Research Article

JOURNAL OF HEPATOLOGY

Prenatal acetaminophen induces liver toxicity in dams, reduces fetal liver stem cells, and increases airway inflammation in adult offspring

Khalil Karimi[1,*], Timo Keßler[1,†], Kristin Thiele[2,†], Katherina Ramisch[2], Annette Erhardt[1], Peter Huebener[3], Roja Barikbin[1], Petra Arck[2], Gisa Tiegs[1,*]

[1]*Institute of Experimental Immunology and Hepatology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany;* [2]*Laboratory for Experimental Feto-Maternal Medicine, Department of Obstetrics and Fetal Medicine, University Medical Center Hamburg-Eppendorf, Hamburg, Germany;* [3]*I. Medical Clinic, University Medical Center Hamburg-Eppendorf, Hamburg, Germany*

Background & Aims: During pregnancy, acetaminophen is one of the very few medications recommended by physicians to treat fever or pain. Recent insights from epidemiological studies suggest an association between prenatal acetaminophen medication and an increased risk for development of asthma in children later in life. The underlying pathogenesis of such association is still unknown.

Methods: We aimed to develop a mouse model to provide insights into the effect of prenatal acetaminophen on maternal, fetal and adult offspring's health. The toxic effect of acetaminophen was studied in mice on 1) maternal liver; mirrored by biomarkers of liver injury, centrilobular necrosis, and infiltration of granulocytes; 2) fetal liver; reflected by the frequency of hematopoietic stem cells, and 3) postnatal health; evaluated by the severity of allergic airway inflammation among offspring.

Results: We observed an increased susceptibility towards acetaminophen-induced liver damage in pregnant mice compared to virgins. Moreover, hematopoietic stem cell frequency in fetal liver declined in response to acetaminophen. Furthermore, a greater severity of airway inflammation was observed in offspring of dams upon prenatal acetaminophen treatment, identified lung infiltration by leukocytes and eosinophil infiltration into the airways.

Conclusion: Our newly developed mouse model on prenatal use of acetaminophen reflects findings from epidemiological studies in humans. The availability of this model will allow improvement in our understanding of how acetaminophen-related hepatotoxicity is operational in pregnant individuals and how an increased risk for allergic diseases in response to prenatal acetaminophen is mediated. Such insights, once available, may change the recommendations for prenatal acetaminophen use.

Introduction

Physicians frequently prescribe acetaminophen (*N*-acetyl-para-aminophenol; APAP) during pregnancy for treatment of fever and pain and indeed, many pregnant women use APAP more than once during pregnancy [1]. APAP hepatotoxicity is the most common cause of death due to acute liver failure in the developed world and is increasingly recognized as a significant public health problem [2–4]. In mammals, APAP is metabolized primarily in the liver into toxic and non-toxic metabolites. The initial event in APAP-induced hepatotoxicity is the formation of the toxic metabolite *N*-acetyl-*p*-benzoquinone imine (NAPQI). This leads to the depletion of hepatic glutathione which in turn leads to an increase in protein adduct formation that ultimately results in the activation of death signaling pathways in hepatocytes and consequently hepatocellular necrosis [5]. Besides the direct hepatocytotoxicity, APAP induces idiosyncratic liver damage [6], linked to the activation of the innate immune response, such as up regulation of inflammatory cytokines and activation of various innate immune cells [5,7].

Recent evidence shows that prenatal factors such as medication, but also stress perception and life style may affect fetal development [8,9]. In this context, the increased risk for allergy and asthma in children later in life is particularly noteworthy. It is well known that the risk of allergy and asthma are associated with susceptibility genes. However, there is emerging evidence that exposure to environmental challenges increases the risk for asthma later in life, irrespective of a hereditary component. In this context, the prenatal period is particularly sensitive to environmental challenges [10,11]. Results from epidemiological studies and meta-analyses have recently become available

Keywords: Hepatotoxicity; Pregnancy immune adaptation; Hematopoietic stem cells; Eosinophil infiltration; Allergic inflammation.

