Cyclic GMP Mediates Apoptosis Induced by Sulindac Derivatives via Activation of c-Jun NH_2-Terminal Kinase 1[1]

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ABSTRACT

Sulindac sulfone (Exisulind) induces apoptosis and exhibits cancer chemopreventive activity, but in contrast to sulindac, it does not inhibit cyclooxygenases 1 or 2. We found that sulindac sulfone and two potent derivatives, CP248 and CP461, inhibited the cyclic GMP (cGMP) phosphodiesterases (PDE) 2 and 5 in human colon cells, and these compounds caused rapid and sustained activation of the c-Jun NH_2-terminal kinase 1 (JNK1). Rapid activation of stress-activated protein/ERK kinase 1 (SEK1) and mitogen-activated protein kinase kinase kinase (MEKK1), which are upstream of JNK1, was also observed. Other compounds that increase cellular levels of cGMP also activated JNK1, and an inhibitor of protein kinase G (PKG), Rp-8-pCPT-cGMPS, inhibited JNK1 activation by the sulindac sulfone derivatives. Expression of a dominant-negative JNK1 protein inhibited CP248-induced cleavage of poly(ADP-ribose) polymerase, a marker of apoptosis. Thus, it appears that sulindac sulfone and related compounds induce apoptosis, at least in part, through activation of PKG, which then activates the MEKK1-SEK1-JNK1 cascade. These studies also indicate a role for cGMP and PKG in the JNK pathway.

INTRODUCTION

The nonsteroidal anti-inflammatory drug sulindac inhibits chemical carcinogenesis in rodent models and causes regression of adenomas in patients with familial adenomatous polyposis coli of the colon (1–3). Sulindac is a prodrug that is metabolized to a pharmacologically active sulfide derivative that inhibits Cox[3]-1 and Cox-2 activity and thereby inhibits prostaglandin synthesis. However, the sulindac metabolite sulindac sulfone (Exisulind) also inhibits chemical carcinogenesis in rodents, although it does not inhibit Cox-1 or Cox-2. Like sulindac sulfide, sulindac sulfone also inhibits growth and induces apoptosis in a variety of human tumor-derived cell lines (1–4), and it also inhibits the growth of human prostate cancer cells in nude mice (5). These and other findings suggest that reduction of prostaglandin levels is not necessary for the antineoplastic activity of this class of drugs (2, 3).

The precise mechanism by which sulindac compounds induce apoptosis is not known. Recent studies suggest that it may involve increased production of ceramide (6), a known inducer of apoptosis, and/or inhibition of the peroxisome proliferator-activated receptor δ (7). An alternative mechanism is based on our finding that sulindac sulfone inhibits the activity of the cGMP-specific PDE2 and PDE5 (PDE2/5; Ref. 8). Representative data for PDE5 are shown in Table 1. The IC_{50} for sulindac sulfone was ~113 mM. On the basis of this finding, two sulindac sulfone derivatives, CP248 and CP461, were synthesized and found to be highly potent inhibitors of PDE2/5 (Table 1 and Ref. 8). *In vivo* inhibition of PDE2/5 by sulindac sulfone and its derivatives induces an increase in intracellular levels of cGMP (8). cGMP has been implicated previously in growth inhibition and apoptosis in various cell types, including pancreatic B cells and nerve cells (9, 10). Taken together, these findings suggest that elevation of intracellular levels of cGMP may trigger apoptosis, but the downstream signaling pathways are not known. Therefore, we examined the effects of sulindac derivatives on the JNK pathway in human colon cancer cells. We focused on this pathway because of its important role in mediating cellular stress responses and apoptosis induced by several other agents (11, 12).

