Enhancement of the Antineoplastic Effect of Mitomycin C by Dietary Fat

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ABSTRACT

In the present study, we investigated the effects of high dietary fat on the growth of MX-1 heterotransplanted in athymic mice and its response to mitomycin C (MC) treatment. We found that high fat intake (25% corn oil, w/w) significantly increased tumor growth, but at the same time it also increased the tumor response to MC treatment compared to the control low fat diet (5% corn oil, w/w). In the tumors from mice fed either low (5% w/w) or high (25% w/w) fat, MC treatment induced oxidative challenge, indicated by significantly increased tumor total superoxide dismutase, catalase, glutathione peroxidase, and glutathione S-transferase activities, as well as increased tumor lipid peroxidation. On the other hand, glutathione reductase activity was inhibited by MC treatment. Some of the enzymes which are known to activate MC, such as cytochrome b5 reductase and DT-diaphorase, were also induced in the tumor by high dietary fat intake. The enzyme activities in hepatic tissues were also altered by dietary fat and MC treatment but to a lesser extent. We conclude that high dietary fat intake could enhance the chemotherapeutic effect of MC by increasing MC-activating enzyme activities. The observed increase in lipid peroxidation after MC treatment in MX-1 human mammary carcinoma implanted in the nude mice could result from the observed inhibited glutathione reductase activity. It is tempting to speculate that this might be another antineoplastic mechanism for MC in addition to its known role as a bioreductive alkylating agent. Alternatively, glutathione reductase may be a target for bioreductive alkylaion.

INTRODUCTION

Altering the levels and/or types of dietary fat markedly influences the development of mammary tumors in experimental animal models (1, 2). High dietary fat levels have been shown to promote the development of mammary tumors with PUFAs2 being found to be more effective than saturated fats. Mammary tumorigenesis in rats and mice has been positively correlated with the content of linoleic acid (18:2; n-6) in the diet, but negatively with the content of n-3 fatty acids (3–7). The mechanisms of dietary effects on mammary tumor development are not clear at the present time, but several possibilities have been proposed, including effects on the endocrine system, the immune system, eicosanoid production, cellular membranes, cell-to-cell interactions, and oxidative stress (for review see Refs. 1 and 8). Our laboratory has reported previously that dietary fish oil (10% w/w) compared to dietary corn oil (10% w/w) not only decreased the rate of tumor growth but also increased the susceptibility of MX-1 human mammary carcinoma grown in athymic mice to doxorubicin and MC chemotherapy (9). In follow-up preliminary investigations we found that high-fat diets increased the MC antitumor effect toward MX-1 grown in athymic mice. Moreover, when fed the same amount of fat (10%, w/w), the longer the feeding period, the greater effect of MC (10). These findings suggest that dietary fat intake could modulate the effect of MC and that the effect is cumulative. It has been reported that dietary fat could enhance a variety of enzyme activities, especially those responsible for metabolizing xenobiotics such as cytochrome P-450s (11–13). In this study we examined the effect of high dietary fat (25% corn oil, w/w) on MX-1 human mammary carcinoma growth and on MC chemotherapy. On the basis of the hypothesis that high dietary fat enhances the MC antitumor effect by increasing MC bioreductive activating enzyme activities, we focused on the activities of several of these enzymes, such as cytochrome b5 reductase (14), cytochrome C reductase (15, 16), DT-diaphorase (16, 17), and xanthine oxidase/dehydrogenase (18, 19). We also tested the antioxidant enzyme activities in low- and high-fat diet groups and monitored the alterations of these enzyme activities after MC treatment as well as to evaluate the hypothesis that increased PUFA content in the diet predisposes the tumor to oxidative stress and therefore to a greater chemotherapeutic response.

