ABSTRACT

Alcohol can cross the placental blood-barrier and can also be secreted into breast milk. This can affect developing foetuses and/or nursing neonates negatively, thus impacting on metabolic health in early or later life. Zingerone (ZO) has anti-oxidant, anti-diabetic, anti-inflammatory, hypolipidaemic and hepato-protective properties. I hypothesised that neonatal oral administration of ZO could programme for protection against alcohol-induced metabolic derangements in suckling Sprague-Dawley (SD) rat pups mimicking human neonates that indirectly consume alcohol through their mother's breast milk.

The first experiment evaluated ZO's potential to protect suckling rat pups against alcohol-induced metabolic derangements. Seventy 10-day old SD rat pups (males = 35; females = 35) were randomly assigned to four groups and administered treatments daily from postnatal (PND) 12-21: group 1-nutritive milk (NM), group 2-1 g/kg body mass ethanol (Eth), group 3- 40 mg/kg body mass ZO and group 4 - NM+Eth+ZO. Terminal body mass, blood glucose concentration, lipid profile and hepatic antioxidant status were determined. Zingerone and ethanol had no effect on pups' growth performance, blood glucose, total cholesterol, HDL- and LDL-cholesterol and hepatic thiobarbituric acid (TBARs), superoxide dismutase and catalase concentrations ($p > 0.05$). Ethanol decreased plasma triglyceride concentration in female rat pups ($p = 0.04$) but increased hepatic cytochrome P450E21 (CYP2E1) and decreased total glutathione (tGSH) concentration in male rat pups ($p < 0.05$). Zingerone increased tGSH in male rat pups ($p = 0.003$). A combination of ZO and ethanol increased ($p = 0.047$) hepatic CP2E1 concentration in male rat pups compared to control but had no effect ($p = 0.717$) on tGSH concentration. Neonatal orally administered ethanol induced hepatic oxidative stress which ZO, administered during the suckling period, failed to protect against.

In experiment II, 123 SD rat pups (males = 60; females = 63) were administered the same neonatal interventions as in experiment I but from PDN22 they were grown to adolescence (PND45) with *ad libitum* access to normal rat chow and tap water. From PND 46-100, rats from each of the four neonatal groups were divided into two subgroups: subgroup I had tap water and subgroup II had ethanol solution as drinking fluids, for eight weeks. Body mass, feed, fluid and caloric intake were measured. Blood glucose concentration, plasma alanine transaminase and aspartate transaminase (ALT and AST) activities, adiponectin (ADP), leptin (LEP) and insulin (INS), tumour necrosis factor-α (TNF-α), interleukin-6 (IL-6) and cytochrome P4502E1 (CYP2E1) concentrations were measured. HOMA-IR was computed. Visceral fat mass, hepatic fat content and histomorphometry were assessed. Hepatic TBARs and mRNA expressions of *peroxisome proliferator activator receptor-alpha* (*PPAR-α*), *sterol regulatory element binding protein 1c* (*SREBP1c*), *nuclear factor kappa beta* (*NF-Kβ*) and *TNF-α* were measured. Ethanol consumption in adulthood decreased feed and fluid intake but increased calorie intake and plasma CYP2E1 concentration ($p < 0.05$ vs control). It decreased blood glucose concentration of male rats ($p = 0.026$). A late single- and a double-alcohol hit had no effect on body and visceral fat mass of the rats ($p > 0.05$). Neonatal orally administered zingerone and ethanol and consumption of ethanol in adulthood had no effect on body mass, plasma lipid profile, adiponectin, leptin and insulin concentrations, HOMA-IR, AST and ALT activities, IL-6, TNF-α and hepatic TBARS and mRNA expression of *NF-KB* and *TNF-α* ($p > 0.05$). A late single hit with ethanol increased hepatic fat content of male rats only ($p = 0.014$). A double and or late single ethanol hit increased liver fat content in female rats ($p < 0.05$). Both a late single and double ethanol hit downregulated *PPAR-α* but upregulated *SREBP1c* expression in male and female rats ($p < 0.05$) and it caused the development of large droplet macrosteatosis. A combination of neonatal orally administered ZO and a late single ethanol hit

decreased visceral fat mass of female rats ($p = 0.045$ vs control) but it did not affect the blood glucose concentration of male rats ($p > 0.05$). Neonatal orally administered ZO with either a late single- or a double-ethanol hit caused hepatic macrosteatosis, but it had no effect on mRNA expression of *PPAR-α* of the rats ($p > 0.05$). However, neonatal orally administered ZO in combination with a late single ethanol hit did not affect *SREBP1c* expression of the rats but a combination of neonatal orally administered ZO with a double ethanol hit increased *SREBP1c* expression of female rats ($p = 0.005$).

The responses of the rats to interventions showed sexual dimorphism: ethanol consumption in adulthood decreased blood glucose concentration of male rats only and an early single ethanol hit caused microsteatosis only in female rats. Zingerone protected male rats against ethanol-induced hepatic fat accumulation. It attenuated the ethanol-induced upregulation of hepatic *SREPB1c* expression in males but not in females. Ethanol (late single and/or double hit) downregulated the hepatic *PPAR-α* expression in the rats which was mitigated by ZO. Neonatal orally administered ZO attenuated the late single- and double-hit ethanol-induced macrosteatosis in the rats. Thus, neonatal orally administered ZO can potentially be used as a prophylactic agent against ethanol-induced hepatic lipid accumulation in males and steatosis in both males and females.