MATERIALS AND METHODS

Cell Cultures. SW480 and HT29 human colon cancer cells were grown in DMEM medium with 10% fetal bovine serum.

cGMP PDE Assays. HT29 cells were grown to confluence in RPMI 1640 containing 2 mM glutamine, 25 mM HEPES, and 5% FBS. The cells were harvested using Pancreatin,

Received 5/8/00; revised 7/3/00; accepted 7/5/00.
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[1] This work was supported by awards from Cell Pathways, Inc., the T. J. Martell Foundation, and the National Foundation for Cancer Research (to I. B. W.).
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[3] The abbreviations used are: Cox, cyclooxygenase; cGMP, cyclic GMP; PDE, phosphodiesterase; CP248, (*Z*)-5-fluoro-2-methyl-1-(3,4,5-trimethoxybenzylidene)-3-(*N*-benzyl)-indenylacetamide; CP461, (*Z*)-5-fluoro-2-methyl-(4-pyridylidene)-3-(*N*-benzyl)-indenylacetamide hydrochloride; JNK, c-Jun NH_2-terminal kinase; DN, dominant negative; MEKK, mitogen-activated protein kinase kinase kinase; SEK, stress-activated protein/ERK kinase 1; HA, hemagglutinin antigen; PKG, protein kinase G; dbcGMP, dibutyrylguanosine 3′:5′-cyclic monophosphate; dbcAMP, dibutyryladenosine 3′:5′-cyclic monophosphate; PARP, poly(ADP-ribose) polymerase.

Table 1 Inhibition of cGMP PDE by sulindac sulfone and its derivatives

cGMP PDE assays were performed with either the entire 100,000 × *g* supernatant fraction of HT29 colon cancer cells or a partially purified PDE5 peak obtained by DEAE anion exchange chromatography (33). Both preparations displayed similar IC_{50}s with the known PDE5 inhibitors E4021 and zaprinast (3 nM and 1.5 μM, respectively) and with the compounds listed in the table. Similar results were obtained with extracts of SW480 cells, which contain both PDE2 and PDE5 (data not shown).

Drug	IC_{50} (μM)
Sulindac sulfone	113 ± 18 (n = 6)
CP461	3.5 ± 3 (n = 2)
CP248	0.39 ± 0.08 (n = 8)

homogenized in 8 mM Tris-Ac (pH 7.4) containing 5 mM MgAc, 0.1 mM EDTA, 0.8% Triton X-100, and protease inhibitors, and the extracts were centrifuged at 100,000 × *g*. The supernatant fraction was used as such or separated on an 180 ml Tris-AcrylM column with a 0.1 M NaAc gradient at a flow rate of 1 ml/min using a AKTA FPLC instrument (Pharmacia). Cyclic GMP PDE activities were determined with 0.25 μM cGMP as the substrate, and IC_{50}s ±SE were calculated using Prizm (Graph-Pad) nonlinear, variable slope curve-fitting routines with 8–12 drug concentrations for each curve. For additional details, see Thompson *et al.* (13).

JNK1 Assays. The cells were lysed in a lysis buffer [20 mM Tris-HCl (pH 7.5), 0.5% NP40, 250 mM NaCl, 3 mM EDTA, 3 mM EGTA, 2 mM DTT, 0.5 mM phenylmethylsulfonyl fluoride, 1 μg/ml leupeptin, 20 mM β-glycerophosphate, and 25% glycerol], and then JNK1 was immunoprecipitated with an anti-JNK1 antibody (Santa Cruz) for 2 h and assayed for *in vitro* kinase activity with GST-c-Jun(1–79) (New England Biolab) as the substrate in a kinase reaction buffer [20 mM HEPES (pH 7.5), 10 mM $MgCl_2$, 1 mM DTT, 20 μM ATP, 20 mM β-glycerophosphate, and 1 μCi [γ-^{32}P]ATP] for 20 min, as described previously (14). The reaction mixture was then subjected to SDS-PAGE. The intensities of the bands were determined with a PhosphorImager (Molecular Dynamics**)**, and the treated sample:control untreated sample ratio was expressed as "relative kinase activity" or "fold activation." The experiments were repeated three times with similar results.

Caspase-3 Assays. The cells were lysed in an extraction buffer [50 mM HEPES KOH (pH 7.4), 0.1% 3-[(3-cholamidopropyl)dimethylamino]-1-propanesulfonate, 1 mM EDTA, 10 mM DTT, 100 mM NaCl, and 10% glycerol], and extracts (50 μg) were assayed for caspase-3 activity with Ac-DEVD-AFC (Alexis) as the substrate, in the presence or absence of the caspase-3 inhibitor Ac-DEVD-CHO (Alexis), as described previously (15). Caspase-3 activity was calculated by subtracting the AFC fluorescence (excitation, 400 nm; emission, 505 nm) in the presence of Ac-DEVD-CHO from the AFC fluorescence in the absence of Ac-DEVD-CHO.

