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Dietary low-dose sucrose modulation of IQ-induced genotoxicity in the colon and liver of Big Blue™ rats

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Abstract

Earlier studies have indicated that sucrose increases 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ)-induced aberrant crypt foci in the colon. In this study, we investigated the role of sucrose in IQ-induced genotoxicity of the colon mucosa and liver. Big Blue™ rats were fed with IQ (20 ppm in feed) and/or sucrose (3.45 or 6.85 wt.% in feed) for 3 weeks. IQ increased DNA strand breaks in the colon, whereas the mutation frequency was increased in the liver. The level of IQ-induced DNA adducts was elevated in both colon mucosa cells and liver. In the liver, high sucrose intake increased the level of DNA adducts above that of IQ and low sucrose intake. Oxidative DNA damage detected in terms of 7-hydro-8-oxo-2′-deoxyguanosine by HPLC-EC, or endonuclease III or formamidopyrimidine DNA glycosylase sensitive sites were unaltered in the colon and liver. Expression of *ERCC1* and *OGG1* mRNA levels were unaffected by IQ or sucrose feeding. Biomarkers of oxidative stress, including Vitamin C, malondialdehyde and protein oxidations (γ-glutamyl semialdehyde and 2-amino adipic semialdehyde) were unaltered in plasma and in liver. In conclusion, sucrose feeding increases IQ-induced genotoxicity in liver but not in colon, suggesting different mechanisms for sucrose and IQ in colon mutagenesis.
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Keywords: BigBlue™ rat; DNA adducts; Mutations; Strand breaks; DNA repair; Apoptosis

1. Introduction

It is commonly acknowledged that one-third of all cancers are caused mainly by dietary factors [1]. Most studies of carcinogenesis are carried out as single compound investigations despite the fact that the human exposure situation is multifactorial. Heterocyclic aromatic amines (HAA) are a class of dietary

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carcinogens that may be formed during cooking of meat and fish as condensation products of creatine (or the cyclic form, creatinine) with amino acids and proteins [2]. 2-Amino-3-methylimidazo[4,5-*f*]quinoline (IQ) and other HAA are among the most potent mutagens in the Ames test, and are carcinogenic in several target organs in long-term rodent feeding bioassays. IQ is the most potent carcinogen of the HAA tested in animals with evidence for carcinogenicity in the liver, small and large intestine, Zymbal gland, and the skin of male rats [3]. It is widely accepted that the HAA are carcinogenic by forming adducts between metabolically activated metabolites and DNA [4].

The role of sucrose in colon carcinogenesis is a matter of controversy. Epidemiological studies suggest that high consumption of sucrose is associated with colon cancer [5]. A few experimental observations suggest that sucrose intake increases the number of aberrant crypt foci induced by IQ or azoxymethane [6,7]. 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 azoxymethane-induced carcinogenesis [8]. 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 change in mutation frequency was observed in the liver [9]. The level of oxidative DNA damage and mRNA expression of the DNA repair enzymes *ERCC1* and *OGG1* were unaltered by sucrose feeding. This indicates a genotoxic mechanism of sucrose, and 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 IQ-induced genotoxicity. Especially we wanted to investigate if simultaneous administration of sucrose and IQ was associated with markedly higher DNA damage and mutation frequency, and whether or not sucrose had any effect on DNA repair, cell proliferation, and apoptosis. To this end, the genotoxic effects of IQ 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, lipid peroxidation (malondialdehyde), and protein oxidation (γ-glutamyl semialdehyde and 2-amino adipic semialdehyde).

2. Materials and methods

The study was set up as a two-way factorial design with two concentrations sucrose in the diet (3.45 and 6.85%), and two doses of IQ (0 and 20 ppm). The four groups were designated control (3.45 wt.% sucrose, $n = 8$), sucrose (6.85 wt.% sucrose, $n = 6$), IQ (20 ppm IQ in the feed and 3.45 wt.% sucrose, $n = 6$), and IQ/sucrose (20 ppm IQ in the feed and 6.85 wt.% sucrose, $n = 8$). The feed was prepared as a powdered diet containing sucrose and IQ. The composition of the diet has been described in detail elsewhere [9]. 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 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 55% humidity. IQ was purchased from Toronto Research Chemicals, North York, Ont., Canada. The doses of sucrose and IQ were selected based on earlier studies: IQ (20 ppm) was known to induce mutations in the liver but not the colon [10], whereas the doses of sucrose were below that required to increase the mutation frequency in the colon [9]. The diets were administrated for a total of 21 days. At day 21, all rats were healthy, and each individual animal was weighed, anaesthetized in a mixture of 40% carbon dioxide and 60% oxygen, and decapitated.

