Avenanthramides Inhibit Proliferation of Human Colon Cancer Cell Lines In Vitro

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A high intake of whole grain foods is associated with reduced risk of colon cancer, but the mechanism underlying this protection has yet to be elucidated. Chronic inflammation and associated cyclooxygenase-2 (COX-2) expression in the colon epithelium are causally related to epithelial carcinogenesis, proliferation, and tumor growth. We examined the effect of avenanthramides (Avns), unique polyphenols from oats with anti-inflammatory properties, on COX-2 expression in macrophages, colon cancer cell lines, and on proliferation of human colon cancer cell lines. We found that Avns-enriched extract of oats (AvExO) had no effect on COX-2 expression, but it did inhibit COX enzyme activity and prostaglandin E2 (PGE2) production in lipopolysaccharide-stimulated mouse peritoneal macrophages. Avns (AvExO, Avn-C, and the methylated form of Avn-C (CH3-Avn-C)) significantly inhibited cell proliferation of both COX-2-positive HT29, Caco-2, and LS174T, and COX-2-negative HCT116 human colon cancer cell lines, CH3-Avn-C being the most potent. However, Avns had no effect on COX-2 expression and PGE2 production in Caco-2 and HT29 colon cancer cells. These results indicate that the inhibitory effect of Avns on colon cancer cell proliferation may be independent of COX-2 expression and PGE2 production. Thus, Avns might reduce colon cancer risk through inhibition of macrophage PGE2 production and non-COX-related antiproliferative effects in colon cancer cells. Interestingly, Avns had no effect on cell viability of confluence-induced differentiated Caco-2 cells, which display the characteristics of normal colonic epithelial cells. Our results suggest that the consumption of oats and oat bran may reduce the risk of colon cancer not only because of their high fiber content but also due to Avns, which attenuate proliferation of colonic cancer cells.

INTRODUCTION

Epidemiological data and animal studies have shown that consuming whole grains is inversely associated with the risk of colorectal cancer (1–5). The nutrient components and the mechanisms for the protective effect of whole grains against colon cancer development have yet to be elucidated. Several meta-analyses of case-controlled studies suggest that high fiber content, which can improve the gut environment by diluting carcinogens in the colon and decrease transit time, might contribute to this protection (2,6–9). However, a prospective study of nurses reported no protective effect of dietary fiber from cereals, fruits, or vegetables against colon cancer (10). More recently, a large, population-based cohort study showed that after additional adjustment for cereal fiber, consumption of whole grains still decreases the risk of colon cancer by 25% (11). Thus, components other than dietary fiber in whole grains may contribute to their ability to protect against colon cancer (7,11). Since the health benefits of whole grains are in part attributed to their unique phytochemical composition (12), understanding the contribution of specific components in whole grains other than fiber is deemed necessary.

Cyclooxygenase (COX)-2, either undetectable or expressed at low levels in most normal tissue (13), is highly expressed in 80–85% of human adenocarcinomas, colon tumors, and...
mediated prostaglandin E₂ (PGE₂) signaling is causally related to epithelial carcinogenesis in the gastrointestinal tract (15,16). Although PGE₂ inhibits cell death, it stimulates cell migration, proliferation, and tumor-associated neovascularization (17). Thus, COX-2 is regarded as a good target for chemoprevention of colon cancer (18,19). Accordingly, both antioxidants and anti-inflammatory agents are shown to inhibit the initiation and development of cancer including colon cancer in humans and in animal models (18,20–23). Intake of non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin has been suggested to reduce the risk of colorectal cancer probably through inhibiting COX-2 expression (18,24–29). It has also been reported that dietary polyphenols such as resveratrol, curcumin, and epigallocatechin gallate (EGCG) inhibit the growth of several human colorectal cancer cell lines through inhibiting COX-2 expression (30–36).

