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Hepatic effects of tartrazine (E 102) after systemic exposure are independent of oestrogen receptor interactions in the mouse

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ABSTRACT

Tartrazine is a food colour that activates the transcriptional function of the human oestrogen receptor alpha in an in vitro cell model. Since oestrogens are cholestatic, we hypothesised tartrazine will cause periportal injury to the liver in vivo. To test this hypothesis, tartrazine was initially administered systemically to mice resulting in a periportal recruitment of inflammatory cells, increased serum alkaline phosphatase activity and mild periportal fibrosis. To determine whether an oestrogenic effect may be a key event in this response, tartrazine, sulphonated metabolites and a food additive contaminant were screened for their ability to interact with murine oestrogen receptors. In all cases, there were no interactions as agonists or antagonists and further, no oestrogenicity was observed with tartrazine in an in vivo uterine growth assay. To examine the relevance of the hepatic effects of tartrazine to its use as a food additive, tartrazine was orally administered to transgenic NF-κB-Luc mice. Pre- and concurrent oral treatment with alcohol was incorporated given its potential to promote gut permeability and hepatic inflammation. Tartrazine alone induced NF- κB activities in the colon and liver but there was no periportal recruitment of inflammatory cells or fibrosis. Tartrazine, its sulphonated metabolites and the contaminant inhibited sulphotransferase activities in murine hepatic S9 extracts. Given the role of sulfotransferases in bile acid excretion, the initiating event giving rise to periportal inflammation and subsequent hepatic pathology through systemic tartrazine exposure is therefore potentially associated an inhibition of bile acid sulphation and excretion and not on oestrogen receptor-mediated transcriptional function. However, these effects were restricted to systemic exposures to tartrazine and did not occur to any significant effect after oral exposure.

1. Introduction

Many consumer products including food and personal care items contain endocrine disrupting chemicals (EDCs) which may potentially interfere with the endocrine system in animals and humans (Diamanti-Kandarakis et al., 2009; Zoeller et al., 2012). A large number of EDCs have oestrogenic properties in that they mimic the biological effects of endogenous oestrogens. These chemicals are termed xenoestrogens and they may modulate endogenous oestrogen activity by interfering with endogenous oestrogen signalling or by disrupting synthesis, metabolism and transport of oestrogens (Shanle and Xu, 2011). A common mechanism in modulating oestrogen signalling is through interactions of xenoestrogens with the nuclear oestrogen receptors (ERs), often because they possess structural similarities to endogenous oestrogens (McKenna and O'Malley, 2002).

The ERs belong to the superfamily of steroid hormone nuclear receptors (Tsai and O'Malley, 1994; Hammes and Levin, 2007; see also Nuclear Receptor Signalling Atlas https://www.nursa.org/nursa/index.jsf). Two isoforms of the ER exist; the ERα (Green et al., 1986) and ERβ (Mosselman et al., 1996; Kuiper et al., 1996; Moore et al., 1998). Both ER isoforms are ligand-activated by oestrogens such as endogenous 17β-estradiol (E2) and mediate ER-regulated changes in gene expres-

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; E2, 17β oestradiol; EtOH, ethanol; hER, human oestrogen receptor; mER, murine oestrogen receptor; OO, olive oil; OSPCA, 5-oxo-1-(4-sulphophenyl)-2-pyrazoline-3-carboxylic acid (a major contaminant of the food additive); PBS, phosphate buffered saline; SA, sulphanilic acid; SA-NAc, sulphanilic acid N-acetate; SCAP, 1-(4-sulphophenyl)-3-carboxy-4-amino-5-pyrazolone; SPH, sulphophenylhydrazine; SSY, sunset yellow; T, Tartrazine; Tg, Tg(NF-κB) mice; w/t, wild type mice

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sion by interacting with specific DNA sequences (EREs) (Tsai and O'Malley, 1994; Hammes and Levin, 2007). In order to screen for chemicals having agonistic or antagonistic oestrogenic activity, a human-based reporter gene assay was developed (Axon et al., 2012). Employing this assay, the food colour tartrazine (also known as E 102) was identified as an activator of the human ERα in vitro (Datta and Lundin-Schiller, 2008; Axon et al., 2012).

The liver is a hormonal target for oestrogens via ERα (Ahlbory-Dieker et al., 2009) and determines the circulating levels of oestrogens via metabolic conversion of oestrogens to inactive products (Bondesson et al., 2015; Tsuchiya et al., 2005; Ziegler et al., 2015). Thus, significant inhibition of hepatic oestrogen metabolism through liver disease can result in feminisation in men (Burra, 2013). The liver is also a target organ for the toxic effects of high levels of oestrogens. Elevations in circulating oestrogens are hepatotoxic due to a disruption of bile flow and/or alteration in bile constituents (cholestasis) through a potential combination of ERα-dependent suppression of transporter expression (Yamamoto et al., 2006), ERα-dependent stimulation of canalicular transporter endocytic internalization (Barosso et al., 2012) and/or other signalling pathways such as GPR30 (Zucchetti et al., 2014). Cholestasis leads to an accumulation of bile acids in the liver, which is toxic and results in liver cell death (Woolbright and Jaeschke, 2012). In susceptible individuals, the elevations in circulating oestrogens in pregnancy or through use of contraceptives can be sufficient to lead hepatic failure and death in the absence of liver transplantation (Ozkan et al., 2015).