MEKK1 Assays. SW480 cells were transiently transfected with an HA epitope-tagged MEKK1 by Lipofectin (Life Technologies, Inc.). After 24 h, the cells were lysed in a lysis buffer [20 mM Tris-HCl (pH 7.5), 0.5% NP40, 250 mM NaCl, 3 mM EDTA, 3 mM EGTA, 2 mM DTT, 0.5 mM phenylmethylsulfonyl fluoride, 1 μg/ml leupeptin, 20 mM β-glycerophosphate, and 25% glycerol], and then MEKK1 was immunoprecipitated with an anti-HA antibody (Berkeley Antibody Co.), and *in vitro* kinase assays were performed with GST-SEK1(K129R) (Calbiochem) as the substrate in a kinase reaction buffer [20 mM HEPES (pH 7.5), 10 mM $MgCl_2$, 1 mM DTT, 20 μM ATP, 20 mM β-glycerophosphate, and 1 μCi [γ-^{32}P]ATP] for 20 min, as described previously (16). The pCEP4-HA-MEKK1 plasmid was kindly provided by M. H. Cobb (University of Texas Southwestern Medical Center).

Generation of Stable Cell Lines That Overexpress DN-JNK1. SW480 cells were transfected with either the control vector pcDNA3 alone or pCMV-DN-JNK1 together with pcDNA3 (10:1 ratio). The pCMV-DN-JNK1 plasmid encodes a FLAG epitope-tagged, kinase-inactive, DN-JNK1. Stably transfected cells were selected with neomycin for 2 weeks, and the neomycin-resistant colonies were pooled and characterized further. The pCMV5-M2-DN-JNK1 plasmid was kindly provided by A. Minden (Columbia University).

RESULTS

Sulindac Derivatives Activate JNK1. SW480 human colon cancer cells were treated with either the solvent DMSO or sulindac derivatives (sulindac sulfide, 50–500 mM; sulindac sulfone, 100–600 mM; CP248, 0.1–5 mM; or CP461, 1–50 mM) for 1 h and assayed for JNK1 activation. These concentrations were chosen because they provided optimal induction of apoptosis. Endogenous JNK1 was immunoprecipitated with anti-JNK1 antibody, and *in vitro* kinase assays were performed with GST-c-Jun(1–79) as the substrate. As shown in Fig. 1*A*, all of the sulindac derivatives caused activation of JNK1. The fold-induction was quantitated by PhosphorImager analysis, and the results are indicated in Fig. 1*A*. Even at very low doses the potent sulindac derivatives CP248 and CP461 activated JNK1 more strongly than did sulindac sulfide or sulindac sulfone. The relative potencies of these compounds for JNK1 activation correlated with their potencies for PDE5 inhibition (Table 1) and, as shown below, for caspase-3 activation. Similar effects were observed in other colon cancer cell lines including HCT116 and HT29, as well as MCF-7 breast cancer and both LNCaP and PC3 prostate cancer cell lines (data not shown). A time course study indicated that when SW480 cells were treated with CP248 (1 mM), JNK1 activation was sustained for at least 24 h (Fig. 1*B*). We then confirmed the apoptotic activity of these sulindac derivatives by measuring caspase-3 activity after treating SW480 cells with similar concentrations of sulindac sulfide, sulindac sulfone, CP248, or CP461 for 24 h. Protein extracts were then prepared, and caspase-3 activity was measured with Ac-DEVD-AFC as the substrate, in the presence or absence of the caspase-3 inhibitor Ac-DEVD-CHO. Fig. 1*C* shows that the treatment of SW480 cells with all of these sulindac derivatives led to activation of caspase-3. Activation of caspase-3 was detected as early as 2 h after treatment with the sulindac derivatives, and this was sustained for at least 24 h (data not shown). Parallel studies on morphological evidence of apoptosis were also done on cells stained with Hoechst 33258, and this revealed nuclear condensation and fragmentation after 12 h of treatment and was sustained for at least 2 days (data not shown). Similar

