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The food processing contaminant glyoxal promotes tumour growth in the multiple intestinal neoplasia (Min) mouse model

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ABSTRACT

Glyoxal is formed endogenously and at a higher rate in the case of hyperglycemia. Glyoxal is also a food processing contaminant and has been shown to be mutagenic and genotoxic in vitro. The tumourigenic potential of glyoxal was investigated using the multiple intestinal neoplasia (Min) mouse model, which spontaneously develops intestinal tumours and is susceptible to intestinal carcinogens.

C57BL/6J females were mated with Min males. Four days after mating and throughout gestation and lactation, the pregnant dams were exposed to glyoxal through drinking water (0.0125%, 0.025%, 0.05%, 0.1%) or regular tap water. Female and male offspring were housed separately from PND21 and continued with the same treatment. One group were only exposed to 0.1% glyoxal from postnatal day (PND) 21.

There was no difference in the number of intestinal tumours between control and treatment groups. However, exposure to 0.1% glyoxal starting *in utero* and at PND21 caused a significant increase in tumour size in the small intestine for male and female mice in comparison with respective control groups. This study suggests that glyoxal has tumour growth promoting properties in the small intestine in Min mice.

1. Introduction

The α-oxoaldehyde compound glyoxal is formed endogenously in all humans during normal metabolism, and at a higher rate in the case of hyperglycemia (Thornalley et al., 1999). Glyoxal is also a food processing contaminant and is probably formed in food through several different pathways such as the Maillard reaction (Gobert and Glomb, 2009), caramelization (Arribas-Lorenzo and Morales, 2010), lipid peroxidation (Fujioka and Shibamoto, 2004) and microbial activity/fermentation (Yamaguchi et al., 1994). Glyoxal is found in a vast number of food items and beverages, such as bread, cookies, yoghurt, sardine oil, coffee, tea, beer and wine (Arribas-Lorenzo and Morales, 2010; Barros et al., 1999; De Revel and Bertrand, 1993; Hirayama et al., 1984; Nagao et al., 1986; Yamaguchi et al., 1994).

The glutathione-dependent glyoxalase system has been suggested as the predominant detoxification pathway for α-oxoaldehydes, which converts glyoxal to glycolate (Abordo et al., 1999; Thornalley, 1998). Also, an in vitro study indicated that aldehyde dehydrogenase is also an important detoxification enzyme for glyoxal (Yang et al., 2011). The α-oxoaldehydes, including glyoxal, are highly reactive (Thornalley, 2005), and can bind to proteins, lipids and nucleic acids resulting in the formation of advanced glycation endproducts (AGEs) (Singh et al., 2001). A-oxoaldehyde-derived AGEs have been associated with the progression of diabetic complications (Ahmed, 2005; Vlassara and Palace, 2002) and is also thought to play a role in metabolic and vascular diseases and ageing (Rabbani and Thornalley, 2015).

A number of in vitro studies have shown that glyoxal is directly genotoxic and mutagenic without metabolic activation. In these in vitro studies, glyoxal has been shown to bind to DNA and form adducts (Kasai et al., 1998; Olsen et al., 2005), mutations (Murata-Kamiya et al., 2000; Suwa et al., 1982), chromosomal aberrations (Nishi et al., 1989), sister chromatid exchanges (Tucker et al., 1989), and DNA single strand breaks (Garberg et al., 1988; Roberts et al., 2003; Ueno et al., 1991).

In vivo studies with glyoxal have produced varying results.

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Positive genotoxicity findings include formation of imidazopurinones adducts (Thornalley et al., 2010), increase in DNA single-strand breaks in the liver and pyloric mucosa (Furihata et al., 1989; Ueno et al., 1991), and induction of unscheduled DNA synthesis in the pyloric mucosa of the stomach of male F344 rats 3 h after administration of doses of 150–400 mg/kg body weight by gastric intubation (Furihata et al., 1985). However, glyoxal was negative in the micronucleus test in mouse after oral application (Hoechst, 1986) and in a short-term liver foci assay (Hasegawa and Ito, 1992). Glyoxal showed tumour-promoting activity in a two-stage glandular stomach carcinogenesis model in male Wistar rats (Takahashi et al., 1989), while a skin tumour-promoting study in the rat produced negative results (Miyakawa et al., 1991).

The aim of this study was to examine the tumourigenic potential of glyoxal using the multiple intestinal neoplasia (Min) mice. This mouse model is heterozygous for a mutation in the tumour suppressor gene *Apc*, which leads to spontaneous development of numerous tumours, mainly in the small intestine, but also in the colon (Moser et al., 1995). This mouse model also develops flat aberrant crypt foci (ACF) in the colon, which is believed to be precancerous lesions showing a continuous development from the monocryptal stage to tumours (Paulsen et al., 2001). Since the Min mouse model has previously been shown to be most susceptible to intestinal carcinogens early in life (Steffensen et al., 2001), the mice were exposed already from *in utero*.

