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GENOTOXICITY

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Dietary elevated sucrose modulation of diesel-induced genotoxicity in the colon and liver of Big Blue rats

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Abstract Earlier studies have indicated that sucrose possesses either co-carcinogenic or tumor-promoter effects in colon carcinogenesis induced by genotoxic carcinogens. In this study we investigated the role of sucrose on diesel exhaust particle (DEP)-induced genotoxicity in the colonic mucosa and liver. Big Blue rats were fed with DEP (0.8 ppm in feed) and/or sucrose (3.45% or 6.85% w/w in feed) for 3 weeks. DEP increased both DNA strand breaks and DNA adducts in colon. Interestingly, sucrose also increased the level of bulky DNA adducts in colon. DEP and sucrose had no effect on DNA strand-breaks and DNA adducts in liver. DEP and sucrose treatment did not have any effect on mutation frequency in colon and liver. Oxidative DNA damage detected as 8-oxodG (8-oxo-7,8-dihydro-2′-deoxyguanosine) and endonuclease III or formamidopyrimidine DNA glycosylase sensitive sites was unaltered in colon and liver. The mRNA expression levels of the DNA repair enzymes *N*-methylpurine DNA glycosylase (*MPG*), 8-oxoguanine DNA glycosylase (*OGG1*) and *ERCC1* (part of the nucleotide excision repair complex) measured by reverse transcription-polymerase chain reaction were increased in liver by DEP feeding. In colon, expression was unaffected by DEP or sucrose feeding. Among biomarkers of oxidative stress, including vitamin C, malondialdehyde and protein oxidations (γ-glutamyl semialdehyde and 2-amino adipic semialdehyde) in plasma and liver, only malondialdehyde was increased in plasma by sucrose/DEP feeding. In conclusion, sucrose feeding did not increase DEP-induced DNA damage in colon or liver.

Keywords Sucrose · Diesel exhaust particles · Big Blue rats · Colon · Liver

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Introduction

Exposure to diesel exhaust particles (DEP) in air pollution is commonly associated with increased risk of lung cancer although the mechanism of action is disputed. The aggregated data from several animal inhalation or intratracheal instillation studies have shown that DEP may cause generation of DNA adducts (Wong et al. 1986; Bond et al. 1990; Mauderly et al. 1994; Sato et al. 2000; Møller et al. 2003a). Also, oxidative DNA damage has been increased in the lung tissue of animals exposed to DEP by inhalation or intratracheal instillation (Ichinose et al. 1997; Tsurudome et al. 1999; Iwai et al. 2000; Sato et al. 2000; Møller et al. 2003a). Most studies have been concentrated on the effects of DEP in the lungs, though it is most likely that a large part of inhaled DEP is removed by mucociliary transport in the bronchial tree and swallowed. Furthermore, food such as vegetables may be contaminated with DEP. This indicates that DEP may mediate toxic effects via gastrointestinal exposure. Previously, we have shown that dietary administration of DEP to Big Blue rats resulted

in markedly higher levels of DNA adducts and strand breaks in the liver and colon, whereas the level of oxidative DNA damage was unaltered, probably because of increased *ERCC1* and *OGG1* mRNA expression (Dybdahl et al. 2003).

In this study we investigated the interaction of DEP exposure and sucrose intake. It is commonly acknowledged that the diet or dietary factors are responsible for one-third of all cancers. Most studies of carcinogenesis are carried out as single compound investigations despite the fact that the human exposure situation is multifactorial. Some observations suggest that high sucrose intake increases the number of aberrant crypt foci in colon induced by 2-amino-3-methylimidazo [4,5-*f*]quinoline (IQ) or azoxymethane (Stamp et al. 1993; Molck et al. 2001). A recent report suggested that sucrose possesses tumor-promotor activity in azoxymethane-induced colon cancer, and may also act as a co-initiator if administered during the initiation of carcinogenesis (Poulsen et al. 2001). In a previous set of experiments we found that dietary intake of sucrose, above 10% sucrose by weight, increased the mutation frequency in the colon of Big Blue rats, whereas no mutations were observed in the liver (Dragsted et al. 2002). The level of oxidative DNA damage, bulky DNA adducts and expression of the DNA repair enzymes *ERCC1* and *OGG1* were unaltered by sucrose feeding in the colon and liver. This indicates a genotoxic mechanism of sucrose and that sucrose feeding may increase the formation of DNA damage and mutations by co-administration of genotoxic carcinogens.