All measurements in this study have been described in detail elsewhere [9,10]. Briefly, the level of oxidative DNA damage was assessed in terms of 7-hydro-8-oxo-2′-deoxyguanosine (8-oxodG) by HPLC-EC, and strand breaks, endonuclease III and FPG sensitive sites were detected by the comet assay. Expressions of *ERCC1* and *OGG1* mRNA relative to 18S were determined by real time RT-PCR. The level of IQ-induced DNA adducts was investigated by ^{32}P-post-labeling with butanol enrichment and determined by quantitation of two spots on autoradiograms of thin-layer chromatography plates as indicated in Fig. 1. The spots detected on the thin chromatography plates here are not linked to specific types of DNA adducts, because studies on specific DNA adducts detected by

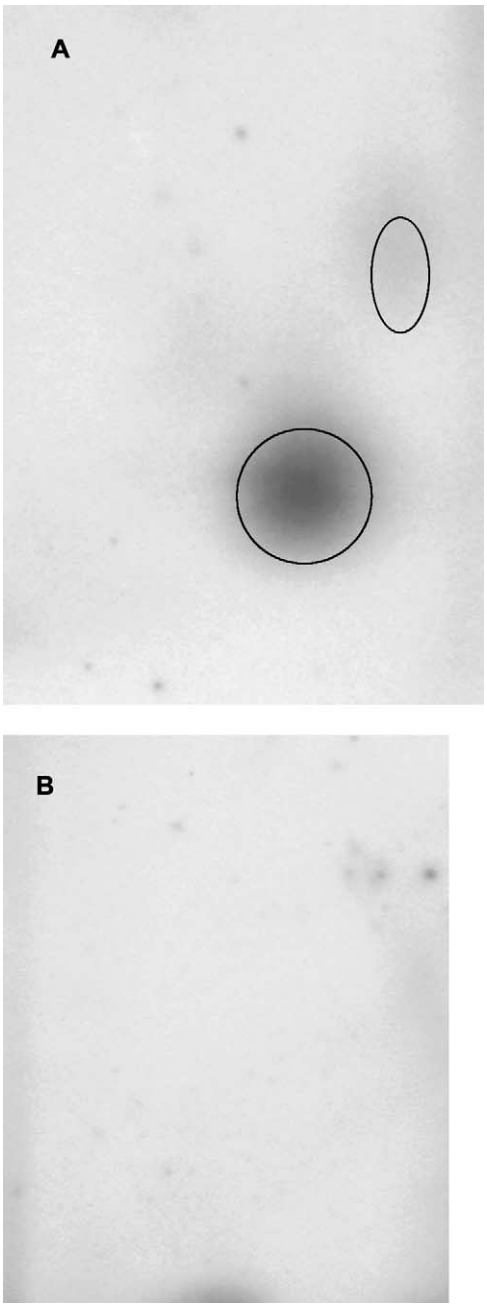

Fig. 1. Autographs of TLC plates. Two spots are indicated by circles in samples from rats fed with IQ (A) and from control rats (B).

^{32}P-post-labeling have employed the nuclease P1 version rather than butanol extraction as enrichment procedure [11,12]. The mutation frequency was assessed at the *cII* transgene. In the liver, cell proliferation was assayed immunohistochemically as the PCNA labeling index, and apoptosis was detected by the TUNEL assay. Plasma biomarkers of oxidative stress included Vitamin C concentration, lipid peroxidation (malondialdehyde), and protein oxidation (2-amino adipic semialdehyde) as described previously. Protein oxidation in the liver was determined by 2-amino adipic semialdehyde and γ-glutamyl semialdehyde levels.

The data were tested for homogeneity of the variance within groups using Levene's test and for normality using Shapiro–Wilk's *W*-test. The log-transformations, using the base of 10, were performed for the liver mutation frequency and liver PCNA labeling index, which did not have homogeneity of variance. The effects of biomarkers were tested for interactions by two-way ANOVA, using 5% as significance level. If the test for interaction was not significant, the effect of single factors (sucrose and IQ) was tested at 5% significance level. For biomarkers being statistically significant, interactions or single-factor effects were analyzed by post hoc ANOVA analysis as the least significant difference. In case of a single-factor effect, the 95% confidence interval was based on the whole data set (i.e. the variation in the non-significant factor was included in the residual variation). IQ-induced DNA adducts were only detectable in the IQ-treated groups by ^{32}P-post-labelling (no spots were visible in control or sucrose group). Thus, differences between the two IQ-treated groups were tested by a Student's *t*-test. The statistical analysis was performed in Statistica 5.0 for Windows, StatSoft Inc. (1997), Tulsa, OK, USA.