MATERIALS AND METHODS

Materials. MC, purified erythrocyte SOD (4000 units/mg protein), buttermilk xanthine oxidase (0.72 units/mg protein), Bakers' yeast GR (190 units/mg protein), and all other reagents for enzyme assays were purchased from Sigma Chemical Co. (St. Louis, MO). The total protein assay reagent kit was obtained from Pierce Chemical Co. (Rockford, IL). Dietary components were purchased from Dyets, Inc. (Bethlehem, PA).

Animals and Diets. Twenty-four young adult female athymic (nude) mice (female heterozygous BALB/c nu/nu) were used. The mice were housed under aseptic conditions (germ-free laminar-flow hood, sterilized cages, bedding, and water) at 22°C. Autoclaved laboratory mouse chow (Dyets, Inc.) was fed ad libitum before the experimental diets were fed. The experimental diets were composed of the casein-based, semipurified AIN-76A diet (20, 21), containing 25% corn oil (w/w), HF or 5% corn oil (w/w, LF) (Table 1). The diets were prepared every 3 days and kept in the freezer at −23°C until used.

The animals were assigned randomly to the HF or LF diet, with 12 mice in each of the 2 experimental groups. The dietary consumption was recorded daily by weighing the amount of uneaten food. The mice were supplied with fresh diet everyday and the nonconsumed food was discarded. The daily caloric intake was not significantly different between the HF and LF dietary groups (P > 0.05; Table 1).

Tumor Implantation and MC Treatment. Mice were fed their respective experimental diets for 20 days prior to tumor inoculation. A tumor fragment (1–2 mm3) of MX-1 human mammary carcinoma was implanted s.c. on the right fore flank of each nude mouse. The tumor weights were measured weekly or semweekly by measuring the three perpendicular diameters with calipers. Tumor weight was calculated as

\[
\text{Tumor weight (mg) = } \frac{A \times B \times C}{2}
\]

where A, B, and C represent the three perpendicular dimensions of the tumor in millimeters (9).

Ten days after tumor implantation, one-half of the animals in each dietary group were given MC (0.5 mg/kg body weight) i.p. once a week for 4 weeks (HF + MC and LF + MC groups). Tumor and body weights were recorded every 3–4 days. The percentage T/C ratio was calculated from the mean tumor weight of the treated group (+MC) divided by the mean tumor weight of the control group (HF or LF) multiplied by 100 (9).

Twenty-four h after the 4th MC treatment, all the mice were sacrificed by cervical dislocation following anesthetization under a stream of CO2. Liver and tumor were removed immediately. The tumor and liver were homogenized in 3 ml of 50 mM phosphate buffer (pH 7.0). The homogenate was centrifuged at 10,000 × g for 30 min. and the supernatant retained for enzymatic and lipid peroxidation assays.

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1 To whom requests for reprints should be addressed.

2 The abbreviations used are: PUFAs, polyunsaturated fatty acids; CAT, catalase; GR, glutathione reductase; GSH, glutathione; GSHp, glutathione peroxidase; GST, glutathione S-transferase; GSTp, GST peroxidase; HF, high fat diet; LF, low fat diet; MC, mitomycin C; T/C, target/control; SOD, superoxide dismutase; ODC, ornithine decarboxylase.
Enzyme Assays. Cytochrome b₅ reductase activity was measured by the method described by Yubisui et al. (22). The enzyme activity was measured by monitoring the disappearance of NADH absorption at 420 nm (ε = 1.02 mm⁻¹ cm⁻¹) at 25°C (22). DT-diaphorase activity was measured as the dicumarol-sensitive reduction of 2,6-dichlorophenolindophenol, according to the method of Ernst (23) and modified by Benson et al. (24). Ornithine decarboxylase activity was measured spectrophotometrically according to the method described by Ngo et al. (25). Xanthine oxidase/dehydrogenase activities were assayed with the formation of uric acid from xanthine at 293 nm (26). The activities of cytochrome C reductase were performed according to the method of Masters et al. (27) and Yasukochi et al. (28). The reaction mixture contained 0.3 M potassium phosphate buffer (pH 7.7) with 0.1 mM EDTA, 40 μM cytochrome C, and 0.6 mM potassium cyanide. The reaction was measured at 550 nm. SOD activity was measured using the method described by Oberely and Spitz (29). CAT activity was determined by measuring the sample catalyzed disappearance of H₂O₂ spectrophotometrically at 240 nm (30). GSTPx activity was measured similarly as GSHPx (32). OR activity was measured as described by Racker (33). GST activity was determined by the method according to Mannervik and Guthenberg (34).