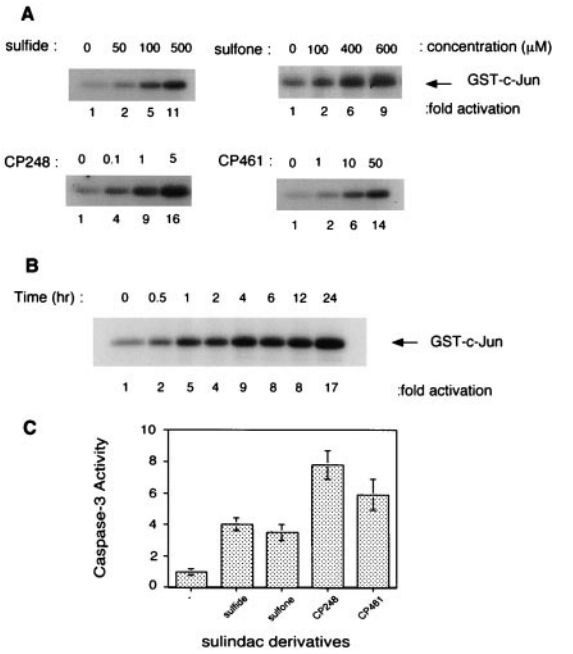

Fig. 1 Activation of JNK1 and caspase-3 in SW480 colon cancer cells exposed to sulindac derivatives. *A*, SW480 cells were grown in DMEM medium with 10% fetal bovine serum, and during exponential growth they were treated with either 0.1% DMSO (−) or the indicated concentrations (μM) of sulindac sulfide, sulindac sulfone, CP248, or CP461 for 1 h. The cells were lysed, and then JNK1 was immunoprecipitated with an anti-JNK1 antibody (Santa Cruz Biotechnology) and assayed for *in vitro* kinase activity with GST-c-Jun(1–79) (New England Biolab) as the substrate, as described previously (14). The experiments were repeated three times with similar results. Fold activation was measured with a PhosphorImager (Molecular Dynamics). *B*, SW480 cells were treated with CP248 (1 μM) for the indicated time periods, and then *in vitro* JNK1 kinase assays were done as described above. *C*, SW480 cells were treated with sulindac sulfide (200 μM), sulindac sulfone (600 μM), CP248 (1 μM), or CP461 (10 μM) for 24 h. The cells were then lysed, and extracts were assayed for caspase-3 activity with Ac-DEVD-AFC (Alexis) as the substrate, in the presence or absence of the caspase-3 inhibitor Ac-DEVD-CHO (Alexis), as described previously (15). Caspase-3 activity was calculated by subtracting the AFC fluorescence (excitation, 400 nm; emission, 505 nm) in the presence of Ac-DEVD-CHO from the AFC fluorescence in the absence of Ac-DEVD-CHO. *Bars*, SD.

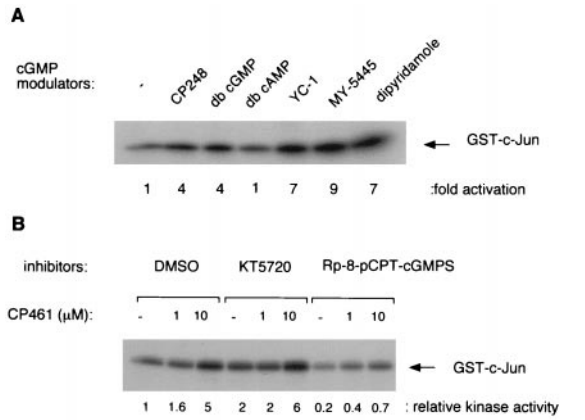

Fig. 2 Activation of JNK1 by cGMP modulators and the role of PKG. *A*, SW480 cells were treated with either 0.1% DMSO (−) or CP248, 0.1 μM; dbcGMP, 500 μM; YC-1, 50 μM; MY-5445, 50 μM; dipyridamol, 10 μM; or dbcAMP, 500 μM (Alexis) for 1 h. The cells were lysed, and JNK1 was immunoprecipitated, and assayed for *in vitro* kinase activity as described in Fig. 1*A*. *B*, SW480 cells were pretreated with either 0.1% DMSO, KT5720 (2 μM), or Rp-8-pCPT-cGMPS (2 μM; Alexis) for 2 h and then treated with CP461 (0, 1, or 10 μM) for 1 h. The cells were lysed and assayed for JNK1 activation as described above.

