Deoxycholic Acid and Selenium Metabolite Methylselenol Exert Common and Distinct Effects on Cell Cycle, Apoptosis, and MAP Kinase Pathway in HCT116 Human Colon Cancer Cells

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The cell growth inhibition induced by bile acid deoxycholic acid (DCA) may cause compensatory hyperproliferation of colonic epithelial cells and consequently increase colon cancer risk. On the other hand, there is increasing evidence for the efficacy of certain forms of selenium (Se) as anticancer nutrients. Methylselenol has been hypothesized to be a critical Se metabolite for anticancer activity in vivo. In this study, we demonstrated that both DCA (75–300 µmol/l) and submicromolar methylselenol inhibited colon cancer cell proliferation by up to 64% and 63%, respectively. In addition, DCA and methylselenol each increased colon cancer cell apoptosis rate by up to twofold. Cell cycle analyses revealed that DCA induced an increase in only the G1 fraction with a concomitant drop in G2 and S-phase; in contrast, methylselenol led to an increase in the G1 and G2 fractions with a concomitant drop only in the S-phase. Although both DCA and methylselenol significantly promoted apoptosis and inhibited cell growth, examination of mitogen-activated protein kinase (MAPK) pathway activation showed that DCA, but not methylselenol, induced SAPK/JNK1/2, p38 MAPK, ERK1/2 activation. Thus, our data provide, for the first time, the molecular basis for opposite effects of methylselenol and DCA on colon tumorigenesis.

INTRODUCTION

A typical part of the Western diet is a high fat intake that leads to increased levels of fecal bile acids; and these bile acids, primarily deoxycholic acid (DCA) in humans, are believed to be tumor promoters of colon cancer (1–3). Apoptosis has a major role in the elimination of DNA damaged cells. Previous studies showed that damage in colon crypts caused by certain mutagenic/carcinogenic agents increased the apoptosis frequency, but surviving mutagen-damaged cells would lead to the emergence of crypts that were composed wholly of cells with a different, mutated phenotype (4,5). Similarly, several lines of evidence have suggested that cell growth inhibition induced by DCA may cause compensatory hyperproliferation of a subpopulation of colonic epithelial cells resistant to DCA's inhibitory effect (6,7). These surviving cells are somehow mutated and are resistant to the bile acid-induced cell cycle arrest and apoptosis and consequently increase cancer risk. Thus, DCA may exert its tumor-promoting activity by modulating cellular signaling and gene expression, which ultimately alters colon cell proliferation. However, the mechanism by which DCA and other bile acids promote colon tumorigenesis remains to be characterized (6).

In contrast, selenium (Se) is an essential trace element currently being considered as a chemopreventive agent for the treatment of cancer (8–10). Epidemiological evidence indicates that Se status is inversely associated with cancer risk, and results from intervention studies have shown that high Se intakes effectively reduce the risk of mammary, prostate, lung, colon, and liver cancer (8–11). In experimental animals, anticarcinogenic effects have been consistently associated with Se at supranutritional intakes (>1 mg/kg diet) that are at least 10 times those required to prevent clinical signs of deficiency and to support near-maximal tissue activities of selenoenzymes (9,12–14). In this context of cancer chemoprevention, methylselenol has also been hypothesized to be a critical Se metabolite for anticancer activity in vivo (9,13). Thus, there is much interest in promoting Se-enriched food intake to reduce cancer risk.

In view of our previous data that submicromolar concentrations of methylselenol inhibited tumor cell proliferation (15) and the fact that DCA, a putative tumor promoter, also inhibited cancer cell proliferation (16), it becomes important to dissect the common and different signaling pathways by which DCA and methylselenol each modulate colon cancer cell proliferation in the same colon cell line in a given experiment. Furthermore, in vivo data suggest that over a period of years of consumption of a high-fat diet, DCA-resistant mutant colon cells may repopulate the colonic mucosa and develop colon cancer (6,7). In agreement with this, there was an increased frequency of
apoptosis at colon crypts during the first 15 wk when mice were fed with a Western-type diet (17). Interestingly, these mice fed with a high-fat diet no longer had greatly increased levels of apoptosis but had much increased frequencies of atypical nuclei at later ages, which would result in cancer development (17). We hypothesize that methylselenol inhibits colon cancer cell proliferation through a mechanism that is different from the one used by DCA, which is the molecular basis for opposite effects of methylselenol and DCA on colon tumorigenesis.

