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Review

Pharmacodynamics and Toxicodynamics of Drug Action: Signaling in Cell Survival and Cell Death[1]

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In therapeutic response to drugs, the plasma concentration range leads to the establishment of a safe and effective dosage regimen. Our hypothesis is that by studying drug concentration-dependent effect on signal transduction mechanisms, a better understanding of the beneficial pharmacodynamic and adverse toxicodynamic responses elicited by the drug may be achieved. Using two classes of chemopreventive compounds (phenolic antioxidants and isothiocyanates), we illustrate the potential utility of two signal transduction pathways elicited by these agents to predict the pharmacodynamic effect (induction of Phase II drug metabolizing enzymes) and the potential toxicodynamic response (stimulation of caspase activity and cytotoxic cell death). At lower concentration, phenolic antioxidants and isothiocyanates activate mitogen-activated protein kinase (MAPK; extracellular signal-regulated protein kinase 2, ERK2; and c-Jun N-terminal kinase 1, JNK1) in a concentration-and time-dependent manner. The activation of MAPK by these compounds may lead to the induction of cell survival/protection genes such as *c-jun, c-fos*, or Phase II drug metabolizing enzymes. However, at higher concentrations, these agents activate another signaling molecule, ICE/Ced3 cysteine protease enzymes (caspases) leading to apoptotic cell death. The activation of these pathways may dictate the fate of the cells/tissues upon exposure to drugs or chemicals. At lower concentrations, these compounds activate MAPK leading to the induction of Phase II genes, which may protect the cells/tissues against toxic insults and therefore may enhance cell survival. On the other hand, at higher concentrations, these agents may activate the caspases, which may lead to apoptotic cell death, and have toxicity. Understanding the activation of these and other signal transduction events elicited by various drugs and chemicals may yield insights into the regulation of gene expression of drug metabolizing enzymes and cytotoxicity. Thus, the study of signaling events in cell survival (hemeostasis) and cell death (cytotoxicity) may have practical application during pharmaceutical drug development.

KEY WORDS: MAPK; caspases; chemopreventive agents; phase II drug metabolizing enzymes; apoptosis.

INTRODUCTION

In therapeutic response to and adverse effect of drugs, the plasma concentration range or the therapeutic window leads to the establishment of a safe and effective dosage regimen. Therapeutic windows defined whether there is null, beneficial, or toxic effects of the drugs. Upper limit of the plasma concentration may be either a decrease in the effectiveness without noticeable signs of increasing toxicity or the possibility of severe toxicity. The hypothesis is that by studying the drug concentration-effect on the signal transduction mechanisms, a better understanding of the beneficial pharmacodynamic and adverse toxicodynamic responses elicited by the drug may be achieved. Using two classes of chemopreventive compounds, phenolic antioxidants and isothiocyanates, illustrate the potential utility of two signal transduction pathways elicited by both agents to predict the pharmacodynamic effect (induction of drug metabolizing enzymes) and the potential toxicodynamic response (stimulation of caspase activity and cytotoxic cell death).

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ABBREVIATIONS: BHA, 2(3)-*tert*-butyl-4-hydroxyanisole; BHQ, *tert*-butyl-hydroquinone; GTP, green tea polyphenols; PEITC, phenethyl isothiocyanate; SUL, sulforaphane; TAM, tamoxifen; DES, diethylstilbestrol; QUI, quinacrine; BaP, benzo[a]pyrene; MAPK, mitogen-activated protein kinase; ERK2, extracellular signal-regulated protein kinase 2; JNK1, c-jun N-terminal kinase 1; SAPK, stress-activated protein kinase; MAPKKK, MAP kinase kinase kinase; PKC, protein kinase C; Hog1, high osmolarity glycerol response-1; CSBP, cytokine-suppressive anti-inflammatory drug-binding protein; RK, reactivating kinase; ICE/Ced-3; Interleukin-1β (IL-1β) Converting Enzyme/Caernohabditis elegans ced-3 gene; NAC, N-acetyl-L-cysteine; GSH, glutathione; V-E, vitamin E; ROS, reactive oxygen species; DCF-DA, 2′,7′-dichlorofluorescin-diacetate; DAPI, Diamidino-2-phenylindole; PARP, poly-ADP-ribopolymerases; CYP, cytochrome P450; GST, glutathione S-transferases; NQO, DT-diaphorase or NAD(P)H:quinone oxidoreductase or NAD(P)H: menadione reductase; NAT, N-acetyl-transferease; EPH, epoxide hydrolases; ST, sulfotransferases; and UGT, UDP-glucuronosyltransferases; ; HO, heme oxygenase; ARE/EpRE, antioxidant response element/electrophile response element; XRE/AhRE, xenobiotics response element/aromatic hydrocarbon response element; TPA; 12-*O*-tetradecanoate 13-acetate.