The aim of this study was to investigate the role of sucrose in DEP-induced genotoxicity. Especially, we wanted to investigate whether simultaneous administration of sucrose and DEP was associated with markedly higher DNA damage and mutation frequency, and whether sucrose together with DEP had any effect on DNA repair and apoptosis. To this end, the genotoxic effects of DEP and sucrose were investigated in Big Blue rats by means of a panel of biomarkers detecting DNA damage, expression of DNA repair genes, and mutations in colon and liver. Markers of DNA damage included strand breaks and bulky adducts. Also, a panel of biomarkers for oxidative stress was included, encompassing vitamin C, and protein oxidation (γ-glutamyl semialdehyde and 2-amino adipic semialdehyde).

Materials and methods

Chemicals and animal treatment

The study was set up as a two-way factorial design with low doses of sucrose and DEP. The four groups were designated control (3.45% w/w sucrose, $n=8$), sucrose (6.85% w/w sucrose, $n=6$), DEP (0.8 ppm DEP and 3.45% w/w sucrose in the feed, $n=8$), and DEP/sucrose (0.8 ppm DEP in the feed and 6.85% w/w sucrose, $n=8$). Concurrently, a separate group received 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ, 20 ppm in the feed) as a positive control for the mutation assay and is reported elsewhere (Møller et al. 2003b). Male Big Blue (Fischer) rats, approximately 8 weeks of age (250 g) were purchased from Stratagene (AH Diagnostics, Århus, Denmark), and acclimatized for 7 days while being maintained on Altromin pellets (Lage, Germany). They were kept in a 12-h light and 12-h dark cycle at an average temperature of 22°C and at 55% humidity. The diet was similar to that used previously (Dragsted et al. 2002) (Table 1). DEP were Standard Reference Material 1650 from the National Institute of Standards and Technology (NIST; Gaithersburg, MD, USA), representing combustion particles from a heavy-duty diesel engine (Huggins et al. 2000). The doses of sucrose and DEP selected were based on earlier studies: DEP at doses above 0.2 ppm were known to increase strand breaks and DNA adducts in colon mucosa cells (Dybdahl et al. 2003), whereas the doses of sucrose were below that required to induce genotoxicity in the colon and liver (Dragsted et al. 2002). The diets were administrated for a total of 21 days as recommended for expression of mutants by the manufacturer.

Institutional guidelines for animal welfare and experimental conduct were followed.

Analytical procedures

The analytical procedures, except that for *N*-methylpurine DNA glycosylase (*MPG*) mRNA quantification, have been described in detail elsewhere (Dragsted et al. 2002; Møller et al. 2002). Briefly, the level of oxidative DNA damage was assessed in terms of 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) per 10^6 deoxyguanosine (dG) by high-performance liquid chromatography coupled to electrochemical detection, and strand breaks, endonuclease III (ENDOIII, oxidized pyrimidines) and formamidopyrimidine DNA glycosylase (FPG, oxidized purines) sensitive sites were detected by the comet assay as the percentage DNA in the tail of 50 randomly selected cells using the Kinetics v4.0 software system. The level of DEP-induced DNA adducts was investigated by ^{32}P-postlabeling with butanol enrichment and determined by quantitation of autoradiograms of thin layer chromatography plates as depicted in Fig. 1 (Dragsted et al. 2002; Møller et al. 2002). Expressions of *ERCC1*, *MPG* and *OGG1* mRNA relative to 18S were determined by real-time reverse transcription-polymerase chain reaction (RT-PCR). *MPG* mRNA levels were quantified using the primers: 5′-AGT TGT GTT CCA TCA GCA AGT CG-3′ and 5′-GGC