The mitogen-activated protein kinase (MAPK) pathway constitutes an important group of signaling mediators that govern cellular processes such as proliferation, differentiation, and cell death. The MAPK signaling pathways mainly consist of 3 sub-families: the stress-activated protein kinase/c-Jun NH4-terminal kinase 1/2 (SAPK/JNK1/2), the p38 mitogen-activated protein kinase (p38 MAPK), and the extracellular-regulated kinase 1/2 (ERK1/2) (18). Although the role of MAPK activated during colon tumorigenesis is not clear, human colon tumors have shown a moderate increase in MAPK levels relative to normal colon mucosa, and colon tumor tissue showed especially high ratios of MAPK activation (19,20). In addition, blockade of the MAPK pathway suppresses growth of colon tumors as much as 80% in mice with colon carcinomas of both mouse and human origin (21). In the present study, we have focused on the comparative effects of DCA and methylselenol on cell growth, cell cycle, apoptosis, and MAPK activities in colon cancer cells.

**MATERIALS AND METHODS**

**Cell Cultures**

HCT116 colorectal carcinoma cells were obtained from American Type Culture Collection and maintained in DMEM (Invitrogen, Carlsbad, CA) with 10% fetal bovine serum (FBS; Sigma Chemical Co., St. Louis, MO). Stock cells were passaged twice weekly at 80% confluency [0.25% trypsin (Invitrogen), 1 mM EDTA, in CaMg-free Hanks balanced salt (Sigma), and 10% FBS]. Cultures were tested and found to be mycoplasma free (22). Experiments were performed between passages 23 and 50. Stock cells were grown in standard media supplemented with 10% FBS.

**Methylselenol Generation**

Purified methioninase (METase) of recombinant METase based on the gene from *Trichomonas vaginalis* produced in *Escherichia coli* was purchased from Wako Pure Chemical Industries, Ltd. (Richmond, VA). The enzyme (METase) preparations were reconstituted in phosphate-buffered saline (PBS) and aliquoted and stored at −80°C. For cell treatments, the enzyme substrate seleno-L-methionine (SeMet; Sigma Chemical Co., St. Louis, MO) for the enzyme was added first into the culture medium. Immediately after the addition of the substrate, METase was added directly into the cell-culture medium.

**Cell Cycle Analysis**

Cell cycle was analyzed using flow cytometry with propidium iodide (PI) staining. HCT 116 cells were trypsinized and washed once with PBS and fixed in 70% (vol/vol) ethanol at −20°C. After fixation, cells were washed with PBS and stained with 50 μg PI/ml containing 6,000 U RNase A/l. The DNA contents of cells were determined by flow cytometry. Data were stored as list mode files of at least 10,000 single cell events and analyzed by EPICS profile II and ModFit LT software (Coulter Corp., Miami, FL and Topsham, ME).

**Apoptosis Analysis**

Apoptosis was analyzed using a Guava Nexin Kit (Guava Technologies, Inc., Hayward, CA). HCT 116 cells were trypsinized and suspended in growth media (DMEM with 10% FBS). In the apoptotic cells, molecules of phosphatidylserine (PS) are translocated to the outer surface of the cell membrane where Annexin V can readily bind them. Annexin V is a calcium-dependent phospholipid binding protein with high affinity for PS, a membrane component normally localized to the internal face of the cell membrane. At least 2,000 single cell events per sample were analyzed by the Guava PCA System (Hayward, CA).