Received 22 April 2014; received in revised form 17 November 2014; accepted 9 December 2014; available online 18 December 2014

* Corresponding authors. Address: Institute of Experimental Immunology and Hepatology, University Medical Center Hamburg-Eppendorf, Martinistrasse 52, 20246 Hamburg, Germany. Tel.: +49 40 741058731; fax: +49 40 741057150.

E-mail addresses: k.karimi@uke.de (K. Karimi), g.tiegs@uke.de (G. Tiegs).

† These authors contributed equally to this work.

Abbreviations: APAP, acetaminophen; gd, gestational day; GSH, glutathione; OVA, ovalbumin; BAL, Bronchoalveolar lavage; HSCs, hematopoietic stem cells; ALT, alanine aminotransferase; PAS, periodic acid-Schiff reagent; AHR, airway hyper-responsiveness.

[12–15] and provide considerable evidence for an association between the prenatal use of APAP and an increased risk of the children to develop asthma later in life [1,16,17].

APAP freely crosses the placenta and thus the fetus can be exposed and may also experience APAP-associated liver damage. During fetal development, the liver is the major source of hematopoietic stem cells (HSCs) before the bone marrow takes over, and thus, APAP-mediated challenges to this pivotal cell subset may have long-lasting consequences.

Despite the growing recognition of an association between prenatal use of APAP and subsequent risk for asthma in the children, the underlying mechanism of this APAP-mediated increased risk of allergy and asthma is still unknown. This is largely due to the very limited availability of experimental models, which may allow dissecting immediate effects and long-term consequences of prenatal APAP medication for mother and child. One study, which dates back to the nineteen eighties, describes an increased liver toxicity upon administration of high dose APAP in pregnant mice [18], but lacks to provide insights on non-toxic APAP doses. Here we aimed to test the effect of non-toxic APAP doses on liver damage and inflammation in pregnant and non-pregnant mice. Furthermore, we evaluated the effect of these APAP doses on fetal immune development and pregnancy outcome and investigated if an increased risk for asthma, as observed in epidemiological studies, could also been detected in mice.

Materials and methods

Mice

7–10 week old females or 12 week old males C57BL/6J mice were obtained from Charles River Laboratories, Sulzfeld, Germany. Animals were housed in individually ventilated cages in the mouse facility of the University Medical Center Hamburg-Eppendorf and received humane care according to the guidelines of the National Institutes of Health in Germany. Following mating and upon detection of a vaginal plug in the morning, females considered to be at gestational day (gd) 0.5.

APAP treatment

Mice were fasted prior to the APAP injection for 16 h with food withdrawn at 5:00 pm on gd 11.5 to equalize glutathione levels. On gd 12.5 APAP in Phosphate Buffered Saline (PBS) was injected intraperitoneally (i.p.) and the control animals received PBS only. Animals were either euthanized after 3 h or on gds 13.5 or 16.5 to analyze maternal and fetal tissues or allowed to give birth to examine severity of airway inflammation among the offspring.

Tissue sampling

Mice were anesthetized lethally by intravenous injection of 150 mg/kg methohexital and 15 mg/kg heparin. Cardiac blood samples were taken to examine plasma activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and bilirubin. To isolate non-parenchymal liver cells, isolated livers were passed through 70 μm nylon meshes. Hepatocytes were removed by centrifugation (800 g, 20 minutes) in isotonic 37% Percoll solution (Amersham-Biosciences, Freiburg, Germany) containing 100 U/ml heparin. Erythrocytes were lyzed by a buffer containing 139 mM NH_4Cl and 19 mM Tris.

Fetal livers were harvested on gd 16.5 using a S6E Stereo Zoom Microscope (Leica, Bensheim, Germany) and uterine-draining lymph nodes were harvested on gd 13.5 and single cell suspensions were prepared by passing the tissues through a 40 μm cell strainer. After centrifugation at 450 g for 8 minutes at 4 °C, red blood cell (RBC) lysis was performed using RBC lysis buffer (eBioscience, SanDiego, CA, USA). The number of viable leukocytes was obtained by counting the cells using a Neubauer chamber upon adding Trypan Blue stain (0.4%, Life Technologies GmbH, Darmstadt, Germany).