Table 1 Animal feed composition in the control and sucrose-rich diet

Feed component	Component concentration (g/kg feed)	
	Control diet	Sucrose diet
Protein (casein)	180	174
Fats (soya oil)	50	48
Carbohydrate		
Potato starch	306	295
Corn starch	306	295
Dextrin	34	33
Sucrose	34	67
Fiber (cellulose)	50	48
Mineral mixture[a]	40	39

[a]Mixture contains (mg/kg): Ca^{2+}(4970), P (phosphate, 3100), K^+ (3610), S (sulfate, 300), Na^+ (2530), Cl^- (1490), Mg^{2+} (600), Fe^{2+} (34), Zn^{2+} (30), Mn^{2+} (10), Cu^{2+} (7), I^- (200), Mb (molybdate, 0.15), Se (selenite, 0.15), Si (2.5), Cr^{4+} (1.0), F^- (1.0), Ni^{2+} (0.5), B (borate, 0.5), Li^{2+} (0.1), V (vanadate, 0.1), Co^{2+} (0.07), thiamine (5), riboflavine (6), pyridoxol (8), folate (2), biotin (0.3), cyanocobalamine (0.03), panthotenate (20), choline (hydrogentartrate, 2600), inositol (400), nicotinamide (40), phylloquinine (1000), *p*-aminobenzoic acid (40), methionine (1000), L-cysteine (2000), vitamin A (5000 IU/kg), vitamin D_3 (1000 IU/kg), vitamin E (DL-α-tocopherol, 50 IU/kg)

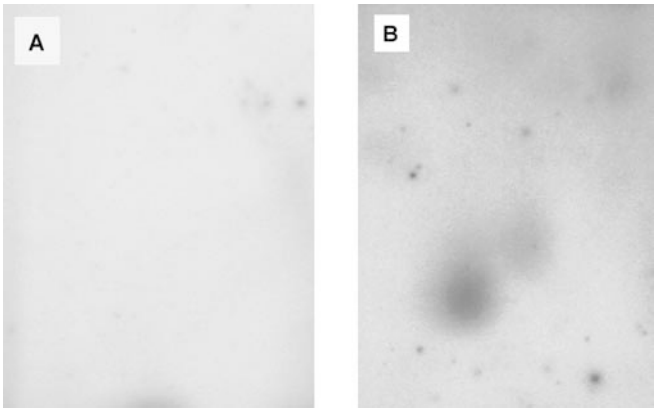

Fig. 1A,B Autoradiographs of thin-layer chromatography plates from **A** control animal and **B** animal exposed to diesel exhaust particles (DEP)

TAG GAC CGG AGT ATT TTG A-3′, and the taqman probe: 5′-FAM- ACC TGT CCC AGA AAT GCA CGG GCT-TAMRA-3′. Determinations were carried out as previously described (Dragsted et al. 2002). The mutation frequency was assessed at the *cII* transgene. In the liver, cell proliferation was assayed immunohistochemically as the PCNA (proliferating cell nuclear antigen) labeling index, and apoptosis was detected by the TUNEL (terminal deoxynucleotidyl transferase dUTP nick end labeling) assay. Plasma biomarkers of oxidative stress included vitamin C concentration, lipid peroxidation (malondialdehyde), and protein oxidation (2-amino adipic semialdehyde), as described previously (Dragsted et al. 2002; Møller et al. 2002). Protein oxidation in the liver was determined by 2-amino adipic semialdehyde and γ-glutamyl semialdehyde levels (Dragsted et al. 2002; Møller et al. 2002).