Avenanthramides (Avns), unique polyphenols found exclusively in oats and oatmeal, exhibit antioxidant and anti-inflammatory activity (37,38). The bioavailability of Avns extracted from oats has been studied in hamsters and humans (39–41). Avn-A, B, and C are the 3 major forms found in oats and oat bran (42), but their content can vary depending on the processing: the content of Avns in oat flakes is reported to be 26–27 mg/kg, whereas oat bran contains fewer Avns (13 mg/kg) (43). Recently, we reported that these compounds suppress serum-induced smooth muscle cell (SMC) proliferation (44). We also found that synthetically prepared methyl-ester of Avn-C (CH₃-Avn-C) is 8 to 10 times more potent than the nonmethylated form in suppressing SMC proliferation (unpublished data). Since COX-2-expressing macrophages are associated with the pathological process of colon cancer, we first examined the effect of Avns on the regulation of COX-2 expression and PGE₂ production in macrophages, which modulate several tumor-associated processes including inflammation, cell proliferation, angiogenesis, and tumor growth. We then investigated the effect of Avns on cell proliferation of several colon cancer cell lines. To our knowledge, no information is available in the literature on Avns’ effect on colon cancer cell proliferation or on the proinflammatory signaling pathway associated with carcinogenesis. Next, we determined if the effect of Avns on the proliferation of colon cancer cell lines was mediated through suppression of COX-2 expression and PGE₂ production (45). To this end, three different forms of Avns (Fig. 1) were tested for their effects on colon cancer cell proliferation: 1) Avns-enriched extract of oats (AvExO) as a natural bioactive compound of oats; 2) synthetically prepared Avn-C, one of the major forms present in oat; and 3) synthetically prepared CH₃-AvnC.

**MATERIALS AND METHODS**

**Materials**

All human colon cancer cell lines and McCoy’s 5 medium were obtained from American Type Culture Collection (ATCC, Rockville, MD). Fetal bovine serum (FBS) and Dulbecco’s modified eagle medium (DMEM) medium were purchased from Gibco (Grand Island, NY). Anti-COX-1, COX-2, antigoat immunoglobulin G (IgG) horseradish peroxidase (HRP), and antimouse-IgG-HRP antibodies were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Arachidonic acid (AA) and anti-β-actin antibody were purchased from Sigma (St. Louis, MO). Tumor necrosis factor alpha (TNFα) and CellTiter 96 Aqueous One Solution Cell Proliferation Assay kits were obtained from Promega Corp. (Madison, WI). Western blotting supplies were obtained from Bio-Rad Laboratories (Hercules, CA). AvExO and synthetic Avn-C were prepared as previously described (42,44). CH₃-Avn-C was prepared as described below.

**Synthesis of CH₃-Avn-C**

Two and a half ml of acetyl chloride was slowly added to 47.5 ml of anhydrous methanol (MeOH). Then Avn-C was added to this solution, kept at room temperature, and protected from light for approximately 24 h. The product was verified by TLC (silica gel 60 F254) using an ethyl acetate:hexane:acetic acid (70:25:5) mobile phase. The reaction solution was dried under vacuum (rotary evaporation), and the product was washed several times with acetone. The product was suspended in 20% MeOH and loaded to a Fisher PrepSep C-18 column pre-equilibrated with 20% MeOH. The column was washed sequentially with 5 volumes of 20% and 40% MeOH before the methyl ester was eluted with 100% MeOH. The pure methyl ester of Avn-C obtained in this manner has a yield of 60% as analyzed by TLC and LC/MS.

**Cell Culture**

Mouse macrophages were isolated and cultured as previously reported (46,47). Cells were pretreated with the indicated concentration of AvExO for 2 h and then stimulated with 5 µg/ml lipopolysaccharide (LPS) for 20 h. The cell culture medium was collected to measure COX activity and PGE₂ production; cell lysates were collected to determine COX-2 protein level.
HT-29 human colon adenocarcinoma cancer cells were cultured in McCoy’s 5A medium; HCT116, and LS174TT, and Caco-2 human colon adenocarcinoma cancer cells were cultured in DMEM. Both media contained 10% FBS and 1% penicillin/streptomycin. Cells were incubated at 37°C in a humidified atmosphere of 5% CO2. To induce differentiation, confluent Caco-2 cells were further cultured for another 10–14 days. Otherwise, cells were maintained in an exponentially growing phase by subculturing them twice a week at a density of 2 × 10^9/10 cm culture dish. AvExO, Avn-C, and CH3-Avn-C were dissolved in dimethyl sulfoxide (DMSO) and added to the cell culture medium with a maximum, final DMSO concentration of 0.2%. This concentration showed no cytotoxicity as determined by trypan blue exclusion assay, and it had no effect on cell proliferation as described below. The same amount of DMSO was also added to the cell culture medium for the control group.

