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Acetaminophen hepatotoxicity in mice: Effect of age, frailty and exposure type

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ABSTRACT

Acetaminophen is a commonly used analgesic that can cause severe hepatotoxicity in overdose. Despite old age and frailty being associated with extensive and long-term utilization of acetaminophen and a high prevalence of adverse drug reactions, there is limited information on the risks of toxicity from acetaminophen in old age and frailty. This study aimed to assess changes in the risk and mechanisms of hepatotoxicity from acute, chronic and sub-acute acetaminophen exposure with old age and frailty in mice. Young and old male C57BL/6 mice were exposed to either acute (300 mg/kg via oral gavage), chronic (100 mg/kg/day in diet for six weeks) or sub-acute (250 mg/kg, t.i.d., for three days) acetaminophen, or saline control. Pre-dosing mice were scored for the mouse clinical frailty index, and after dosing serum and liver tissue were collected for assessment of toxicity and mechanisms. There were no differences with old age or frailty in the degree of hepatotoxicity induced by acute, chronic or subacute acetaminophen exposure as assessed by serum liver enzymes and histology. Age-related changes in the acetaminophen toxicity pathways included increased liver GSH concentrations, increased NQO1 activity and an increased pro- and anti-inflammatory response to acetaminophen in old age. Frailty-related changes included a negative correlation between frailty index and serum protein, albumin and ALP concentrations for some mouse groups. In conclusion, although there were changes in some pathways that would be expected to influence susceptibility to acetaminophen toxicity, there was no overall increase in acetaminophen hepatotoxicity with old age or frailty in mice.

1. Introduction

Acetaminophen is a commonly used analgesic that can cause severe hepatotoxicity in overdose. The pharmacokinetic and toxicological mechanisms of an acute over-exposure to acetaminophen in young adults have been well established (Larson, 2007; Jaeschke et al., 2012). Following an acute overdose of acetaminophen, there is saturation of the conjugation metabolism pathways in the liver, resulting in more drug being oxidized into the toxic metabolite, N-acetyl-p-benzoquinone imine (NAPQI), by the cytochrome P450 (CYP450) enzyme, CYP2E1 (Holt and Ju, 2006). Glutathione (GSH) becomes depleted, resulting in build-up of the toxic metabolite, NAPQI in the liver. NAPQI causes direct mitochondrial damage (Jaeschke, 1990), and the induction of an inflammatory response (Antoine et al., 2008), which can result in cell death, predominantly centrilobular necrosis (Mitchell et al., 1973), with some evidence for apoptosis (Possamai et al., 2013).

With old age, and frailty, pharmacokinetic and pharmacodynamic changes occur that may affect the pharmacology and toxicology of acetaminophen (Mitchell et al., 2011a). Despite the fact that old age and frailty are associated with significant physiological changes, extensive utilization of drugs, including acetaminophen, (Pearson et al., 2007; Koponen et al., 2013), and a high prevalence of adverse drug reactions (Burgess et al., 2005), there are very limited studies on the changing risks of toxicity from acetaminophen in old age and frailty.

A study of hospitalized patients found that frail older people taking therapeutic doses of acetaminophen for five days had higher serum

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acetaminophen concentrations than younger patients, without increased liver enzymes (Mitchell et al., 2011b). A study of acetaminophen self-poisoning in Denmark, found that although the majority of acetaminophen induced hepatotoxicity occurs in young adults, the majority of deaths associated with acetaminophen exposure occur in old age (Schmidt, 2005). Clinical studies have shown an increased risk of liver failure after staggered over-exposures to acetaminophen (Craig et al., 2012; Ferner et al., 2011). Older people are more likely, than younger people, to have chronic low-dose exposure or accidental over-exposure to acetaminophen (Kane et al., 2012). However, the changing risk of toxicity to these types of acetaminophen exposures in old age has not been investigated.

There have been several animal studies, using Fischer 344 rats that have shown a decreased risk of acute acetaminophen hepatotoxicity in old age (Rikans and Moore, 1988), due to a reduction in CYP2E1 activity (Mach et al., 2014) or reduced transfer of acetaminophen into the liver (Mitchell et al., 2011c). Studies in Sprague Dawley rats, however, found an increased susceptibility to hepatotoxicity in old age (Tarloff et al., 1996). This may have been related to higher dosing in old age due to weight-gain in this ageing model (Mach et al., 2014). A recent study suggested that mice are a more clinically relevant model of acetaminophen toxicity than rats, due to similar susceptibility and mechanisms of damage (McGill et al., 2012). The only mouse studies we are aware of on the effect of old age on acetaminophen toxicity, did not investigate direct toxicity outcomes, but did find decreased liver glutathione concentrations in old age which may imply increased susceptibility to toxicity in old age (Chen et al., 1990; Al-Turk and Stohs, 1981). Here we seek to establish for the first time the risk of acetaminophen toxicity in old age and frailty in mice.

Animal studies on the effect of chronic or sub-acute acetaminophen exposure are limited. Several studies showed that low-dose acetaminophen administered daily did not cause liver toxicity in the absence of other risk factors in young mice (Yisarakun et al., 2014; Kondo et al., 2012; De Meijer et al., 2012). Studies of moderate dose acetaminophen pre-treatment have found it either to protect against (Ghanem et al., 2009; O'Brien et al., 2000; Shayiq et al., 1999), or increase the risk of toxicity (Kim et al., 2009) from further acetaminophen doses. There have been no animal studies on chronic or sub-acute acetaminophen exposure in old age.