Statistics

The data were tested for homogeneity of variance within dose groups using Levene's test and for normality using Shapiro-Wilk's *W*-test. Log-transformations, using the base of 10, were performed for adducts in colon and *OGG1* mRNA in liver, which did not have homogeneity of variance. The effects of biomarkers were tested for interactions by two-way ANOVA, using 5% probability as the significance level. If the test for interaction was not significant, the effect of single factors (sucrose and DEP) was tested at the 5% probability significance level. For biomarkers being statistically significant, interactions or single-factor effects were analyzed by post hoc ANOVA analysis as the least significant difference (LSD). In case of a single factor effect, the 95% confidence interval (CI) was based on the whole data set (i.e., the variation in the non-significant factor was included in the residual variation). The statistical analysis was performed in Statistica 5.5 for Windows (1997) (StatSoft, Inc., Tulsa, OK, USA).

Results

At the end of the 21-day feeding period all animals were healthy. The mean energy intakes were not statistically significant among the groups (the means ± SD for the energy intake were 4626 ± 337 MJ for control, 5406 ± 460 MJ for DEP, 5312 ± 310 MJ for sucrose, and 5760 ± 318 MJ for sucrose/DEP; $P > 0.05$ for interaction and $P > 0.05$ for single-factor effects). Correspondingly, the mean bodyweight gain was not statistically different among the groups (the mean weight gains ± SD were 44 ± 8 g for control, 33 ± 14 g for DEP, 30 ± 13 g for sucrose, and 38 ± 15 g for sucrose/DEP; $P > 0.05$ for interaction and $P > 0.05$ for single-factor effects).

The level of DNA adducts, strand breaks, and mutation frequencies are outlined in Fig. 2. Increased levels of DNA adducts were observed in both colon and liver cells in all exposed groups compared with the control group. The DNA adduct levels in the liver indicated single-factor effects of DEP with a 2.0-fold increase (95% CI 1.8–2.3, $P < 0.0001$ for difference between animals treated with and without DEP), and of sucrose with a 1.3-fold increase (95% CI 1.16–1.43, $P < 0.01$ for difference between animals treated with and without sucrose). In the colon, sucrose and DEP increased the adduct levels compared with control by 2.6- and 3.5-fold, respectively (sucrose 95%CI 1.6–3.7, and DEP 95%CI 2.5–4.4), whereas the sucrose/DEP group showed a negative interaction compared with the