**RESULTS**

**Tumor Growth Studies**

In the typical American diet, more than 40% of calories are derived from fat (36). In the present study, we used a diet containing 25% (w/w) corn oil as the high-fat diet (46% of calories), which approximates the typical American diet. Fig. 1 illustrates that feeding a HF diet (25%) to the host animals significantly increased the growth rate of MX-1 human mammary carcinoma, when compared to feeding a LF diet (5%). From 0—24 days after tumor implantation tumor growth was faster in the HF group than in the LF group. Twenty-five days after tumor implantation, tumor growth in the HF animals decreased, presumably due to the large tumor volume, allowing the tumors from the LF group to decrease the differences in tumor size between the two groups. To assess the effect of dietary fat on MC chemotherapeutic response, one-half of the mice (6 animals) in each dietary group were administered MC at the dose of 0.5 mg/kg body weight. As shown in Fig. 1, the tumors in the HF group were more sensitive to MC than those in the LF group. Although the tumor sizes were almost 2-fold larger in the HF group than in the LF group at the beginning of MC treatment, with T/C ratios for the HF group (201 ± 45 mg in the HF group and 94 ± 21 mg in the LF group), the tumors were only half the size in the HF group than those in the LF group at the end of MC treatment, with T/C ratios for the HF and LF groups of 5 and 12, respectively. These results suggest that feeding HF diet (in this study, corn oil) could enhance the chemotherapeutic effect of MC. Fig. 1A shows the body weight changes after

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**Table 1 Composition of experimental diets**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Low fat (g)</th>
<th>High fat (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn oil</td>
<td>2.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Casein</td>
<td>0.30</td>
<td>0.30</td>
</tr>
<tr>
<td>Sucrose</td>
<td>0.20</td>
<td>20.0</td>
</tr>
<tr>
<td>Cellulose</td>
<td>3.50</td>
<td>5.00</td>
</tr>
<tr>
<td>AIN vitamin mix</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>AIN mineral mix</td>
<td>3.50</td>
<td>3.50</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>Total Weight</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Caloric density (kcal/g)</td>
<td>3.85</td>
<td>4.85</td>
</tr>
<tr>
<td>% of caloric from fat</td>
<td>11.69</td>
<td>46.39</td>
</tr>
<tr>
<td>Daily diet consumed (g/mouse)</td>
<td>4.36 ± 0.89</td>
<td>3.54 ± 0.80</td>
</tr>
<tr>
<td>Daily caloric intake (kcal/mouse)</td>
<td>16.79 ± 3.43</td>
<td>17.17 ± 3.88</td>
</tr>
</tbody>
</table>

*a* Formulated in accordance with the American Institute of Nutrition (AIN) recommendation (20, 21).

*b* Calculated as 4.0 kcal/g for starch, sucrose, and casein, and 9.0 kcal/g for corn oil.

