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Research Paper

Paracetamol Medication During Pregnancy: Insights on Intake Frequencies, Dosages and Effects on Hematopoietic Stem Cell Populations in Cord Blood From a Longitudinal Prospective Pregnancy Cohort

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ABSTRACT

Background: Paracetamol is the first choice for antipyretic or analgesic treatment throughout pregnancy. Products with Paracetamol are readily available over the counter and therefore easily accessible for self-medication. Epidemiological data on Paracetamol intake pattern during pregnancy and its potential immunological effects are sparse. We aimed to analyze a possible association between Paracetamol medication and numbers of hematopoietic stem cells (HSC) in cord blood.
Methods: The objective was addressed in the PRINCE (PRENATAL DETERMINANTS OF CHILDREN'S HEALTH) study, a population-based prospective pregnancy cohort study initiated in 2011 at the University Medical Center in Hamburg, Germany. 518 healthy pregnant women with singleton pregnancies were recruited during the first trimester. Three examinations were scheduled at the end of the 1st (gestational week 12–14), the 2nd (gestational week 22–24) and the 3rd trimester (gestational week 34–36). For 146 of these women, cord blood flow cytometry data were available. Paracetamol intake was assessed for each trimester of pregnancy.
Findings: Among the 518 enrolled women, 40% took Paracetamol as main analgesic treatment during pregnancy. The intake frequency and dosage of Paracetamol varied between the women and was overall low with a tendency towards higher frequencies and higher dosages in the third trimester. Paracetamol intake, particularly during the third trimester, resulted in decreased relative numbers of HSCs in cord blood, independent of maternal age, first-trimester BMI, parity, gestational age and birth weight (-0.286 (95% CI -0.592, 0.021), $p = 0.068$).
Interpretation: Prenatal Paracetamol intake, especially during the third trimester, may be causally involved in decreasing HSCs in cord blood.

1. Introduction

While fever and pain are debilitating and endangering, especially during pregnancy, medication might evoke long-term side effects in the offspring. Although a multitude of fever and pain relievers is available, only few are considered to be safe during pregnancy. Since 1893, *N*-acetyl-*p*-aminophenol (Paracetamol, Tylenol, APAP) became the first-line and widely used medication for pain and fever (Brune et al., 2015). Paracetamol is an over the counter (OTC) drug and sold under different brand names, pure or in combination with vitamin C or caffeine. Paracetamol is apparently safe at therapeutic doses and neither an opioid nor an inhibitor of coagulation. Nevertheless, Paracetamol is able to pass the placenta freely, thus exerting a direct effect on the fetus. Several retrospective and cohort studies addressed the question whether the intake of Paracetamol during pregnancy affects pregnancy

Abbreviations: FACS, fluorescence-activated cell sorting; gw, gestational week; HSC, hematopoietic stem cells; OTC, over the counter; PRINCE, PRENATAL DETERMINANTS OF CHILDREN'S HEALTH.

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outcome or children's health. Here, the impact of prenatal Paracetamol on neuronal development and children's behavior is currently intensively discussed (Stergiakouli et al., 2016; Saunders and Habgood, 2017; Damkier et al., 2017). Moreover, a Danish cohort study from 2008 revealed a positive correlation between prenatal Paracetamol exposure and children's asthma (Rebordosa et al., 2008). These findings are supported by the work of Shaheen et al., linking Paracetamol to antioxidant gene polymorphisms and wheezing when taken in late pregnancy (Shaheen et al., 2002, 2010). Recently, Magnus et al. published a remarkable Child Cohort Study which demonstrated that pre- and postnatal exposure to Paracetamol is associated with asthma development (Magnus et al., 2016). A meta-analysis revealed that Paracetamol intake during the first trimester seems to elevate the risk of childhood asthma (Cheelo et al., 2015). Nevertheless, association between Paracetamol and risk of childhood asthma varies between the studies and lacks insights on causality, e.g. an alteration of immune ontogeny in response to Paracetamol (Cheelo et al., 2015). Compelling evidence from mouse models showed a reduction of fetal liver HSCs, which could be associated with an increased risk of airway inflammation in the offspring (Karimi et al., 2015; Thiele et al., 2015).

2. Aim

The aim of our present study was to determine the intake pattern of Paracetamol during pregnancy in a longitudinal study. Furthermore, we aimed to investigate possible effects and critical periods of Paracetamol intake on fetal immune ontogeny by analyzing the association between Paracetamol and HSC frequencies in cord blood.

