Additive Interaction of Oxaliplatin and 17-Allylamino-17-demethoxygeldanamycin in Colon Cancer Cell Lines Results from Inhibition of Nuclear Factor κB Signaling

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Abstract

Elucidation of the mechanism by which oxaliplatin induces cell death is essential to enhancing its action. We investigated the effects of oxaliplatin and 17-allylamino-17-demethoxygeldanamycin (17-AAG) in a panel of four colon adenocarcinoma cell lines. Cytotoxicity assays demonstrated at least additivity in three of the cell lines. Activation of the c-Jun NH₂-terminal kinase pathway by oxaliplatin does not determine cytotoxicity. Activation of p38 was shown to be a key proapoptotic mediator of oxaliplatin-induced cell death. Modulation of extracellular signal-regulated kinase and AKT signaling had no impact on oxaliplatin toxicity in these cells. Nuclear factor (NF)-κB was constitutively active in all of the cell lines and was inhibited by 17-AAG. Down-regulation of NF-κB transcription by pharmacological inhibitors enhanced oxaliplatin cytotoxicity. These data support an interaction between 17-AAG and components of the NF-κB pathway in the modulation of oxaliplatin sensitivity in colon cancer cells.

Introduction

Oxaliplatin is the first of several analogs from the diaminocyclohexane platinum family to be successfully developed in the clinic. In a proof of principle that the diaminocyclohexane ligand would confer altered tumor specificity, oxaliplatin has been found to be active in the treatment of colorectal cancer, a disease in which cisplatin has no activity. The anticancer effects of oxaliplatin appear to be improved when it is combined with other anticancer agents, such as thymidylate synthase inhibitors and other antimetabolites, topoisomerase 1 inhibitors, and taxanes (reviewed in Refs. 1 and 2). The basis for the interaction of oxaliplatin with these other cytotoxics is unknown, and the pathways through which cells may be sensitized to oxaliplatin remain to be described.

In previous work (3), we have shown cell line-dependent interactions between cisplatin and the benzoquinone ansamycins geldanamycin and 17-allylamino-17-demethoxygeldanamycin (17-AAG) in colon cancer cells. The ansamycins act by blocking the binding of ATP to the chaperone protein Hsp90 (M, 90,000 heat shock protein), an action that results in destabilization of the Hsp90-client protein complexes and subsequent proteasomal degradation of the client proteins ErbB2, Raf, Akt, and others (reviewed in Refs. 4 and 5). The inhibitory effects of geldanamycin on transcriptional activation through activator protein 1 (AP-1) and nuclear factor (NF)-κB have also been shown (6, 7). Previously, we demonstrated the role of p53/Fas/caspase 8/caspase 3 pathways in cisplatin-induced apoptosis in the HCT116 cell line (3). In an extension of this work, we found that the differential effects of the combination of 17-AAG and cisplatin among cell lines depend on quantitative inhibition of signaling through c-Jun NH₂-terminal kinase (JNK) by 17-AAG.

In this study, we demonstrated that the combination of oxaliplatin and 17-AAG is at least additive in colon cancer cell lines, and that the effect of oxaliplatin is independent of the JNK pathway activation, whereas signaling through p38 appeared to be an important mediator of cytotoxicity. Finally, we report that in the human colon cancer cell lines, 17-AAG inhibits the activity of transcription factor NF-κB through the abrogation of upstream components of the NF-κB pathway, and that this phenomenon shifts the balance from cell survival to cell death in response to oxaliplatin treatment.