The recent development of frailty assessment tools for animals, allows the study of not only age-related changes, but also frailty-related changes in animals models for the first time. Whitehead et al. (2014) developed and validated a mouse frailty index, which utilizes 31 simply assessed clinical measures to assess frailty in C57BL/6J mice. This tool has not yet been used to investigate changes in drug toxicity with frailty.

Our study aims to assess changes in the risk and mechanisms of hepatotoxicity from acute, chronic and sub-acute acetaminophen exposure with old age and frailty in C57BL/6 mice. Understanding the risk of acetaminophen hepatotoxicity, from different exposure types, and in old age and frailty is important for optimizing the safe effective use of acetaminophen in older people.

2. Methods

2.1. Animals

Three cohorts of young and old male C57BL/6 mice were used to test the different acetaminophen exposures. Cohort one was made up of young (age = 7.3 ± 0.3 months, n = 23) and old (age = 18.9 ± 2.3 months, n = 25) male Cre control C57BL/6 transgenic littermates, and C57BL/6 wild-types, aged at the National Institute on Ageing (NIA, Baltimore, MD). Mice were fed a 2018 Teklad Global 18% Protein Rodent diet (Harlan laboratories). A second cohort of young (age = 10 ± 0.0 months, n = 12) and old (age = 23.7 ± 0.02 months, n = 16) male C57BL/6 mice were also aged at the NIA (Baltimore, MD). They were fed a standard AIN-93G diet (Harlan laboratories). A third cohort of young (age = 4.0 ± 0.3 months, n = 21) and old (age = 26.8 ± 0.5 months, n = 17) male C57BL/6 mice were obtained from, and aged at the Kearns Facility (St Leonards, NSW, Australia). Mice were fed a standard Rat and Mouse Premium Breeder Diet (Gordon's Specialty Stockfeeds, NSW, Australia).

All mice were group housed in cages of 4–5 with ad libitum access to food and water. Animal rooms were maintained on a 12 h light/dark cycle at 20–22 °C, and 30–70% humidity. Animals were randomly assigned to treatment or control groups prior to the treatment day. All animal protocols were approved by the Animal Care and Use Committee of the National Institute on Ageing (429-TGB-2017 and 405-TGB-2016) or the Northern Sydney Local Health District Animal Ethics Committee (1206-014A).

2.2. Frailty index assessment

The Mouse Clinical Frailty Index (Whitehead et al., 2014) is a simple, noninvasive tool for the assessment of frailty in mice. A frailty index score is calculated for each mouse using a 31 item frailty index. Old mice from cohort two were assessed for frailty, after randomization to treatment group, the day before being dosed with acetaminophen. Frailty testing was carried out by two raters (AK and SM). Old mice from cohort three were assessed for frailty, after randomization to treatment group, the day before being started on their acetaminophen or control diet. Frailty testing was carried out by two raters (AK and JM). The raters were blinded to each others scores. The final reported frailty index scores were calculated from the mean of the two raters' scores.

2.3. Acetaminophen treatment and tissue collection

For cohorts one and two, there were two exposure groups: an acute high dose acetaminophen group and a control group. Animals were fasted overnight (16 h), then dosed with 300 mg/kg acetaminophen (Panadol Color-free Baby Drops, 100 mg/mL, GlaxoSmithKline, Australia) (acute group) or saline vehicle (control group) via oral gavage between 8 and 10 am. This paracetamol dose was chosen as a standard dose to induce toxicity in C57BL/6 mice (McGill et al., 2012). Food was returned to the mice 2 h after dosing. Six hours after dosing mice were anaesthetized with an i.p. injection of ketamine (75 mg/kg, DVR Pharmacy, Bethesda MD) and xylazine (10 mg/kg, DVR Pharmacy Bethesda MD). A midline laparotomy was performed and blood taken from the Inferior Vena Cava. The portal vein was then cannulated with a 21G intravenous catheter (BD, Sydney, Australia) through which the liver was perfused in-situ at 1–1.5 mL/min/g of liver with oxygenated Krebs–Henseleit bicarbonate buffer (95% O_2–5% CO_2, 37 °C), to remove blood from the liver. Sections of the liver were snap frozen in liquid nitrogen for subsequent enzyme activity assays, RNA extraction and fixed in 10% neutral formalin for subsequent histopathological analysis. See Appendix Fig. A.1 for flow chart.

For cohort three, there were three exposure groups: a control group, a chronic acetaminophen group, and a sub-acute acetaminophen group. All mice were randomized to either a control diet (control and sub-acute groups), or control diet supplemented with acetaminophen at a concentration of 1.33 g/kg feed (chronic group), both diets fed ad libitum. Mice remained on their respective diets for six weeks (food was weighed every 2 weeks). The sub-acute acetaminophen group was then administered, via oral gavage, acetaminophen (Panadol Color-free Baby Drops, 100 mg/mL, GlaxoSmithKline, Australia), 250 mg/kg three times per day (at 8 am, 1 pm, 6 pm) for two days. On the third day the mice were fasted at 6 am, then gavaged with two more doses of acetaminophen (250 mg/kg), at 8 am and 1 pm. The control diet group and the chronic group, received the same regimen of saline dosing. Three hours after the final acetaminophen or saline dose, all mice were anaesthetized with an i.p. injection of ketamine (75 mg/kg, Cenvet Australia, #K1500) and xylazine (10 mg/kg, Cenvet Australia, #X5010), and blood