Chemoprevention of Colon Cancer by Organoselenium Compounds and Impact of High- or Low-Fat Diets

Bandaru S. Reddy, Abraham Rivenson, Karam El-Bayoumy, Pramod Upadhyaya, Brian Pittman, Chinthalapally V. Rao*

Background: Observational and experimental studies have suggested that dietary supplementation with selenium can inhibit the development of colon cancer. However, many forms of selenium are toxic. Consequently, the development of efficacious compounds with low toxicity has been pursued. Purpose: Two synthetic organoselenium compounds, p-methoxy-benzyl selenocyanate (p-methoxy-BSC) and 1,4-phenylenebis(methylene)selenocyanate (p-XSC), were tested for their ability to inhibit colon carcinogenesis in rats that were treated with the carcinogen azoxymethane or saline, and for their ability to inhibit colon carcinogenesis in laboratory animals. Methods: Groups of 5-week-old male F344 rats (42 animals/group) were fed either a high-fat diet or a low-fat diet with or without added p-methoxy-BSC (10 or 20 parts per million [ppm]) or p-XSC (20 ppm). Two weeks later, 30 animals in each group received a subcutaneous injection of azoxymethane (15 mg/kg body weight); 1 week later, they received a second injection. The remaining 12 rats in each group received two injections of saline. Three days after the second injection of carcinogen or saline, animals being fed diets with p-methoxy-BSC or p-XSC were switched to corresponding organoselenium-free low- or high-fat diets for the remainder of the study to determine the effects of the selenium compounds on the initiation phase of colon carcinogenesis. At that time, groups of animals that had been maintained on organoselenium-free low- or high-fat diets were switched to diets containing p-methoxy-BSC or p-XSC until the end of the study to determine the effects of these compounds on the postinitiation phase of colon carcinogenesis. All animals were killed during the 38th week after azoxymethane or saline treatment, and histopathologic analysis of the colon tumors was performed. Colon tumor incidence and multiplicity were analyzed statistically. Results: No obvious toxic effects were observed following dietary administration of 10 or 20 ppm p-methoxy-BSC or 20 ppm p-XSC. Administration of 20 ppm p-methoxy-BSC in a high-fat diet during the initiation and postinitiation phases of colon carcinogenesis significantly (statistically) reduced colon tumor incidence; 10 ppm p-methoxy-BSC in a high-fat diet significantly reduced colon tumor incidence but only when it was given during the postinitiation phase. Colon tumor incidence was also significantly reduced when 20 ppm p-XSC was given in a high-fat diet during the initiation phase of colon carcinogenesis. When 20 ppm p-XSC was administered in either a high-fat diet or a low-fat diet during the postinitiation phase, both colon tumor incidence and multiplicity were significantly reduced; the greatest reductions were in animals fed a low-fat diet. Conclusions: In this model system, p-methoxy-BSC and p-XSC are effective agents for the chemoprevention of colon cancer. The effects of p-XSC were enhanced in animals fed a low-fat diet. [J Natl Cancer Inst 1997; 89:506-12]

Colorectal cancer is one of the leading causes of cancer death in the Western world, including North America (1). Several ecologic and case–control studies (2-4) have found a significant positive association between total intake of fat, especially of animal fat, and colorectal cancer mortality. Laboratory animal model studies (4) have provided evidence that not only the amount but also the types of dietary fat are important factors in determining the tumor-promoting effect of dietary fat. Such studies have also pointed to an inverse association between dietary selenium and both colon cancer risk in humans (5) and chemically induced colon carcinogenesis in laboratory animals (6,7). Humans ingest primarily organic forms of selenium, such as selenomethionine and selenocysteine. Chemoprevention studies (8), however, have not revealed any significant differences between the inorganic and natural sources of selenium. Yet, chronic feeding of inorganic and certain organic forms of selenium at levels above 5 parts per million (ppm) produced toxic effects (9). Therefore, substantial efforts have been made to develop organic selenium compounds with maximal chemopreventive efficacy and the lowest possible toxicity (8).