*c* Calculated daily by subtracting the amount of uneaten food from the amount of the added the day before.
ACTIVATION OF MITOMYCIN C BY DIETARY FAT

Fig. 2. Effects of high dietary fat (25% corn oil, w/w), low dietary fat (5% corn oil, w/w), and the treatment of each group with MC (0.5 mg/kg body weight, i.p.) on the tumor cytochrome b5 reductase (A), cytochrome C reductase (B), xanthine oxidase (C) and xanthine dehydrogenase (D) activities. Bars, SD of six independent determinations. Statistical significant differences were: A, in cytochrome b5 reductase activity between HF + MC and LF + MC groups (a, P < 0.001), between HF + MC and HF groups (b, P < 0.01), between LF + MC and LF groups (c, P < 0.05), and between HF and LF groups (d, P < 0.001); C, in xanthine oxidase activity between HF + MC and LF + MC groups (a, P < 0.01) and between HF + MC and HF groups (b, P < 0.01); D, in xanthine dehydrogenase activity between HF + MC and LF + MC groups (a, P < 0.01) and between LF + MC and LF groups (b, P < 0.01).

Fig. 3. Effects of high dietary fat (25% corn oil, w/w), low dietary fat (5% corn oil, w/w), and the treatment of each group with MC (0.5 mg/kg body weight, i.p.) on tumor DT-diaphorase activity. Bars, SD of six independent determinations. Statistical significant differences were between HF + MC and LF + MC groups (a, P < 0.01) and between HF and LF groups (b, P < 0.01).

Tumor Enzyme Activities

MC Bioreductive-activating Enzymes. MC is a naturally occurring antibiotic which exhibits activities against a variety of animal and human tumors. It is well known that MC undergoes both one- and two-electron reduction by a wide variety enzymes to form an activated form which is responsible for its antitumor activity (37—40). In the present study tumor MC activating enzyme activities were significantly changed by either high fat intake or MC treatment (Fig. 2). Cytochrome b5 reductase activity (Fig. 2A) was increased by HF intake (P < 0.001) and was further increased by MC treatment. Cytochrome C reductase activity was not significantly changed by either HF intake or MC treatment (Fig. 2B). Xanthine oxidase activity was not changed by diet alone but was significantly increased in the HF group after MC treatment (Fig. 2C). Xanthine dehydrogenase activity was increased by MC treatment in both dietary fat groups (Fig. 2D). Tumor DT-diaphorase activity was significantly increased by high dietary fat intake (P < 0.01) and MC treatment had no further effect on its activity (Fig. 3). These results indicated that HF and/or MC treatment could change tumor drug activating enzyme activities which may at least be partially responsible for the greater sensitivities of the tumor to MC treatment in the HF + MC group.

Tumor Antioxidant Enzyme Activities. The one-electron reduction of MC produces a semiquinone intermediate which may give rise to reactive oxygen species through interactions with molecular oxygen and could in turn account for some of the toxic activities in tumor cells (37—40). It has been proposed that dietary fat could cause oxidative damage in tumor cells (41, 42). Fig. 4A shows that GSHPx activity was significantly increased by MC treatment after high fat intake compared to either the HF group or the LF + MC group. There was no difference in GSHPx activity between the different dietary groups without MC treatment. GST activity was not affected by high-dietary fat intake alone but was significantly increased in the HF group after MC treatment compared to the LF + MC group. There was no difference in GSTP activity between the different dietary groups without MC treatment. GST activity was not affected by high-dietary fat intake alone but was significantly increased in the HF group after MC treatment compared to the LF group (Fig. 4B). GSTP activity was not altered by high levels of fat intake but was induced by MC treatment in both dietary groups (Fig. 4C). Interestingly, tumor GR activity was not increased by MC treatment, but was inhibited by MC treatment in both dietary groups (P < 0.05; Fig. 4D). Tumor SOD and CAT activities (Fig. 5) were significantly higher in the group fed a HF diet, and MC treatment further enhanced the activities in both dietary groups. These findings suggest that high-dietary fat intake induces SOD and CAT activities in tumor tissues, especially after a high intake of fats rich in PUFAs like corn oil, which is rich in 18:2, n-6. This could be due to the increased formation of reactive oxygen species by either high-dietary fat or MC treatment.

Tumor ODC Activity. In this study ODC activity was not significantly affected by dietary fat intake. It was inhibited by MC treatment (Fig. 6).