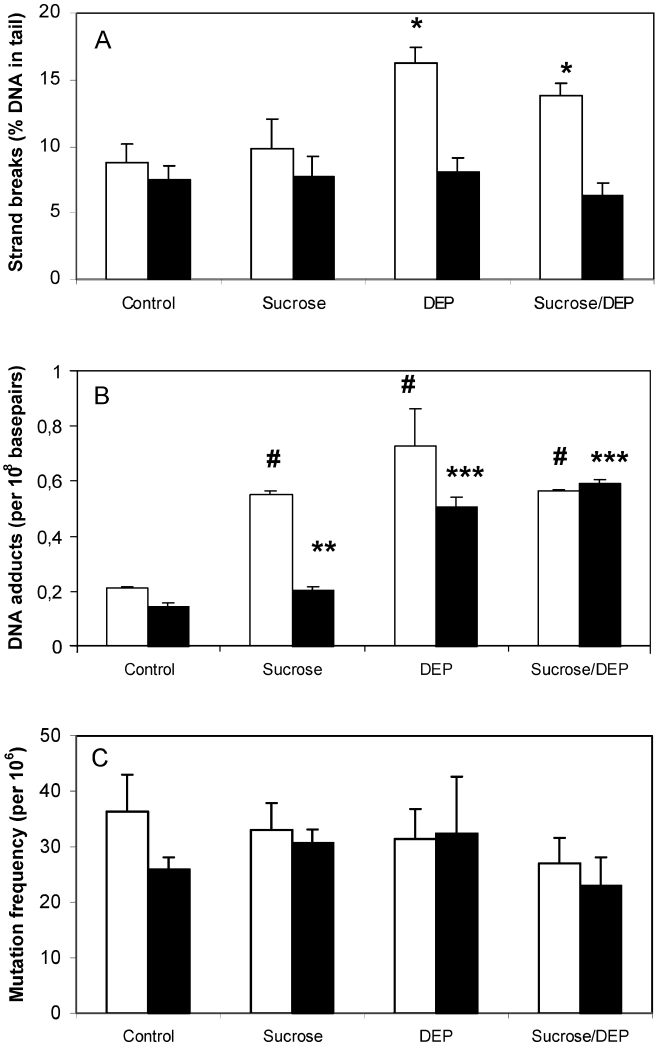

Fig. 2A–C Strand breaks (**A**), DNA adducts (**B**), and mutations (**C**) induced by diesel exhaust particles (DEP) and sucrose in the colon (*open bars*) and liver (*solid bars*) of Big Blue rats. Bars denote the mean with SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with the control; $^{\#}P < 0.0001$ for interaction effect (DNA adducts in the colon)

DEP and sucrose groups ($P < 0.0001$ for interaction). DEP increased strand breaks in the colon by 1.67-fold (95% CI 1.35–1.95, $P < 0.05$ for difference between animals treated with and without DEP), independent of the concentration of sucrose in the feed. Oxidative DNA damage, detected as 8-oxodG, ENDOIII, or FPG-sensitive sites were unaltered in the colon and liver (Table 3). The mutation frequency was not increased in the colon or the liver as a result of sucrose or DEP administration (Fig. 2).

The expression levels of the *ERCC1*, *OGG1*, and *MPG* DNA repair genes were quantified by real-time RT-PCR (Table 2). In the colon, the expression of *ERCC1*, *OGG1* and *MPG* was unaltered in both the sucrose- and DEP-treated rats. In the liver DEP enhanced the expression of all three DNA repair genes. The expression of *OGG1* and *MPG* was only affected in the DEP-treated rats, whereas high sucrose intake and DEP was associated with lower *ERCC1* expression than in the DEP group receiving low sucrose ($P < 0.05$ for interaction; Table 2).

Malondialdehyde in the plasma was increased in the high sucrose/DEP-treated rats 1.6-fold (95% CI 1.1–2.1) compared with the sucrose alone group, and 1.7-fold (95% CI 1.2–2.2) compared with the DEP group receiving low sucrose ($P < 0.05$ for interaction; Table 3).

Apoptosis was increased in the liver by DEP treatment ($P < 0.001$; Table 2).

General biomarkers of oxidative stress, including total and oxidized vitamin C, γ-glutamyl semialdehyde, and 2-amino adipic semialdehyde, were unaltered in plasma and liver (Table 3).

Discussion

A low concentration of DEP increased the level of strand breaks and bulky DNA adducts in the colon,

Table 2 Expression of mRNAs for DNA repair genes *ERCC1*, *MPG* and *OGG1* (per 10^{-6} 18S mRNAs) and apoptosis (percentage of total cells staining positive) in Big Blue rats fed with diesel exhaust particles (DEP, 0.8 ppm) and sucrose 3.45% (Control) or 6.85% in feed for 3 weeks. Data represent means ± SD

Tissue	Parameter	Dietary group				Statistics[a]
		Control ($n=8$)	Sucrose 6.85% ($n=6$)	DEP ($n=8$)	DEP/Sucrose 6.85% ($n=8$)	
Colon	*ERCC1*	97 ± 61	165 ± 134	140 ± 86	182 ± 146	NS
	MPG	1.1 ± 0.2	1.9 ± 1.0	1.0 ± 0.7	1.7 ± 1.4	NS
	OGG1	52 ± 24	59 ± 43	56 ± 24	67 ± 38	NS
Liver	*ERCC1*	9 ± 2	9 ± 2	42 ± 15	24 ± 7	Interaction ($P < 0.05$)
	MPG	0.25 ± 0.03	0.27 ± 0.07	0.38 ± 0.08	0.28 ± 0.11	DEP ($P < 0.01$)
	OGG1	4 ± 1	3 ± 1	8 ± 2	6 ± 2	DEP ($P < 0.001$)
	Apoptosis	0.8 ± 0.3	1.1 ± 0.1	2.1 ± 0.3	1.9 ± 0.2	DEP ($P < 0.001$)

