



Acetaminophen and pregnancy: short- and long-term consequences for mother and child

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ABSTRACT

Counter-intuitively, over-the-counter medication is commonly taken by pregnant women. In this context, acetaminophen (APAP, e.g. Paracetamol, Tylenol) is generally recommended by physicians to treat fever and pain during pregnancy. Thus, APAP ranks at the top of the list of medications taken prenatally. Insights on an increased risk for pregnancy complications such as miscarriage, stillbirth, preterm birth or fetal malformations upon APAP exposure are rather ambiguous. However, emerging evidence arising from human trials clearly reveals a significant correlation between APAP use during pregnancy and an increased risk for the development of asthma in children later in life. Pathways through which APAP increases this risk are still elusive. APAP can be liver toxic and since APAP appears to freely cross the placenta, therapeutic and certainly toxic doses could not only affect maternal, but also fetal hepatocytes. It is noteworthy that during fetal development, the liver transiently functions as the main hematopoietic organ. We here review the effect of APAP on metabolic and immunological parameters in pregnant women and on fetal development and immune ontogeny in order to delineate novel, putative and to date underrated pathways through which APAP use during pregnancy can impair maternal, fetal and long term children's health. We conclude that future studies are urgently needed to reconsider the safety and dosage of APAP during pregnancy and – based on the advances made in the field of reproduction as well as APAP metabolism – we propose pathways, which should be addressed in future research and clinical endeavors.

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1. Introduction

The most commonly used over-the-counter (OTC) pain medication is acetaminophen (APAP; e.g. Tylenol, Paracetamol), along with aspirin and non-steroidal anti-inflammatory drugs (NSAIDs). Surprisingly, these OTC medications are also commonly taken by pregnant women

(Black and Hill, 2003). Especially APAP ranks at the top of medication taken by pregnant women, although numbers vary between studies from 20% to 75% (Werler et al., 2005; Table 1). These variations may be explained by study sizes and participant clientele, but large studies ($n > 10,000$) support that the frequency of pregnant women taking APAP at least once during pregnancy is as high as 20–30%. Clearly, this high frequency of APAP use during pregnancy is explained by the fact that APAP is a highly effective antipyretic and analgetic drug. Further, the use of APAP is often recommended by doctors during all stages of pregnancy in order to treat pain or fever. Therefore, APAP is generally perceived as a 'safe' medication during pregnancy. Also, until 2008, the Food and Drug Administration (FDA)

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Table 1

Overview of published evidence on prenatal APAP challenge in various species.

Dosage		Gestational age of APAP challenge	Readout parameters/observation ^a		Reference
Mg (application route)	Category ^b		Maternal health	Fetal outcome/offspring health	
Mouse					
130, 615 and 1210 mg/kg/day (dietary)	Therapeutic and toxic	Throughout gestation	• Reduced survival	• Low litter size	Wright (1967)
100–400 mg/kg (intraperitoneal)	Therapeutic and toxic	Late gestation	Observed for 300 and 400 mg/kg: • Increased hepatotoxicity • Increased serum GPT • Higher incidence of liver necrosis • Increased mortality	ND	Larrey et al. (1986)
357, 715 and 1430 mg/kg/day (dietary)	Toxic	Throughout gestation	ND	• Low litter size at 1430 mg • Unaltered fetal weight • No morphological changes • Retarded postnatal growth • Increased percentage of abnormal sperm in male offspring at 1430 mg	Reel et al. (1992)
800 and 1430 mg/kg (intragastrically)	Toxic	Pre-implantation	• Decreased liver GSH • Unaltered ovarian GSH • Increased mortality (1430 mg/kg)	• Unaltered fetal GSH • Increased fetal weight at 1430 mg	Laub et al. (2000)
Rat					
125 and 250 mg/kg/day (gavage)	Therapeutic and subtoxic	Mid and late gestation	ND	Observed for 125 mg/kg: • Reduced fetal length • Increased fetal loss • Unaltered fetal weight	Lubawy and Garrett (1977)
10, 100 and 1000 mg/kg (orally)	Therapeutic and toxic	Late gestation	ND	• Decreased DA/PA ratio: • 25% at 100 mg; 80% at 1000 mg	Momma and Takeuchi (1983)
14 mg/kg (orally)	Therapeutic	Late gestation	ND	• Decreased ductal diameter of the heart • Increased right ventricular volume of the heart	Momma and Takao (1990)
3.5, 35 and 350 mg/kg/day (orally by stomach tube)	Therapeutic and subtoxic	Mid gestation	ND	• Reduced fetal length at 350 mg • Morphological liver changes occasionally in all groups	Buridan et al. (2001)
150, 250 and 350 mg/kg/day (by gavage)	Therapeutic and subtoxic	Late gestation (GD 13–21)	ND	• Reduced AGD in male fetuses • Reduced testosterone production in ex vivo fetal rat testes (1 μ M APAP)	Kristensen et al. (2011)
Human					
Not reported	Therapeutic	1. trimester ($n = 13/1.369$) ^d , ($n = 226/50.282$) ^d , ($n = 493/6.837$) ^d , ($n = 697/6.509$) ^d and ($n = 7.499/16.110$) ^d	ND	• Unaltered odds ratio for congenital abnormalities	Nelson and Forfar (1971), Heinonen et al. (1977), Jick (1981), Aselton et al. (1985), and Feldkamp et al. (2010)