**Cell Proliferation Assay**

Cell proliferation was measured using CellTiter 96 Aqueous One Solution Cell Proliferation Assay kit. Cells were seeded into 96-well plates at a density of 3,000 cells/well/100 µl and incubated for 6 h for cells to adhere to the wells. Cells were then treated with different concentrations of AvExO, Avn-C, and CH3-Avn-C for 48 h. To obtain differentiated Caco-2 cells, which are generally used as models of human intestinal epithelial cells (48–50), confluent Caco-2 cells were cultured for an additional 2 wk before treating with Avns. Cell viability was measured according to the assay kit instructions. Cell proliferation data were expressed as the percentage of untreated cells.

**Measurement of COX Activity**

Total cellular COX activity of mouse macrophages and human colon cancer cell lines HT29 and Caco-2 cells was determined as previously reported (51,52). Briefly, after treatment with or without 40 ng/ml TNFα for 23 h or 5 µg/ml LPS for 20 h, the cells were incubated in the presence of 30 µM exogenous AA for 10 min. At the end of the reaction, aspirin (2 mM; Sigma) was added to stop COX activity, and the supernatants were collected to measure COX activity as indicated by the conversion of AA to PGE2. Cells were lysed in 1 M NaOH for total cellular protein analysis using a bicinchoninic acid (BCA) protein assay kit (Pierce).

**Measurement of PGE2 Production**

The cell culture medium of HT29 cells and Caco-2 cells as well as the medium of mouse macrophages was collected to measure basal PGE2 levels. The PGE2 levels in the cell culture medium were measured using radioimmunoassay as previously reported (53).

**Western Blot Assay**

HT29 and Caco 2 cells were seeded into 60-mm culture dishes and cultured to 70% confluence. FBS in the medium was reduced from 10% to 1% to minimize the effects of growth-promoting substances normally present in serum. For determining COX-1 and COX-2 expression levels, the cells were treated with different concentrations of AvExO and CH3-Avn-C for 24 h. Mouse macrophages, which were treated with different concentrations of Avns and stimulated with LPS, were collected to measure COX-2 protein levels. Western blotting was performed as previously described (47,54). Briefly, total cellular lysates were collected, and protein concentrations were determined using a bicinchoninic acid (BCA) protein assay kit (Pierce). Each protein sample was electrophoresed in 12% SDS-polyacrylamide gel and transferred to nitrocellulose membranes. The membranes were blocked with TST (50 mM Tris-HCl [pH 7.5], 150 mM NaCl, and 0.02% Tween-20) containing 2.5% milk proteins before incubating overnight at 4°C with the primary antibodies against COX-1 or COX-2. The membranes were then washed 4 times with TST and incubated with an HRP-conjugated secondary antibody for 1 h at room temperature. The protein bands were visualized using an Immun-Star HRP substrate kit (Bio-Rad Laboratories, Hercules, CA) according to manufacturer’s instructions, followed by exposure to X-ray film.

**Statistical Analysis**

All data shown are representatives of at least 3 independent experiments. Treatment differences were analyzed by 1-way ANOVA followed by Tukey’s test. Data are presented as means ± SD. Differences were considered significant at P < 0.05.

**RESULTS**

Avns Inhibited COX Activity and PGE2 Production But Had No Effect on COX-2 Expression in Mouse Peritoneal Macrophages

Since COX-2 expressing macrophages are associated with the pathological process of colon cancer, we used the peritoneal macrophages as a model system to examine whether Avns modulate COX-2 expression in mouse primary macrophages. The expression of COX-2 protein by peritoneal macrophages was not significantly affected by AvExO (Fig. 2A and 2B). However, AvExO dose-dependently downregulated COX activity (Fig. 2C) and PGE2 production (Fig. 2D). These observations indicate that the effect of Avns on PGE2 production in mouse macrophages is posttranslationally.

Avns Inhibited the Proliferation of Human Colon Cancer Cell Lines

After 48 h of treatment, all 3 forms of Avns suppressed proliferation of Caco-2 cells during the growing phase, which displays cancer cell characteristics (Fig. 3a, 3c, 3e). The effective inhibitory doses of AvExO (Fig. 3a) and Avn-C (Fig. 3c)
FIG. 2. Effect ofavenanthramides enriched extract of oats (AvExO) on regulation of cyclooxygenase (COX)-2 expression, COX activity, and prostaglandin E2 (PGE2) production in lipopolysaccharide (LPS)-stimulated mouse macrophages. AvExO does not affect COX-2 expression. Mouse macrophages treated with AvExO and LPS were collected. Cell lysates were used to determine COX-2 protein levels using Western blotting as described inMaterials and Methods.A: The representative results from two independent experiments are shown. The bands in A were densitometrically analyzed, and B: the ratio ofCOX-2 toβ-actinis plotted in a bar graph. C: AvExO dose-dependently decreased COX activity and D: PGE2 production. Mouse macrophages were pretreated with AvExO for 2 h and then stimulated by LPS (5 µg/ml) for 20 h. In COX activity assay, the mouse macrophages were incubated in the presence of arachidonic acid (30 µM). The cell culture medium was collected, and PGE2 production was measured as described inMaterials and Methods. Each bar on the graph shows the mean±SD ofresults from three independent experiments. Treatment differences were analyzed by 1-way ANOVA followed by Tukey’s test. Bars not sharing the same letter are significantly different from each other (P < 0.05).