findings were obtained with HCT116 and HT29 cells (data not shown).

We should emphasize that in the present studies, and in all previous cell culture studies on sulindac sulfide and sulindac sulfone, rather high concentrations (100–600 mM) are required to exert various biological effects. Because the IC_{50} for sulindac sulfone in subcellular assays for inhibition of PDE5 was also rather high (113 mM; Table 1), this phenomenon is not entirely attributable to poor cellular uptake of the drug. Nevertheless, these compounds are active, after oral administration, in humans and rodents, perhaps because they are concentrated in specific tissues.

cGMP Modulators Activate JNK1 through a cGMP/PKG Pathway. We then examined the effects of an elevation of cellular levels of cGMP on JNK1 activity in SW480 cells. The intracellular level of cGMP is positively regulated by guanylate cyclase and negatively regulated by PDE2/5 (17, 18). We treated SW480 cells with various cGMP modulators for 1 h and then collected protein extracts for JNK1 assays (Fig. 2*A*). db-cGMP (500 μM), a cell-permeable cGMP analogue, activated JNK1 in SW480 cells, but the cell-permeable cAMP analogue dbcAMP (500 μM) was inactive. YC-1 (50 μM), a guanylate cyclase activator, also activated JNK1. MY-5445 (50 μM) and dipyridamol (10 μM), PDE5-specific inhibitors (19, 20), also activated JNK1 in SW480 cells. Similar activation of JNK1 by these cGMP modulators was observed in HCT116 and HT29 cells (data not shown). These results show that compounds that would be expected to increase cellular levels of cGMP, by various means, lead to activation of JNK1 in colon cancer cells. The signal appears to be specific for cGMP and not cAMP because dbcGMP but not dbcAMP activated JNK1 in these cells.

PKG is one of the major cellular targets of cGMP, and the binding of cGMP, or the above-mentioned analogue, activates PKG kinase activity, both *in vivo* and *in vitro* (21, 22). However, the precise role of PKG in signal transduction pathways is not known. Because our results indicated that factors that increase cGMP lead to activation of JNK1, we tested whether a PKG-specific inhibitor, Rp-8-pCPT-cGMP (23), could inhibit the ability of a sulindac sulfone derivative to induce JNK1 activation. As a control, KT5720 was used as a protein kinase A-specific inhibitor. SW480 cells were treated with either DMSO, KT5720 (2 mM), or Rp-8-pCPT-cGMPS (2 mM) for 2 h and then treated with either DMSO or CP461 (1 or 10 mM) for 1 h. Cell extracts were collected and assayed for JNK1 activation. As shown in Fig. 2*B*, Rp-8-pCPT-cGMP strongly inhibited CP461-induced JNK1 activation, whereas KT5720 had no inhibitory activity. Taken to-