Not reported	Therapeutic	1. trimester (<i>n</i> = 58/7.530) ^d All trimester ^c (<i>n</i> = 123/13.452) ^d	ND	• Unaltered odds ratio for congenital abnormalities	Thulstrup et al. (1999)
Not reported	Therapeutic	1./2. trimester – until 5. month (<i>n</i> = 173/421) ^d	ND	• Unaltered odds ratio for IQ and attentional deficits in children	Streissguth et al. (1987)
Not reported	Therapeutic	1. trimester (<i>n</i> = 23/2.684) ^d	ND	• Increased odds ratio for neural tube defects	Lynberg et al. (1994)
Not reported	Therapeutic	1. trimester (<i>n</i> = 285/1077) ^d	ND	• Unaltered odds ratio for neural tube defects	Shaw et al. (1998)
Not reported	Therapeutic	1. trimester (<i>n</i> = 1.126/2.218) ^d , (<i>n</i> = 506/1.004) ^d	ND	• Increased odds ratio for gastroschisis	Werler et al. (1992, 2002)
Not reported	Therapeutic	1. trimester (<i>n</i> = 84/330) ^d	ND	• Unaltered odds ratio for gastroschisis	Torfs et al. (1996)
Not reported	Therapeutic	1. trimester (<i>n</i> = 283/1.036) ^d (<i>n</i> = 463/860) ^d	ND	• Unaltered odds ratio for cardiac malformations	Zierler and Rothman (1985) and Cleves et al. (2004)
Not reported	Therapeutic	1. trimester (<i>n</i> = 177/689) ^d 2. trimester (<i>n</i> = 219/689) ^d 3. trimester (<i>n</i> = 173/689) ^d	Unaltered odds ratio for stillbirth	ND	Pastore et al. (1999)
Not reported	Therapeutic	20–32 weeks (<i>n</i> = 4266/9.400) ^d and (<i>n</i> = 3.824/8.511) ^d	ND	• Increased odds ratio for asthma and wheezing in children at the age of 30–42 months	Shaheen and Yamaura (2002) and Shaheen et al. (2005)
Not reported	Therapeutic	All trimesters ^c (<i>n</i> = 311/4.116) ^d	ND	• Increased odds ratio for multiple-trigger wheeze and ICS-treated wheeze at the age of 4.5 years	Goksor et al. (2011)
Not reported	Therapeutic	1. trimester (<i>n</i> = 26.945/90.459) ^d 2. trimester (<i>n</i> = 20.290/90.459) ^d 3. trimester (<i>n</i> = 25.701/90.459) ^d	ND	• Increased odds ratio for asthma and wheezing in children at the age of 18 month and 7 years	Rebordosa et al. (2008a)
Not reported	Therapeutic	All trimester ^c (<i>n</i> = 913/1.719) ^d	ND	• Increased odds ratio for wheezing in preschool children	Garcia-Marcos et al. (2009)
Not reported	Therapeutic	All trimester ^c (<i>n</i> = 103/301) ^d	ND	• Increased odds ratio for asthma and wheezing in children at the age of 5	Perzanowski et al. (2010)
Not reported	Therapeutic	1. and 3. trimester (<i>n</i> = 1.032/1.505) ^d	ND	• Unaltered odds ratio for asthma and wheezing in children	Kang et al. (2009)
Not reported	Therapeutic	1. trimester (<i>n</i> = 52/3.221) ^d	ND	• Unaltered odds ratio for renal malformations	Abe et al. (2003)
Not reported	Therapeutic	1. trimester (<i>n</i> = 7.007/12.311) ^d	ND	• Increased odds ratio for amniotic band syndrome	Werler et al. (2003)
Not reported	Therapeutic	All trimester ^c (<i>n</i> = 172/934) ^d	Unaltered odds ratio for miscarriage	ND	Li et al. (2003)
Not reported	Therapeutic	All trimester ^c (<i>n</i> = 173/38.151) ^d	Reduced risk of preterm birth	ND	Czeizel et al. (2005)
Not reported	Therapeutic	3. trimester (<i>n</i> = 28.484/98.140) ^d	Increased risk of preterm birth in preeclampsia	ND	Rebordosa et al. (2009)

Table 1 (Continued)