Liver Enzyme Activities

Dietary fat intake may not only alter tumor enzyme systems, but it may also change liver enzyme activities. It has been reported that high
fat intake could change a broad range of hepatic enzyme activities (12, 13). In this study, we found that some hepatic enzyme activities were changed by either high fat intake or MC treatment, but to a lesser extent when compared to the tumors (Table 2). The MC bioreductive-activating enzymes were not significantly changed by either dietary fat intake or MC treatment. GSHPx activity was significantly increased by MC treatment only in the HF group. After MC treatment, there was a significant difference in GSHPx activity between the HF and LF groups ($P < 0.01$; Table 2). Hepatic GST and GSTPx activities were not affected by either high dietary fat intake or MC treatment. Liver GR activity was significantly inhibited by MC treatment in the HF group ($P < 0.01$), but was not significantly inhibited in the LF + MC group. Hepatic SOD activity was slightly increased by feeding a high-fat diet and using MC treatment (HF + MC) but the observed differences are not statistically different. CAT activity was not affected by the HF diets but was significantly higher in the HF group after MC treatment. These findings indicate that liver has a more complete antioxidant defense system than the tumor and is less susceptible to the oxidative challenge, such as caused by a high intake of PUFAs or by MC treatment. Hepatic ODC was significantly inhibited by MC treatment ($P < 0.01$), same as in the tumors.

**Lipid Peroxidation**

The data presented in Fig. 7 show that in liver, thiobarbituric reactive substances formation was not affected by either high-dietary fat or MC treatment. As seen with the antioxidant enzyme activities, the more complete prevention system in the liver protects it from the oxidative challenge by either HF intake or MC treatment. Conversely, in tumor tissue MC treatment significantly increased malondialdehyde formation ($P < 0.01$), again indicating that MC induces oxidative stress in the tumor which in part may be responsible for its antineoplastic response.

**DISCUSSION**

Dietary fat intake plays an important role in the regulation of metabolism, clearance, and toxicity of xenobiotics via modulation of xenobiotic metabolism enzyme systems. This kind of influence may alter the therapeutic effects of drugs and the toxicity or carcinogenicity of environmental chemicals (for review see Ref. 11). It has been reported that dietary fat has changed hepatic cytochrome P-450s and other monoxygenase activities (11—13, 43, 44). Mitomycin C is an antitumor quinone which undergoes bioreductive metabolism to generate a reduced electrophilic species which can alkylate DNA (37—40). Enzymes which have been shown to be capable of catalyzing the reductive activation of MC include cytochrome b5 reductase (14), cytochrome C reductase (15, 16), xanthine oxidase/dehydrogenase (18, 19), and DTDiaphorase (16, 17). Alteration of these enzyme activities can directly
change the antitumor effect of MC. Our laboratory first reported that a significant difference was between HF + MC and HF groups (a, P < 0.01) and between ornithine decarboxylase activity. Bars, SD of six independent determinations. Statistical

As a result, it will also alter the susceptibility of the cell to lipid peroxidation. Slaga et al. (46) reported that benzoyl peroxide, a free radical-generating compound, is a promotor in 7,12-dimethylbenzanthracene induced tumorigenesis in mouse skin. Marmett et al. (47) provided evidence that the hydroperoxy and hydroxy autoxidation products of arachidonic and linoleic acids stimulate colonic DNA synthesis and ODC activity. More recently, Djuric et al. (41) reported that a low-fat diet could reduce the oxidative damage of DNA in human peripheral nucleated blood cells. Feeding monkeys with diets either rich in n-6 fatty acids or rich in n-3 fatty acids, KAASGAARD et al. (42) found that the animals fed a menhaden oil diet, which is rich in n-3 fatty acids, had a high level of lipid peroxidation in their liver. At the same time, GSHPx activity was also enhanced. Similar results were also reported in tumor tissues (48, 49).