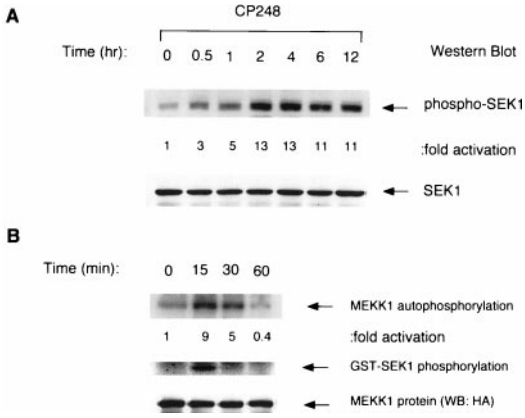

Fig. 3 Activation of the MEKK1-SEK1 pathway by the sulindac sulfone derivative CP248. *A*, SW480 cells were treated with CP248 (1 μM) and collected at the indicated time points, and cell lysates were assayed for SEK1 activation by Western blotting with an anti-phospho-SEK1 (Thr-223) antibody (New England Biolab), as described previously (34). Fold increase in phosphorylation was measured by densitometry. The experiment was repeated three times with similar results. *B*, SW480 cells were transiently transfected with an HA epitope-tagged MEKK1, and the transfected cells were then treated with CP248 (1 μM) for the indicated time periods. MEKK1 was immunoprecipitated with an anti-HA antibody (Berkeley Antibody Co.), and *in vitro* kinase assays were performed with GST-SEK1(K129R) (Calbiochem) as the substrate.

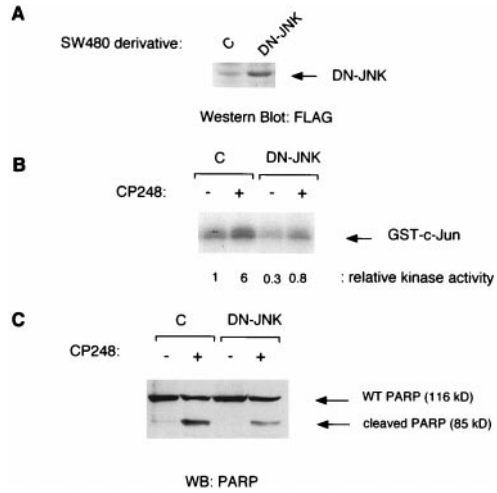

Fig. 4 Inhibition of CP248-induced PARP cleavage by DN-JNK1. *A*, Cell extracts from SW480 derivatives that had been transfected with either a control plasmid (C) or a DN-JNK1 (*DN-JNK*) plasmid were prepared and subjected to Western blot analysis with an anti-FLAG antibody (Kodak). For additional details, see text. *B*, control (*C*) or DN-JNK derivatives of SW480 cells were treated with CP248 (1 mM) for 1 h. The cells were lysed, and then endogenous JNK1 was immunoprecipitated with an anti-JNK1 antibody and assayed for *in vitro* kinase activity with GST-c-Jun(1–79) as the substrate, as described above. The experiments were repeated three times with similar results. Relative kinase activity was measured with a PhosphorImager. *C*, the control (*C*) or DN-JNK derivatives of SW480 cells were treated with CP248 (1 mM) for 2 days. Then both the floating and attached cells were collected, and the cell lysates were assayed for PARP cleavage by Western blotting (*WB*) with an anti-PARP antibody (PharMingen).

gether, these results show that this sulindac sulfone derivative activates JNK1 through a cGMP/PKG pathway.

Sulindac Derivatives Activate SEK1. To further characterize the signal transduction pathway involved in the above-described JNK1 activation, we tested whether SEK1, the protein kinase immediately upstream of JNK1 (24), was also activated by a sulindac derivative. Activation of SEK1 occurs through phosphorylation of two residues of this protein, Ser-219 and Thr-223, and by the protein kinase MEKK1 (16). SW480 cells were treated with CP248 (1 μM) for various times, up to 6 h, and extracts were analyzed by Western blot analysis using a phospho-Thr-223-specific SEK1 antibody. The treatment with CP248 induced increased phosphorylation of SEK1, within 30 min, without changing the total cellular level of the endogenous SEK1 protein (Fig. 3*A*). By 2 h, there was a 13-fold induction, and this effect persisted for at least 12 h (Fig. 3*A*). Similar results were obtained with sulindac sulfone and CP461. Treatment of the cells with only the DMSO solvent did not induce phosphorylation of SEK1 (data not shown).