Dosage		Gestational age of APAP challenge	Readout parameters/observation ^a		Reference
Mg (application route)	Category ^b		Maternal health	Fetal outcome/offspring health	
Not reported	Therapeutic	1. trimester (n = 28.484/98.140) ^d 2. trimester (n = 20.501/98.140) ^d 3. trimester (n = 25.792/98.140) ^d	Unaltered odds ratio for miscarriage, preterm birth and stillbirth	• Unaltered odds ratio for low birth weight and SGA	Rebordosa et al. (2008b)
Not reported	Therapeutic	1. trimester (n = 56/398) 2. trimester (n = 95/398)	ND	• Increased odds ratio for congenital cryptorchidism especially in the 2. trimester	Kristensen et al. (2011)
Overdose	Toxic	All trimester (n = 60) ^e	Spontaneous miscarriages (n = 8) Stillbirth (n = 1) Elective pregnancy terminations (n = 9)	• Live born children without visible malformation (n = 41) • Live born children with malformations (n = 1)	Riggs et al. (1989)
Overdose	Toxic	All trimester (n = 300) ^e	Spontaneous miscarriages (n = 16) Stillbirth (n = 2) Elective pregnancy terminations (n = 54)	• Live born children (n = 219) without visible malformation • Live born children with malformations (n = 11)	McElhatton et al. (1997)
Assumed: 1200 mg plus 240 mg codeine per day	Therapeutic, chronic abuse	Throughout gestation acute event: 3. trimester (n = 1) ^e	Abnormal liver function: increased AST, ALT, LDH and bilirubin Slightly prolonged coagulation times (PT and PTT increased)	• CT scan: infratentorial hemorrhage • Increased serum ALT • Elevated bleeding times (PT and PTT increased)	Kurzel (1990)
8–9 g per day for 10–14 days	Toxic	2. trimester (n = 1) ^e	Fulminant hepatic failure resulting in liver transplantation	• Intra-uterine fetal demise 2 weeks after liver transplantation • MRI of the fetal corpus: extensive peri-cerebral and intraventricular hemorrhage with periventricular leukomalacia	Thornton and Minns (2012)

The following abbreviations were used: ND, not determined; GPT, glutamic-pyruvic transaminase; GSH, glutathione; DA/PA, ductus arteriosus/pulmonary artery; AGD, anogenital distance; ICS, inhaled corticosteroid; SGA, small for gestational age; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; PT, prothrombin time; and PTT, partial thromboplastin time.

^a Observations were made for all doses used, if not specified otherwise.

^b The category 'therapeutic dosage' was defined as <100 mg/kg in mice, 250 mg/kg in rats and <60 mg/kg in humans; 'toxic dosage' was defined as >200 mg/kg in mice, 400 mg/kg in rats and >150 mg/kg in humans.

^c No differentiation was made by the authors respective trimester.

^d Number of parentheses show: (number of study participants using APAP/total number of participants in respective cohort).

^e Number of parentheses show: (total number of participants in respective cohort).

in the United States had assigned APAP to 'Pregnancy Category B' during all three trimesters (Black and Hill, 2003; www.fda.gov/Drugs), which supports the notion that APAP can be considered as risk-free for mother and fetus. In contrast, i.e. aspirin was classified as 'Pregnancy Category D', based on observations of an increased risk, for e.g. neonatal intracranial hemorrhage (Rumack et al., 1981), increased perinatal mortality, decreased birth weight and prolonged gestation and labor (Black and Hill, 2003). Interestingly, in 2008, the FDA has begun to reconsider the classification categories related to the use of medication during pregnancy to inform more accurately and consistently about risk and benefit. However, updated information on, e.g. the recommendations for the use of APAP during pregnancy are rather difficult to obtain, neither medication leaflets, nor the FDA website currently provide clear statements.

Whilst an increased risk for adverse pregnancy outcome upon therapeutic use of APAP is not supported by a number of clinical trials, these findings are ambiguous and some trials do unveil a link between pregnancy-related complications such as miscarriage, preterm birth, low birth weight, and fetal malformations (cf. Table 1). However, potential mechanisms through which the risk for such pregnancy complications is communicated are still unknown.

APAP can freely cross the placenta (Byer et al., 1982; Weigand et al., 1984). Hence, it is not only the pregnant woman, but also the growing fetus that needs to be considered when discussing possible side effects and dosages limitations of APAP during pregnancy. The frequent and extensive use of APAP during pregnancy urgently requires a timely re-evaluation, with a special focus of potential effects of APAP on the maternal immune and endocrine adaptation to pregnancy as well as an impact of APAP on fetal development. This necessity to re-evaluate the use of APAP during pregnancy and its potential impact on fetal immune ontogeny and organ development is strongly supported by recent observations arising from epidemiological studies, revealing, e.g. an increased risk for asthma development in children born to mothers who took APAP during pregnancy (Eyers et al., 2011; Shaheen et al., 2002, 2005; Garcia-Marcos et al., 2009, cf. Table 1). Until now the mechanism of this APAP dependent increased risk toward asthma development in the offspring is unknown. It has been speculated that APAP depletes the anti-oxidant glutathione (GSH) in the alveolar fluid where it seems to be increased in asthma patients (Farquhar et al., 2010). However, extracellular GSH is unstable and not directly taken up by neighboring cells as an anti-oxidant (Hahn et al., 1978) in order to prevent, e.g. inflammation. Moreover, APAP is not primarily metabolized in the lung and, even more important, only high toxic but not therapeutic doses of APAP deplete GSH in liver parenchymal cells (see below). In addition, a shift toward a Th2 cytokine response as it has been shown to be mediated by antigen-presenting cells expressing low levels of GSH, was supposed as mechanism for asthma sensitization by APAP (Farquhar et al., 2010). However, again it seems unlikely that APAP is metabolized toward its GSH-depleting metabolite (see below) in antigen-presenting cells. Therefore it is an inevitable goal to elucidate the cellular and molecular mechanisms of

APAP sensitization toward asthma in order to be able to lower the risk of asthma development and probably other immune disorders of children whose mothers took APAP during pregnancy.

