Dietary Modulation of DNA Adduct Formation of the Food Mutagen 2-Amino-3-methylimidazo-[4,5-f]quinoline in the Male Fischer 344 Rat

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In numerous in vivo systems it has been shown that diets high in menhaden oil (a fish oil high in ω-3 fatty acids) can inhibit the carcinogenic process. In the present study, we have assessed the effects of a diet containing menhaden oil on 2-amino-3-methylimidazo[4,5-f]quinoline (IQ)-DNA adduct formation in target tissues of the male Fischer 344 rat. Young adult male Fischer 344 rats were maintained on either a) an AIN-76A diet containing 5% corn oil, b) an isocaloric AIN-76A diet modified to contain 2% corn oil and 19% menhaden oil (MO diet), or c) a regular laboratory rodent diet (chow diet) for 6 weeks prior to receiving a single oral dose of 10 or 50 mg IQ/kg. Groups of four animals were killed 1 or 6 days after IQ administration. Using 32P-postlabeling assays, IQ-DNA adducts were isolated and quantitated in the liver, small intestine, and large intestine. Adduct patterns were similar in all cases. Adduct levels, expressed as relative adduct labeling values (RAL x 108), were related to dose in all three tissues, with liver levels up to 10-fold higher than the large intestine and up to 20-fold higher than the small intestine. On day one, liver adduct levels in animals on the AIN-76A diet were similar to those in animals on the chow diet, while those in animals on the MO diet were approximately 2-fold lower. On day six, these differences were much lower, probably as a result of the more efficient removal of liver adducts in animals on AIN-76A or chow diets. In the intestines, removal of adducts in animals on either AIN-76A or MO diet was slower than that in the liver, while that in animals on the chow diet was similar to that in the liver. While dietary MO is generally thought to inhibit experimental tumorigenesis at the promotional stage, the present results show that MO diets may also inhibit the initiation process by inhibiting the initial rate of IQ-DNA adduct formation. It also appears, however, that MO diets may impair the process of adduct removal. —Environ Health Perspect 102(Suppl 6):57-60 (1994)

Key words: 2-amino-3-methylimidazo[4,5-f]quinoline, IQ, 32P-postlabeling, DNA adducts, menhaden oil, chemoprevention, carcinogenesis, heterocyclic amines, food mutagens

Introduction

2-Amino-3-methylimidazo[4,5-f]quinoline (IQ) is a potent mutagenic compound which was first isolated from broiled sun-dried sardines (1). It is also one of several aminoidazoloazines that can be isolated from cooked meat (2). IQ is carcinogenic in Fischer 344 rats, affecting principally the Zymbal gland, liver, small and large intestine, and in CDF1 mice where it affects the liver, lungs, and stomach (3). In addition, IQ induces mammary tumors in female Sprague-Dawley rats (4) and hepatocellular carcinoma in cynomolgus monkeys (5).

IQ is activated to N-hydroxy-IQ, a reaction catalyzed by cytochrome P450I1A2 in animal cells (6) as well as in human cells (7). N-Hydroxy-IQ (N-OH-IQ) reacts with DNA in vitro to yield DNA adducts (8,9), but in the cell, various etiosterefication reactions are thought to cause further enhancement of its reactivity (8,10). Using 32P-postlabeling methodology, we have isolated and quantitated specific IQ-DNA adducts formed in Salmonella after exposure of the bacteria to IQ in the presence of an activating system and in various organs of the Fischer 344 rat, CDF1 mouse, and cynomolgus monkey after administration of IQ in vivo (11-14). In each case the adduct pattern was identical to that obtained after reaction of N-OH-IQ with calf thymus DNA and the major adduct (adduct 1) cochromatographed with synthetic standard N-(deoxyguanosin-8-yl)-IQ (11).

The chemopreventive effect of fish oil-containing diets in experimental carcinogenesis is well established (15). It is believed that ω-3 fatty acids, which are particularly ubiquitous in fish oil, are responsible for the chemopreventive effect. While it is accepted generally that fish oil affects tumor formation by inhibiting the promotional phase of the process, recent evidence suggests that dietary menhaden oil may also affect the initiation phase (16). In this study, we have examined the effect of a diet high in menhaden oil on IQ-DNA adduct formation in several target organs of the male Fischer 344 rat.