Materials and Methods

Cell Lines and Reagents. The colon cancer cell lines HT29, HCT116, DLD1, and SW480 were purchased from American Type Culture Collection (Manassas, VA). Cell cultures were maintained as described previously (3). 17-AAG was kindly provided by Dr. Edward Sausville (Developmental Therapeutics Program, National Cancer Institute, Bethesda, MD). Oxaliplatin was purchased from LTK Laboratories (St. Paul, MN), dissolved in sterile PBS, aliquoted, and stored at −20°C. Chemical inhibitors for JNK (SP600125), p38 (SB203580), and phosphatidylinositol 3′-kinase (PI3K) (LY294002) from Biomol (Plymouth Meeting, PA), the mitogen-activated extracellular protein kinase inhibitor U0126 (Promega Corp., Madison, WI), and inhibitors of NF-κB, caffeic acid phenethyl ester and helenalin (BioSource International, Inc., Camarillo, CA), were dissolved as recommended by the manufacturers, aliquoted, and stored at −20°C.

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Bromide (MTT) Assay and Isobologram Analysis. MTT assays and isobologram analysis were carried out as described in Ref. 3. Cells were plated in 96-well plates and treated with various concentrations of oxaliplatin and 17-AAG alone and in combination or with oxaliplatin in the presence of chemical inhibitors. Cytotoxicity was measured using a standard MTT assay after drug exposure for 72 h. Data were derived from at least three independent experiments (in triplicate) for the oxaliplatin/17-AAG combination and from two independent experiments for oxaliplatin with signaling inhibitors. IC₅₀ values for oxaliplatin, 17-AAG, or oxaliplatin in the presence of chemical inhibitors were determined as described by Tsai et al. (8). The cytotoxicity of oxaliplatin and 17-AAG in combination was quantified according to the method of Chou et al. (9).

Colony Forming Assay. For clonogenic assays, cells were plated in 6-well plates at a density of 250 cells/well, and after 24 h, oxaliplatin and 17-AAG or chemical inhibitors, alone or in combination, were added for 72 h. Oxaliplatin and 17-AAG were used at the concentrations corresponding to IC₅₀ values for each drug, derived from the clonogenic assays. After addition of fresh media, cells were cultivated for 7–10 days; colonies (>50) were then fixed in 75% ethanol, stained with Coomasie Blue (Sigma), and counted manually. The combination index for the clonogenic assay was determined as the ratio of the cumulative percentage of cells killed by each drug or signaling inhibitor

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alone to the percentage of cells killed by the combination. All experiments were performed in duplicate at least three times.

Drug and Inhibitor Concentrations. Oxaliplatin, 17-AAG, and signaling inhibitors were used at concentrations established in preliminary experiments: they were sufficient to cause detectable responses in signaling and apoptotic pathways after 24 h of treatment (oxaliplatin and 17-AAG) or sufficient inhibitory activity in the absence of significant cytotoxicity after 72 h of incubation (inhibitors). By these criteria, all of the chemical inhibitors were used at a concentration of 3 μM (except helenalin, which was used at 0.3 μM) and added 1 h before oxaliplatin. For Western blot analysis and caspase assays, cells were plated 24 h before the addition of drugs and cultured for another 24 h. Oxaliplatin was used at concentrations equivalent to 25 × IC50 derived from MTT assay: 15 μM for HT29, SW480, and HCT116 cell lines and 50 μM for DLD1 cell line. 17-AAG was used at 200 nM for HT29 cells and 500 nM for the rest of the panel (equivalent to 20 × IC50 and 10 × IC50, respectively). 0.01% DMSO was used as a control. For analysis of DNA fragmentation, a 24-h exposure to both drugs at concentrations of 2.5 × IC50 was carried out: oxaliplatin was used at 1.5 μM for HT29, SW480, and HCT116 cell lines and 5 μM for DLD1 cell line; 17-AAG was used at 30 nM for HT29 cells and 150 nM for the rest of the panel.

Quantitative Analysis of the DNA Fragmentation. A photometric two-step ELISA (Cell Death Detection ELISA; Roche Applied Science, Indianapolis, IN) that detects cytoplasmic histone-associated DNA fragments was used according to the manufacturer’s instructions. Aliquots of cytoplasmic lysates, corresponding to 4000 cells/reaction, were analyzed. Color development was quantified using a Universal Microplate Reader (Bio-Tek Instruments, Inc.) at 405 nm. Comparison of the absorbance of the treated sample with an untreated control determined the extent of DNA fragmentation. All experiments were performed three times in duplicate.