2. Drug transport across the placenta and its impact on fetal development

During pregnancy the fetus is provided with nutrients, hormones, and oxygen by the mother to ensure fetal growth and development. Here, the placenta represents the interface between mother and fetus and is responsible for the regulation of substance transfers (Fowden et al., 2008; Lewis et al., 2012; Moffett and Loke, 2006). Hence, besides controlling the transfer of nutrients and oxygen, the placenta also acts as a barrier to protect the fetus from challenges. In the placenta, the syncytiotrophoblast and fetal capillary endothelium express a number of drug transporters, which control fetal exposure to drug substrates including APAP (Iqbal et al., 2012). Research aiming to understand the role of placental drug transporters in controlling drug exposure of the fetus in general is largely underrepresented in coordinated research endeavors, but urgently required. One rationale for this urgency is that the average childbearing age of women in Western Societies steadily increases and an increased maternal age is more often associated with chronic diseases that may require treatment. Here, medication or other toxic agents such as caffeine may potentiate side effects if taken in combination with APAP (Lum and Wells, 1986; Burdan, 2004). Further, pregnancies arising from in vitro fertilization currently experience an unprecedented rise and require hormonal supplementation especially during early pregnancy. Thirdly, some drug may be taken during pregnancy, i.e. APAP.

Studies in mice aiming to identify the effect of APAP on drug transporter gene expression revealed a differential modulation, resulting, e.g. in a reduced expression of organic anion-transporting polypeptides and an increased expression of multidrug resistance-associated proteins (MRPs) as well as multidrug resistance proteins (Aleksunes et al., 2007). Drug transporters are also found in human placenta, including MRPs (Vahakangas and Myllynen, 2009). Using trophoblast cell lines, MRPs have been suggested to contribute to the elimination of APAP sulfate across the placenta (Mitra and Audus, 2010). However, the placental barrier still appears to be leaky for APAP, since APAP and its metabolites could be detected in infant's urine after the mother had taken APAP a few hours before delivery (Levy et al., 1975). This observation emphasizes the need to look at fetal hepatotoxic and teratogenic effect of APAP use during pregnancy.

Over the past decades, experimental rodent models aiming at unveiling the effect of APAP on oocyte, embryonic and fetal development are surprisingly sparse (Table 1). With regard to human pregnancies, a number of studies were carried out, with a great variety of read out parameters addressed in mother and (unborn) child (Table 1). As some of these findings are conflicting, an in depth evaluation of the risk for mother and child needs to be re-assessed, best by harmonising study designs and read

out parameter. Generally, crude read out parameters were chosen in these studies, e.g. maternal liver enzyme activity or the incidence of pregnancy complications or fetal malformations. Surprisingly, none of the studies focused on the effect of APAP on the maternal and/or fetal immune system. Noteworthy, the fetal liver is likely the key target of pre-natal APAP exposure, as APAP is known to be hepatotoxic and the fetal liver is the origin of hematopoietic stem cells (HSC) in humans as well as mice during fetal development, until the bone marrow takes over to provide HSC around birth (Solano et al., 2011). An APAP impairment of the HSC pool may affect immune development in other fetal organs to which HSC seed, e.g. the thymus.

3. Acetaminophen: toxicity, pharmacology and its impact on the maternal liver

APAP toxicity is the most common cause of acute liver failure in the US and Europe. Liver toxicity may be due to not only intentional suicides but also to unintended overdosing (Lee, 2010). As approximately half of the cases are unintentional or involve chronic ingestion (Kaplowitz, 2005), the FDA recently considered reducing the maximum single and daily dose (Lee, 2010). Moreover, one third of the individuals seem to have increased susceptibility to APAP-induced liver injury which has recently been suggested to be encoded by a single-nucleotide polymorphism in the *cd44* gene in both, animals and humans (Harrill et al., 2009).

At low doses, APAP is deactivated by glucuronylation and sulfatation into phase II metabolites that are excreted in the kidneys (Fig. 1 summarizes the metabolism and liver toxicity of APAP). A proportion of the drug undergoes metabolic activation to the toxic metabolite *N*-acetyl-*p*-benzoquinone imine (NAPQI) by CYP2E1, CYP1A2, and CYP3A4 finally inactivated by hepatic glutathione (GSH) and excreted as mercapturic acid conjugate in the kidney. When APAP doses exceed the threshold to toxicity, NAPQI formation depletes hepatic GSH (reviewed in Park et al., 2005; Jaeschke et al., 2012). Therefore, early administration of *N*-acetylcysteine (NAC) serves as a rescue for APAP hepatotoxicity as it represents a precursor amino acid for GSH biosynthesis (Prescott et al., 1977). NAC also crosses the placenta, since it was detectable in the cord blood of three viable infants delivered from pregnant women with APAP toxicity (Wilkes et al., 2005; Horowitz et al., 1997). Therefore, NAC might also be hepatoprotective to the fetus due to binding of toxic metabolites. However, when the capacity of GSH for NAPQI elimination is exceeded, it reacts with protein sulfhydryl groups which leads to the formation of protein adducts (Fig. 1), resulting in a loss of activity of several liver enzymes (Park et al., 2005). Although GSH depletion has been shown to be the obligatory step for covalent binding and toxicity (Jollow et al., 1973), additional hepatotoxic mechanisms have been reported. These include mitochondrial oxidative stress and peroxynitrite formation, inhibition of mitochondrial respiration, JNK activation, mitochondrial translocation of Bax, release of cytochrome c, formation of the mitochondrial permeability transition pore, and DNA fragmentation (Liu and Kaplowitz, 2006; Jaeschke et al., 2012). Although the latter mechanisms point to apoptotic cell death induced