Materials and Methods

IQ was a gift from Richard H. Adamson of the National Cancer Institute. The purity of IQ was >99.7% as established by HPLC using a Partisphere C8 column (Whatman) eluted isocratically with methanol-water (60:40).

Young adult Fischer 344 rats (187-206 g) were obtained from Harlan Sprague-Dawley (Indianapolis, IN). The animals were kept on corncobb bedding in polycarbonate cages covered with bonnets in a temperature-controlled (22 ± 2°C) and humidity-controlled (30-60%) room. Food and water were provided ad libitum.

Pelleted rodent laboratory chow (#5001, Purina Mills, St. Louis, MO) was stored at room temperature. Menhaden oil (MO) was obtained from the National Oceanic and Atmospheric Administration Fish Oil Biomedical Test Material Program (Southeast Fisheries Science Center, Charleston Laboratory, Charleston, SC). The oil was preserved by the addition of antioxidants (Eastman Chemical Products, Kingsport, TN): 0.1% (w/w) Tenoxx 20A (containing 20% t-butyldihydroxyquinone),
0.115% (w/w) Kodak 5-67 (containing 67.2% α-tocopherol, and 0.31% (w/w) Tenox GT-1 (containing 30–32% γ-tocopherol, 7.5% α-tocopherol, and 7.5% δ-tocopherol). Powdered AIN-76A diet, which contains 5% corn oil, was the control diet. All diets were prepared in the laboratory, purchasing ingredients from Teklad Test Diets (Madison, WI). Prior to mixing the AIN-76A diet, the three antioxidant preparations (Tenox 20Α, Kodak 5-67, and Tenox GT-1) were mixed with the corn oil component such that their final concentration per kg diet was the same as in the MO diet. The MO diet was similar to the AIN-76A diet except that it contained 19% MO and 2% corn oil (a source of linoleic acid). The extra calories introduced when 19% MO and 2% corn oil were added to the powdered AIN-76A diet instead of the 5% corn oil were compensated by a corresponding caloric reduction in the carbohydrate (sucrose) content. Diet components were kept at room temperature, except for the antioxidants, corn oil, and AIN-76A vitamin mix, which were kept at 4°C. MO and prepared diets were stored at −20°C in air-tight containers of 0.5 to 1.0 kg each. Animals received fresh food (AIN-76A and MO diet) every third day. It has been shown that, without added antioxidants, MO diets auto-oxidize rapidly (17), but that in the presence of the above antioxidants MO diets are stable at room temperature for at least 8 days when protected from direct light through the use of bonnets covering the animals’ cages (18).

After 6 weeks on the experimental diets, the animals (four rats/dose/time point) received a single oral dose of IQ (10 or 50 mg/kg). One or 6 days thereafter the animals were killed by CO₂ asphyxiation. The liver from each rat was chopped into small pieces (<2 mm²) and a random sample of approximately 0.5 g was taken for DNA isolation. The small and large intestine were removed, cut open, and rinsed with phosphate-buffered saline containing 1 mM EDTA to remove their contents. The epithelial layer was then scraped off, and a 0.5-g aliquot was used for isolation of DNA, using a modification of the direct salt precipitation procedure (19) as described before (20). DNA was quantitated by measuring the absorbance at 260 nm using a value of 20 A₂₆₀ absorbance units/mg DNA.

IQ-DNA adducts were isolated by ³²P-postlabeling, using the intensification version of the assay as described before (14). The extent of adduct formation was expressed as relative adduct labeling (RAL) values, which were calculated by dividing the counts per minute (cpm) per adduct by the sum of the cpm in the adduct and the normal nucleotides, making adjustments for dilution and aliquot factors. <RAL> values obtained under intensification conditions were converted to the actual RAL values using the previously determined intensification factors for each adduct (12).

Analysis of variance was used to test for the effect of diet on RAL values and Duncan's multiple range test was used to test for differences between the individual diets.