Rapidly evolving progress in chemoprevention research has brought about innovative approaches to the prevention of colon cancer (10). Studies in our laboratory (8,11-15) have indicated that certain synthetic organoselenium compounds hold great promise as chemopreventive agents, since they have been found to be superior to historically used selenium compounds such as sodium selenite. For example, in rats, benzyl selenocyanate (BSC), but not its sulfur analogue (i.e., benzyl thiocyanate), inhibited azoxymethane (AOM)-induced colon carcinogenesis when administered during the initiation or postinitiation stages. To enhance the che-

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See “Notes” following “References.”
mopreventive index of BSC, we conducted structure–activity assays and found that 1,4-phenylenebis(methylene)selenocyanate (p-XSC) was far less toxic, yet more effective, than BSC in inhibiting colon, mammary, and/or lung carcinogenesis (12-16). These early efficacy studies with p-XSC have led to preclinical toxicology studies being initiated by the National Cancer Institute, Bethesda, MD. In our efforts to optimize chemopreventive efficacy while keeping toxicity low, several derivatives of BSC and p-XSC, including o-, m-, and p-nitro- and methoxy-isomers of BSC and o-, p-, and m-isomers of XSC, were evaluated for their potential chemopreventive activities in colon carcinogenesis, using AOM-induced colonic aberrant crypt foci and colonic mucosal cell proliferation as biomarkers (17,18). The p-methoxy-BSC and p-XSC compounds were found to be the most effective agents in inhibiting these putative biomarkers (17,18).

Our study was designed to evaluate p-methoxy-BSC for its potential chemopreventive properties when administered in a high-fat diet during the initiation or postinitiation phases of colon carcinogenesis in rats. The rationale for the high-fat diet was to simulate a Western-style diet. We had observed previously that p-XSC inhibited AOM-induced colon carcinogenesis in rats fed a high-fat diet (14). Because of the known tumor-promoting effects of high dietary fat, we also examined how fat intake would have an impact on the chemopreventive efficacy of p-XSC. This protocol is very important because secondary prevention of colon cancer by administration of chemopreventive agents alone may reduce the risk of colon cancer somewhat among high-risk individuals consuming a Western-style diet, but it is likely to be most effective along with lifestyle changes, such as maintaining a low intake of dietary fat.

Materials and Methods

Animals, Diets, and Carcinogen

Weanling male F344 rats were purchased from Charles River Breeding Laboratories (Kingston, NY). AOM was obtained from Ash Stevens (Detroit, MI). All ingredients for semipurified diets were obtained from Dyets, Inc. (Bethlehem, PA), and stored at 4 °C prior to preparation of the diets. The compositions of low-fat and high-fat semipurified diets are shown in Table 1 (19).

Table 1. Composition of experimental diets

<table>
<thead>
<tr>
<th>Diet ingredients</th>
<th>Low fat*</th>
<th>High fat†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>20.0</td>
<td>23.50</td>
</tr>
<tr>
<td>n- Methionine</td>
<td>0.3</td>
<td>0.35</td>
</tr>
<tr>
<td>Corn starch</td>
<td>52.0</td>
<td>32.9</td>
</tr>
<tr>
<td>Dextrose</td>
<td>13.0</td>
<td>8.32</td>
</tr>
<tr>
<td>Alphacel‡</td>
<td>5.0</td>
<td>5.9</td>
</tr>
<tr>
<td>Corn oil</td>
<td>5.0</td>
<td>23.5</td>
</tr>
<tr>
<td>Mineral mix</td>
<td>3.5</td>
<td>4.11</td>
</tr>
<tr>
<td>Vitamin mix</td>
<td>1.0</td>
<td>1.18</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>0.2</td>
<td>0.24</td>
</tr>
<tr>
<td>p-methoxy-BSC§</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>p-XSC§</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

*This diet was formulated on the basis of the American Institute of Nutrition standard reference diet, with modification of varying sources of carbohydrate (19).
†In this diet, corn oil was added at the expense of starch. The composition of high-fat diets was adjusted so that animals in all dietary groups would consume approximately the same amounts of protein, minerals, vitamins, fiber, and calories (19).
‡Source of fiber.
§p-methoxy-benzyl selenocyanate (p-methoxy-BSC) was added to the high-fat diet at concentrations of 10 and 20 parts per million (ppm), whereas 1,4-phenylenebis(methylene)selenocyanate (p-XSC) was added to the low-fat and the high-fat diets at a level of 20 ppm.