3. Material and Methods

3.1. Study Design and Population

The PRINCE study is conducted at the University Medical Center Hamburg-Eppendorf (UKE) and was initiated in 2011. Inclusion criteria were maternal age of 18 years or higher and a viable singleton pregnancy at gestational week 12–14. Women with chronic infections (HIV, hepatitis B/C), known substance abuse, who were smoking, had multiple pregnancies or pregnancies conceived after assisted reproductive technologies were excluded. Pregnant women were invited to three antenatal visits, once per trimester (gestational weeks 12 to 14, 24 to 26, and 34 to 36). Data on the assessment of relevant covariables is described in detail elsewhere (Diemert et al., 2017). All study subjects signed informed consent forms and the study protocol was approved by the ethics committee of the Hamburg Chamber of Physicians (PV3694).

At the time of analyses, the PRINCE study sample consisted of 620 women. To be included in the present analysis, data on analgesic intake had to be available for each trimester, which was the case for 518 women. For analyses, only those women with no analgesic intake at all were compared to those with Paracetamol medication. Hence, women relying only on other analgesic medication such as ibuprofen were excluded from further analyses (final $n = 483$). Cord blood could be obtained from women delivering at the UKE (30% overall) and analyzed for hematopoietic stem cell ($n = 146$) (Fig. 1).

3.2. Assessment of Analgesic Medication

At each study visit, medication intake was assessed via a questionnaire assisted interview from the study gynecologists. Women were asked to reflect in detail which analgesic they took since the beginning of their pregnancy or the last study visit, respectively. For each analgesic taken, information was asked regarding the brand name, specific date(s) of intake, intake duration and dosage. For the present analyses, total intake dosage as well as duration of intake were summed up for each trimester as well as for the entire pregnancy. Furthermore, categorical variables were computed: Based on the standard concentrations (500 mg Paracetamol/tablet) available in Germany, women were divided into four a priori defined groups according to Paracetamol intake: ≤500 mg, >500–1500 mg, >1500-4000 mg, and >4000 mg per day. Regarding intake duration, women were divided into groups of onetime, occasionally (irregular acetaminophen intake), weekly (regular intake at least for two weeks per trimester), and daily (regular intake at least for three continuous days per trimester).

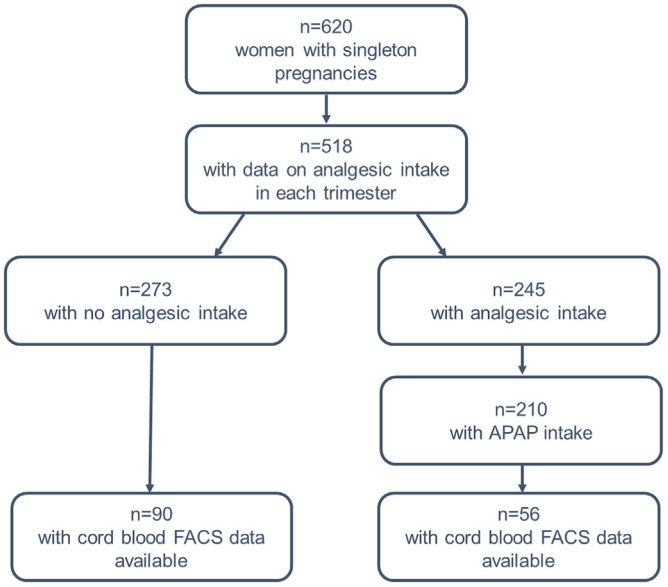

Fig. 1. Flow chart of the study sample. Abbreviations used: FACS, fluorescence-activated cell sorting.

3.3. Cord Blood Analyses

If the woman gave birth at the UKE and signed consent, cord blood was collected. Samples were processed within 24 h after delivery, and 50 μl whole blood were stained with an antibody mixture as described elsewhere (Diemert et al., 2016). Total HSCs were identified using two independent gating strategies in the CD45-positive mononuclear cell subset (mother population) using CD34 and CD133 as markers on cells with intermediate CD45 expression (CD45int). HSCs are defined as CD45int $CD34^+$ cells, while "total HSCs" encompass in addition to the $CD34^+$ cells the 'early HSC', defined as the $CD34^-$ $CD133^+$ (Supplemental Fig. 1).

3.4. Statistical Analyses

Descriptive statistics were used to present analgesic intake in the cohort: Group comparisons were made using *t*-test or ANOVA for normally distributed continuous variables, Kruskal-Wallis test for not normally distributed continuous variables and Chi-square test for categorical variables.

In a first step assessing the association between Paracetamol intake and HSCs, binary variables (Paracetamol intake in pregnancy yes/no) were analyzed using univariate regression models. As variables for HSC populations were not normally distributed, they were logarithmized prior to analyses. If an association was indicated in this first analysis by a statistical trend, further analyses were run to investigate whether a certain period during pregnancy was of particular importance. To achieve this, the structured approach to hypotheses involving binary exposures over the life course, presented by Smith et al. (2015), was used: As we aimed to assess the association between repeatedly measured Paracetamol intake over the course of pregnancy and cord blood HSC populations, applying our research question to

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