by APAP, caspase-3 was not activated following APAP overdose, pan-caspase inhibitors failed to prevent APAP-induced hepatotoxicity in mice (Lawson et al., 1999) and the histology is rather necrosis than apoptosis (Jaeschke et al., 2011).

Following initial death, necrotic hepatocytes release DNA fragments, formyl peptides, high mobility group box-1 (HMGB1), and heat-shock proteins, that can act as damage-associated molecular patterns (DAMPs) known to evoke an inflammatory response by stimulation of Toll-like receptors (Fig. 1; Ju, 2012). For example, DNA fragments, in particular mitochondrial DNA, activate TLR9, which plays a critical role for inflammasome activation during APAP hepatotoxicity (Imaeda et al., 2009). Moreover, low levels of endogenous LPS present in the hepatic circulation have been implicated in APAP hepatotoxicity as APAP-induced liver injury was attenuated in mice lacking LPS-binding protein (LBP) or treated with a LBP inhibitory peptide (Su et al., 2005, 2010). However, the role of this down-stream activation of the innate immune response in the liver during APAP toxicity is controversially discussed with respect to the induction of collateral tissue damage versus repair mechanisms (Kaplowitz, 2005; Liu and Kaplowitz, 2006; Jaeschke et al., 2012). Both, DAMPs and LPS activate the Kupffer cells, which may have dual functions by releasing both pro- as well as anti-inflammatory cytokines and chemokines at different stages during inflammation and resolution of inflammation. For example, in the model of concanavalin A-induced liver injury which is initiated by T cell activation, depletion of Kupffer cells results in protection from acute liver damage by preventing the release of the cytotoxic mediator TNF α (Schümann et al., 2000). However, Kupffer cells significantly contribute to the production of the immune-regulatory cytokine IL-10, which mediates tolerance induction in response to liver inflammation in this model (Erhardt et al., 2007). Therefore, Kupffer cells are well known key players to counter-regulate inflammatory processes by initiating anti-inflammatory and immunoregulatory mechanisms including IL-6 and IL-10 release (Fig. 1). The most reliable experiment regarding the role of Kupffer cells during APAP hepatotoxicity was carried out using depletion of these cells by clodronate liposomes, which resulted in the aggravation of liver damage (Ju et al., 2002). Hence it seems likely that again IL-10 regulated the initial inflammatory response whereas IL-6 produced by Kupffer cells mediated liver regeneration.

There is evidence in the literature that NK cells and NKT cells produce IFN γ upon APAP injection, which mediates inflammation and liver damage. This was evidenced by cell depletion experiments and the use of IFN γ ^{-/-} and NKT cell-deficient CD1d^{-/-} mice (Liu et al., 2004; Ishida et al., 2002). IFN γ induces the expression of several chemokines, which might induce the recruitment of other leukocytes into the liver (Fig. 1). The high plasticity of these cells might again result in a conversion of pro-inflammatory cells to immune regulatory cells in the immune-privileged milieu of the liver (Erhardt et al., 2011; Tiegs and Lohse, 2010; Crispe, 2003). Accordingly, the chemokine receptors CCR2, CXCR2 and CXCR3 seem to play a hepatoprotective role in APAP-induced liver injury (Bone-Larson et al., 2000, 2001).