The organoselenium compounds were incorporated into the diets by use of a V-blender (Patterson-Kelley Co., East Stroudsburg, PA) after each compound was premixed with a small quantity of diet in a food mixer. The control and experimental diets containing selenium compounds were prepared weekly in our laboratory and stored in a cold room. The organoselenium content of the experimental diets was determined periodically (every 4 weeks) on multiple samples taken from the top, middle, and bottom portions of individual diet preparations to ensure uniform distribution (14).

Organoselenium Compounds

The p-methoxy-BSC and p-XSC compounds were synthesized as described by Tamura et al. (20) and El-Bayoumy et al. (13), respectively. The purity of synthesized organoselenium compounds was in each case more than 99%, as ascertained by high-performance liquid chromatography analysis. The dose of p-XSC added to the diet was based on our previous study (14), in which 20 ppm p-XSC (10 ppm as selenium) induced significant inhibition of colon carcinogenesis in our model system. Moreover, based on studies (17,18) of cell proliferation and aberrant crypt foci as well as body weight gain, the levels of p-methoxy-BSC were evaluated at 10 and 20 ppm (equivalent to 4.1 and 8.2 ppm selenium, respectively). The low-fat and high-fat control diets contained about 0.1 ppm selenium in the form of Na2SeO3.

Experimental Procedure

Experiments were designed to study the efficacy of two levels of p-methoxy-BSC administered along with the high-fat diet and p-XSC administered together with high- or low-fat diets. The administration of selenium compounds occurred during either the initiation or the postinitiation phase of AOM-induced intestinal carcinogenesis in male F344 rats.

The bioassay protocols were approved by the Institutional Animal Care and Use Committee of the American Health Foundation. Animals received proper care in accordance with institutional guidelines and guidelines specified in the “Guide for Care and Use of Laboratory Animals” (U.S. Department of Health and Human Services publication No. 85-23, 1985).

A total of 360 male F344 rats received at weaning were quarantined for 10 days and had access to a modified American Institute of Nutrition AIN-76A control diet (14). After quarantine, all animals intended for p-methoxy-BSC and p-XSC studies were randomly distributed (21) so that the body weights in each group were equally distributed (30 animals for each AOM-treated group and 12 animals for each saline-treated group). The animals were then transferred to a holding room. The experimental groups in p-methoxy-BSC and p-XSC studies are shown in Table 2. The same high-fat control diet group served as a control group for both p-methoxy-BSC and p-XSC studies (Table 2). The animals were housed in plastic cages (three to a cage) equipped with filter tops under controlled conditions of a 12-hour-light and 12-hour-dark cycle at 50% relative humidity and at 21 °C. Experimental diets were prepared by adding test agents to the control diets as described above. At 5 weeks of age, groups of animals were fed the low- or high-fat diets or one of the experimental diets containing p-methoxy-BSC or p-XSC (Table 1). Two weeks later, all animals (30 per group) except the vehicle-treated rats (12 per group) received subcutaneous injections of 15 mg AOM/kg body weight once weekly for 2 successive weeks. Vehicle-treated groups received an equal volume of normal saline. Three days after the second injection of AOM or normal saline, groups of animals receiving the experimental diets containing 20 ppm p-XSC or 10 or 20 ppm p-methoxy-BSC were switched to their respective organoselenium-free low- or high-fat diets for the remainder of the study so that the effect of selenium during the initiation stage could be observed. At that time, groups of rats that had been maintained on organoselenium-free low-fat or high-fat diets and were intended for postinitiation studies were given their respective experimental diets containing p-XSC and p-methoxy-BSC until the...
termination of the study to measure the effects of the selenium compounds during the postinitiation stage. Body weights were recorded every 2 weeks until the 16th week and then every 4-6 weeks until the study was ended 38 weeks after the AOM treatment.

