Acrolein Causes Inhibitor κB-independent Decreases in Nuclear Factor κB Activation in Human Lung Adenocarcinoma (A549) Cells*

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Acrolein is a highly electrophilic α,β-unsaturated aldehyde to which humans are exposed in various situations. In the present study, the effects of sublethal doses of acrolein on nuclear factor κB (NF-κB) activation in A549 human lung adenocarcinoma cells were investigated. Immediately following a 30-min exposure to 45 fmol of acrolein/cell, glutathione (GSH) and DNA synthesis and NF-κB binding were reduced by more than 80%. All parameters returned to normal or supranormal levels by 8 h post-treatment. Pretreatment with acrolein completely blocked 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced activation of NF-κB. Cells treated for 1 h with 1 mM diethyl maleate (DEM) showed a 34 and 53% decrease in GSH and DNA synthesis, respectively. DEM also reduced NF-κB activation by 64% at 2 h post-treatment, with recovery to within 22% of control at 8 h. Both acrolein and DEM decreased NF-κB function by 50% at 2 h after treatment with TPA, as shown by a secreted alkaline phosphatase reporter assay. GSH returned to control levels by 8 h after DEM treatment, but proliferation remained significantly depressed for 24 h. Interestingly, DEM caused a profound decrease in NF-κB binding, even at doses as low as 0.125 mM that had little effect on GSH. Neither acrolein nor DEM had any effect on the levels of phosphorylated or nonphosphorylated inhibitor κB-α (IκB-α). Furthermore, acrolein decreased NF-κB activation in cells depleted of IκB-α by TPA stimulation in the presence of cycloheximide, demonstrating that the decrease in NF-κB activation was not the result of increased binding by the inhibitory protein. This conclusion was further supported by the finding that acrolein modified NF-κB in the cytosol prior to chemical dissociation from IκB with detergent. Together, these data support the conclusion that the inhibition of NF-κB activation by acrolein and DEM is IκB-independent. The mechanism appears to be related to direct modification of thiol groups in the NF-κB subunits.

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Acrolein, an α,β-unsaturated aldehyde, is a highly electrophilic (1), volatile liquid with a pungent and irritating odor. It is produced by a wide variety of both natural and synthetic processes including incomplete combustion or pyrolysis of organic materials such as fuels, wood, synthetic polymers, food, and tobacco. In addition, patients treated with the cytostatic agent cyclophosphamide are exposed to acrolein as a metabolite of the parent drug (2).

Extensive research has been done on the acute biochemical effects of acrolein. However, the effects of subacute exposures have been little studied, particularly at the molecular level. Recent work has shown that acrolein can inhibit cell proliferation at doses that do not cause lethality (3), and such information may have major significance in terms of signal transduction pathways as well as, perhaps, in the control of cell division and apoptosis. As a metabolite of cyclophosphamide, acrolein may also play a role in the unique antineoplastic efficacy of this drug through molecular effects associated with low acrolein doses.

Myriad adverse cellular effects are seen following exposure to acrolein, including growth inhibition, alterations in the levels of glutathione (GSH), protein sulfhydryls, and thiol-containing enzymes, and increased cell membrane permeability (4–8). The primary source of acrolein’s reactivity is its α,β-unsaturated carbon–carbon bond. This molecule will react via a Michael addition in the presence of a nucophile to form an alkylated adduct. Acrolein’s potential role as a carcinogen is based on the observation that it binds GSH (9) and nuclear chromatin (10) and can form a number of adducts with DNA (11–13).

Some researchers have suggested that acrolein’s antiproliferative effects may be the result of its binding to RNA polymerase, thereby serving as a transcriptional restraint (14). However, the fact that GSH appears to play some role in cell division (15, 16) raises the possibility that acrolein-mediated alterations in this tripeptide may also be an important factor. Our previous data (3, 17) demonstrated that inhibiting the proliferation of human lung adenocarcinoma A549 cells with acrolein correlated with acrolein-induced changes in GSH.