Caspase Assays. Apoptotic caspase colorimetric protease assay kits were purchased from BioSource International, Inc. Assays were carried out according to the manufacturer’s recommendations, as described previously (3). All experiments were performed at least three times in duplicate.

Protein Extract Preparation and Western Blotting. Protein extracts were prepared as described previously (6). Western blotting was carried out according to a standard procedure using horseradish peroxidase-conjugated secondary antibodies (Santa Cruz Biotechnology, Santa Cruz, CA) and the ECL+ Plus detection system (Amer sham, Arlington Heights, IL). Immunoblotting with β-actin antibodies was performed to confirm equal protein loading. The following primary antibodies were used: mouse monoclonal antibodies against phospho-extracellular signal-regulated kinase (ERK) and phospho-c-Jun; rabbit polyclonal antibodies against ERK, JNK, and p38; and goat polyclonal antibodies against β-actin (all from Santa Cruz Biotechnology); and rabbit polyclonal antibodies against Akt and IκB-α (all from Cell Signaling Technology). Mouse monoclonal antibodies against mitogen-activated extracellular protein kinase (MEKK3) and cellular inhibitor of apoptosis 1 were purchased from PharMingen (BD Biosciences, San Diego, CA).

Transfection and Luciferase Reporter Assay. All reporter plasmids were purchased from Stratagene (La Jolla, CA). Cells were transfected using FuGENE 6 transfection reagent (Roche) with AP-1-Luc reporter or NF-κB-Luc reporter, in combination with pRL-CMV vector purchased from Promega. Twenty-four h after transfection, cells were treated with drugs as described above, lysed, and subjected to luciferase assays using the Dual-Luciferase Reporter Assay System (Promega), according to the manufacturer’s protocol, on the TD20/20 tube luminometer (Turner Design, Sunnyvale, CA). The relative light units were calculated as ratio of Firefly luciferase luminescence to Renilla luciferase luminescence. Nonspecific luciferase activation determined in parallel experiments with pCIS-CK (control vector) was deducted. Comparison of the relative light units of the treated samples with the relative light units of untreated controls reflected the degree of induction of transcriptional activity. All experiments were performed three times in duplicate.

Results and Discussion

Oxaliplatin Demonstrates at Least Additive Effects in Combination with 17-AAG in Colon Adenocarcinoma Cell Lines. Four colon cancer cell lines were used in this study. HCT116 is mismatch repair deficient but has intact p53 function. HT29 and SW480 have impaired p53 function but intact mismatch repair, whereas DLD1 has both lesions. These lines have the following IC50 values in MTT assays to oxaliplatin: HCT116, 0.64 μM; HT29, 0.58 μM; SW480, 0.49 μM; and DLD1, 2.05 μM. The pattern of sensitivity of these cell lines to oxaliplatin differs from that to cisplatin (3), suggesting differences in the mechanisms of cytotoxicity. IC50 values for 17-AAG are 52.6 nM for HCT116, 8.9 nM for HT29, 62.5 nM for SW480, and 42.5 nM for DLD1. Thus, HT29 is markedly more sensitive to 17-AAG in comparison with the other cell lines, whereas DLD1 is substantially more resistant to oxaliplatin.

The effects of combining oxaliplatin and 17-AAG were analyzed using two approaches: MTT assays, followed by isobologram analysis (isobolograms not shown); and clonogenic assays. Combination indices were calculated based on the results of both. The MTT assays demonstrated additivity in HCT116, SW480, and DLD1 but antagonism in HT29 (Table 1). By contrast, analysis of the clonogenic assay data showed additivity in three cell lines and, by conventional definition, synergy in HCT116. The latter assay is thought to afford a more accurate assessment of cell survival because this methodology eliminates the contribution of cells that have been growth arrested or have committed to cell death before actual apoptosis.