were similar, ranging from 80 µM to 160 µM. However, CH3Avn-C (Fig. 3e) was more potent than AvExO and Avns-C at inhibiting the proliferation of these colon cancer cells.

One important property of Caco-2 cells is that after these cells are maintained for about 2 wk in a confluent condition, they differentiate to a phenotype with the normal characteristics of colonic epithelial cells (55). These cells provide an ideal cell culture model for comparing the differential effects of chemotherapeutic agents on normal colonic epithelial cells vs. cancer phenotypes. Using this characteristic of Caco-2 cells, we found that these compounds had no effect on the viability of differentially, normal colonic epithelial cells (Fig. 3b, 3d, 3f) in contrast to the effect on nondifferentiated cells.

The growth inhibitory effect of AvExO, Avn-C, and CH3-Avn-C on human colon cancer cell lines HCT116, LS174, and HT-29 was also examined. We found that all tested forms of Avns significantly inhibited proliferation of HT29 (Fig. 4a, 4d, 4g), LS174T (Fig. 4b, 4e, 4h), and HCT116 (Fig. 4c, 4f, 4i) colon cancer cells. The effective inhibitory doses of AvExO and Avn-C for the 3 cell lines were similar, ranging from 120 µM to 160 µM. However, CH3-Avn-C was more potent than AvExO and Avns at inhibiting proliferation of these colon cancer cells. CH3-Avn-C significantly inhibited proliferation of HT29, LS174T, and HCT116 at concentrations of 40, 80, and 40 µM, respectively. The tested Avns significantly inhibited proliferation of HCT116 cells, which do not express COX-2 mRNA or protein (56); therefore, our results suggest that the inhibitory effect of Avns on colon cancer cell proliferation is independent of the COX-2 pathway.

Avns Had No Effect on COX-1 and COX-2 Protein Expression in Human Colon Cancer Lines

HCT116 has been described as COX-2 negative (56); thus, we did not use HCT116 cells. Instead we used only two, extensively studied colon cancer cell lines, HT29 and Caco-2 cells, to determine the inhibitory effect of Avns on COX-1 and COX-2 expression by these cells. Since we found that the efficacy of Avn-C in the inhibition of colon cancer cells was similar to that of AvExO, we did not test Avn-C; rather, we tested AvExO and CH3-Avn-C for their effect on COX-1 and COX-2 expression. Avns treatment at the concentrations tested had no significant effect on either COX-1 or COX-2 expression in Caco-2 cells (Fig. 5A). Similar results were also observed in HT29 cells (Fig. 5B).

The conflicting results for PGE2 production (the indication of COX activity) in both HT29 and Caco-2 cells have been reported in the literature. Some results have shown that the PGE2 level in both HT29 and Caco-2 cell culture media was not detectable (57–59), whereas others have demonstrated that high levels of PGE2 production in HT29 cells have been detected (59–62). Therefore, we examined the effect of Avns on COX activity in HT29 and Caco-2 cells, human colon cancer cell
FIG. 3. Avenanthramides (Avns) inhibit cell proliferation of growing phase Caco-2 cells but have no effect on differentiated Caco-2 cells. Growing phase Caco-2 cells (3a, 3c, and 3e) and confluence-induced differentiated Caco-2 (3b, 3d, and 3f) cells were treated with Avns enriched extract of oats (AvExO; 3a and 3b), Avn-C (3c and 3d), or CH3-Avn-C (3e and 3f) at different concentrations as indicated for 48 h. Cell proliferation was determined using MTT assay. Data were means ± SD from at least three independent experiments. Treatment differences were analyzed by 1-way ANOVA followed by Tukey’s test. *, different from corresponding control, P < 0.05.

lines. Surprisingly, the basal COX activity of HT29 and Caco-2 cells, as assessed by the production of PGE2 in the presence of exogenous AA, was below detection limit.

