analysis.

TABLE 1. *Cumulative Dermal Exposure to Pyrene (PYR) and Benz[a]pyrene (B[a]P) during Topical Treatment with Coal Tar Containing Ointments (CTO)*

Type of CTO	Number of treatments	Total amount of ointment applied (g)	PAH content (μg/g ointment)		Topically applied amount (mg)	
			PYR	B[a]P	PYR	B[a]P
Dark	9	1,018	965	455	982	463
Light	14	3,738	3.0	0.13	11	0.5
Total	23	4,756	–	–	993	464

Urine and breast milk was analyzed for both glucuronide-conjugated and free 1-hydroxypyrene using the method of Jongeneelen et al (7) and urinary creatinine was analyzed using the method according to Jaffe (8).

Mother's milk was analyzed by mixing 2.5 mL of the homogenized sample with 2.5 mL of ethanol (Labscan, Dublin, Ireland). After 10 minutes of ultrasonic treatment, 10 mL of ether (Labscan) was added for extraction of PYR and B[a]P. This extraction was repeated and the ether aliquots were combined, shaken, and centrifuged at 2,000 rpm. The clear supernatant was decanted and the ether was evaporated to dryness using a gentle stream of nitrogen at 30°C. The residue was dissolved in 2 mL of methanol (Labscan) in an ultrasonic bath for 5 minutes. After centrifugation, the supernatant was transferred to a vial and analyzed on high performance liquid chromatography (SP8800, Spectra Physics, Utrecht, The Netherlands) equipped with a fluorescence detector (FP 920, Jasco, Tokyo, Japan). Pyrene was detected at an excitation wavelength (λ_{ex}) of 264 nm and emission wavelength (λ_{em}) of 381 nm. Benz[a]pyrene (B[a]P) was detected at 296 nm (λ_{ex}) and 407 nm (λ_{em}), respectively. This method was validated by addition of known quantities of PYR and B[a]P to a mixture of breast milk samples from four healthy nonsmoking volunteers (29–33 yrs old). Recovery of PYR and B[a]P was 60% to 70% in the low range (5–90 nmol/L). Matrix-mixed standards prepared in the same range gave a calibration curve with a R^2 of 0.998.

The breast milk samples of the nontreated volunteers and of the patient contained no detectable concentrations of PYR and B[a]P (limits of determination 0.0035 and 0.054 μmol/L, respectively). Also 1-OHP was not detected in the volunteers or the patient before commencement of the treatment. However, breast milk samples collected on days 5, 15, and 36 after the start of treatment contained some traces of 1-OHP at levels of 0.00076, 0.00028, and 0.00059 μmp; 1-OHP/L.

The urine samples of the mother contained 0.12 μmol/mol before and 39.3 (range 1.3–133) μmol 1-OHP/mol creatinine after the start of the treatment (Fig. 1). The urinary excretion of 1-OHP was still 1.3 μmol/mol creatinine 62 days after cessation of the CTO treatment. 1-OHP was detected in all of the five urine samples collected from the child that received breast feeding. The initial value (before the start of treatment) was 1.63 μmol/mol creatinine. The subsequent values were 9.96, 97.1, 8.67, and 11.3 μmol/mol creatinine (see Fig. 1 for a graphic representation).

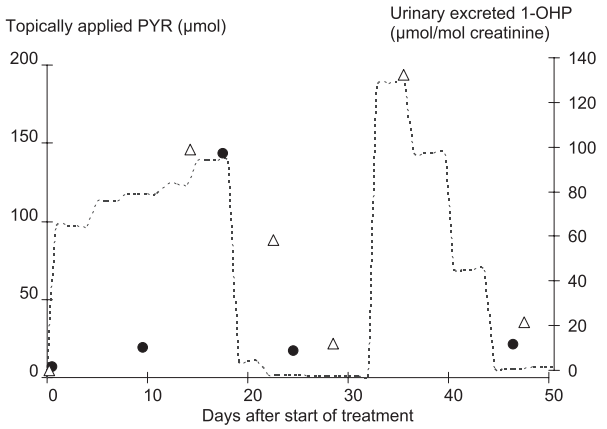

Figure 1. Total amount of topically applied pyrene (PYR) in coal tar containing ointments (CTO) (—) and urinary excretion of 1-hydroxypyrene (1-OHP) by the mother (△) and her child (•) during the entire period of treatment.