3. Results

Rats fed IQ alone consumed less energy than the other groups (5554 ± 380, 5199 ± 429, 5312 ± 310, 5489 ± 131 MJ (mean ± S.D.) for the control, IQ, sucrose, and IQ/sucrose group, respectively, $P < 0.05$ for the interaction between IQ and sucrose groups). Correspondingly, the body weight gain after 3 weeks of feeding was lower in the group fed IQ alone than the other groups (43 ± 9, 27 ± 12, 30 ± 13, 37 ± 15 g

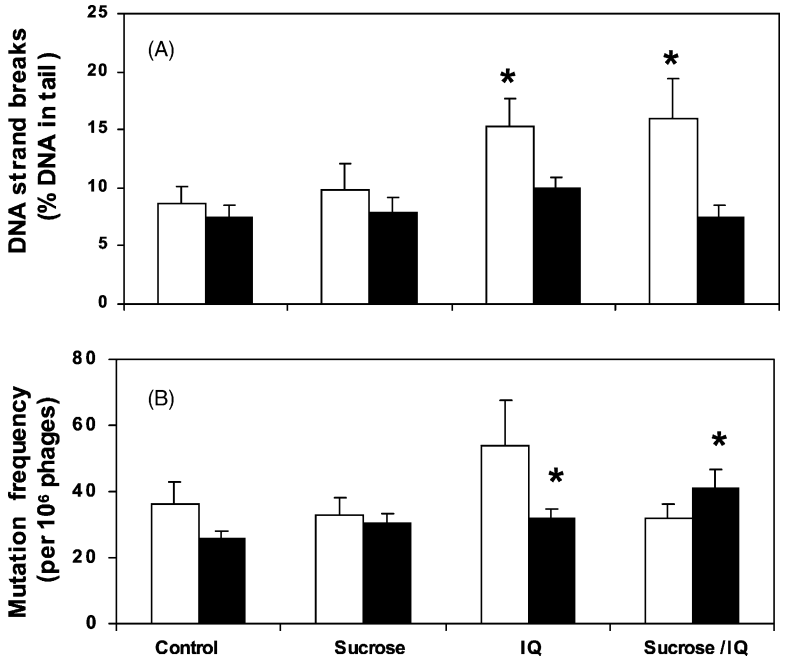

Fig. 2. Strand breaks (A) and mutation frequency at the *cII* transgene (B) induced by IQ and sucrose in the colon (open bars) and liver (solid bars) of Big Blue™ rats. Bars denote the mean and S.E.M. The number of animals in the control ($n = 8$), sucrose ($n = 6$), IQ ($n = 6$), and IQ/sucrose ($n = 8$) groups, except for strand breaks in the colon that had lower group sizes, e.g. control ($n = 6$), sucrose ($n = 4$), IQ ($n = 4$), and IQ/sucrose ($n = 7$). The level of strand breaks was increased by 72% (95% CI: 8–135%) in the colon of IQ-treated animals. The mutation frequency increased by 32% (95% CI: 2–63%) in the liver of IQ-treated animals. $^{*}P < 0.05$ compared to the control.

(mean ± S.D.), $P < 0.05$ for the interaction between IQ and sucrose groups).

The level of strand breaks and mutations induced by IQ and sucrose are shown in Fig. 2. Administration of IQ increased the level of strand breaks by 72% (95% CI: 8–135%, $P < 0.05$) in the colon, whereas IQ increased the mutation frequency in the liver by 32% (95% CI: 2–63%, $P < 0.05$). Although the mutation frequency was highest in the IQ/sucrose group, there was no significant effect or interaction with sucrose. Two distinct IQ-induced DNA adducts were observed in the IQ-treated rats, whereas no IQ–DNA adducts were detected in the groups receiving only sucrose (control and sucrose groups). The levels of IQ-induced DNA adducts is outlined in Table 1. A high concentration of sucrose in the diet appeared to increase the total DNA adduct level in the liver ($P < 0.001$, Student's *t*-test). Although the same tendency was observed in the colon, it was not statistically significant at the 5% level ($P = 0.37$, Student's *t*-test). In general, the level of DNA adducts was relatively low, although above the detection limit (arbitrary, set to 0.1 adducts per 10^8 basepairs).