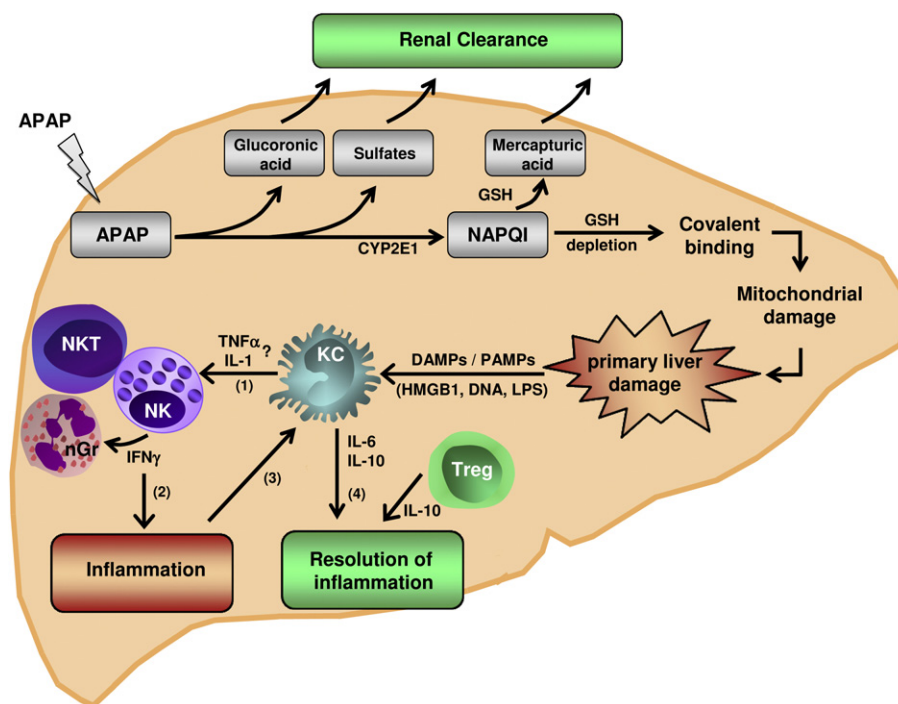


Fig. 1. A schematic overview depicting metabolism and liver toxicity of acetaminophen accompanied by the activation of the immune response [steps (1)–(4)] upon primary liver injury. (APAP, acetaminophen; NAPQI, *N*-acetyl-*p*-benzoquinone imine; GSH, glutathione; DAMPs, damage-associated molecular patterns; PAMPs, pathogen-associated molecular patterns; HMGB1, high mobility group box-1; LPS, lipopolysaccharide; KC, Kupffer cell; NK, natural killer cell; NKT, natural killer T cell; Treg, regulatory T cell; nGr, neutrophil granulocyte; NO, nitric oxide; TNF α , tumor necrosis factor- α ; IL-1, interleukin-1; IL-6, interleukin-6; IL-10, interleukin-10; and IFN γ , interferon- γ .)

However, it seems that NK and NKT cells are responsible for the accumulation of neutrophils in livers of APAP-treated mice (Liu et al., 2004; Ishida et al., 2002). Indeed, the role of neutrophils for liver damage in APAP-induced liver injury has been demonstrated by several groups (Ishida et al., 2002; Liu et al., 2004; Marques et al., 2012).

Although APAP is widely used in pregnant women, only very few reports analyzed its effects on metabolism, hepatotoxicity, and maternal health during pregnancy in experimental rodent models (Table 1: mouse and rat; Wright, 1967; Larrey et al., 1986; Laub et al., 2000). For example in experiments performed in rats (Table 1), the effect of APAP on the maternal system was largely neglected. In an experimental mouse model and in cultured two-cell stage embryos, Laub et al. (2000) demonstrated decreased GSH levels in the maternal liver, but not in ovaries and embryos upon challenge with hepatotoxic doses of APAP. Larrey et al. showed that hepatotoxicity of 300–400 mg/kg APAP administered intraperitoneally was aggravated in pregnant mice measured by increased serum transaminase activities as well as higher incidence of liver necrosis and mortality. Moreover, liver GSH depletion was accelerated and more prominent in pregnant mice (Larrey et al., 1986). This could be explained by the high concentrations of estradiol during pregnancy, which activate the constitutive androstane receptor (CAR; Koh et al., 2012), which is critically involved in APAP metabolism (Zhang et al., 2002) and enhances promoter activity of CYP450 isoenzymes. However, insights on mitochondrial

dysfunction, release of DAMPs and the subsequent activation of the innate immune system during APAP use in pregnancy are largely elusive.

4. Impact of acetaminophen on the pregnancy-adapted immune system

Although – or possible due to the fact that – APAP is on the market for approximately 50 years, its mechanisms of action are still not well understood. In the early 70s, it was demonstrated that APAP is far more effective against cyclooxygenase-1 (COX-1) enzyme in brain compared to spleen (Flower and Vane, 1972), suggesting a rather central than a peripheral effect of APAP. Several years later, cyclooxygenase-2 was discovered; however, it did not solve the APAP mystery with respect to its poor anti-inflammatory capacities. Unlike the selective COX-2 inhibitors, APAP is not successful in patients with rheumatoid arthritis (Graham and Scott, 2005). In 2002, another COX splice isoform was identified and named cyclooxygenase-3 (COX-3; Chandrasekharan et al., 2002). However, in rodents and humans, COX-3 seems to play a minor role with respect to prostaglandin-mediated fever and pain and therefore is unlikely to be clinically relevant (Kis et al., 2005). Cyclooxygenase enzymes catalyze the first step of metabolizing arachidonic acid to prostaglandin H₂ (PGH₂). In contrast to non-steroidal anti-inflammatory drugs (NSAIDs) and selective COX-2 inhibitors, APAP seems to act on the peroxidase reaction. However, high levels of

peroxides present in inflamed tissue might diminish the effect on prostaglandin synthesis mediated by APAP (Hinz and Brune, 2012). Therefore, the suggestion that APAP has no peripheral anti-inflammatory capacities has prevailed over the last years. Although the analgesic effect of APAP might be central and is due to activation of descending serotonergic pathways (Anderson, 2008; Graham and Scott, 2005), several publications hypothesized that APAP also exerts inhibitory effects on peripheral COX enzymes. Indeed, it was demonstrated that APAP selectively suppresses the peripheral prostaglandin E_2 (PGE_2) release and increases the COX-2 gene expression in a clinical model of acute inflammation (Lee et al., 2007). Interestingly, COX-2-deficient mice are highly susceptible to APAP-induced liver injury, since the prostaglandin responsiveness is altered in these mice (Reilly et al., 2001). These results suggest that COX-derived mediators such as prostaglandins might also exert hepatoprotective effects (Quiroga and Prieto, 1993). Indeed, this urgently requires an in depth re-evaluation of the cyclooxygenase-dependent effects of APAP, especially during pregnancy due to the immunosuppressive status in this outstanding phase.

