PROGRESS REPORT

North Central Region Canola Research Program
(January 2009)

Title: CANOLA OIL REDUCES BREAST CANCER RISK

Investigators: Chung S. Park, PI, North Dakota State University, Fargo
Kyongshin Cho, Graduate Student
Lawrence Mabasa, Graduate Student
Andrea Fowler, Research Technician

The final report of the study will be completed coinciding with the scheduled termination date (06/30/2009) of the current project.

A. Rationale and Objective

Epidemiological and experimental studies suggesting that omega-3 fatty acid and oleic acid may exert protective effects against mammary tumors have generated a lot of interest in vegetable oils with a high level of either one of these forms of fatty acids. One vegetable oil of interest is that of the canola seed. Currently, the United States is the seventh largest canola producer and processor in the world. North Dakota produces about 90% of this canola, with smaller amounts grown in Minnesota and a few other states. In the last few years, consumption of canola oil has increased because of associated health benefits. The most interesting feature of canola oil is its fatty acid profile. It has the lowest concentration of saturated fatty acids (7%) of all eight major vegetable oils, it is high in monounsaturated fatty acids (61%), and it has a favorable omega-6 to omega-3 fatty acid ratio (1.9:1). Considering the recommended 2:1 ratio of omega-6 to omega-3 fatty acids for the reduction of breast cancer risk along with the known effects of monounsaturated fatty acids, particularly oleic acid, in the down-regulation (suppression) of cancer-related oncogenes, canola oil has a uniquely balanced fatty acid composition and could be beneficial in reducing breast cancer risk.

While canola oil has been linked to many health benefits, its association with cancer risk has been overlooked. We wish to develop a better understanding of a potential mechanism responsible for the inhibitory effects of canola oil on human breast cancer cell growth. Completion of the proposed study will have a positive impact on both human nutrition (health) and the canola industry. The specific aims of this project were to determine the extent to which exposure to canola oil affects human breast cancer cell growth. This study entails determining if canola oil: 1) suppresses human breast cancer cell proliferation, 2) increases anticancer caspase-3 activity, and 3) increases tumor suppressor p53 activity.

B. Experimental Approach

The basic strategies were as follows. First, we determined the optimal concentration of canola oil that causes the maximum inhibition of human breast cancer cell growth. Second, based on the level of canola oil determined from the cell proliferation study, we examined caspase-3 and p53 assays, the key enzyme and protein involved in the apoptotic pathway of cells.

Cell Culture: Three cell lines (two breast cancer cell lines, MCF-7 and T47D, and a normal mammary cell line, MCF-10A) were obtained from the American Type Culture Collection (ATCC, Manassas, VA). Cells were cultured in a 5% CO_2-humidified atmosphere at 37°C and maintained in basal medium (Dulbecco's Modified Eagle's Medium for MCF-7, MCF-10A; Roswell Park Memorial Institute 1640 for T47D; Gibco Invitrogen, Carlsbad, CA) supplemented with 10% heat-inactivated fetal bovine serum (FBS, Gibco Invitrogen) and 1% antibiotic-antimycotic (Gibco Invitrogen) as recommended by the supplier.

Cell Proliferation Assay: Cells were cultured in either control medium or canola oil supplemented medium (treatment). Treatment culture media (DMEM and RPMI 1640) contains 6, 10, 14, or 18 µL of canola oil (for a total volume of 100 µL). Cell proliferation was measured using MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium] colorimetric assay (CellTiter 96 AQueous One Solution Reagent; Promega, Madison, WI). Briefly, cells were seeded at a density of 1 X 10^5 cells/mL in flat-bottomed 96-well microtiter plates with the desired concentrations of canola oil (6, 10, 14, or 18 µL/well). After 1, 2, 3, 4 and 5 day incubations, MTS was added to each well and the plate was incubated further for 1 to 4 hours at 37°C in a 5% CO_2 incubator. The absorbance was measured at 490 nm with a microtiter plate reader (Molecular Devices, Sunnyvale, CA).

Caspase-3 Apoptosis Assay: Cells were cultured at a density of 1 X 10^5 cells/mL in flat-bottomed 24-well plates in media containing the optimum canola oil concentration determined from the cell proliferation experiment. The activity of caspase-3 was measured by the colorimetric assay with CaspACE assay system (Promega, Madison, WI). Briefly, cultured cells were washed twice with PBS and resuspended in cell lysis buffer (Promega). Cell lysates were incubated with colorimetric substrate, N-acetyl-Asp-Glu-Val-Asp-amino-*p*-nitroanilide (Ac-DEVD-pNA). After a 4 hour incubation, the release of *p*-nitroaniline from Ac-DEVD-pNA was measured at 405 nm using a microtiter plate reader (Molecular Devices).