The level of oxidative DNA damage detected in terms of 8-oxodG, endonuclease III, formamidopyrimidine DNA glycosylse sensitive sites, as well as ex-

Table 1
Level of IQ-induced DNA adducts in colon mucosa cells and hepatocytes[a,b]

Treatment	Colon epithelium	Liver
IQ	0.42 ± 0.16	0.34 ± 0.10
IQ + sucrose	0.54 ± 0.29	0.71 ± 0.07[c]

[a] The IQ group (20 ppm) consisted of six animals and IQ/sucrose group of eight animals. There were no IQ-induced DNA adducts detected in the groups receiving only sucrose (3.45 or 6.85 wt.%).

[b] The data are expressed as adducts per 10^8 basepairs (mean ± S.D.).

[c] $P < 0.001$ compared to IQ group.

Table 2
Biomarkers of oxidative stress in the colon, liver, and plasma of BigBlue^{TM} rats treated with sucrose and IQ (mean ± S.D.)

Group	Control	Sucrose	IQ	IQ/sucrose
Colon				
ENDOIII sites (percent tail DNA)	5.4 ± 4.6 (6)	5.4 ± 4.3 (4)	4.0 ± 10.4 (4)	0.6 ± 2.9 (7)
FPG sites (percent tail DNA)	6.5 ± 5.7 (6)	6.6 ± 2.9 (4)	5.3 ± 3.6 (4)	1.9 ± 7.0 (7)
8-oxodG/10^6 dG	2.9 ± 0.9 (8)	3.4 ± 1.6 (6)	3.1 ± 1.1 (6)	3.1 ± 1.1 (8)
Liver				
ENDOIII sites (percent tail DNA)	4.6 ± 4.6 (8)	3.2 ± 3.4 (6)	0.8 ± 3.4 (8)	6.5 ± 6.5 (8)
FPG sites (percent tail DNA)	11.6 ± 8.5 (8)	9.6 ± 10.2 (6)	14.2 ± 10.9 (8)	13.6 ± 11.1 (8)
8-oxodG/10^6 dG	4.7 ± 0.7 (8)	5.1 ± 1.7 (6)	4.4 ± 1.5 (6)	5.0 ± 0.7 (8)
2-Amino adipic semialdehyde (pmol/mg)	90 ± 19 (6)	85 ± 15 (6)	84 ± 8 (6)	78 ± 10 (7)
γ-Glutamyl semialdehyde (pmol/mg)	103 ± 30 (6)	109 ± 12 (6)	101 ± 17 (6)	103 ± 21 (7)
Plasma				
2-Amino adipic semialdehyde (pmol/mg)	134 ± 34 (6)	132 ± 24 (5)	151 ± 22 (6)	131 ± 33 (7)
Malondialdehyde (nmol/mg)	425 ± 186 (8)	357 ± 172 (6)	347 ± 195 (6)	557 ± 262 (8)
Total Vitamin C (mg/l)	35 ± 7 (8)	29 ± 5 (6)	35 ± 7 (6)	35 ± 9 (8)
Reduced Vitamin C (mg/l)	27 ± 8 (8)	20 ± 5 (6)	27 ± 7 (6)	26 ± 8 (8)

pression of *OGG1* and *ERCC1*, were unaltered both in the colon and liver (Tables 2 and 3). As can be discerned from Table 3, the expression of both *ERCC1* and *OGG1* was higher in the colon than in the liver.

Rats fed IQ had 50% (95% CI: 25–75%, $P < 0.0001$) increased level of apoptosis in the liver (Table 3). IQ-induced alterations of the liver cell proliferation appeared to depend on simultaneous sucrose feeding (Table 3). The interaction indicated that IQ feeding in combination with low sucrose intake resulted in low cellular proliferation, whereas the PCNA labeling index was similar between control rats and rats fed high sucrose and IQ (approximately two-fold (95% CI: 0.2–5-fold) higher level of PCNA in the IQ group compared to the IQ/sucrose group). Reliable determinations of cell proliferation and apoptosis in the colon mucosa were not feasible because of technical problems.

Biomarkers of oxidative stress including malondialdehyde, total and oxidized Vitamin C, γ-glutamyl semialdehyde, 2-amino adipic semialdehyde were unaltered in plasma and liver (Table 2).