Sulindac Derivatives Activate MEKK1. We then examined the effect of a sulindac sulfone derivative on MEKK1, a protein kinase immediately upstream of SEK1 (25, 26). SW480 cells were transiently transfected with a HA epitope-tagged MEKK1, and the transfected cells were then treated with CP248 (1 μM) for 0, 15, 30, or 60 min. MEKK1 was immunoprecipitated with an anti-HA antibody, and *in vitro* kinase assays were performed with GST-SEK1(K129R) as the substrate in the presence of [γ-^{32}P]ATP. CP248 induced rapid (within 15 min), strong, and transient activation of MEKK1 activity, as determined by MEKK1 autophosphorylation and also by the phosphorylation of GST-SEK1 (Fig. 3*B*). The amounts of immunoprecipitated MEKK1 remained constant. We also observed cleavage of the endogenous MEKK1 protein by Western blot analysis, within 24 h after CP248 treatment of the cells (data not shown). This is a characteristic effect of caspase activation (27).

JNK1 Activation Is Required for CP248-induced Apoptosis. Finally, we investigated whether activation of the JNK pathway is required for the induction of apoptosis by these sulindac derivatives. SW480 cells were transfected with either the control vector pcDNA3 alone or pCMV-DN-JNK1 together with pcDNA3 (10:1 ratio). The pCMV-DN-JNK1 plasmid encodes a FLAG epitope-tagged, kinase-inactive, DN-JNK1. Stably transfected cells were selected with neomycin for 2 weeks, and the neomycin-resistant colonies were pooled and characterized further. Western blot analysis confirmed that the FLAG-tagged, DN-JNK1 was expressed in the DN-JNK derivatives of SW480 cells (Fig. 4*A*). We found that activation of endogenous JNK1 by CP248 was markedly inhibited in the DN-JNK cells when compared with the control cells (Fig. 4*B*). The control (C) or DN-JNK derivatives of SW480 cells were also treated with CP248 (1 mM) for 2 days. Then both the floating and attached cells were collected, and cell lysates were assayed for PARP cleavage by Western blotting with an anti-PARP antibody. PARP is a M_r 116,000 nuclear enzyme that converts NAD to nicotinamide and protein-linked ADP-ribose polymers, which are important for DNA repair and genomic maintenance. In cells that are undergoing apoptosis, the M_r 116,000 PARP protein is cleaved by caspase-3 into M_r 85,000 and M_r 25,000 fragments,

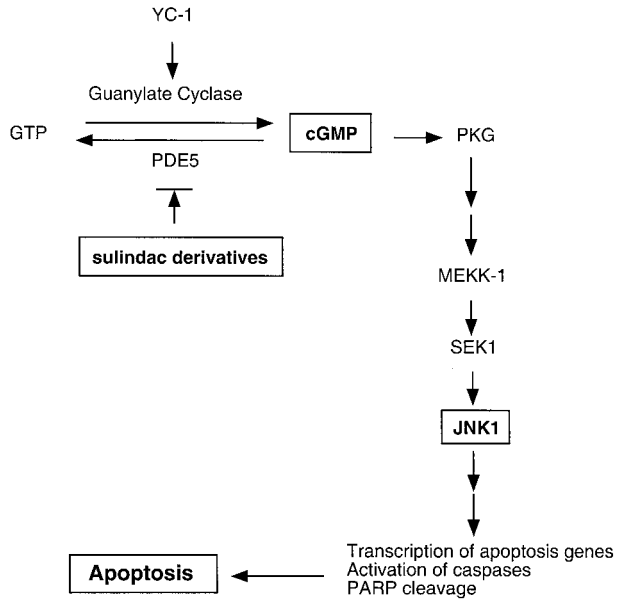

Fig. 5 Hypothetical scheme of the apoptotic signal transduction pathway activated by sulindac derivatives. Sulindac derivatives induce an increase in intracellular levels of cGMP through inhibition of PDE2/5. This activates PKG, which leads, through unknown mechanisms, to activation of the MEKK-1/SEK1/JNK1 pathway. Activation of JNK1 then plays a critical role, perhaps together with other signals, in the activation of caspases, PARP cleavage, and other events that mediate apoptosis. By activating guanylate cyclase, YC-1 mimics the effects of the sulindac derivatives.