Pregnancy is associated with a protective immune milieu (see below), which is established by progesterone (reviewed in Pincus and Arck, 2012). Although APAP does not induce CYP450 isoenzymes, it has been described that therapeutic doses of APAP lower female sex hormone levels in humans (Cramer et al., 1998). Moreover, APAP elicited anti-estrogenic responses in mice including inhibition of progesterone receptor up-regulation in the uterus and in the liver (Patel and Rosengren, 2001). Hence, it could be speculated that APAP impairs pregnancy-associated immune tolerance by skewing the immune system toward an increased activation of inflammation and a decrease of regulatory T cells. This skewed immune response could also affect the fetus leading to enhanced susceptibility toward allergic reactions of the offspring (reviewed in Pincus and Arck, 2012).

During pregnancy, the maternal immune system has to deal with an immunological challenge: on the one hand, paternal allo-antigens have to be tolerated by the maternal immune system and rejection of the semi-allogenic embryonic graft has to be prevented; on the other hand, the capability to mount an immune response against infectious and noxious agents has to be maintained during pregnancy to ensure maternal and hence, fetal survival and protection. As immune adaptations to pregnancy are covered by numerous recent reviews, we here wish to focus on maternal adapted mechanisms during pregnancy that could be affected by APAP.

Studies in non-pregnant mice have shown that APAP suppresses the humoral and cellular immunity (Ueno et al., 2000). Interestingly, this phenomenon seems to be independent of liver injury in mice (Yamaura et al., 2002). APAP-induced immuno-suppression is characterized by decreased antibody production, reduced frequencies of total thymocytes (especially in the immature $CD4^+CD8^+$ thymocyte pool), total splenocytes (especially in the B cell pool), and hepatic lymph node cells measured 24 h upon a hepatotoxic APAP injection. Interestingly, cell counts quickly recovered in the spleen, but not in the thymus,

even not within 5 days upon APAP challenge (Masson et al., 2007). Moreover, APAP challenge induces an impaired proliferation capacity of splenocytes upon mitogenic re-stimulation (Masson et al., 2007; Yamaura et al., 2002). Furthermore, it is suggested that APAP-induced hepatocyte death might trigger the activation of resident macrophages and lymphocytes, release of inflammatory mediators, and recruitment of further inflammatory cells including neutrophils and mononuclear cells (Jaeschke, 2005; Liu and Kaplowitz, 2006). Recently, it has also been demonstrated that liver DCs undergo maturation by up-regulating MHC II, co-stimulatory molecules, and Toll-like receptors upon APAP challenge, whereas splenic DCs did not. Moreover, DC depletion resulted in an aggravated hepatotoxicity. The authors concluded that APAP-primed liver DCs have the capacity to prevent NK cell activation and induce neutrophil apoptosis (Connolly et al., 2011). During normal pregnancy, DCs have a tolerogenic phenotype induced by tolerogenic stimuli such as progesterone, Galectin-1, vitamin D, and – most interesting for the present problem and research objective – PGE_2 . Subsequently, mediators and cells of fetal immune tolerance are generated, i.e. IL-10 and Tregs. However, since APAP usage suppresses PGE_2 release, DC maturation might be promoted as shown in the murine study (Connolly et al., 2011), which possibly results in the loss of fetal tolerance.

Given the importance of regulatory T cells in pregnancy maintenance, it may be tempting to speculate that APAP may alter frequency or function of this pivotal cell subset. However, it is still elusive if APAP directly influences regulatory T cells. To unveil such putative influence, the synergistic role of TGF- β and PGE_2 in Treg conversion/expansion and suppressive capacity might be an interesting approach (Baratelli et al., 2005). Besides an effect on regulatory T cells, APAP challenge induces hepatocyte cell death followed by the activation of resident immune cells in the liver including NKT cells (Jaeschke, 2005; Liu and Kaplowitz, 2006). Interestingly, alpha-galactosylceramide-mediated stimulation of CD1d-restricted NKT cells induces pregnancy loss in early gestation by a perforin-dependent mechanism (Boyson et al., 2006). Hence, APAP-mediated activation of NKT cells might also have adverse side-effects on pregnancy outcome. This might be of particular importance for sensitization of the offspring toward asthma, as NKT cells not only produce IFN γ , which mediates Th1-related immune responses and, via mast cell activation, airway hyper-responsiveness, neutrophilic and eosinophilic inflammation and airway remodeling in a chronic asthma model (Yu et al., 2011), but also IL-4, which is indeed critical for asthma development. However, to our knowledge the role of NKT cell derived IL-4 has never been investigated so far in this context.