**Western Blotting Analysis**

After DCA and methylselenol treatment for 3.5 or 16 h, adherent cells were scraped, pooled with the detached cells in 5 ml media, and then these cells were collected by centrifugation at 350 g for 10 min at 4°C; at least 3 independent experimental cell sample sets were collected. As previously described (23), the cell pellet was washed once in ice-cold PBS and lysed in an assay buffer [50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 2 mM EDTA, 50 mM NaF, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 1 mM DTT, 5 mM sodium orthovanadate, 1 mM phenylmethylsulfonyl fluoride, and 38 mg/ml aprotonin (added fresh)]. After a brief sonication, the cell lysate was centrifuged at 14,000 g for 30 min at 4°C. The supernatant was designated as whole cell protein extract and kept at −80°C. The protein concentration was quantified by the Bradford dye-binding assay (Bio-Rad laboratories, Richmond, CA). Equal amounts of protein extract (~ 40 μg) were resolved over 4–20% Tris-glycine gradient gels under denaturing and reducing conditions and electroblotted onto PVDF membranes (Invitrogen, Carlsbad, CA). Membrane blots were blocked in PBS—0.05% Tween (vol/vol) supplemented with 1% (wt/vol) nonfat dry milk (BioRad, Hercules, CA) at 4°C for overnight. Membranes were probed with antibodies against SAPK/JNK1/2, p38 MAPK, and ERK1/2 or the corresponding phospho-specific antibodies (Cell Signaling Technology, Danvers, MA) and then incubated with an antimouse (1:3000 dilution) HRP-conjugated secondary...
antibody (Santa Cruz Biotechnology, Santa Cruz, CA) in blocking solution for 1 h at RT. Blots were washed as above and proteins were detected by using an ECL plus kit (Amersham Pharmacia Biotech, Piscataway, NJ) with the Molecular Dynamics Image-Quant system (Sunnyvale, CA).

Statistical Analysis
Results are given as means ± SD. Data were analyzed using mixed model analyses of variance (ANOVAs), blocking on group and allowing heterogeneous variances. If the overall ANOVA was statistically significant, then Dunnett’s test for multiple comparisons to a control was used to analyze treatment means. Differences with a P value ≤ 0.05 were considered statistically significant.

RESULTS
Distinct Effects of DCA and Methylselenol Each on Morphological Responses and Cell Growth
When treated with 0, 75, 150, or 300 µmol/l DCA, cells were increasingly retracted, their membranes leaked, and they detached from the bottom of the culture dish as individual cells (Fig. 1A, panel a, b, c, d) in a dose-dependent manner. These cellular morphological changes were the most obvious in those cells treated with 300 µmol/l DCA (Fig. 1A, panel d). In a parallel experiment, 5 µmol/l SeMet alone (Fig. 1B, panel b) or METase (40 U/l) plus its regular substrate l-methionine (Met) at 5 µmol/l (Fig. 1B, panel c) had no apparent effect on cellular morphology when compared with untreated cells (Fig. 1B, panel a). However, when METase (40 U/l) was added into the medium with SeMet (1.25, 2.5, or 5 µmol/l), cells were increasingly elongated, retracted, and detached from the bottom of the culture flask, and many detached cells were not individual cells but appeared as a “multi-cell sheet” or “anoikis” (24) (Fig. 1B, panel d, e, f). These morphological responses were the most prominent in those cell treated with methylselenol generated by incubating METase (40 U/l) with 5 µmol/l SeMet (Fig. 1B, panel f). The cell growth rate was inhibited by 5%, 19% (P < 0.01), and 64% (P < 0.0001), respectively, in the cells treated with 75, 150, or 300 µmol/l DCA when compared with those cells treated with 0 µmol/l DCA (Fig. 1C). Similarly, the cell growth rate was inhibited by 8% and 20% (P < 0.0001) and 63% (P < 0.0001), respectively, in the cells treated with methylselenol generated by incubating METase (40 U/l) with 1.5, 2.5, or 5 µmol/l SeMet when compared with control cells (no combination of METase and SeMet; Fig. 1D).

Effect of DCA and Methylselenol Each on Apoptosis
Apoptosis rate was increased by 5.5%, 42.7%, and 223.5% (P < 0.0001), respectively, in the cells treated with 75, 150, and 300 µmol/l DCA (Fig. 2A) when compared with that of 0 µmol/l DCA treated cells. Similarly, apoptosis rate was increased by 22.7%, 42.9% (P < 0.02), and 204.1% (P < 0.0001), respectively, in the cells treated with methylselenol generated by

FIG. 1A and 1B. Effect of DCA vs. methylselenol on cell morphology. Panel A: Representative phase-contrast photomicrographs of HCT116 cells after 16-h exposure to increasing doses of DCA (a, 0 µmol/l; b, 75 µmol/l; c, 150 µmol/l; d, 300 µmol/l). Panel B: Representative phase-contrast photomicrographs of HCT116 cells after 16-h exposure to increasing doses of methylselenol generated by incubating 40 U/l METase with µmol/l SeMet. a: Untreated cells as control. b: 5 µmol/l SeMet alone as control. c: 40 U/l METase with 5 µmol/l Met as control. d, e, and f: 40 U/l METase with increasing doses of SeMet (d, 1.25 µmol/l; e, 2.5 µmol/l; f, 5 µmol/l).
incubating METase (40 U/l) with 1.5, 2.5, or 5 \( \mu \text{mol/l} \) SeMet when compared with that of control cells (no combination of METase and SeMet; Fig. 2B).