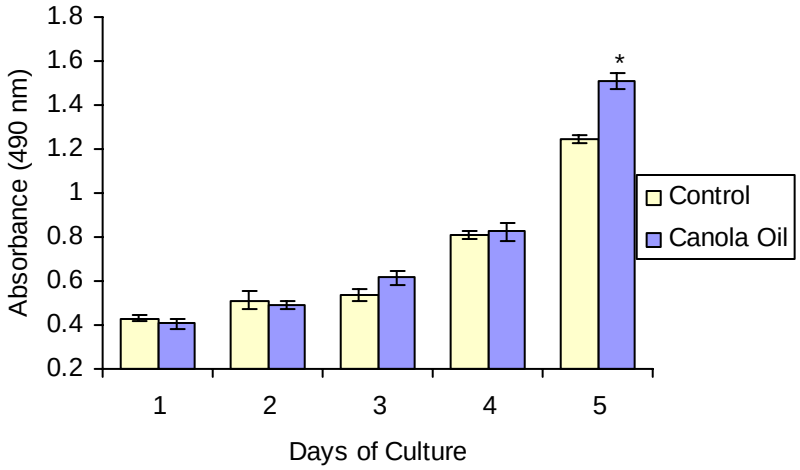
p53 Enzyme Immunometric Assay (EIA): p53 concentration was determined by using an enzyme immunometric assay kit (TiterZyme EIA Kit, Assay Designs, Ann Arbor, MI). Briefly, cultured cells were washed twice with PBS and resuspended in cell lysis buffer (Assay Designs). The cell lysates were added to a plate preimmobilized to p53 by polyclonal antibody. After the plate was incubated overnight at 4°C, it was washed as described in the protocol provided by Assay Designs. A rabbit polyclonal antibody to p53 conjugated to Horseradish peroxidase was reconstituted in the buffer provided with

the EIA kit and added to the plate. Substrate was then added to react with the labeled antibody. Stop solution was added after incubation in the dark at room temperature for 30 min. The absorbance will be measured at 450 nm using a microtiter plate reader (Molecular Devices).

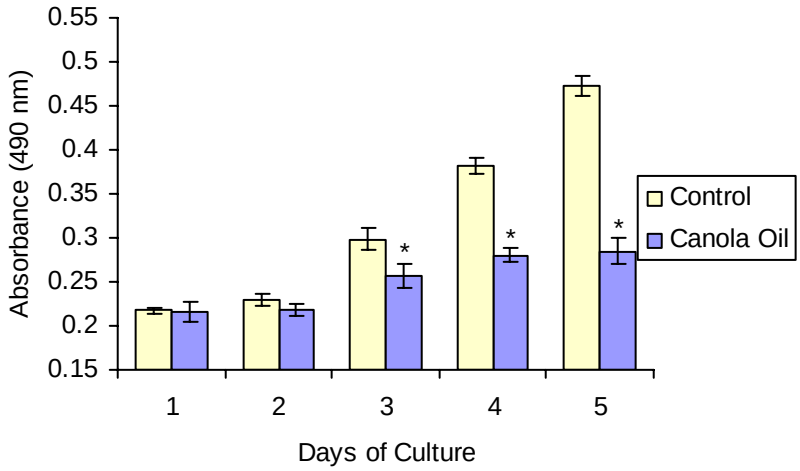
C. Results

This study determined the effect of canola oil on growth and apoptosis of established human breast cancer cell lines, T47D and MCF-7. Canola oil significantly inhibited the growth of both T47D (39.6% on day 5, $P < 0.05$) and MCF-7 (14.9% on day 5, $P < 0.05$) cancer cells, although the pattern of these two cell lines was different (Figure 1). The proliferation of T47D and MCF-7 breast cancer cell lines was significantly reduced by canola oil, while the proliferation data showed no difference on a normal breast cell line, MCF-10A. The caspase-3 data as well as cell proliferation suggest cancer suppressive effects of canola oil in relation to apoptosis. Canola oil significantly increased the level of both T47D (77.5% on day 5, $P < 0.05$) and MCF-7 (20.3% on day 5, $P < 0.05$) cancer cells (Figure 2), but not MCF-10A, a normal cell line.