Next, we examined the basal level of PGE2 production in HT29 and Caco-2 cells. The basal PGE2 level in the culture medium of HT29 cells was below detection limit, which is consistent with the reported results that have shown that HT29 cells cultured for 24 h produced no demonstrable PGE2 (58,59,63,64). The basal PGE2 level in the culture medium of Caco-2 cells was also undetectable. We further tested the effect of TNFα on PGE2 production in HT29 cells. TNFα treatment for 23 h also failed to induce HT29 cells to produce PGE2. We did not test the effect of TNFα on PGE2 production in Caco-2 cells any further because it was reported that TNFα did not induce COX-2 in Caco-2 cells (58).

DISCUSSION

Chronic inflammation of the gastrointestinal tract is believed to be the main factor in the initiation of colon cancer (65). Generally, macrophages constitute a major component of the tumor stroma (66). Although conventionally considered to be cytotoxic effector cells, macrophages have recently been described as promoters of tumor progression (66). In colorectal carcinomas, although COX-2 is expressed predominantly by epithelial cells and implicated in tumor progression, tumor-associated
FIG. 4. Avenanthramides (Avns) inhibit proliferation of colon cancer cell lines. HT29 (4a, 4d, 4g), LS174 (4b, 4e, 4h), and HCT116 (4c, 4f, 4i) cells in growing phase were treated with AvExO (4a, 4b, 4c), Avn-C (4d, 4e, 4f), or CH3-Avn-C (4g, 4h, 4i) at different concentrations as indicated for 48 h. Cell proliferation was determined using MTT assay. Data were means ± SD from at least three independent experiments. Treatment differences were analyzed by 1-way ANOVA followed by Tukey’s test. *, different from corresponding control, \(P < 0.05\).

macrophages may also express COX-2 when they are activated and when they influence proliferation, angiogenesis, and tumor growth (67,68). In this study, we found a differential effect of Avenanthramides on COX activity in different cell types. Avns inhibited COX activity and PGE2 production in LPS-stimulated mouse peritoneal macrophages in a dose-dependent manner without affecting COX-2 expression. These results are consistent with previous reports that have shown that the level of COX-2 protein expression and COX enzyme activity can be modulated independently (52,62,69), which indicates that Avns inhibit COX activity and PGE2 production in mouse macrophages through posttranslational regulation of COX enzymes. Since COX-2-expressing macrophages are associated with the pathological process of colon cancer, these results suggest that Avns might reduce the risk of colon cancer in vivo partly by inhibiting COX activity in macrophages.

Because COX-2 is expressed at high levels in intestinal tumors in humans and rodents (70) and PGE2, a prominent product of the COX-2 enzyme, COX activity plays an important role in colorectal tumorigenesis and the promotion of colorectal tumor cell growth (71,72). Since we also found that Avns inhibited COX activity and PGE2 production in mouse peritoneal macrophages, we then tested whether Avns had an inhibitory effect on COX-1 and COX-2 expression as well as COX activity and PGE2 production in HT29 and Caco-2 cells, two extensively studied colon cancer cell lines. We found that AvExO and CH3-Avn-C had no effect on COX-1 and COX-2 expression in Caco-2 or HT29 cells. Furthermore, we did not detect PGE2 in the cell culture medium of HT29 cells. Even when supplemented with AA or stimulated with TNF\(\alpha\), HT29 cells did not produce detectable levels of PGE2. These results are consistent with the reports that have shown that HT29 cells produce no demonstrable PGE2 levels (57–59,63,64) because COX-2 protein in HT29 cells is catalytically inactive (73), although COX-2 protein is strongly expressed. We found that basal PGE2 levels in the cell culture media of both proliferating and differentiated Caco-2 cells were either undetectable or very low. This is consistent with published studies showing that Caco-2 cells produce little PGE2 (74,75).

Whereas LS174T, HT29, and Caco-2 cells express both COX-2 mRNA and protein (60,64,76), HCT116 cells have been reported not to express COX-2 mRNA or protein (56). Our results show that Avns inhibited cell proliferation of both COX-2-positive colon cancer cell lines (Caco-2, LS174T, and HT29 cells) and the COX-2-negative HCT116 cell line. These results suggest that the growth inhibitory effects of Avns might be independent of the COX-2 pathway.