Results and Discussion

The IQ-DNA adduct pattern obtained was similar in all three tissues (Figure 1) and was identical to that observed earlier in similarly treated Fischer 344 rats (14). The pattern was independent of the diet and organs from vehicle-treated animals showed no IQ-related adducts (data not shown). Adduct formation was dose-dependent in all three organs (Figures 2–4), and hepatic adduct levels measured 1 day after IQ administration to animals on either the AIN-76A diet or the regular chow diet were 8- to 11-fold higher than in the large intestine and 14-fold to more than 50-fold higher than in the small intestine (Figures 2–4). These data are in agreement with our previous findings (14).

In the liver of animals on the AIN-76A diet, adduct levels measured 1 day after IQ administration were similar to those in animals on the regular chow diet (Figure 2). This also applied both to the small intestine (Figure 3) and large intestine (Figure 4). It should be emphasized that adduct levels in the intestines represent minimal values, as no attempt was made in these studies to correct for differences in cell turnover rates.

Feeding rats a 19% MO diet reduced hepatic IQ-DNA adduct formation on day one to approximately half that observed with the other diets (Figure 2). This effect was not apparent in the intestines (Figures 3, 4). In the small intestine, however, adduct levels measured 1 day after a dose of 10 mg IQ/kg were higher in animals on AIN-76A diet than in animals on the other diets (Figure 3). Such a difference was not apparent at the 50 mg/kg dose (Figure 3). These differences between the liver and the intestines may be a reflection of the different
INHIBITION OF IQ-DNA ADDUCTS BY DIETARY MENHADEN OIL

Figure 3. Total 2-amino-3-methylimidazo[4,5-f]quinoline (IQ)-DNA adducts in the small intestine. See legend to Figure 2 for further details. * indicates significantly different (p<0.01) from corresponding value with menhaden oil (MO) or pelleted rodent chow diet (CHOW); # indicates significantly different (p<0.05) from the corresponding value with MO or AIN-76A diet; † indicates significantly different (p<0.01) from the corresponding value with AIN-76A or the CHOW diet.

Figure 4. Total 2-amino-3-methylimidazo[4,5-f]quinoline (IQ)-DNA adducts in the large intestine. See legend to Figure 2 for further details.

Table 1. The effect of diet composition on the rate of removal of IQ-DNA adducts in the male Fischer 344 rat.

| Organ | IQ dose, mg/kg | % of day 1 values*  |
|-------|---------------|----------------------|
| Liver | 50            | Chow                 | MO | AIN-76A | 19% MO |
|       | 29.8          | 20.1                 | 35.9 |        |        |
|       | 10            | 40.4                 | 13.4 | 21.0    |        |
| Small | 50            | 16.4                 | 42.5 | 62.2    |        |
| intestine | 10          | 30.5                 | 64.3 | 96.2    |        |
| Large | 50            | 21.0                 | 48.7 | 34.1    |        |
| intestine | 10          | 27.6                 | 59.3 | 43.6    |        |

Abbreviations: IQ, 2-amino-3-methylimidazo[4,5-f]quinoline; MO, menhaden oil. IQ-DNA adduct levels on day 6 expressed as a percentage of those on day 1. Data taken from Figures 2 to 4.

rates of cellular uptake of ω-3 fatty acids (or other fish oil components) between the organs, or may result from effects of ω-3 fatty acids on IQ-activating enzymes such as cytochrome P450 or esterases. Fish oil is known to exert specific and profound effects on triglyceride formation and secretion by the liver (21), but the relationship of this effect to carcinogen activation is not known. While ω-3 fatty acids are not considered essential fatty acids, except perhaps in the brain and retina (22), it is interesting to note that ω-3 fatty acids in fish oil can markedly depress both the Δ6 and Δ9 microsomal desaturases (23), which are enzymes involved in the generation of precursors such as arachidonic acid for prostaglandin and leukotriene formation. Prostaglandin H synthase (PHS) is an endoperoxidase that catalyzes the initial steps leading to the biosynthesis of prostaglandins. PHS converts arachidonic acid to prostaglandin H₂ and concomitantly generates an activated form of oxygen. Accordingly, a number of studies have shown that PHS can mediate arachidonic acid-dependent, one-electron co-oxidation of several classes of compounds, including poly cyclic aromatic hydrocarbons and aromatic amines (24). In view of the finding that IQ can be activated, at least in vitro, by PHS-mediated one-electron oxidation (25,26), it appears possible that fish oil may suppress this activation pathway, thereby reducing adduct formation. Eicosapentaenoic acid and docosahexaenoic acid, two of the main ω-3 fatty acids present in MO, are known inhibitors of oxidative metabolism of arachidonic acid by the cyclooxygenase pathway (23), but it is not known to what extent the PHS-mediated pathway of IQ activation is active in vitro.