A. MCF-10A



B. T47D



C. MCF-7

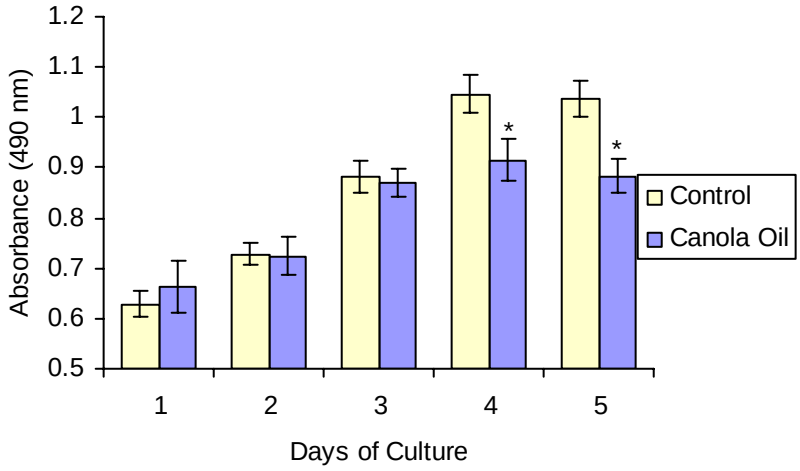
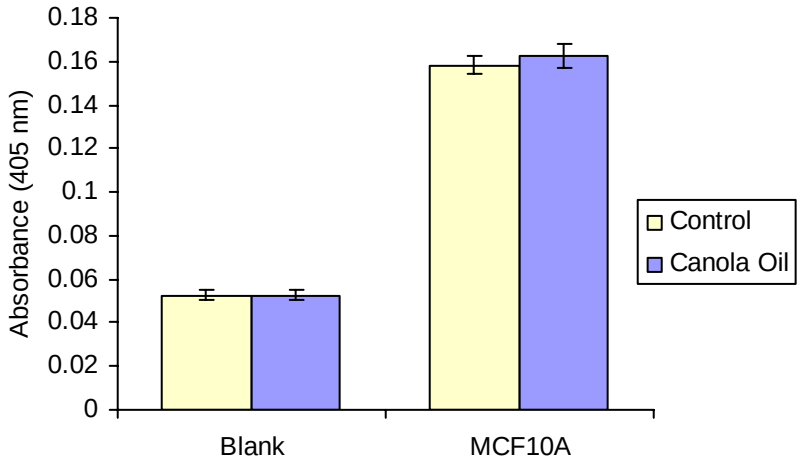
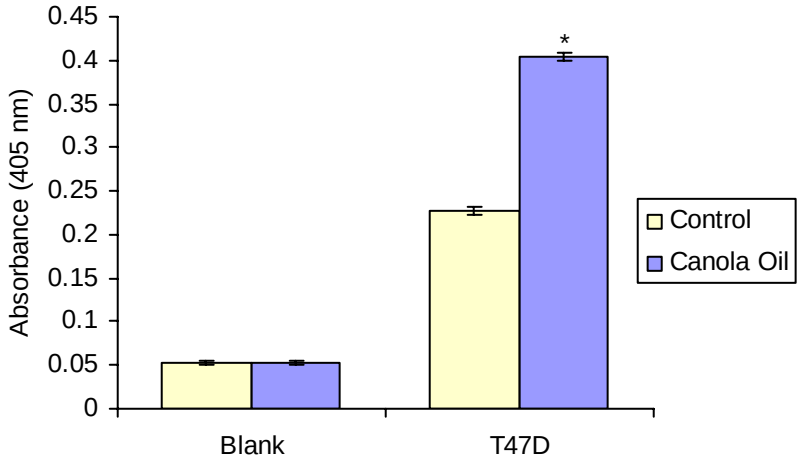


Figure 1. The time-dependent cell proliferation (MTS assay) of three cell lines (two breast cancer cell lines, MCF-7 and T47D, and a normal mammary cell line, MCF-10A) that were treated with 10% v/v of canola oil. Asterisks indicate the difference between control and treatment ($P < 0.05$). Values are expressed by absorbance at 490nm (means ± SEM, $n = 8$).