2. Materials and methods

2.1. Chemicals

Glyoxal solution 40 wt % in H_2O (Cas nr: 1077-22-2, product nr: 128465) was purchased from Sigma-Aldrich (Leirdal, Norway).

2.2. Animals and treatment

Forty-six C57BL/6J (B6) wild type (wt) females were purchased from The Jackson Laboratory (Bar Harbor, ME). Ten B6 multiple intestinal neoplasia (Min) males originally purchased from The Jackson Laboratory (Bar Harbor, ME), were bred at the Norwegian Institute of Public Health, Oslo, Norway.

The B6 wt females were housed two in each cage and mated with the heterozygous B6 Min males, which will give two possible genotypes in the offspring, wt or Min. After four days, the males were moved to another cage, and the dams were exposed to different concentrations of glyoxal through drinking water (0.0125%, 0.025%, 0.05%, 0.1%) or regular tap water (control group). The litter size and weight was measured at postnatal day (PND) 7. The litters were separated from the dams at PND21, and female and male offspring were housed separately and continued with the same treatment. One group of mice that were not exposed *in utero* or during lactation, were exposed to 0.1% glyoxal in drinking water starting at PND21. After weaning, the F0 wt females were again mated with the Min males. The second litter were assigned to another treatment group to minimise the cage-effect.

Water and rodent diet (Harlan Teklad 2018 (E), Global (Harlan Teklad U.K., Bicester, Oxfordshire, U.K.)) were given ad libitum and the feed intake was monitored weekly. The glyoxal solutions were changed at every 7–14 days depending on consumption. The animals were housed in plastic cages with a 12 h light/dark cycle. Ear cartilage was used for the genotyping of the litters as described previously (Svendsen et al., 2012). Only Min mice were used in the study.

2.3. Scoring of flat ACF and tumors

The Min mice were sacrificed at the age of ten weeks to assess the intestinal tumourigenesis. The collection and preparation of the small intestine and the colon was done as previously described (Olstorn et al., 2007). The entire small intestine (divided in three sections) and the entire colon were evaluated by scoring the number, size, and location of the lesions in an inverse light microscope. The size of the tumors was scored as mm^2, and the location was recorded as cm distal from the ventricle or caecum for the small intestine and colon, respectively. In the colon, flat ACF were characterized by their bright blue staining, moderate enlarged or small crypts not elevated from the surrounding epithelium and their compressed round or elongated luminal openings. The size of an ACF was scored as the number of crypts/lesion. A colonic tumor was defined as a lesion with >10 aberrant crypts or when it was not possible to distinguish the number of crypts.

2.4. Statistical analyses

Data were analysed using SigmaStat (SigmaStat software; Jandel Scientific, Erkrath, Germany). Data for number of tumours, average size of tumours, and number of flat ACF were analysed by two-way ANOVA or two-way ANOVA on ranks (used when data did not fulfil the assumptions of normality) followed by pair-wise comparison with Holm-Šídák method to identify which groups differed from one another. Differences in incidence between the groups were tested by Z-test. A P-value of <0.05 was considered significant.

3. Results

3.1. Impact of maternal glyoxal exposure on litter size and pup weight

Exposure to glyoxal *in utero* did not have any effect on litter size ($p = 0.978$), which varied between mean number of 5.4–5.8 pups per litter for the different groups. Also, there was not a statistical difference for the weight of the one-week-old pups between the control group which had an average pup weight of 3.7 g/pup and the treatment groups where the pup weights varied between 3.1 and 4.3 g ($p = 0.123$) (data not shown).

3.2. Body weight, food intake, water consumption, and estimated exposure of glyoxal

Throughout the experiment, the addition of glyoxal to the drinking water reduced the water intake, although the difference between the treatment groups and control groups were not statistically significant for all the time points. The water consumption decreased with increasing concentration of glyoxal (Fig. 1). The supplementation of glyoxal in water resulted in an estimated glyoxal exposure varying from 22 to 145 mg/kg/bw and 27–164 mg/kg bw for females (Table 1) and males (Table 2), respectively.

Although glyoxal exposure did not have an effect on the weight of one-week-old pups, the 0.1% glyoxal exposure for both males and females and 0.025% for females significantly retarded body weight gain, and the mice in these groups had significantly lower body weights at PND21 compared to the mice in the control group (data not shown). However, the negative effect of glyoxal on weight development was already diminished at the age of four weeks, and there were no significant differences in body weight between the control and treatment groups for the rest of the experiment period (data not shown).

In line with the decreased body weight gain, food intake from