The rationale for assessing tumors at 38 weeks after AOM treatment in this study was based on our previous investigations of the efficacy of organoselenium compounds, including p-XSC, and other nutritional factors (11,14). Therefore, the current study was designed to be similar to our previous investigations so that the results could be compared.

Animals that were dying or moribund were killed and necropsied. Surviving animals were killed and necropsied as scheduled. All organs, including the intestines, were examined grossly with the aid of a dissection microscope. Intestines were fixed in 10% neutral-buffered formalin, embedded in paraffin blocks, and processed by routine histologic methods with the use of hematoxylin–eosin staining. The histologic criteria for intestinal tumor classification were those described previously (22). According to those criteria, most of the colon tumors in this study were either invasive or noninvasive adenocarcinomas.

**Statistical Analysis**

Tumor incidence (i.e., the percentage of animals with tumors) among the groups was compared by use of the chi-squared test. To account for the number of comparisons made, the type I error rate (α) was adjusted by use of the Bonferroni correction (23). Differences in tumor multiplicity (i.e., the number of tumors per animal) were compared among the groups by use of a one-way analysis of variance (ANOVA) followed by Dunnett’s multiple comparisons procedure (24), in which several treatment groups are compared with a control group. In the analyses for p-methoxy-BSC, each experimental group was compared with the high-fat control group. For p-XSC, each high-fat experimental group was compared with the high-fat control group, and each low-fat experimental group was compared with the low-fat control group. Since one of our major aims was to determine the impact of fat intake on the efficacy of p-XSC, the high-fat control group was compared with the low-fat control group as well as with the low-fat p-XSC group. Body weights among the groups were also compared by use of ANOVA. Dose–response relationships in tumor incidence were tested by logistic regression analysis. Reported P values are two-sided.

**Results**

Body weights of animals treated with vehicle only or with AOM and given the low-fat diet with or without organoselenium or the high-fat diet with or without organoselenium were comparable (Table 2). As expected, the body weights of saline- or AOM-treated animals fed the high-fat diet were slightly but not statistically significantly higher than those of animals fed the low-fat diet (Table 2). In vehicle-treated rats, the administration of p-XSC or p-methoxy-BSC did not produce any gross changes attributable to toxicity in either the liver, the kidneys, or the lungs.

Table 3 summarizes the effect of p-methoxy-BSC on colon carcinogenesis. The incidence of adenocarcinomas was significantly inhibited in rats receiving 20 ppm p-methoxy-BSC in a high-fat diet during the initiation (P = .009) and postinitiation (P = .009) period when compared with the incidence in animals fed the high-fat control diet. Administration of 20 ppm p-methoxy-BSC during the initiation period significantly inhibited the development of invasive adenocarcinomas (P = .045), but the inhibition of invasive adenocarcinomas was not statistically significant (P = .19) when the same concentration of p-methoxy-BSC was administered during the postinitiation period, although it did reach 45% inhibition.
These findings suggest that p-methoxy-BSC may prevent and/or delay the progression from noninvasive to invasive adenocarcinomas, although it appears to have a minimal effect on the transition from normal mucosa to noninvasive adenocarcinomas. Administration of 10 ppm p-methoxy-BSC during the postinitiation period also significantly inhibited the incidence of adenocarcinomas of the colon (P = .033). We have also calculated the chemopreventive index (15), which is defined as the maximum tolerated dose (MTD) divided by the dose that produces a 50% inhibition in tumor yield, for 20 ppm p-methoxy-BSC administered during the postinitiation period. The MTD of p-methoxy-BSC, which was evaluated previously, was 10.3 ppm as selenium, and the numbers of colon tumors in the high-fat control group and the group receiving 20 ppm p-methoxy-BSC (8.2 ppm as selenium) were 49 and 21, respectively, resulting in a chemopreventive index of 1.25. The dose–response effect of p-methoxy-BSC, analyzed by the logistic regression method (data not shown), indicates that administration of 10 or 20 ppm p-methoxy-BSC during the initiation and postinitiation periods significantly inhibited the incidence of colon adenocarcinomas in a dose-dependent manner (P = .019, β = −.86; and P = .0142, β = −.78, respectively).