[a]Analyzed by two-factor ANOVA: *Interaction* indicating a statistically significant interaction between sucrose and DEP, *DEP* indicating a single-factor effect of DEP, and *NS* being not statistically significant

Table 3 Biomarkers of oxidative stress in the colon, liver, and plasma of Big Blue rats treated with sucrose and diesel exhaust particles (DEP). Data are means ± SD, with *n* values given in parentheses (*ENDOIII* endonuclease III, *FPG* formamidopyrimidine DNA glycosylase, *8-OxodG* 8-oxo-7,8-dihydro-2′-deoxyguanosine)

Tissue	Parameter	Dietary group				Statistics[a]
		Control	Sucrose 6.85%	DEP	DEP/Sucrose 6.85%	
Colon	ENDOIII sites (%tail DNA)	5.4 ± 4.6 (6)	5.4 ± 4.3 (4)	3.7 ± 3.6 (8)	0.7 ± 5.4 (7)	NS
	FPG sites (%tail DNA)	6.5 ± 5.7 (8)	6.6 ± 2.9 (4)	5.1 ± 5.0 (8)	3.9 ± 6.4 (7)	NS
	8-OxodG per 10^6 dG	2.9 ± 0.9 (8)	3.4 ± 1.6 (6)	3.1 ± 1.8 (8)	2.3 ± 0.5 (8)	NS
Liver	ENDOIII sites (%tail DNA)	4.6 ± 4.6 (8)	3.4 ± 3.4 (6)	5.1 ± 3.4 (8)	5.7 ± 5.7 (8)	NS
	FPG sites (%tail DNA)	11.6 ± 8.5 (8)	9.6 ± 10.2 (6)	12.8 ± 9.3 (8)	13.8 ± 10.9 (8)	NS
	8-OxodG per 10^6 dG	4.7 ± 0.7 (8)	5.1 ± 1.7 (6)	4.5 ± 1.7 (6)	4.7 ± 1.2 (8)	NS
	2-Amino adipic semialdehyde (pmol/mg)	90 ± 19 (6)	85 ± 15 (6)	90 ± 17 (8)	83 ± 7 (7)	NS
	γ-Glutamyl semialdehyde (pmol/mg)	103 ± 30 (6)	109 ± 12 (6)	111 ± 23 (8)	102 ± 17 (7)	NS
Plasma	Malondialdehyde (nmol/mg)	425 ± 186 (8)	357 ± 172 (6)	341 ± 108 (8)	568 ± 197 (8)	Interaction ($P < 0.05$)
	2-Amino-adipic semialdehyde (pmol/mg)	134 ± 34 (6)	132 ± 24 (5)	129 ± 51 (6)	148 ± 52 (7)	NS
	Total vitamin C (mg/l)	35 ± 7 (8)	29 ± 5 (6)	28 ± 7 (8)	32 ± 9 (8)	NS
	Reduced vitamin C (mg/l)	27 ± 8 (8)	20 ± 5 (6)	20 ± 9 (8)	24 ± 8 (8)	NS

[a]Analyzed by two-factor ANOVA: *Interaction* indicating a statistically significant interaction between sucrose and DEP, and *NS* being not statistically significant

whereas only DNA adducts were increased in the liver. DEP and sucrose treatment did not induce oxidative DNA damage in terms of 8-oxodG-, ENDOIII-, and FPG-sensitive sites. The increased expression of liver *OGG1*, *ERCC1*, and *MPG* in the DEP-treated rats suggests an upregulation of DNA repair capacity in the liver.