The bioavailability of Avns extracted from oats has been studied in hamsters and humans (39–41). The maximum plasma concentrations for Avn-A and Avn-B from hamsters administered 0.25 g oat bran phenol-rich powder were 0.04 and 0.03 \(\mu\)mol/l, respectively (39). In humans, maximum plasma concentrations...
of Avns (free + conjugated) after consumption of 0.5 and 1 g AvExO were 112.9 and 374.6 nmol/l for Avn-A, 13.2 and 96.0 nmol/l for Avn-B, and 41.4 and 89.0 nmol/l for Avn-C, respectively (40). It has also been reported that only a small part of ingested polyphenols are absorbed, suggesting that compounds may have only a local effect on the epithelial cells of the colon (77,78). Therefore, we examined Avns, unique polyphenols in oats, in their natural forms as well as their synthetically prepared form (Avn-C) and its modified form (CH3-Avn-C), on the cell proliferation of several human colonic cancer cell lines including Caco-2, HCT116, LS174T, and HT29 cells. Our results showed that Avns suppressed proliferation of these colon cancer cell lines. Among the Avns tested, CH3-Avn-C had a more robust inhibitory effect than AvExO and Avn-C on the proliferation of colon cancer cells. This differential effect of CH3-Avn-C can be attributed to the presence of a single methyl ester group in the chemical structure of Avns (Fig. 1), which might increase its lipid solubility and bioavailability, making it readily incorporated into the cell membrane and allowing it to hinder the molecular pathways that are involved in cell proliferation.

Although we did not investigate the effect of Avns on signaling pathways involved in cell proliferation, our earlier studies on vascular SMCs have indicated that Avns may arrest the cell cycle at the G1 phase by upregulating the p53-p21cip1 pathway and by inhibiting phosphorylation of retinoblastoma protein (pRB) (79). We have also reported that CH3-Avn-C inhibits proteasome activity and increases the overall levels of high mass, ubiquitin-conjugated protein in endothelial cells (80). It is known that p53 is degraded by the ubiquitin proteasome pathway (81). Thus, inhibition of proteasome activity and the subsequent stabilization of p53 protein and cell cycle arrest might be a plausible mechanism for Avns’ inhibition of colon cancer cell proliferation. Along this line, it has been reported that other polyphenols such as curcumin also protect the degradation of p53 and increase the half-life of p53 through the impairing the ubiquitin-proteasome system (82). It is known that cyclin D/Cdk4/Cdk6 is associated with colon cancer (83–86), and cyclin D/Cdk4 complex phosphorylates pRB. Western blot analysis also revealed that primary regions of advanced colorectal cancer tissues showed overexpression on the average in cyclin D1 and Cdk4 (87). Polyphenols, such as quercetin and resveratrol, could downregulate cyclin D1/Cdk4 complex in colon cancer cell lines (30,88). Therefore, the effects of Avns on colon cancer cell proliferation might be mediated through p53-p21cip1 pathway and/or cyclin D1/Cdk4/pRB pathway. These and other mechanism(s) are currently under investigation in our laboratory.

In summary, our results suggest that Avns may potentially impede the development of colon cancer through their effect on macrophages and their direct inhibition of colon cancer cell proliferation and inflammation. These effects of Avns on colon cancer cell lines were not due to their inhibition of the COX pathway. AvExO suppresses LPS-induced COX activity and PGE2 production in mouse macrophages without significant effect on COX-2 expression. Our data also showed that the proliferation of several colon cancer cell lines may be suppressed in a dose-dependent manner in three tested forms of Avns: AvExO, which is a natural bioactive compound in oats; and two synthetically prepared forms of Avns, Avn-C, and methyl-ester of Avn-C. These unique polyphenols, however, have no cytotoxic effects on differentiated colon cancer cells, which represent the characteristics of normal, colonic epithelial cells. Apart from the health benefits of fiber in oats, the anti-inflammatory and antiproliferative properties of Avns of oats may also contribute toward reducing the risk of colon cancer. Avns, in particular CH3-Avn-C, which is the most potent Avns in the inhibition of both colon cancer cell proliferation and inflammation markers, may provide a basis for developing natural sources of chemopreventive and chemotherapeutic agents for the treatment of colon cancer.

Acknowledgments

This manuscript is based on the work supported by the U.S. Department of Agriculture, under agreement No. 58-1950-7-707. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S. Department of
Agriculture. We would also like to thank Stephanie Marco for her assistance in the preparation of this manuscript.

REFERENCES
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