thus resulting in loss of normal PARP function (28). This inactivation of PARP apparently prevents depletion of cellular levels of NAD and ATP, which are thought to be required for later events in apoptosis (28). As shown in Fig. 4*C*, CP248 induced significant PARP cleavage in the control cells, but this cleavage was markedly inhibited in the DN-JNK cells. Caspase-3 assays and morphological studies also confirmed that DN-JNK cells are more resistant to CP248-induced apoptosis (data not shown). SW480 cells that express a DN-MEKK1 were also more resistant to CP248-induced apoptosis when assayed for PARP cleavage and capsase-3 activity (data not shown). CP248 caused only weak activation of ERK2 and p38, and inhibitors of these kinases did not protect cells from CP248 induced apoptosis (data not shown). This weak activation of ERK2 and p38 by CP248 was not inhibited in the DN-JNK cells, thus indicating the specificity of the DN-JNK construct for JNK1 (data not shown). Therefore, the cGMP/PKG/JNK1 pathway plays a critical role in the apoptosis induced by this sulindac sulfone derivative in SW480 cells.

DISCUSSION

The above studies demonstrate that sulindac derivatives and other cGMP-inducing agents activate the JNK1 pathway of signal transduction and provide evidence that this pathway plays a critical role in the apoptosis induced by these compounds. A hypothetical scheme based on the present results is shown in Fig. 5. Sulindac sulfone, and the two potent derivatives CP248 and CP461, inhibit cGMP-specific PDE2/5 (Table 1), thus causing increased cellular levels of cGMP (8). This leads to activation of PKG, most likely PKG1α and 1β, but this remains to be determined. Further studies are required to determine whether PKG directly phosphorylates and thereby activates MEKK1 or another signaling molecule that is further upstream. In any case, the activation of PKG leads, within 30–60 min, to persistent phosphorylation and activation of SEK1 which, in turn, leads to rapid and persistent activation of JNK1. Presumably, the activation of JNK1 then leads to activation of caspases, the cleavage of PARP, and the transcription of genes that also contribute to the program of apoptosis, as described previously for other apoptotic agents that activate JNK1. Several investigators have reported that JNK1 is involved in apoptotic signaling pathways triggered by various agents, including UV and γ radiation (29), benzyl isothiocyanate (11), and the DNA topoisomerase inhibitor β-lapachone (15). Activated JNK1 activates the AP-1 transcription factor and thereby induces several genes involved in apoptosis (12). It also phosphorylates bcl-2 and thus inactivates its antiapoptotic activity (30–32). It seems likely that the activation of PKG also influences other pathways that may contribute to the growth-inhibitory and apoptotic effects of these sulindac derivatives, but this remains to be determined. Furthermore, our studies do not exclude the possibility that specific nonsteroidal anti-inflammatory drugs exert their antitumor effects through alternative mechanisms, *i.e.*, inhibition of Cox-2, increased production of ceramide, or inhibition of peroxisome proliferator-activated receptor δ (6, 7). Previous studies have implicated cGMP and PKG in the apoptotic process induced by nitric oxide (9, 10), and PKG has been implicated previously in induction of the c-*fos* gene (33). Our studies implicate PKG in the JNK1 pathway of signal transduction, thus expanding the role of PKG in signal transduction and the control of gene expression.

ACKNOWLEDGMENTS

We are grateful to Wang-Qui Xing for valuable technical assistance.

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Erratum

In the article by Soh *et al*., which appeared in the October, 2000, issue of *Clinical Cancer Research* (pp. 4136–4141), the concentrations of sulindac sulfide, sulindac sulfone, CP248, CP461, KT5720, and Rp-8-pCPT-cGMPS in the text and figures should always be "μM" instead of "mM." On page 4137, lines 4 and 5 should read "18 ml Tris-AcrylM column with a 0-1 M NaAc gradient" instead of "180 ml Tris-AcrylM column with a 0-1 M NaAc gradient."

Correction

In the article by U. Vaishampayan *et al.*, which appeared in the November, 2000 issue of *Clinical Cancer Research* (pp. 4205–4208), the first author's name should read "Ulka Vaishampayan," instead of "Uika Vaishampayan," and the sixth author's name should read "John Wright," instead of "Jeremy Wright."

Clinical Cancer Research

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Clin Cancer Res 2000;6:4136-4141.