In this study, we observed an increase in tumor activities of SOD, CAT, GSH, and GSTP by feeding the HF diet. Tumor lipid peroxidation was also increased by the HF diet, a finding consistent with others (48, 49). Conversely, we did not find significant changes in hepatic xenobiotic metabolism and antioxidant enzyme activities or lipid peroxidation, as reported by CHEN et al. (48) and Gonzalez et al. (49). This might be due to other or more active protective mechanisms being present in liver. An interesting discovery in the present study is that MC treatment can cause significant oxidative challenge in the tumor as indicated by the increased activities of GSHPx, GSTP, SOD, CAT, and increased lipid peroxidation. This observation could at least in part be related to the enhancement of MC bioreductive-activating enzyme systems. After activation, MC can form a semiquinone intermediate which can interact with molecular oxygen to produce toxic reactive oxygen species. The increased reactive oxygen species in tumor tissues could cause significant oxidative challenge and cytotoxicity. It is also possible that the significant oxidative challenge after MC treatment may be due to the observed inhibition of GR in tumor tissue. Under normal conditions GR

change the antitumor effect of MC. Our laboratory first reported that a 10% fish oil diet made MX-1 human mammary carcinoma more susceptible to MC and doxorubicin (9). In this study, we found that the MC antitumor effect was enhanced by feeding the host animals a HF diet (25% corn oil, w/w). At the same time, the activities of cytochrome b5 reductase and DT-diaphorase in the MX-1 tumor were also increased by feeding the HF diet. This suggests that dietary fat can manipulate the MC antitumor effect by enhancing tumor bioreductive enzyme systems that may be responsible for drug activation.

Tumor cells generally display a low endogenous level of lipid peroxidation which has been attributed to the low content of PUFAs in membrane phospholipids (45). The unsaturation level of dietary fat can affect the degree of unsaturation of membrane phospholipids (6). As a result, it will also alter the susceptibility of the cell to lipid peroxidation. Slaga et al. (46) reported that benzoyl peroxide, a free radical-generating compound, is a promotor in 7,12-dimethylbenzanthracene induced tumorigenesis in mouse skin. Marmett et al. (47) provided evidence that the hydroperoxy and hydroxy autoxidation products of arachidonic and linoleic acids stimulate colonic DNA synthesis and ODC activity. More recently, Djuric et al. (41) reported that a low-fat diet could reduce the oxidative damage of DNA in human peripheral nucleated blood cells. Feeding monkeys with different diets either rich in n-6 fatty acids or rich in n-3 fatty acids,
plays an important role to regenerate reduced GSH. Depletion of GSH by either increasing its oxidation or inhibiting its regeneration will cause oxidative stress (50). It is reasonable to assume that the increased oxidative stress induced in MC treatment contributes to its antineoplastic activity.

ODC is known as a biomarker for tumor growth (51). In this study we observed an inhibition of tumor and liver ODC activity by MC which correlated with the inhibition of tumor growth. Recently, McCann and Pegg (51) suggested that ODC might be used as a target for cancer chemotherapy. Due to our observations that high-dietary fat did not affect tumor ODC activity, while tumor growth was affected by high dietary fat, it is questionable whether ODC can be used as a biomarker for tumor growth or as a target for cancer chemotherapy. These aspects require further investigation.

In summary, feeding a HF diet can increase the response of MX-1 human mammary carcinoma grown in athymic nude mice to MC treatment. This may be caused by the observed increase in MC bioreductive-activating enzyme activities in the tumor. MC significantly induces oxidative stress within the tumor, which is exacerbated in the HF group. The GR inhibition and concomitant increase in lipid peroxidation may contribute to the antineoplastic activity of MC. The findings reported herein demonstrate that it is possible to manipulate the responsiveness of tumors to chemotherapy by dietary manipulation, an area that requires further investigation.

REFERENCES


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