### Differential Effects of DCA and Methylselenol Each on Cell Cycle Progression

G1 phase cell distribution was increased by 24.6% and 76.5% \((P < 0.0008)\), respectively; G2 phase cell distribution was decreased by 19.9% and 34.3%, respectively; and S phase cell distribution decreased by 9.5% and 41.1% \((P < 0.012)\) respectively, in the cells treated with 75 or 150 \( \mu \text{mol/l} \) DCA when compared with that of 0 \( \mu \text{mol/l} \) DCA-treated cells (Table 1).

Similarly, G1 phase cell distribution was increased by 33.6% \((P < 0.0004)\) and 56.1% \((P < 0.0001)\), respectively; however, G2 phase cell distribution was also increased by 48.3% \((P < 0.0002)\) and 61.2% \((P < 0.0003)\), respectively; and S phase cell distribution decreased by 32.4% \((P < 0.0001)\) and 48.7% \((P < 0.0001)\) respectively, in the cells treated with methylselenol incubating METase (40 U/l) with 1.5 or 2.5 \( \mu \text{mol/l} \) SeMet when compared with that of control cells (no combination of METase and SeMet; Table 2).

### Differential Effect of DCA vs. Methylselenol on MAPK Signaling

To determine the role of MAPK cascades in colon cell cycle arrest and apoptosis, we examined phosphorylation status of MAPK proteins after cells had been treated with DCA or methylselenol for 3.5 or 16 h, although total SAPK/JNK1/2, p38 MAPK, and ERK1/2 protein expression did not differ (Fig. 3). First, when compared with those cells treated with 0 \( \mu \text{mol/l} \) DCA, the active phosphorylated SAPK/JNK1/2 was increased by 46.5-fold \((P < 0.0001, n = 4)\) and 16.4-fold...
TABLE 1

Effect of deoxycholic acid (DCA) for 16 h on HCT-116 cell growth and cycle phase distributions

<table>
<thead>
<tr>
<th>DCA (µmol/l)</th>
<th>G1 phase</th>
<th>G2 phase</th>
<th>S phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>34.5 ± 1.61</td>
<td>16.6 ± 1.62</td>
<td>48.7 ± 0.68</td>
</tr>
<tr>
<td>75</td>
<td>42.9 ± 2.96</td>
<td>13.3 ± 1.55</td>
<td>44.1 ± 2.45</td>
</tr>
<tr>
<td>150</td>
<td>60.9 ± 7.89**</td>
<td>10.8 ± 0.78</td>
<td>28.6 ± 8.64*</td>
</tr>
</tbody>
</table>

*Values are means ± SD, n = 4. HCT116 cells treated with 300 µmol/l DCA were not suitable for cell cycle analysis because there was a significant amount of apoptosis. Asterisks indicate different from control (0 µmol/l DCA); *, P < 0.05; **, P < 0.001.

(P < 0.0001, n = 3) in the cells treated with 300 µmol/l DCA for 3.5 h and 16 h, respectively (Fig. 3, Table 3). In contrast, there were no inducible signals of the active phosphorylated SAPK/JNK1/2 at 3.5 h or 16 h time points in the cells treated with methylselenol generated by incubating METase (40 U/l) with 1.5, 2.5, or 5 µmol/l SeMet (Fig. 3, Table 4). Second, the active phosphorylated p38 MAPK was increased by 2.3-fold (P < 0.05, n = 3), 3.6-fold (P < 0.01, n = 3), and 6.5-fold (P < 0.0001, n = 3) in the cells treated with 75 and 300 µmol/l DCA, respectively, for 3.5 h when compared with those cells treated with 0 µmol/l DCA (Fig. 3, Table 3). Similarly, the active phosphorylated p38 MAPK was decreased by 72.0% (P < 0.01, n = 3) at 16 h time point but not 3.5 h time point in the cells treated with methylselenol generated by incubating METase (40 U/l) with 5 µmol/l SeMet when