Table 3
Expression of DNA repair genes, apoptosis and PCNA in Big Blue^{TM} rats fed with IQ and sucrose for 3 weeks (mean ± S.D.)

Group	Control	Sucrose	IQ	IQ/sucrose
Colon				
ERCC1 (per 10^6)	57 ± 38	70 ± 48	105 ± 49	72 ± 29
OGG1 (per 10^6)	52 ± 24	59 ± 43	63 ± 12	55 ± 27
Liver				
Apoptosis	1.1 ± 0.5	1.1 ± 0.1	1.5 ± 0.3[a]	1.7 ± 0.3[a]
PCNA labeling index (%)[b]	1.2 ± 1.3	0.7 ± 0.4	0.4 ± 0.4	1.4 ± 1.1
ERCC1 (per 10^6)	19 ± 6	32 ± 12	25 ± 9	30 ± 9
OGG1 (per 10^6)	7 ± 2	11 ± 2	10 ± 4	10 ± 5

The number of animals in the control ($n = 8$), sucrose ($n = 6$), IQ ($n = 6$), and IQ/sucrose ($n = 8$) groups.

[a] $P < 0.0001$ compared to control and sucrose treatment (the difference between the IQ groups and controls were 50% (95% CI: 25–75%).

[b] $P < 0.05$ for the interaction between sucrose and IQ. The interaction indicates a two-fold (95% CI: 0.2–5-fold) higher level of PCNA labeling index in the IQ/sucrose group compared to the IQ group.

4. Discussion

In this study, we have found that low doses of sucrose had no significant effect on IQ-induced DNA strand breaks or mutagenesis. IQ alone induced strand breaks in the colon, and mutation frequency in the liver. IQ–DNA adducts were not observed in the colon of sucrose-fed rats, whereas IQ-induced DNA adducts were higher in the liver of IQ/sucrose-fed rats. Thus, in general, sucrose appears not to increase IQ-induced genotoxicity in the colon mucosa, whereas sucrose increased IQ-induced genotoxicity in the liver to a limited extent. Considering that sucrose increases the colon mutation frequency [9], it is likely that sucrose and IQ are mutagenic by different mechanisms.

The exact mechanism of sucrose in chemical-induced carcinogenesis remains to be elucidated, although mechanisms involving insulin resistance or focal defects in the epithelium may provide clues to a role of sugar in colon cancer [13]. It has been hypothesized that ROS play a crucial role in the carcinogenic mechanism involving a focal epithelial defect [13]. According to this model, focal epithelial barrier defects induce an inflammatory reaction and electrolyte imbalance that both are associated with increased generation of ROS [13]. We found no difference in the level of oxidative DNA damage in colon epithelial cells and liver tissue between any of the groups. Also biomarkers of oxidative stress in the circulatory compartment were unaffected. This suggests that a generalized oxidative stress process apparently does not occur in the colon, although we cannot rule out that the existence of small spots of colon epithelium containing high levels of oxidative DNA damage. However, the present data is in agreement with our previous results showing that the mutagenic mechanism of sucrose in the colon was unlikely to be mediated by an oxidative pathway [9].

It has been described that high bolus intake of sucrose increases the colonic crypt cell proliferation [6]. It is likely that tissues with rapid proliferation are more apt to form tumors because dividing cells have sufficient supply of growth factors. Also, the high cell proliferation may increase the access of genotoxic compounds to relevant target genes. This type of epigenetic action of sucrose could explain the co-carcinogenecity and the elevated mutation frequency of high dose sucrose in the colon. Unfortunately, we were unable to determine the proliferation in the colon epithelium. In the liver, the cell proliferation was reduced in both the sucrose and the IQ groups, whereas the IQ/sucrose group had virtually similar PCNA labeling index as the control group. Thus, the increased mutation frequency cannot be explained by elevated liver cell proliferation in the IQ-treated rats compared to the control rats. Also, liver apoptosis was only observed in the IQ-treated rats suggesting that genotoxicity and mutagenicity are parallel with increased apoptosis frequency.

In conclusion, this study has shown that low doses of sucrose may modify the level of DNA adducts in colon and liver, yet sucrose does not affect the level of oxidative DNA damage, strand breaks, DNA repair, or mutagenic potential of IQ in the colon and liver of Big Blue^{TM} rats. These data suggest that sucrose affects IQ-induced colon carcinogenesis by a tumor promotor effect rather being co-carcinogenic.

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