Although a cause-and-effect relationship between acrolein-induced changes in GSH and proliferation has not been shown, it is apparent that acrolein can alter redox-regulated cellular pathways. Nuclear factor-κB (NF-κB) is one of the most widely studied molecules affected by cellular redox status. It was first identified as a factor that activated the Ig κ-light chain intron enhancer during B-lymphocyte development (18). High levels of interest in this transcription factor are based on its broad role in coordinately controlling a number of genes including those encoding inflammatory cytokines, chemokines, interfer-
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The uptake of exogenous \(^{3}H\)-labeled thymidine was measured in cells treated with acrolein, DEM, or vehicle. Cells were pulsed for 2 h with 2.5 \(\mu\)Ci/ml \([^{3}H]\)thymidine before isolating the DNA (28). DNA was quantitated by fluorescence after treatment with ethidium bromide (29).

**Cell Counts**—Cell counts at the time of treatment (48 h post-seeding) were obtained using the CyQuant cell proliferation assay. This assay has a linear detection range of 50–50,000 cells/200 \(\mu\)l and is dependent on a green dye (CyQuant-GR) that fluoresces when bound to cellular nucleic acids. Cell monolayers were washed twice with phosphate-buffered saline, trypsinized, suspended in phosphate-buffered saline, and counted at 200 \(\times\) 10\(^4\). The supernatant was carefully removed and the cells frozen at \(-80^\circ\) C. At the time of the assay, cells were thawed at room temperature and lysed in buffer containing the CyQuant-GR dye prepared according to manufacturer’s instructions. Fluorescence was measured (excitation, 480 nm; emission, 520 nm) and compared with a standard curve for cell number determination.

**Total Glutathione Measurement**—Previous work in our laboratory showed that acrolein treatment does not significantly alter the level of glutathione disulfide (17). Therefore, only total glutathione (GSH + glutathione disulfide) was measured by HPLC (30) or enzymatically (31). Briefly, cells were seeded at 5000 cells/cm\(^2\) in six-well plates and treated with acrolein or DEM. Cell monolayers were washed twice with PBS and lysed with 1 ml of 20 mM EDTA followed by sonication for 1 min. Samples were analyzed using a modified Lowry assay (32) and 25 \(\mu\)l of the lysate were added to 25 \(\mu\)l of 25 mM NaH\(_2\)PO\(_4\), pH 7.0. Samples were then processed, derivatized with o-phenthaldehydes, and analyzed as described previously (17). For the enzymatic assay, 100 \(\mu\)l of cell lysate were combined with 600 \(\mu\)l of 0.2 \(\mu\)M KH\(_2\)PO\(_4\) and 5 mM EDTA (pH 7.4) and analyzed. Total protein in the lysates was determined (32) and compared with a bovine serum albumin standard curve.

**Electrophoretic Mobility Shift Assays**—Electrophoretic mobility shift assays were carried out after the method of Denison et al. (33) as modified by Bowes et al. (34). Briefly, cells were rinsed twice and lysed in ice-cold HEGD (25 mM HEPES, pH 7.6, 1.5 mM EDTA, 10\% glycerol, 1 mM dithiothreitol, 0.1 mg/ml phenylmethylsulfonyl fluoride, 0.75 mM aprotinin, 0.1 mM benzamidine, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benza...
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Table I

[\(^{3}H\)Thymidine incorporation

| Time (h) | 45 fmol of acrolein/cell | 1 mM DEM* |
|---------|-------------------------|-----------|
| 2       | 30 ± 16                 | 47 ± 1    |
| 4       | 47 ± 1                  | 69 ± 3    |
| 8       | 130 ± 2                | 78 ± 2    |
| 24      | 89 ± 1                 | 54 ± 2    |

*All values are significantly different from vehicle control (p < 0.05).

Results

Acrolein and DEM Reduce Proliferation in A549 Cells—Six-well plates were seeded at 5000 cells/cm² in six-well plates and incubated for 48 h at 37 °C in 5% CO₂ before treatment. Following treatment with 45 fmol of acrolein/cell (30 min) or 1 mM DEM (1 h), cells were incubated for 24 h with medium containing 2.5 μCi/ml [\(^{3}H\)thymidine before harvesting. Data are expressed as the mean percent of [\(^{3}H\)thymidine incorporation relative to vehicle-treated cells ± S.E. (n = 3).