Table 4 summarizes the incidence and multiplicity of AOM-induced colonic adenocarcinomas in rats maintained on low- or high-fat diets and p-XSC. As expected, a low intake of dietary fat resulted in a significant decrease in the incidence (P = .012) and multiplicity (P = .017) of adenocarcinomas of the colon when compared with the results for the high-fat diet. Administration of a high-fat diet and p-XSC during or after the initiation period significantly decreased the incidence of adenocarcinomas (P = .026 and P = .014, respectively). Both the incidence and multiplicity of noninvasive adenocarcinomas were significantly inhibited when p-XSC was administered during the postinitiation period (P = .013 and P = .019, respectively). Also, significant suppression of the incidence (P = .035) and multiplicity (P = .033) of adenocarcinomas—of specifically noninvasive carcinomas—was observed in rats fed the p-
XSC-containing low-fat diet during the postinitiation period when compared with the findings for rats fed the low-fat diet alone. It is interesting that administration of p-XSC along with a low-fat diet during the postinitiation period reduced the incidence \( P = .000006 \) and multiplicity \( P = .000002 \) of adenocarcinomas by 70% and 81.5%, respectively, when compared with the results obtained for the high-fat control group. This inhibition was again strongest in terms of noninvasive adenocarcinomas \( P{=} .00004 \). The fact that the administration of p-XSC with both low- or high-fat dietary regimens during the postinitiation phase significantly suppressed the incidence and multiplicity of noninvasive adenocarcinomas suggests that this compound can effectively inhibit the progression from noninvasive to invasive tumors. This finding has practical implications in the chemoprevention of colon cancer. The chemopreventive indices for p-XSC when administered in high-fat and low-fat diets were 2.25 and 3.35, respectively. These results were based on the following: The total numbers of tumors were 49 and 26 in the high- and low-fat control diet groups, respectively, and 24 and 9 in the high-fat plus p-XSC and low-fat plus p-XSC dietary groups, respectively; the MTD of p-XSC was 45 ppm (22.5 ppm as selenium). The high index values for p-XSC signify that this compound is well tolerated at doses required for chemoprevention and are superior to the value for p-methoxy-BSC. Also, the high index value for p-XSC when administered in a low-fat diet (3.35) compared with its high-fat counterpart (2.25) provides evidence that the chemopreventive effect of this agent is enhanced when it is given in a low-fat diet.

**Discussion**

As a part of a large-scale investigation directed toward the development of less toxic but highly effective organoselenium compounds as chemopreventive agents, we examined the colon tumor-inhibiting properties of a novel organoselenium compound, p-methoxy-BSC. We also examined whether the chemopreventive indices of p-methoxy-BSC and p-XSC were comparable. Another major aim of this study was to determine whether the efficacy of p-XSC chronically administered during the postinitiation period could be enhanced further by administering the compound with a low-fat diet. To our knowledge, this study is the first one to demonstrate the efficacy of this approach. The administration of p-XSC along with a low-fat dietary regimen inhibited the incidence (70%) and multiplicity (81%) of adenocarcinomas compared with the findings in animals maintained on a high-fat diet. This finding underscores the idea that a reduction in dietary fat may offer an important adjunct to chemopreventive efficacy in human colorectal cancer prevention trials involving organoselenium compounds. This compound should be explored in a broad range of human trials involving other chemopreventive agents.