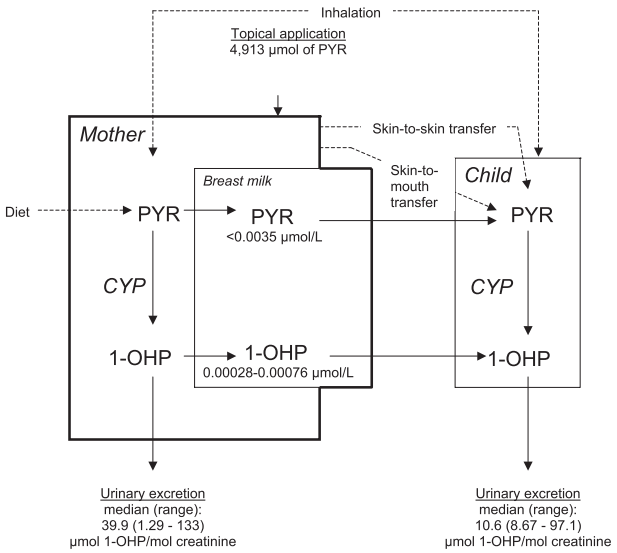

Figure 2. Schematic diagram showing possible and confirmed routes of transfer of PYR and its metabolite 1-hydroxypyrene (1-OHP) from a breastfeeding mother to her child. Solid lines represent transfer that was quantified in this study. Dotted lines are possible routes of transfer not supported by measurements. Only a very small quantity of 1-OHP is transferred by breastfeeding. It must be assumed that the urinary excretion of 1-OHP by the child is the result of metabolism of pyrene (PYR) by the child. This means that the child is at risk, since oxidative metabolism is a bioactivation step for polycyclic aromatic hydrocarbons (PAH). Uptake of PYR by inhalation or PYR or 1-OHP from the breast milk does not explain the substantial excretion of 1-OHP by the child. It must be assumed that PYR is primarily transferred by direct skin-to-skin contact or skin-to-mouth contact.

DISCUSSION

Much more light ointment was used than dark. Nevertheless, the treatment with the light CTO contributed only a very small potion to coal tar exposure (1.1% for PYR and 0.1% for B[a]P).

In a recent contribution of Zanieri et al (9) it was shown that PYR and B[a]P were only detected in a fraction of the breast milk samples collected in Italian women at concentrations in the same range as the limit of detection in this study (0.0035 μmol/L).

Very low concentrations of 1-OHP (two orders of magnitude lower than in urine) were observed in three samples of breast milk (out of 11 samples collected after the start of the treatment). The pattern of observed detectable levels of 1-OHP in breast milk was consistent with the treatment pattern, but in most of the milk samples 1-OHP was found to be below the limit of detection (0.0035 μmol/L).

Despite the limited number of urine samples collected, the patterns of urinary excretion of 1-OHP in both mother and child are consistent with the amounts of PYR topically applied during the treatment. 1-hydroxypyrene (1-OHP) concentrations were in the same range for mother and child, and follow the pattern of CTO applications expressed as topically applied amounts of PYR (Fig. 1). High concentrations of PYR are cleared effectively between days 19 and 32, a period when only the light CTO was used. This initial rapid decline was followed by a slow clearance as indicated by the urinary level of 1.3 μmol 1-OHP/mol creatinine 62 days after cessation of the treatment.

We did not detect PYR in the milk and based on the low levels of 1-OHP observed in the milk samples, it is not likely that the urinary excretion of 1-OHP is explained by the route of breastfeeding (Fig. 2). Instead, the uptake might be explained by transfer from the mother's skin to the mouth of the child (skin-to-mouth contact) or by transfer from the skin of the mother to the skin of the child (skin-to-skin contact). Dermal absorption is a route of uptake that can lead to significant internal exposure (10). The most likely moment of exposure of the child is during breastfeeding. Possible exposure of the child because of inappropriate hygiene of the mother during self-application of the CTO cannot be ruled out but is unlikely because of the guidance that was provided by the hospital clinic.

The baseline 1-OHP excretion of the mother was within the range that was observed in the general population. The range of values observed during the treatment is somewhat lower compared with other patients treated with CTO (11). Two months after the treatment the urinary excretion returned to a level that corresponds to approximately 10-fold the background in adults in The Netherlands (5). The 1-OHP excretion level of the child before the treatment (1.63 μmol/mol creatinine) was

higher than expected (12). Before the start of the study the mother had been using light CTO at home twice a day on her entire body surface (except her head) for several weeks. This would explain the somewhat higher level of urinary excretion of 1-OHP observed in the child prior to the start of treatment of the mother with dark CTO. We presume that during this prestudy period the same mechanism of uptake must have been involved as supposed for the period during which dark CTO was used and urine was collected. As the mother only used light CTO, the uptake by the child was considerably lower than during the study period.

After the start of the treatment of the mother, a five to sevenfold increase was observed on three occasions (days 10, 24, and 45) and one very high value on day 18 (60-fold higher than baseline). This suggests that the child was exposed to PAH on multiple occasions.

As no significant amounts of 1-OHP were transferred to the child with breastfeeding, it must be assumed that the substantial excretion of 1-OHP by the child is the result of metabolism of PYR by the child (Fig. 2). This means that the child was capable of CYP-mediated metabolism of PAH (13–15). The transfer of PYR and the metabolism by the child can be interpreted as a potential risk to the child's health, as oxidative metabolism is a bioactivation pathway for PAH.

It is concluded that if a treatment of a mother who is breastfeeding her child is considered, direct skin-to-skin or skin-to-mouth contact of the child with the treated skin parts should be avoided. The risk of contamination is not restricted to women who breast-feed a child. We recommend that all patients treated with CTO should receive information how to avoid contamination by skin-to-skin or skin-to-mouth transfer. It is the responsibility of the dermatologist to give guidance to treatments and at the outpatient clinic or in the day care unit to cover all treated areas with bandages that do not allow any direct contact with CTO.

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