Acrolein and DEM Attenuate NF-κB Activation—Treating A549 cells with 35 fmol of acrolein/cell caused a significant decrease in NF-κB activation relative to TPA-treated or serum-deprived controls after as little as a 5-min exposure. This binding inhibition increased with the time of exposure. However, NF-κB activation in serum-deprived, vehicle-treated cells also began to decline at 2 h post-treatment (Fig. 2). To minimize the effects of serum deprivation, 30-min acrolein treatments were selected for further studies. With this length of treatment, both constitutive and TPA-stimulated NF-κB activation were inhibited by acrolein (Fig. 2). Nonspecific binding was evident in this gel but did not correlate with either time or acrolein treatment.

A time-response study of NF-κB activation in which cells were treated with 45 fmol of acrolein/cell for 30 min (Fig. 3) showed inhibition and recovery patterns very much like those seen when examining changes in DNA synthesis (Table I) and total GSH (Fig. 1). Acrolein caused a dramatic decline in NF-κB binding at 30 min and at 2 h post-treatment. Some recovery of NF-κB activation was evident at 4 h, and the inhibitory effect of 30 min of acrolein treatment was fully reversed by 8 h post-treatment (Fig. 3).

DEM also caused a decrease in NF-κB activation. There was a clear dose-response relationship with NF-κB binding increasingly reduced after 1-h exposures to DEM doses from 0.125 to 2 mM (Fig. 4). Interestingly, the inhibition seen in NF-κB activation with 0.125 mM DEM was profound, yet little or no change in total GSH was evident at this dose (data not shown). Treating cells with 3.33 pmol of DEM/cell (1 mM) for 1 h resulted in a NF-κB activation time response (Fig. 5) that was almost identical to that obtained following acrolein exposure (Fig. 3), and again paralleled changes in GSH (Fig. 1). NF-κB binding decreased dramatically at 1–2 h post-treatment and showed a recovery to near normal binding by 8–12 h. Nonspecific binding was more intense in this gel, but again did not correlate with either time or DEM treatment.

The SEAP reporter assay confirmed that both acrolein and DEM diminished the transcriptional activity of NF-κB. Two h after adding TPA to cells pretreated for 30 min with either 45 fmol/cell acrolein or 1 mM DEM, SEAP activity was decreased by 51 and 45%, respectively.

Role of IκB in Reduced NF-κB Binding—Changes in NF-κB activation are generally controlled by IκB. In a number of different experiments, there were no consistent changes in the levels of IκB-α up to 2 h after cells were treated for 30 min with...
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45 fmol of acrolein/cell or for 1 h with 3.33 pmol of DEM/cell (Fig. 6, A and B). To further examine this phenomenon, changes in the level of phosphorylated IkB-α were examined following treatment with acrolein. Once again, no changes in the levels of this protein were observed (Fig. 6C), suggesting that acrolein blocks NF-κB activation by an IkB-independent mechanism.

A more thorough analysis of the possibility of IkB-independent changes in NF-κB activation involved examining the degradation of IkB following stimulation with TPA, a tumor promoter that up-regulates protein kinase C. Treatment with 100 ng/ml TPA caused a temporary decrease in the levels of IkB (Fig. 7A). By stimulating cells with TPA in the presence of the protein synthesis inhibitor cycloheximide (CHX), IkB levels were almost completely abrogated at 2 h post-treatment (Fig. 7B). NF-κB activation was also checked at this time to ensure that the treatment with CHX had not affected NF-κB binding. Stimulating cells with TPA in the presence of CHX resulted in maximum NF-κB activation at 2 h post-treatment (Fig. 8), the same time that IkB levels were at their nadir. Finally, cells that had been stimulated with TPA for 1.5 h in the presence of CHX were treated with 45 fmol of acrolein/cell for 30 min. Under these conditions, acrolein still caused a 63% decline in NF-κB activation (Fig. 9), indicating that the effect of acrolein was independent of IkB.