DEP exposure did not increase the mutation frequency in the colon or liver. Previously, we have shown that higher sucrose intake than used in this study increased the mutation frequency in the colon (Dragsted et al. 2002). In addition, we have found that IQ increased the mutation frequency in the liver (Møller et al. 2002). In this study IQ, serving as a positive control, increased the mutation frequency significantly in the liver by 1.32-fold (95% CI 1.02–1.63) (reported in Møller et al. 2003b). This indicates that the assay is capable of detecting mutations, and that the lack of DEP-induced mutations is not due to inappropriate experimental design. It may be speculated that the dose of DEP was too low for mutations to occur. However, we have previously found that DEP in concentrations of 0.2–80 ppm in the diet was not associated with increased mutation frequency in the colon or liver (Dybdahl et al. 2003).

Most of the animal toxicology of DEP has been related to pulmonary effects by either instillation or inhalation. However, considering that a large part of inhaled DEP is removed by mucociliary transport in the bronchial tree and swallowed, gastrointestinal exposure of DEP may be of toxicological relevance. The aggregated data from pulmonary exposure to DEP indicates both increased levels of DNA adducts and oxidative DNA damage (Wong et al. 1986; Bond et al. 1990; Mauderly et al. 1994; Ichinose et al. 1997; Tsurudome et al. 1999; Iwai et al. 2000; Sato et al. 2000; Møller et al. 2003a). The NIST1650 particles used in this study contain several polycyclic aromatic hydrocarbon (PAH) compounds, as well as small amounts of transition metals (Huggins et al. 2000). However, considering that very high dietary iron intake is not associated with oxidative stress in the rat colon, it may therefore be speculated that the transition metal content is insufficient to produce oxidative damage in the colon. The particles or released transition metals may be transported to the liver and exert their toxic effects.

The higher level of DNA adducts and strand breaks in the colon epithelial cells is probably caused by PAH compounds, rather than metal-catalyzed reactions, considering that oxidative DNA damage was unaffected. The lack of oxidative DNA damage and strand breaks in the liver may be due to overall upregulation of the DNA repair system as indicated by *ERCC1*, *MPG*, and *OGG1* expression. Inverse correlation between 8-oxodG and OGG1 repair activity has been reported in animals exposed to DEP and metals (Kasai 2002).

In this study we used relatively low doses of sucrose and DEP. The dose of DEP (0.8 mg/kg feed) used in the present study corresponds to 24 μg/kg bodyweight per day. This is an exposure that may certainly be achieved in an occupational setting, and that may be approached in urban environments with heavy diesel traffic and possibly with consumption of contaminated food. Accordingly, effects of gastrointestinal exposure may be important for risk assessment of diesel exposure. Interactions between dietary sucrose and other genotoxic compounds have indicated increased levels of IQ-induced DNA adducts in colon epithelial cells (Møller et al. 2003b). By contrast, sucrose did not further increase the level of strand breaks in colon epithelial cells by simultaneous ingestion of 1,2-dimethylhydrazine (Rowland et al. 1996) or IQ (Møller et al. 2003b), although both genotoxins generate strand breaks.

The interaction effect of increasing the concentration of sucrose in the feed apparently reduced the effects of DEP on DNA adducts, expression of DNA repair enzymes, and apoptosis, suggesting that sucrose reduces the toxicity of gastrointestinal exposure to DEP. Few studies have focused on interactions between diet and effects of DEP. A high-fat diet has been reported to enhance the oxidative DNA damage induced by intratracheal instillation of DEP in mice (Nagashima et al. 1995). Accordingly, it may be interesting to study interactions between a high-fat diet and gastrointestinal exposure to DEP, as well as between a high-sucrose diet and inhalation exposure to DEP.

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