FIG. 3. Western blot analyses of the effects of DCA vs. methylselenol on SAPK/JNK1/2, p38 MAPK, ERK1/2 phosphorylation status in HCT116 colon cells for 3.5-h or 16-h treatments. A representative Western blotting of 3 independent experiments.
TABLE 3
Effect of deoxycholic acid (DCA) for 3.5 and 16 h, on MAPK phosphorylation status

<table>
<thead>
<tr>
<th>DCA (µmol/L)</th>
<th>0</th>
<th>75</th>
<th>150</th>
<th>300</th>
</tr>
</thead>
<tbody>
<tr>
<td>p-SAPK/JNK1/2 (3.5 h)</td>
<td>1433.6 ± 239.5</td>
<td>1827.1 ± 1744.95</td>
<td>30623.8 ± 24774.0</td>
<td>68044.4 ± 7298.3 **</td>
</tr>
<tr>
<td>p-SAPK/JNK1/2 (16 h)</td>
<td>2321.5 ± 1029.2</td>
<td>4507.0 ± 4166.6</td>
<td>6925.7 ± 2308.4</td>
<td>40366.9 ± 3963.4 **</td>
</tr>
<tr>
<td>p-p 38 MAPK (3.5 h)</td>
<td>41674.5 ± 9409.1</td>
<td>70244.1 ± 11338.5</td>
<td>88135.1 ± 31183.3</td>
<td>183685.1 ± 10249.5 **</td>
</tr>
<tr>
<td>p-SAPK/JNK1/2 (16 h)</td>
<td>80388.5 ± 78912.1</td>
<td>265409.7 ± 64490.8</td>
<td>366964.6 ± 85980.2</td>
<td>602305.0 ± 37076.0 **</td>
</tr>
<tr>
<td>p-p 38 MAPK (16 h)</td>
<td>33537.1 ± 9031.9</td>
<td>36156.7 ± 9520.9</td>
<td>39827.1 ± 3763.9</td>
<td>77741.4 ± 13440.1 **</td>
</tr>
<tr>
<td>p-p 38 MAPK (16 h)</td>
<td>24,149.0 ± 7,630.3</td>
<td>29,270.9 ± 10,469.6</td>
<td>15,020.5 ± 26,834.4</td>
<td>40,538.4 ± 12,163.9</td>
</tr>
</tbody>
</table>

1Values are means ± SD, n = 3.
2Asterisks indicate different from control (0 µmol/L DCA), * P < 0.05; ** P < 0.0005.
3We did not include the intensity units of total SAPK/JNK1/2, p38 MAPK, and ERK1/2 protein expression because they did not differ (See Fig. 3).

Compared with that of control cells (no combination of METase and SeMet; Fig. 3, Table 4). Third, the active phosphorylated ERK1/2 was increased by 1.3-fold (P < 0.0005, n = 3) in the cells treated with 300 µmol/l DCA for 3.5 h time point but not 16 h time point when compared with those cells treated with 0 µmol/l DCA (Fig. 3, Table 3). In contrast, active phosphorylated ERK1/2 was decreased by 87.3% (P < 0.0001, n = 3) at 16 h time point but not 3.5 h time point in the cells treated with methylselenol generated by incubating METase (40 U/l) with 5 µmol/l SeMet when compared with that of control cells (no combination of METase and SeMet; Fig. 3, Table 4).

DISCUSSION
Colon cancer is among the most common human cancers in the United States (25). Dietary factors, including exposure to bile acids have been implicated as important etiological factors in colon cancer (26). It is hypothesized that colon epithelium are likely to be unhealthy in individuals who are at increased risk for colon cancer because of long-term exposure to damaging agents such as bile acids (27). Previous studies have shown that bile acid concentration could reach 1,000 µmol/l in the colon after the consumption of a high-fat meal, and high concentrations of bile acids in solution in colon contents because of high fat diets

TABLE 4
Effect of methylselenol, generated by incubating 40 U/L METase with SeMet for 3.5 and 16 h, on MAPK phosphorylation status