Glutathione assay

Total glutathione (GSH) (GSH + GSSG) was measured in liver homogenates prepared from individual mice using a commercial glutathione assay kit (Cayman Chemical Company, Ann Arbor, MI, USA) as described by the manufacturer's instructions.

Histology

Maternal livers were embedded in GSV-1 tissue-embedding medium (Slee Technik GmbH, Mainz, Germany) and frozen at −75 °C for further analysis. For histological analysis of tissue structure, maternal livers were fixed in 4% formalin and subsequently embedded in paraffin. Sections were stained with hematoxylin and eosin (H&E) following standard protocols and analyzed by light microscopy.

Adult offspring lungs were kept in 4% phosphate buffered formalin for 24 h before transferring them into PBS for a week. The lungs were subsequently embedded in paraffin and 4 μm sections were prepared and either stained with H&E or periodic acid-Schiff reagent (PAS) for histological assessment. Images of sections were obtained with a Mirax Midi Scanner (Zeiss, Göttingen, Germany) and analyzed using the Mirax Viewer Software.

Analysis of pregnancy outcome

Pregnant control and APAP-treated mice were euthanized on gd 13.5 or gd 16.5 and the number of implantations and abortions were documented. On gd 16.5, fetuses were isolated from embryonic membranes to determine the fetal weight. Newborns were weighed the day after birth. Maternal weight assessment was performed before the food withdrawal, on gd 11.5 and considered as a reference value to calculate percentage weight loss or weight gain.

Experimental asthma

This study used an ovalbumin (OVA)-sensitized/challenged mouse model of allergic airway inflammation in order to examine the sensitivity of offspring to allergic asthma. Briefly, 6–8 week old offspring were sensitized by i.p. injection of 50 μg OVA (Grade 5, Sigma-Aldrich, Munich, Germany) adsorbed with 1.5 mg of Alum (Thermo Scientific, Rockford, IL, USA) in saline on day 0 and day 5. On days 12 and 14, mice were challenged intranasally with 75 μg OVA (Grade 6, Sigma-Aldrich) per mouse. Forty eight hours after the last challenge (day 16), mice were subjected to measurements of airway inflammation. OVA/alum-sensitized, saline-challenged mice served as control animals.

Bronchoalveolar lavage

On day 16, 48 h after OVA challenge and prior to harvesting the lungs, two aliquots of 500 μl PBS were injected and withdrawn through a tracheal cannula. Cells were removed from BAL fluid by centrifugation at 500 g for 5 minutes; room temperature and supernatants were stored at −80 °C for further evaluation of cytokine content. The cells were resuspended in PBS and smears of BAL cells were prepared with a Cytospin and stained with Jenner's stain solution and Giemsa solution for differential cell counts. A total of 200 cells were counted for each lavage sample.

Assessment of airway inflammation (H&E)

Following bronchoalveolar lavage, the lungs were inflated with formalin, fixed over night at 4 °C, and were embedded in paraffin. The blocks were cut at the main bronchus and stained with H&E and PAS. Airway inflammation and mucus production was assessed with a scoring system. Blinded observers evaluated tissue sections with 0 equal to no sign of inflammation and no mucus detection on the slide section, 1 equal to low cell infiltration with few number of goblet cells, or 2 high cell infiltrations with many goblet cells on the slide section.

Flow cytometric analysis

Cells were incubated with Fc-block (BD Pharmingen) for 15 minutes prior to antibody staining (see Supplementary data for the list of antibodies). Fixable Viability Dye eFlour506 from eBioscience was used to exclude the dead cells. Most staining conditions involved 8 flurochromes, and data were acquired using a BD LSRFortessa II (BD Biosciences) and analyzed using FlowJo (Tree Star, Ashland, OR, USA).