To verify that the cytotoxicity of the oxaliplatin/17-AAG combination was related to activation of an apoptotic process, we quantified the level of apoptosis induced by these drugs (used at 2.5 × IC50 concentrations) over 24 h by a specific ELISA kit that detects cytoplasmic histone-associated DNA fragments. Fig. 1A shows that in SW480 and HCT116 cells, DNA fragmentation is induced by each drug alone and in combination in proportions reflecting the additive effect of the combination demonstrated in MTT assay for these two cell lines. However, in HT29 and DLD1 cell lines, treatment with oxaliplatin alone or in combination did not induce measurable apoptosis by this technique, again consistent with the MTT assay. It is likely, in light of the clonogenic assay data showing a positive interaction, that in these two cell lines oxaliplatin caused growth inhibition rather than cell death and that this growth delay caused partial inhibition of apoptotic signaling generated by 17-AAG. It has been shown previously in HT29 that the apoptotic activity of the topoisomerase 1 inhibitor irinotecan is inhibited or delayed as a result of 24-h exposure to a low concentration (1.23 μM) of oxaliplatin, which induced a G2/M-phase arrest in this model (10).

To further explore the interactions between oxaliplatin and 17-AAG in combination, we measured the activation of caspases 8, 9, and 3. Because of the lower sensitivity of these assays compared with ELISA, higher drug concentrations (10 × or 20 × IC50 of 17-AAG and 25 × IC50 of oxaliplatin) were used for a 24-h treatment. Enhanced activation of caspase 3 in all four cell lines after combination treatment, compared with the effects of single agents, correlated with the at least additive effect of the combination in the clonogenic assay (Fig. 1B). Caspase 8 activation demonstrated similar results (data not shown).

Table 1 Cytotoxic effects of oxaliplatin and 17-AAG in combination against colon cancer cell lines determined in MTT (CI at IC50) and clonogenic assay (CI at IC25)

<table>
<thead>
<tr>
<th>Cell line</th>
<th>MTT assay</th>
<th>Clonogenic assay</th>
</tr>
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<tbody>
<tr>
<td>SW480</td>
<td>0.915 ± 0.05</td>
<td>0.999 ± 0.25</td>
</tr>
<tr>
<td>HT29</td>
<td>1.24 ± 0.18</td>
<td>0.901 ± 0.15</td>
</tr>
<tr>
<td>HCT116</td>
<td>0.99 ± 0.07</td>
<td>0.88 ± 0.09</td>
</tr>
<tr>
<td>DLD1</td>
<td>1.07 ± 0.05</td>
<td>0.995 ± 0.05</td>
</tr>
</tbody>
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*17-AAG, 17-allylamino-17-demethoxygalanamycin, CI, combination index; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

b CI with values from 0.9 to 1.1 indicate an additive effect of combinations, <0.9 or >1.1 represent synergism or antagonism, respectively. Values represent results from at least three independent experiments.
assay (Fig. 1). Cells were treated for 24 h with oxaliplatin and 17-AAG used at 2.5 \( \mu \text{M} \). We previously alluded to the study (10) of the irinotecan/oxaliplatin combination in HT29 cells, a result that may be playing a role with oxaliplatin. In the previously alluded to study, however, higher concentrations of oxaliplatin (50 \( \mu \text{M} \)) were used, making the experimental conditions significantly different from ours.