<table>
<thead>
<tr>
<th>SeMet (µmol/l)</th>
<th>5</th>
<th>0</th>
<th>1.25</th>
<th>2.5</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Met (µmol/l)</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>METase (40 U/l)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>p-SAPK/JNK1/2 (3.5 h)</td>
<td>1,404.4 ± 297.7</td>
<td>1,520.6 ± 429.6</td>
<td>1,511.5 ± 440.7</td>
<td>1,458.7 ± 410.7</td>
<td>1,489.1 ± 386.8</td>
</tr>
<tr>
<td>p-SAPK/JNK1/2 (16 h)</td>
<td>1,741.7 ± 537.4</td>
<td>1,762.2 ± 471.8</td>
<td>1,782.0 ± 388.7</td>
<td>1,814.4 ± 337.3</td>
<td>1,856.5 ± 371.6</td>
</tr>
<tr>
<td>p-p 38 MAPK (3.5 h)</td>
<td>50,045.6 ± 23,724.5</td>
<td>63,621.6 ± 16,829.6</td>
<td>58,048.8 ± 13,636.7</td>
<td>64,317.3 ± 2,194.6</td>
<td>63,515.4 ± 6,154.8</td>
</tr>
<tr>
<td>p-p 38 MAPK (16 h)</td>
<td>24,149.0 ± 7,630.3</td>
<td>29,270.9 ± 10,469.6</td>
<td>15,020.5 ± 2,922.8</td>
<td>13,032.8 ± 5,051.7</td>
<td>6,760.0 ± 860.2**</td>
</tr>
<tr>
<td>p-ERK1/2 (3.5 h)</td>
<td>55,215.4 ± 6,135.4</td>
<td>54,213.1 ± 22,147.6</td>
<td>47,713.7 ± 20,480.5</td>
<td>66,662.9 ± 5,602.3</td>
<td>81,179.0 ± 18,290.8</td>
</tr>
<tr>
<td>p-ERK1/2 (16 h)</td>
<td>49,646.9 ± 7,808.4</td>
<td>68,280.7 ± 26,901.7</td>
<td>54,056.1 ± 26,834.4</td>
<td>40,538.4 ± 12,163.9</td>
<td>6,325.4 ± 2,313.3**</td>
</tr>
</tbody>
</table>

*Abbreviations are as follows: METase, methioninase; SeMet, seleno-L-methionine; MAPK, mitogen-activated protein kinase; Met, L-methionine; SAPK/JNK1/2, stress-activated protein kinase/c-Jun NH4-terminal kinase 1/2; ERK1/2, extracellular-regulated kinase 1/2. Values are means ± SD, n = 3. We did not include the intensity units of total SAPK/JNK1/2, p38 MAPK, and ERK1/2 protein expression because they did not differ (see Fig. 3). Asterisks indicate different from controls (no combination of METase and SeMet); *, P < 0.05; **, P < 0.0005.
may induce apoptosis in colon epithelial cells (26,27). However, when colon cells are induced to cell cycle arrest or apoptosis due to increased bile acids, surviving cells from adjacent areas form new epithelium (28). It is believed that these surviving cells are somehow mutated and have a survival phenotype that is resistant to the bile acid-induced cell cycle arrest and apoptosis (26–28). A reduction in this inhibitory ability will promote the retention of cells with DNA damage and consequently increase the risk of mutations.

In contrast, several lines of evidence implicate methylselenol as the active in vivo Se metabolite pool for anticancer effects associated with supplements and high Se intakes and that cell cycle arrest/apoptosis was reported to be the major mechanism by which methylselenol exerts its anticancer effects (9,13,15,23,29–33). It has been documented that Se significantly reduced both colon tumor incidence and multiplicity in either a high-fat diet or a low-fat diet in experimental animal studies (34). Thus, the study of comparative effects of methylselenol and DCA at physiological doses (26) on cell proliferation and signaling pathways will further our understanding of functional roles of methylselenol in the inhibition of colon cancer related to high-fat diets.

It has been documented that 300 μmol/l DCA and submicromolar concentrations of methylselenol are well within physiological levels (15,26,27,30). Our data showed that the effect of 0, 75, 150, or 300 μmol/l of DCA on HCT116 cell growth inhibition and apoptotic rate was virtually identical to that of methylselenol generated by incubating METase (40 U/l) with 0, 1.25, 2.5, or 5 μmol/l SeMet. However, DCA and methylselenol employed distinct molecular pathways to modulate HCT116 cell proliferation. For example, at lower doses, 75 or 150 μmol/l, DCA increased only G1 fraction with a concomitant drop in both G2 and S-phase cell fractions, indicating slower cell growth. In contrast, methylselenol generated by incubating METase (40 U/l) with 1.25 or 2.5 μmol/l SeMet, led to an increase in the G1 and G2 fractions with a concomitant drop only in the S-phase. The opposite effect of DCA and methylselenol on G2 cell fractions suggests distinct molecular targets.