We hypothesised that tartrazine is a mouse ER activator and that if sufficient intact food chemical is absorbed and reaches the liver, it would have a cholestatic effect. We show that systemic exposure to tartrazine through intraperitoneal administration resulted in a pathology consistent with a cholestatic effect (although bile flow was not examined). However, in vitro reporter gene screening assays with all the known mouse ERs indicated that neither tartrazine, its sulphonated metabolites nor a major sulphonated contaminant of the food colour activated or antagonised any of the murine ER receptors. This was supported by an in vivo mouse uterine growth bioassay with tartrazine administered systemically. Oral exposure to tartrazine resulted in gut and hepatic inflammation (based on activation of NF-κB transcriptional function), but there was no evidence for any periportal inflammatory cell recruitment or fibrosis via this route of exposure, and co-exposing with ethanol to increase gut permeability to the food additive, inhibited these effects. Since tartrazine, its 4 sulphonated metabolites and a major sulphonated contaminant of the food additive inhibited dopamine sulphotransferase in a dose-dependent manner in hepatic S9 extracts, the hepatic effects of systemic exposure to tartrazine may be associated with an inhibition of bile acid sulphate conjugation. However, this effect is unlikely to occur after oral exposure to tartrazine.

2. Materials and methods

2.1. Materials

The mouse cholangiocyte cell line 603B was a gift from Dr Yedidya Saiman, Mount Sinai School of Medicine, New York. The mouse pancreatic epithelial cell line LTPA was originally obtained from the American Type Culture Collection (ATCC, catalogue CRL-2389, Manassas, Virginia). Tartrazine purity of 85% or greater – which meets the EC specifications for its use as a food additive, E2 and ICI182780 were purchased from Sigma (Poole, UK). The tartrazine metabolites sulphanilic acid (SA) [CAS 121-57-3] and 4-sulphopenylhydrazine (SPH) [CAS 98-71-5] and the contaminant 5-oxo-1-(4-sulphophenyl)-2-pyrazoline-3-carboxylic acid (OSPCA) [CAS 118-47-8, permitted at up to 0.5% in tartrazine preparations when used as a food additive according to EC and JECFA specifications] were purchased from Sigma (Poole, UK). The tartrazine metabolites sulphanilic acid N-acetate (SA-NAc) [CAS 121-62-0] and 1-(4-sulphophenyl)-3-carboxy-4-amino-5-pyrazolone (SCAP) [CAS 2508-84-1] were purchased from Santa Cruz Biotechnology (Dallas, Texas, USA) and custom synthesised by An-gene (Hong Kong) respectively. Analytical data for SCAP is provided in Supplementary Fig. 1.

2.2. Animal studies

C57Bl/6 wild type (wt) mice were purchased from Charles River (Kent, UK). Transgenic NF-κB-Luciferase (tg) mice (bearing a transgene composed of three NF- B sites from the Ig light chain promoter coupled to the gene encoding firefly luciferase) were originally obtained from Dr Harald Carlsen (Oslo University, Norway) and were generated and genotyped as previously described (Wallace et al., 2010). All animals had free access to food and water and conditions were kept on a 12 h light/12 h dark cycle at 47% relative humidity at 23 °C ± 1 °C. All experiments were performed under a UK Home Office licence with Local Ethics Committee approval.

2.2.1. Systemic exposure to tartrazine in adult mice

To investigate the effects of direct tartrazine exposure, male 12 week old mice were dosed with tartrazine at 50 mg/kg bw/day [dissolved in 137 mM NaCl, 2.7 mM KCl, 10 mM phosphate pH 7.4 (PBS)] via 5 intraperitoneal injections per week for 2 weeks before termination 24 h after the last administration. Control mice were administered with the PBS vehicle alone. Mice were exposed to E2 [prepared in ethanol:olive oil (1:20, v/v)] by intraperitoneal injection at a dose of 0.5 mg/kg bw/day for 3 consecutive days before termination 24 h after the last administration. Control mice were administered with ethanol:olive oil (1:20, v/v)] vehicle alone.

2.2.2. In vivo uterine growth bioassay

To test for mouse ERα activation in vivo, 19 day old female wt mice were treated with oestrogen or potential xenooestrogens by daily intraperitoneal injection on 4 consecutive days. Compounds were prepared in PBS or ethanol:olive oil (1:20, v/v) solvent vehicle with control mice administered solvent vehicle alone. On day 5, mice were culled and uteri removed and relative wet weight determined.

2.2.3. Oral exposure to tartrazine in adult mice, effect of alcohol

To investigate effects of oral tartrazine exposure, male adult wt and tg mice were pre-treated with 3 g ethanol per kg bodyweight from a 20% (v/v) ethanol solution twice daily by oral gavage for 14 days to increase gut permeability and/or alter the gut microbiota (Kirpich et al., 2012; Szabo, 2015). The control group was pre-dosed with 6.32 g dextrose (Sigma) per kg bodyweight from a 0.33 g/ml dextrose solution to control for the calorific content of ethanol. Following the 14-day pre-treatment period, mice were administered 50 mg tartrazine per kg bodyweight from a 2.6 mg/ml stock in either 20% (v/v) ethanol solution or in 0.33 g/ml dextrose solution by oral gavage twice daily for 10 consecutive weeks. Mice in the control groups were dosed with ethanol or dextrose solution alone. Body weights were measured once a week. Tg mice were imaged for inflammation by live in vivo imaging on an IVIS spectrum (Caliper Life Sciences) essentially as previously outlined (Wallace et al., 2010). D-luciferin was obtained from Synchem (Altenburg, Germany).

2.3. Cell line culture

603B cells were cultured in low glucose Dulbecco's Modified Eagles Medium (Sigma, Dorset, UK), supplemented with 10% (v/v) foetal bovine serum (Sigma) and 80 U/ml of penicillin and streptomycin. LTPA cells were cultured in the above medium further supplemented with 0.1 mM non-essential amino acids (Gibco, Life technologies, Paisley, UK) and 1 mM sodium pyruvate (Gibco, Life technologies). All cell lines were maintained in a humidified atmosphere at 37 °C in