The literature testifies to the existence of several successful studies of the chemopreventive effects of p-XSC in carcinogenesis \((12-15)\). Dietary supplementation with p-XSC during the initiation and/or postinitiation periods significantly inhibited 7,12-dimethylbenz[a]anthracene-induced mammary carcinogenesis in female rats \((15)\). Also, dietary p-XSC inhibited the formation of DNA adducts in the mammary gland, which may account for its inhibitory effect during the initiation phase of carcinogenesis \((12)\). In another model system, lung tumor development caused by the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in A/J mice was inhibited by p-XSC, even though sodium selenite had no effect \((13)\). The results of our present study and our earlier investigation \((14)\) with the colon cancer model plus studies with mammary \((12)\) and lung \((13)\) cancer models should provide a rational basis for designing chemoprevention strategies in the human setting. In fact, the National Cancer Institute’s Chemoprevention Branch has initiated a program to evaluate p-XSC in preclinical toxicology studies.

Our study also demonstrates that p-methoxy-BSC can effectively inhibit colon carcinogenesis when it is administered during the initiation and/or postinitiation phases of colon carcinogenesis. The compound is well tolerated without any signs of toxicity even when given at 20 ppm, equivalent to 8.2 ppm selenium, in contrast to Na2SeO3 at 4 ppm selenium, which retarded body weight gain in male F344 rats \((10)\). Overall, p-methoxy-BSC and p-XSC can be regarded as organoselenium compounds that can be administered safely in the diet at chemopreventively effective doses without inducing toxicity. However, on the basis of its chemopreventive index, p-XSC is considered a better chemopreventive agent than p-methoxy-BSC.

Although the precise mechanisms by which p-XSC and p-methoxy-BSC inhibit colon carcinogenesis are not fully known, it is likely that these agents operate through distinct mechanisms during the initiation and postinitiation stages of carcinogenesis. An earlier study \((25)\) suggests that the colon tumor-inhibitory effect of the parent compound BSC administered during the initiation phase may be mediated through alteration of the metabolic activation of AOM to reactive metabolites in the liver and the colon. Although the details of this mechanism are still not clear, possible modulation of the metabolism of AOM by p-methoxy-BSC may explain the chemopreventive properties of these agents when administered during the initiation period. It should be recognized, however, that AOM is a model carcinogen and not a naturally occurring agent that causes colon cancer in humans. This possible mechanism of action of organoselenium compounds during the initiation period may also apply to carcinogens that are present in the environment.

The general mechanisms of tumor inhibition that have been proposed for selenium compounds include the inhibition of lipid peroxidation and facilitation of peroxide decomposition, free radical scavenging, the repair of molecular damage, and incorporation into enzymes with protective functions for the cell, e.g., glutathione peroxidase (GSH-Px) \((26)\), an enzyme responsible for preventing oxidative damage due to peroxidation. We have established that dietary BSC and p-XSC increase colonic mucosal GSH-Px activity \((14)\). It is therefore possible that the inhibition of colon carcinogenesis by dietary p-methoxy-BSC and p-XSC can also be mediated through an increase in selenium-dependent GSH-Px activity in the colon.

Alternatively, or additionally, p-XSC and p-methoxy-BSC administered during the postinitiation period may inhibit colon carcinogenesis by blocking colonic cell proliferation and by accelerating apoptosis. In previous studies with rodent models, hyperproliferation of colonic epithe-
cial cells has been induced by the administration of bile acids (27), fatty acids (28), certain carcinogens (28), and a nutritional stress diet (29), whereas oral supplementation of calcium or the chemopreventive agent D,L-α-difluoromethylornithine decreased the proliferation of colonic epithelial cells (29,30). In addition, evidence (31) supports the concept that tumor growth depends on the evasion of normal homeostatic control mechanisms that operate through the induction of cell death by apoptosis. The degree of apoptosis in the tissues of both experimental animals and humans has been associated with the growth potential of cancers (32,33). Several chemopreventive agents and tumor promoters induce or inhibit apoptosis, respectively (34-36).

Studies in our laboratory (18) have revealed that p-XSC and p-methoxy-BSC inhibit cell proliferation in the colonic mucosa. Also, chronic dietary administration of p-XSC increases apoptosis in colon tumor cells (Samaha H, Reddy BS: unpublished observations). Treatment of murine carcinoma cells with p-XSC and sodium selenite caused an increase in cell death by apoptosis, but the effect of p-XSC on apoptosis appeared more pronounced than that of selenite (37). Also, when added to mammary tumor cell lines, p-XSC was capable of inhibiting thymidine kinase, whereas equal concentrations of selenium in the form of sodium selenite had no effect (38).