MAPKs are an important group of signaling molecules that transduce most signals from cell membrane to the nucleus and that govern cell growth, cell cycle, and apoptosis. The SAPK/JNK1/2, p38 MAPK, and ERK1/2 are the 3 most important MAPK cascades that govern cell proliferation and cell death (18,35). In this study, we have shown that SAPK/JNK1/2 was markedly stimulated by 300 μmol/l DCA but not methylselenol at 3.5 h and 16 h time points. Furthermore, our data demonstrated that p38 MAPK was significantly stimulated by DCA at 3.5 h and 16 h time points; ERK1/2 was stimulated by DCA at 3.5 h but not 16 h time point. In contrast, methylselenol did not affect the activation of both ERK1/2 and p38 MAPK at 3.5 h time point. Interestingly, methylselenol inhibited the activation of both ERK1/2 and p38 MAPK at longer exposure (16 h). It is known that ERK1/2 mediates cell proliferation and differentiation and protects cells from apoptotic cell death, whereas p38 MAPK and SAPK/JNK1/2 may promote apoptotic cell death (36). The balance between ERK1/2 cascade and SAPK/JNK1/2 and p38 MAPK cascades has been proposed to be a critical determinant of cell growth or death (37). Our observation that SAPK/JNK1/2 and p38 MAPK cascades but not ERK1/2 activation lasted up to 16 h suggests that increased SAPK/JNK1/2 and p38 MAPK cascades are the net effect at 16 h time point, which is likely to enhance cell apoptosis by DCA. The concurrence of induction of cell growth and cell death signaling by DCA plays an important role in bile acid promotion of colon cancer.

In sharp contrast, methylselenol suppressed ERK1/2 and p38 MAPK cascades at higher doses at 16 h time point. It is hypothesized that cell death induced by bile acids is likely to cause compensatory hyperproliferation of colonic epithelial cells, and these subpopulation of cells are resistant to bile acid induced apoptosis (26–28). Thus, the most intriguing observation is that although experimental doses of DCA and methylselenol in this study had the same potential to induce apoptosis and to inhibit the cell growth, their effects on the molecular targets are distinct. Although it remains to be determined full spectrum of tumor types that rely on MAPK activation for tumor progression, colon tumors are among the population of neoplasms that showed especially high MAPK activation (21). In this study, we demonstrated, for the first time, that DCA activated MAPK but methylselenol inhibited MAPK activation in the same colon cancer cells. This observation is consistent with methylselenol’s anticancer effect because human colon tumors showed a moderate increase in MAPK levels (21). Recently, the Selenium and Vitamin E Cancer Prevention Trial (SELECT) data (38) suggest that Se does not help prevent prostate cancer. This is somehow unexpected because most previous studies had found that Se reduced cancer risk (9–15,29–33). It has been suggested that L-SeMet given in the SELECT trial may have been less active than Se chemical forms (high-Se yeast) given in the Nutritional Prevention of Cancer (NPC) trial (38), which may be at least a part in contributing the failure of L-SeMet to prevent prostate cancer in SELECT trial. The fact that 200 μg Se/day intake of L-SeMet but not high-Se yeast caused a serious health safety issue in SELECT trial further suggests the significant difference of Se chemical forms between L-SeMet and high-Se yeast. This underscores the urgency of understanding Se chemical forms, doses, and their molecular targets. Our present data suggest that the MAPK level of target tissue may be used as a biomarker for studying bioactivity of different Se’s formulations.

In summary, we have shown that DCA and methylselenol each inhibit cell proliferation and induce cell cycle arrest and apoptosis. DCA induced an increase in only the G1 fraction with a concomitant drop in G2 and S-phase; in contrast, methylselenol led to an increase in the G1 and G2 fractions with a concomitant drop only in the S-phase. Furthermore, our data showed DCA but not methylselenol activated SAPK/JNK1/2, p38 MAPK, and ERK1/2. Thus, DCA and methylselenol have common and distinct effects on cell cycle, apoptosis, and signaling pathway in colon cancer cells.
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