**Cytosolic versus Nuclear Effects**—To determine whether acrolein blocked NF-κB activation by acting in the cytoplasm or in the nucleus, cytosolic extracts were obtained from cells pre-treated for 30 min with 45 fmol/cell acrolein or vehicle. Treatment of vehicle extracts with deoxycholate dissociated NF-κB from IkB, yielding binding to the consensus sequence (Fig. 10, lane 4). In contrast, extracts from acrolein-treated cells exhibited greatly diminished binding (Fig. 10, lane 5), suggesting that cytosolic binding occurs. Acrolein may also bind to NF-κB in the nucleus as shown by an experiment in which cells were first treated with TPA to stimulate the nuclear translocation of NF-κB followed by treatment with either acrolein or DEM. This experiment revealed that both acrolein and DEM decreased NF-κB activation under these conditions (data not shown), suggesting that both can act within the nucleus.

Analysis of the inhibition of activation of NF-κB was studied...
further by treating the nuclear extracts of acrolein- and DEM-treated cells with either 100 μM GSH or 1% b-mercaptoethanol (b-ME) in vitro to determine whether the inhibitory effects were reversible. These data show that neither GSH nor b-ME restores NF-kB binding in cells treated with acrolein or DEM (Fig. 11). Pretreatment with TPA before exposing the cells to acrolein or DEM had no effect on the ability of GSH or b-ME to restore binding. Interestingly, the nuclear extracts from untreated control cells showed a 24% increase in NF-kB binding following the addition of b-ME but not GSH.

**DISCUSSION**

The data presented here provide several avenues of support for the hypothesis that both acrolein and DEM inhibit NF-kB
activation by an IκB-independent mechanism. Most importantly, acrolein and DEM block NF-κB binding without altering the cellular levels of either the phosphorylated or nonphosphorylated forms of IκB-α. Acrolein and DEM also block binding in the absence of IκB making it clear that they must be interfering with the activation of NF-κB by some mechanism other than modulation of IκB. Two possible mechanisms exist: the direct inactivation of NF-κB; or the scavenging of nuclear reducing equivalents required for NF-κB binding, such as GSH, thereby lowering its binding affinity for DNA. This latter mechanism is unlikely, however, because the effect of DEM occurred at doses that did not alter GSH. Further, in cells treated with acrolein or DEM alone, or when treatment followed TPA stimulation, NF-κB activation could not be restored by the addition of either GSH or β-ME. An interesting observation was that β-ME increased NF-κB binding in untreated control cells. The nuclear redox status could be such that some molecules of NF-κB are not in the reduced form necessary to facilitate DNA binding. The addition of a strong reducing agent in vitro may have forced the reduction of this pool of NF-κB, resulting in the observed increase in binding.

Acrolein-mediated modifications of NF-κB can occur in the cytosol of intact cells as shown by the inability of detergents to release an active form following acrolein treatment. This finding also indicates that the site of modification is not blocked by IκB, matching previous data from studies with other thiol reactive agents (37, 38). NF-κB-like transcription factors belong to the Rel protein family and have a highly conserved N-terminal region that is critical for DNA binding. Within this region, the Cys62 residue on the p50 subunit and the Cys38 residue on the p65 subunit are essential for DNA binding (26, 39, 40). Thiols present in these regions may form conjugates with acrolein via a Michael addition. A mechanism of this type has been described previously when various compounds directly reacted with the p50 subunit to inhibit NF-κB binding (38). In a similar way, acrolein inhibits the DNA repair enzyme O6-alkylguanine-DNA alkyltransferase by acting at an acrolein-sensitive thiol residue required for the catalytic activity of the enzyme (41).

The translocated NF-κB must have a reduced environment if it is to bind DNA (26, 37, 42). Therefore, in addition to forming conjugates with NF-κB, acrolein may inhibit binding by depleting nuclear GSH. In support of this mechanism, we observed a strong correlation between the time necessary to recover intracellular GSH and the time it takes for NF-κB binding to return to normal following an acrolein insult. However, this seems unlikely to be the sole mechanism based on the inability of deoxycholate to activate NF-κB after acrolein.