It should also be considered that necrosis as a means of cell death may be playing a role with oxaliplatin. In the previously alluded to study (10) of the irinotecan/oxaliplatin combination in HT29 cells, a larger proportion of necrotic than apoptotic cell death occurred in response to oxaliplatin (5 \( \mu \text{M} \) over 24 h). This observation is consistent with an unexpected dissonance in the levels of caspase 3 activation induced by cisplatin and oxaliplatin in these four colon cancer cell lines. As shown previously (3), cisplatin (although less toxic against these cell lines, based on higher IC\(_{50} \) values), when used at 10 \( \times \) IC\(_{50} \) concentration, induced the same or higher relative levels of caspase 3 activation than oxaliplatin at 25 \( \times \) IC\(_{50} \). Therefore, whereas with cisplatin, an apoptotic mechanism of cell death appears to predominate, both apoptotic and necrotic mechanisms have a role in oxaliplatin cytotoxicity.

**Oxaliplatin-Induced JNK and p38 Activation Exerts Different Effects on Colon Cancer Cell Lines: p38 Activation Causes Cell Death.** To further investigate the mechanism of the additive interaction between 17-AAG and oxaliplatin, we used two approaches: first, we used high concentrations of both drugs to allow detection of the effects of 17-AAG on signal transduction pathways related to apoptosis; and second, we investigated the roles of three major kinase cascades relevant to oxaliplatin cytotoxicity by using selective pharmacological inhibitors of these pathways.

Among the cellular responses to treatment with platinum compounds is activation of mitogen-activated protein kinase cascades (reviewed in Ref. 12), especially JNK and p38 (3). The conflicting reports regarding the role of these pathways in mediating cytotoxicity have been a source of controversy (reviewed in Ref. 13). As shown in Fig. 2A, in all four colon cancer cell lines, oxaliplatin induced phosphorylation of both JNK and p38 mitogen-activated protein kinase, with consequent phosphorylation of two major components of the AP-1 transcription factor, c-Jun and activating transcription factor 2. A functional assay using a luciferase reporter confirms the activation of AP-1 signaling by oxaliplatin (Fig. 2B). 17-AAG demonstrated selective effects on oxaliplatin-induced signaling through these pathways: phospho-JNK, c-Jun and activating transcription factor 2 were completely depleted; and AP-1 transactivation was inhibited, whereas p38 phosphorylation was not affected (Fig. 2, A and B). The total protein level of JNK and p38 mitogen-activated protein kinase was unchanged. These observations indicate that in our cellular model, activation of the JNK rather than p38 mitogen-activated protein kinase induces the activation of AP-1 transcriptional factor. The same finding was reported recently for breast carcinoma cells (14).

Previously, we demonstrated that 17-AAG did not inhibit cisplatin-induced p38 activation but showed cell line-dependent effects on the JNK pathway (3) and that the level of JNK inactivation by 17-AAG determined differential cytotoxicities of cisplatin/17-AAG combination in colon cancer cell lines, where complete inhibition of JNK signaling resulted in antagonism. Therefore, the effects of 17-AAG on oxaliplatin signaling through the JNK pathway differ from those with cisplatin, in that a uniform complete inhibition of JNK signaling did not result in antagonism in any of the cell lines.

To explore the role of individual strands of the mitogen-activated protein kinase pathways in oxaliplatin-induced cell death we used selective inhibitors and examined their effects on cytotoxicity of oxaliplatin. As shown in Table 2, in all cell lines, inhibition of JNK by the selective inhibitor SP600125 (15) demonstrated additivity (MTT assay) or very slight antagonism (clonogenic assays). Inhibition of p38 kinase by SB203580, on the other hand, markedly increased cellular resistance to oxaliplatin. This observation strongly suggests that p38 kinase activation is one of the major factors in oxaliplatin-induced cell death in colon cancer cell lines, when the JNK activation might play a role in the both apoptotic and antiapoptotic cellular responses.