A. MCF-10A



B. T47D



C. MCF-7

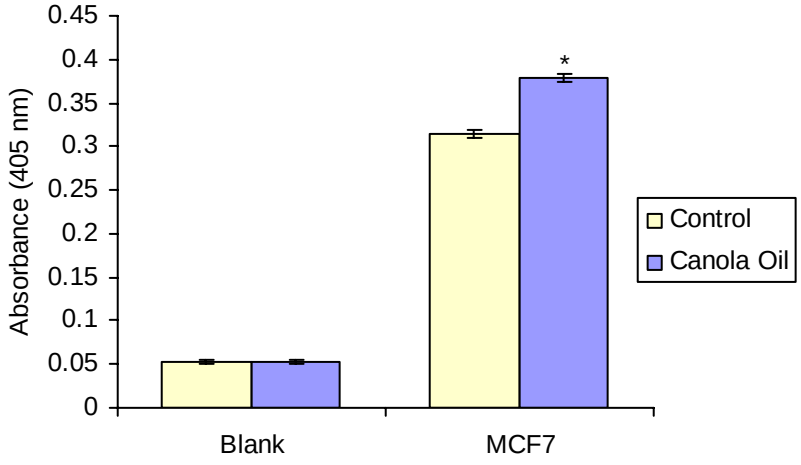


Figure 2. The caspase-3 activity (CaspACE assay) of three cell lines (two breast cancer cell lines, T47D and MCF-7, and a normal mammary cell line, MCF-10A) that were treated with 10% v/v of canola oil. Asterisks indicate the difference between control and treatment ($P < 0.05$). Values are expressed by absorbance at 405nm (means ± SEM, $n = 5$).

D. Summary

Our study represents the first demonstration in a human breast cancer in vitro model that canola oil, rich in oleic acid and omega-3 fatty acids, might be beneficial in reducing breast cancer risk in association to enhanced apoptosis.

E. Significance

The prevention of cancer worldwide is one of the most pressing challenges facing scientists and public health policy-makers, among others. We hope that results from our studies will be useful in dietary counseling in breast cancer prevention and therapy. We also hope that this and similar studies will improve the market (increased demand) for canola oil, especially in North Dakota where 90% of the U.S. canola production is based.

F. Current Research

Our current research is focusing on the hypothesis that due to its fatty acid composition (i.e., omega-3 fatty acid), canola oil may reduce human breast cancer cell growth by modulating the expression of p53, a key gene involved in cancer cell death, as well as the activity of caspase-3 enzyme, one of the crucial genes regulating cancer cell death pathway.

This research (supported from the North Central Region Canola Research Program; FY 2008/09) is scheduled to be complete around June, 2009.

Abstract Title. Molecular Biology of the Cell 19 (suppl 855-6), 2008

Dietary Canola Oil Reduces Susceptibility to Chemically-induced Mammary Carcinogenesis.

C. Park, L. Mabasa, K. Cho, A. Fowler; Animal Sciences, North Dakota State University, Fargo, ND

Increasing evidence indicates that it is not the quantity of lipid but the type of lipid intake that influences breast cancer risk. Canola oil is currently the lowest saturated fat-containing vegetable oil and has a very high proportion of mono- and polyunsaturated fatty acids. We postulated that canola oil, due to its omega-6 to omega-3 fatty acids ratio, may have an anticancer effect against human breast cancer development by modulating the immune response leading to breast cancer reduction. The objective of this study was to determine if dietary canola oil reduces susceptibility to chemically-induced mammary tumorigenesis. Forty-two four-week-old female Sprague-Dawley rats were randomly assigned to either the control diet or diet supplemented with 10.7% canola oil. At 50 days of age, all rats were injected with 50 mg of nitrosomethylurea (NMU) per kg of body weight to induce mammary cancer. Tumor development was observed and tumor size and volume measured. Twelve weeks after NMU administration, spleens from non-tumor bearing rats were collected and then cultured for the determination of immune cell proliferation. The canola oil supplemented diet reduced the tumor incidence by 22% compared to the control diet. The latency period and the average number of mammary tumors per rat were not affected by canola oil supplementation when compared to the control diet. However, mammary tumor volumes were significantly reduced in the canola oil fed group when compared with the control fed group. Canola oil supplementation increased the spleen cell proliferation in the presence of concanavalin A and lipopolysaccharide, suggesting improved activation of splenic T and B cell populations. These results indicate that canola oil rich in oleic acid and omega-3 fatty acids can suppress cancer cell growth, which may be related to an enhanced immune response. Further studies are needed for a better understanding of the mechanisms by which canola oil affects specific immune cell proliferation and tumor growth.