In conclusion, dietary administration of p-XSC and p-methoxy-BSC during the initiation and postinitiation periods inhibits colon tumor incidence in rats maintained on a high-fat diet. Furthermore, the chemopreventive effect of p-XSC is more pronounced when the compound is administered along with a low-fat diet. While our understanding of the mechanisms of the chemopreventive action of organoselenium compounds is evolving, the development of preventive strategies on the basis of experimental studies will serve as a practical approach toward the design of chemoprevention trials in humans. The results described herein for the first time make a strong case for the use of a low-fat dietary regimen along with chemopreventive agents as a desirable approach for primary prevention in the general population and for secondary prevention of colon cancer in high-risk individuals; however, the major emphasis in colon cancer prevention must include significant dietary modification.

References


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Regression of Established Tumors Expressing P-glycoprotein by Combinations of Adriamycin, Cyclosporin Derivatives, and MRK-16 Antibodies

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Background: Overexpression of P-glycoprotein, a transmembrane protein capable of transporting a broad spectrum of anticancer drugs out of cells, likely contributes to tumor drug resistance. Strategies for overcoming this resistance include the use of specific compounds, such as cyclosporin derivatives, that modulate P-glycoprotein function and antibodies that bind to the protein, thereby altering its activity.

Purpose: We examined the antitumor activity of combination treatment with the anti-P-glycoprotein monoclonal antibody MRK-16, a cyclosporin derivative (either cyclosporin A [CsA] or PSC 833), and the anticancer drug Adriamycin (ADM) against human colorectal carcinoma cells in vitro and established xenografts of these cells in vivo.

Methods: The human colorectal carcinoma cell line HCT-15 and its ADM-resistant subline HCT-15/ADM2-2 were used in this study. Cellular staining with a tetrazolium dye was used to assess the antitumor (i.e., antiproliferative) effects of treatment in vitro. Caliper measurement of tumor volumes was used to assess the antitumor effects of treatment in vivo. Cell surface binding of MRK-16 was measured by means of an immunofluorescence assay. Differences in the patterns of tumor cell growth in vitro and tumor growth rates in vivo were evaluated by means of repeated measure analysis of variance. Synergy in the combined effects of treatment was evaluated by means of the fractional product method.

Results: HCT-15 cells were found to express P-glycoprotein intrinsically; HCT-15/ADM2-2 cells expressed approximately five times more P-glycoprotein than the parental cells. HCT-15/ADM2-2 xenografts were also found to be about eight times more resistant to ADM in vitro than the parental cells. Incubation of both cell types in vitro with either MRK-16 and ADM or one of the cyclosporin derivatives and ADM inhibited cell growth minimally; however, ternary treatment with MRK-16, one of the cyclosporin derivatives, and ADM dramatically reduced the growth of both cell types. An analysis of treatment effects indicated that synergistic effects were obtained with ternary treatment. When athymic mice bearing established tumors (either HCT-15 or HCT-15/ADM2-2) were treated similarly with various combinations of the tested agents, the most pronounced antitumor effects were observed with ternary treatment. In some mice bearing HCT-15/ADM2-2 xenografts, ternary treatment led to complete tumor regression. Finally, CsA and PSC 833 were both shown to enhance MRK-16 binding to HCT-15 cells and HCT-15/ADM2-2 cells in vitro. Conclusion: Combination treatment with a cyclosporin derivative and an anti-P-glycoprotein antibody can be effective in circumventing P-glycoprotein-mediated drug resistance.

A major problem in cancer chemotherapy is the emergence of drug-resistant phenotypes during treatment. The most established mechanism contributing to drug resistance is the multidrug resistance mediated by P-glycoprotein (P-170). P-glycoprotein, encoded by the MDRI gene, is a transmembrane protein that transports anticancer drugs out of cells. P-glycoprotein has an affinity for a broad spectrum of anticancer drugs, and overexpression of this transporter makes cells resistant to these drugs.

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