In general, high-fat diets increase the activity of hepatic mixed function oxidases (cytochrome P450), leading to increased rates of in vitro carcinogen metabolism and, in some cases, to increased DNA adduct formation (27). Dietary fish oil induces certain forms of microsomal cytochrome P450 but leaves others unaffected (28-31). The effect of fish oil diets on cytochrome P4501A2, the isozyme catalyzing the formation of N-OH-IQ from IQ (6), is not known, nor are its effects on esterification enzymes or on other P450 isozymes that may be involved in the detoxification of IQ. It appears possible, however, that the ω-3 fatty acids, when incorporated into hepatic microsomal phospholipids (30), may either inhibit the synthesis of cytochrome P4501A2 or adversely affect its catalytic activity, resulting in reduced adduct formation. The 19% MO diet did not affect the relative distribution of the individual IQ-DNA adducts (data not shown), which is in accordance with our finding that the formation of all of the IQ-DNA adducts depends on the cytochrome P450-catalyzed N-hydroxylation of IQ (6,11).

In rats on the regular chow diet, removal of adducts during the 6-day period following IQ administration was slower in the liver than in the intestines, especially at the 50 mg/kg dose (Figure 2, Table 1), confirming our previous findings (14). With the other two diets, however, the reverse applies (Table 1), which is possibly a reflection of diet-related effects on intestinal cell turnover rates or on activity of repair enzymes. Interdiet differences in adduct removal rates in the three organs were most prominent at the 10 mg/kg dose, and the largest differences were between AIN-76A and 19% MO diet on the one hand and regular chow diet on the other hand. At the 10 mg/kg dose, adduct removal in the intestines was much slower in animals on AIN-76A or 19% MO diet than in animals on the regular chow diet (Figure 3, Table 1). In the liver there was little difference (50 mg/kg) or the opposite situation applied (i.e., at 10 mg/kg both the AIN-76A and 19% MO diet accelerated adduct removal [40.4% vs 13.4-21.0%, Table 1]). Thus, it is possible that regular chow diet slows down IQ-DNA adduct repair in the liver while accelerating this process in the intestines. However, it is possible that the type of diet affects the rate of absorption of IQ from the gastrointestinal tract and that the AIN-76A and 19% MO diets increase the rate of reabsorption of IQ via the enterohepatic circulation (32,33). The apparent longer half-life of IQ-DNA adducts in the intestines of rats on the 19% MO diet could indicate that this diet may not be protective in IQ-induced intestinal carcinogenesis. This would oppose the results of Reddy et al. (16), who showed that an 18.5% MO diet inhibited the initiation phase of azoxymethane-induced colon tumors in male Fischer 344 rats. Such a conclusion would be premature, however, in the absence of knowledge of the IQ-induced colon tumor response in animals on 19% MO diets.

Our results show that it is possible that dietary ω-3 fatty acids derived from MO inhibit both carcinogen-DNA adduct formation and removal, and that these processes are organ specific. The ω-3 fatty acids may also inhibit the process of neoplastic transformation. Cultured C₃H 10T1/2 or NIH 3T3 cells transfected with plasmid T24 containing the H-ras oncogene exhibited decreased transformed foci formation after the addition of 100 μM eicosapentaenoic acid or docosahexaenoic acid to the medium (34). Therefore, dietary ω-3 fatty acids may exert beneficial preventive effects through multiple mechanisms.
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