Most if not all agents activating NF-κB tend to trigger the formation of reactive oxygen species or are oxidants by themselves (43). Despite the increased NF-κB activation seen in the presence of oxidants, studies in two human T-cell lines have shown that a reduction in GSH by buthionine sulfoximine inhibits the activation and translocation of NF-κB (25, 44). It was also found that the addition of cysteine caused a reversible oxidation of NF-κB (25), presumably by raising intracellular glutathione disulfide levels. These data may point to the existence of a critical intracellular redox balance. Increasing this reactive oxygen species pool in the cytosol appears to activate NF-κB, whereas increasing it in the nucleus blocks NF-κB binding, which may enhance apoptosis (45, 46). In general, acrolein appears to affect NF-κB binding directly by conjugating its protein subunits and indirectly by alkylating GSH, thereby altering the nuclear redox balance.

In the same way that acrolein affects NF-κB, our data suggest that DEM decreases activation both directly and indirectly. The fact that DEM blocks NF-κB binding in a manner not reversible with either GSH or β-ME indicates that it has direct interactions with the NF-κB subunits. Furthermore, because DEM is known to conjugate GSH through the actions of glutathione S-transferase, it may act on NF-κB indirectly by lowering the nuclear levels of GSH.

The key features of NF-κB transcriptional control are that it is fast, versatile, and involved in many different gene systems (47). Of particular interest to the current study is the involvement of NF-κB with genes that regulate cell proliferation. The importance of NF-κB subunits in cell proliferation is suggested by several studies. Snapper et al. (48) showed that B cells from p50−/− knockout mice proliferated normally in response to some stimuli but showed no response to other stimuli that were mitogenic in control cells. Mice lacking the p50/p105 subunits developed normally but exhibited defects in immune responses involving B cells (49). A more critical role seems to belong to the p65 subunit. Vascular smooth muscle cells treated with p65 antisense oligonucleotides showed a concentration-dependent inhibition of both adherence and proliferation (50). Even more dramatic is the report that p65 (Rel A) null mice exhibited a dramatic phenotype-embryonic lethality, apparently because of widespread apoptosis in the liver (51). This function in the regulation of cell growth may be mediated through c-myc, which is known to have two NF-κB sites in its promoter/enhancer region (52). Furthermore, NF-κB is implicated in the transcriptional regulation of the p53 gene (53).

In the current study, treating cells with 45 fmol of acrolein/cell for 30 min caused an 85% decline in DNA synthesis, which corresponded to a 55% decrease in NF-κB binding and an 87% loss of GSH. In all instances, the measured parameters returned to normal levels or higher by 8 h post-treatment. Although electrophoretic mobility shift assay data are only semi-quantitative, the correlation between acrolein-mediated reductions in NF-κB activation and DNA synthesis support a functional link. It may also be that acrolein inhibits growth protein(s) in a manner similar to the proposed inhibition of NF-κB activation, i.e. by conjugation with thiol residue(s) critical to their function.

Results from DEM studies support the role of NF-κB in mediating changes in DNA synthesis. Although DEM-treated cells posted a full recovery of GSH by 8 h post-treatment, the 8-h levels of DNA synthesis and NF-κB activation reached only
77 and 74% of the level in vehicle-treated cells, respectively. However, NF-κB activation in DEM-treated cells did return to normal by 12 h post-treatment, whereas DNA synthesis remained suppressed to 24 h.

In conclusion, acrolein causes a dramatic decline in NF-κB binding by an IκB-independent mechanism, which most likely involves alkylation of critical thiol sites within the DNA binding domain of the NF-κB subunits. Identification of acrolein/DEM-p50 and/or acrolein/DEM-p65 conjugates will provide conclusive evidence of the mechanism by which these chemicals alter NF-κB binding. It is apparent that GSH is intimately involved with NF-κB activation and that both GSH and NF-κB play a role in cell growth and apoptosis. The observed attenuation of DNA synthesis following acrolein insult is probably the downstream result of the effects of acrolein on GSH and NF-κB. In addition, NF-κB is involved in regulating the expression of several growth-related genes. As a result, the effects of acrolein on these factors could have unrecognized toxic consequences, including a role in the effectiveness of the anticancer drug cyclophosphamide.

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