**Inhibition by 17-AAG of Raf/Mitogen-Activated Extracellular Protein Kinase Kinase/ERK and PI3K/AKT Pathways in Colon Cancer Cell Lines Exerts Minimal Effects on Oxaliplatin-Induced Cell Death.** We then explored the roles of two cellular pathways directly affected by ansamycins, Raf-1/mitogen-activated extracellular protein kinase kinase/ERK and PI3K/AKT (4, 5), in the cellular response to oxaliplatin. As shown in Fig. 2C, the basal level of phosphorylated ERK and AKT indicates a certain level of constitutive protein phosphorylation.
activation of these pathways in all cell lines. This may result from known activating mutations in oncogenes such as K-Ras, one that is common to all of the cell lines studied here. Oxaliplatin itself did not inhibit or induce ERK signaling but did inhibit phosphorylation of AKT. The finding correlates with recently reported data that activation of several protein kinase C isozymes [known as one of the intermediate steps in distributing DNA damage signaling into the cell (reviewed in Ref. 12)] leads not only to activation of the JNK/p38 mitogen-activated protein kinase cascades but also causes dephosphorylation of AKT through a protein phosphatase 2A-dependent mechanism (16). 17-AAG, as shown in Fig. 2C, strongly inhibited phosphorylation of ERK in all cell lines. There was no effect on ERK protein levels. AKT signaling was profoundly inhibited, as evidenced by almost complete inhibition of phosphorylation in all of the lines. In addition, there was a marked effect on AKT protein content. These observations are well correlated with the known mechanisms of direct and indirect effect of ansamycins on AKT (4, 5).

We tested the effects of pharmacological inhibition of both ERK and AKT pathways on oxaliplatin-induced cell death, using either SP600125, specific inhibitor of JNK1/2, or UO126, selective mitogen-activated extracellular protein kinase kinase 1/2 inhibitor. As shown in Table 2, despite an additive effect of both inhibitors in the MTT assay, the clonogenic assay showed that inhibition of the PI3K/AKT pathway was associated with resistance to oxaliplatin rather than with cell protein levels. AKT signaling was profoundly inhibited, as evidenced by almost complete inhibition of phosphorylation in all of the lines. In addition, there was a marked effect on AKT protein content. These observations are well correlated with the known mechanisms of direct and indirect effect of ansamycins on AKT (4, 5).

We tested the effects of pharmacological inhibition of both ERK and AKT pathways on oxaliplatin-induced cell death, using either LY294002, specific inhibitor of PI3K, or UO126, selective mitogen-activated extracellular protein kinase kinase 1/2 inhibitor. As shown in Table 2, despite an additive effect of both inhibitors in the MTT assay, the clonogenic assay showed that inhibition of the PI3K/AKT pathway was associated with resistance to oxaliplatin rather than with cell protein levels. AKT signaling was profoundly inhibited, as evidenced by almost complete inhibition of phosphorylation in all of the lines. In addition, there was a marked effect on AKT protein content. These observations are well correlated with the known mechanisms of direct and indirect effect of ansamycins on AKT (4, 5).

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sensitization. As has been described, AKT transmits survival signals through phosphorylation-dependent suppression of intracellular pro-apoptotic factors mostly related to mitochondrial apoptosis (reviewed in Ref. 17). As noted above, in the colon cancer cell lines, we did not detect the activation of caspase 9 after oxaliplatin treatment. This suggests a relatively insignificant role for the intrinsic apoptotic pathway in oxaliplatin-induced cell death. We believe that this might explain why down-regulation of the PI3K pathway does not increase the cytotoxicity of oxaliplatin in this model of colon cancer.

**17-AAG Inhibits the Transcriptional Activity of NF-κB in Colon Cancer Cell Lines and Consequently Increases Oxaliplatin-Induced Cytotoxicity.** One of the major prosurvival cellular responses is the activation of NF-κB (18). Inhibition of this pathway has already been shown to provide a molecular target for anticancer therapy (reviewed in Refs. 19 and 20). Signaling to NF-κB occurs through the activation of IKKs, which phosphorylate IκB, leading to the release and nuclear translocation of the transcription factor NF-κB; alternatively, NF-κB itself is subject to an IκB-independent regulation through phosphorylation by different kinases that increases NF-κB-induced gene expression (reviewed in Ref. 21). Geldanamycin (7), of which 17 AAG is an analogue, inhibits tumor necrosis factor-induced NF-κB activation through proteasomal degradation of receptor-interacting protein; the role of this tumor necrosis factor receptor 1-interacting protein in DNA damage-induced IKK activation was reported recently (22). We have found that high basal activation of NF-κB in HT29 and HCT116 cell lines is linked to MEKK3 expression.2