APAP use during the third trimester increased the risk of preterm birth in women who also suffered from preeclampsia (Rebordosa et al., 2009). In this context, it has been suggested that the increased risk of preeclampsia and thromboembolic diseases was triggered by reduced prostacyclin (prostaglandin I_2 , PGI_2) production in endothelial cells (O'Brien et al., 1993; Rebordosa et al., 2009, 2010; Zelov, 2008).

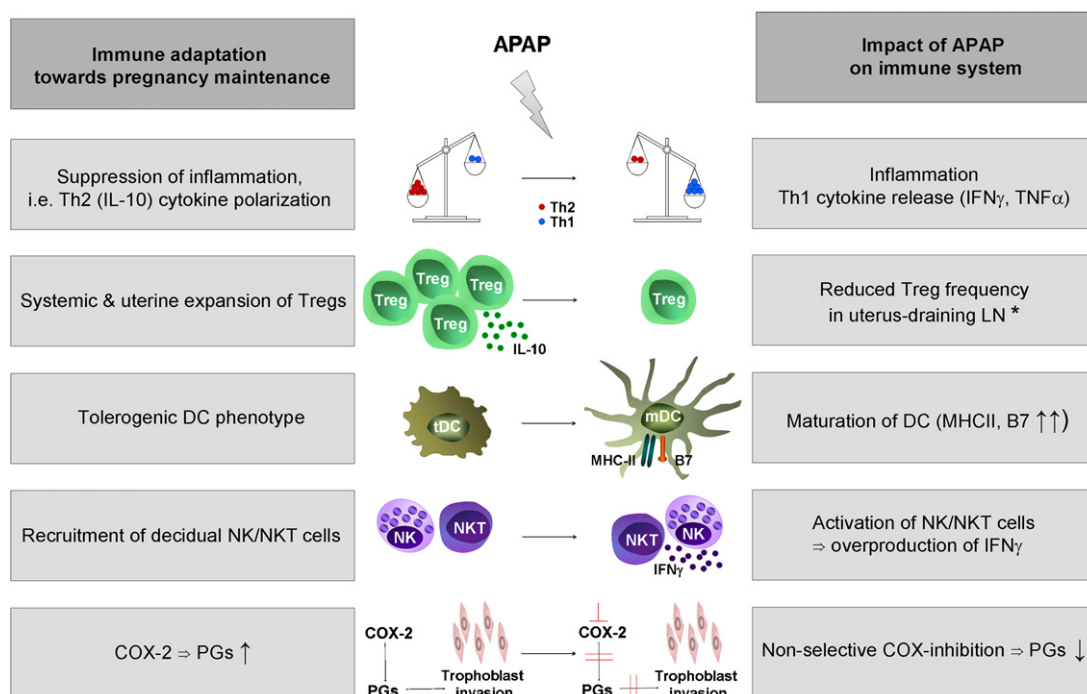


Fig. 2. A schematic overview illustrating the immune adaptation toward pregnancy maintenance and the impact of acetaminophen on the immune system. The central part shows the putative influence of acetaminophen on the immuno-suppressive status during pregnancy. (APAP, acetaminophen; Th1, T helper 1 cell; Th2, T helper 2 cell; Treg, regulatory T cell; iDC, immature dendritic cell; mDC, mature dendritic cell; NK, natural killer cell; NKT, natural killer T cell; COX-2, cyclooxygenase 2; PGs, prostaglandins; and *own unpublished data.)

Further, reduced prostaglandin synthesis in the human endometrium causes poor endometrial receptivity for embryonic implantation, since prostaglandin synthesis seems to be impaired in women with repeated in vitro fertilization (Achache et al., 2010). Therefore, it can be postulated that APAP not only impairs with pregnancy progression, but also hampers fecundity by suppressing PGE₂ release.

The immune adaptation toward pregnancy maintenance, the impact of acetaminophen on the immune system and a putative influence of APAP on the immuno-suppressive status during pregnancy are summarized in Fig. 2. In order to elucidate the impact of APAP on the immune system during pregnancy, we established an experimental mouse model of APAP-induced liver injury in pregnant mice. Interestingly, our pilot data show that even therapeutic dosages of APAP result in a dysregulation of the immune system in the liver and uterus-draining lymph nodes of dams (Kessler et al., 2012). Hence, the disruption of immune-regulatory processes which affect both, a Th1 bias as well as an altered Th2/immunosuppressive response, on the maternal side during pregnancy might also affect and retard fetal development. Here, fetal immune ontogeny may be particularly vulnerable, due to the hepatotoxicity of APAP, as the liver is a pivotal immune organ during fetal development. Hence, an APAP-induced challenge to immune ontogeny may carry over into postnatal life and increase the risk of chronic immune diseases such as asthma in the offspring.

In summary, in depth analyses are needed to identify how prenatal use of APAP affects the mother and child.

Considering the advances that have emerged over the past two decades, future interdisciplinary research studies are urgently needed to test the mechanisms proposed herein through which APAP may alter the maternal and fetal systems, via the immune system.

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