All of these findings led us to investigate the effects of oxaliplatin and 17-AAG on NF-κB activation in colon carcinomas and to evaluate the impact of NF-κB down-regulation on oxaliplatin-induced cytotoxicity. As shown in Fig. 3A, all of the colon cancer cell lines demonstrated constitutive activity of signaling to NF-κB, including active expression of MEKK3 and phosphorylation of IKK subunits. To determine the relevance of these observations for the subsequent activation of NF-κB, we assessed the phosphorylation status of the Rel A/p65 NF-κB subunit, which has been suggested to reflect NF-κB transactivation ability (21), and the expression of a known NF-κB transcriptional target, cellular inhibitor of apoptosis 1 (23). We also used a luciferase reporter assay for a definitive confirmation of NF-κB activation status. As shown in Fig. 3B, NF-κB activation strongly correlated with the levels of IKK and p65 phosphorylation, as well as with cellular inhibitor of apoptosis 1 expression. No significant NF-κB induction after oxaliplatin treatment was detected in three of the four colon cancer cell lines. However, in HCT116, oxaliplatin was an inducer of NF-κB activation, as evident from the reporter assay and immunoblots demonstrating upstream activation of MEKK3, IKKα, and IKKβ and phosphorylation of p65 (Fig. 3, A and B). HCT116 is different from the three other cell lines in its p53 status, and only in this cell line was a high level of cisplatin-induced Fas activation demonstrated (3). We detected a similar activation of Fas when these cells were treated with oxaliplatin (data not shown). As was found recently for colon cancer cells, activated Fas can function not only as an inducer of apoptosis but also as an activator of NF-κB by a mechanism that appears to be distinct from that used by tumor necrosis factor α (24). This suggested that Fas might act as an inducer of apoptosis, as shown in Fig. 3B. These data establish the ability of 17-AAG to down-regulate basal and oxaliplatin-induced NF-κB activation in a cellular model of colon cancer. To determine the importance of this action of 17-AAG for oxaliplatin-induced cytotoxicity, we used two known NF-κB inhibitors, caffeic acid phenethyl ester and helenalin. As shown in Table 2, these two different Rel A/p65 inhibitors demonstrated a synergistic interaction with oxaliplatin in both the MTT and clonogenic assays. Therefore, inhibition by 17-AAG of upstream components of the NF-κB signaling cascade followed by down-regulation of NF-κB transcriptional activity is a major factor determining the positive interaction between oxaliplatin and 17-AAG in combination. One might predict that 17-AAG may be of value in other settings in which NF-κB signaling is a determinant of sensitivity.

NF-κB is constitutively expressed in a wide range of tumor cells, where it commonly promotes cell proliferation and resistance to

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2 C. W. Nho, and P. J. O’Dwyer. NF-κB activation by the chemopreventive diethylnitrosamine exerts through stimulation of MEKK3 signaling, submitted for publication.
apoptosis induced by genotoxic agents (19, 20). Therefore, a reasonable strategy for cancer treatment may be to combine chemotherapy with compounds active against NF-κB. Clinical trials of such interventions are in progress in several tumor types (20). We noted above that the cytotoxic effects of oxaliplatin are enhanced when it is administered in combination with 5-fluorouracil (1, 2). The ability of 5-fluorouracil to suppress the activation of NF-κB has been reported recently (25). These findings support an effort to increase the activity of oxaliplatin in colorectal cancer by such targeted interventions.

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References


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