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During pharmaceutical drug development, many potential drug candidates are "killed" either because of unforeseen toxicity when entering clinical trials, and/or the possibility of severe clinical drug-drug interactions which impede further clinical drug development. For many of these agents, in addition to their specific receptor/enzyme interaction which may be their primary pharmacological effects, they may also induce signal transduction events either specifically or non-specifically leading to various cellular responses including homeostasis, proliferation, differentiation, apoptosis, or necrosis. Of particular interest to pharmaceutical scientists during drug development are the ability of the potential drug candidates to induce various Phase I and/or Phase II drug metabolizing enzymes. Phase I drug metabolizing enzymes primarily consist of the cytochrome P450 (CYP) superfamily, which compose of families and subfamilies of enzymes that are defined on the basis of their amino acid sequence similarities (1,2). Of the 36 gene families described to date, 12 families exist in all mammals which comprise 22 subfamilies (22). In humans, three CYP gene families (i.e., CYP1, CYP2, and CYP3) are thought to play important role in hepatic drug metabolism and pharmacokinetic disposition of drugs.

Phase II drug metabolizing or conjugating enzymes, consisting of many superfamily of enzymes including glutathione S-transferases (GST) (3), DT-diaphorase or NAD(P)H:quinone oxidoreductase (NQO) or NAD(P)H: menadione reductase (NMO) (4), *N*-acetyltransferases (NAT) (5), epoxide hydrolases (EPH) (6), sulfotransferases (SULT) (7), and UDP-glucuronosyltransferases (UGT) (8). In particular, the UGT and SULT, which catalyze glucuronidation and sulfation, play important roles in the excretion and elimination of drugs that contain hydroxyl (OH) functional group either present on the parent molecules and/or after biotransformation by the Phase I enzymes such as the CYP. Hence, regulation of gene expression of various Phase I and Phase II drug metabolizing enzymes have significant impact in the metabolism, pharmacokinetics, toxicodynamics, and drug-drug interactions of many therapeutic drugs.

The molecular signaling mechanisms leading to the transcriptional activation of various Phase I CYP drug metabolizing enzymes (1,2) such as CYP1 (9), CYP2 (10), CYP3 (1,11) and CYP4 (12,13) have been well characterized. However, the molecular signaling events leading to the transcription activation and subsequent induction of some Phase II drug metabolizing enzymes have remained unclear. This is in part due to the diversity of Phase II drug metabolizing enzymes consisting of many superfamilies of enzymes as described above. Secondly, diverse chemicals with seemingly unrelated chemical structures, both naturally occurring and synthetic, which include flavonoids, diphenols/phenolic antioxidants, organic isothiocyanates, diterpenes, indoles, unsaturated lactones, thiocarbamates, barbiturates, planar aromatic hydrocarbons (PAHs), phorbol esters (e.g., 12-*O*-tetradecanoate 13-acetate; TPA), and electrophilic compounds, were found to induce certain Phase II genes expression (3,4,14,15). Further studies of these Phase II genes revealed existence of *cis*-acting regulatory elements, such as the antioxidant response element (ARE)/electrophile response element (EpRE), xenobiotic-responsive element (XRE)/aromatic hydrocarbon responsive element (AhRE), activator protein-1 (AP-1), and nuclear factor-kappa B (NF-κB) in their 5′-flanking regulatory region (3,4,14–17). Recent findings from several laboratories suggest the increasingly important role of the ARE/EpRE in the regulation of expression of many Phase II genes by phenolic antioxidants (3,4,14,15,18,19). Future cloning of ARE-binding proteins (BPs) will clarify the potential transcription factors. In addition, previously, little was known about the upstream signal transduction events leading to the activation of these transcription factors in response to phenolic antioxidants and/or other Phase II gene inducers. Recently, data from our laboratory (20–22) as well as from others (23) provided the first evidence that the mitogen-activated protein kinases (MAPKs) may be implicated as the upstream signal transduction events leading to the activation of ARE/EpRE in response to phenolic antioxidants and/or other Phase II gene inducers.

MITOGEN-ACTIVATED PROTEIN KINASES (MAPKs)

Mitogen-activated protein kinases (MAPKs), characterized as proline-directed serine/threonine kinases (24–26), are important cellular signaling components which convert various extracellular signals into intracellular responses through serial phosphorylation cascades (27). At the present time, there are at least three distinct but parallel MAPK cascades (ERK, JNK, and p38) have been identified in mammalian cells as shown in Fig. 1 (28,29). Each cascade consists of a module of three kinases: a MAPK kinase kinase (MAPKKK), which phosphorylates and activates a MAPK kinase (MAPKK), which, in turn, phosphorylates and activates a MAPK. The best characterized MAPK pathway is a Ras-dependent activation of extracellular signal-regulated protein kinases (ERKs) in response to many growth factors and cytokines. In this pathway, tyrosine-phosphorylated transmembrane receptors associate with the src-homology-2 (SH2) domain of the adapter protein Grb2 (30)

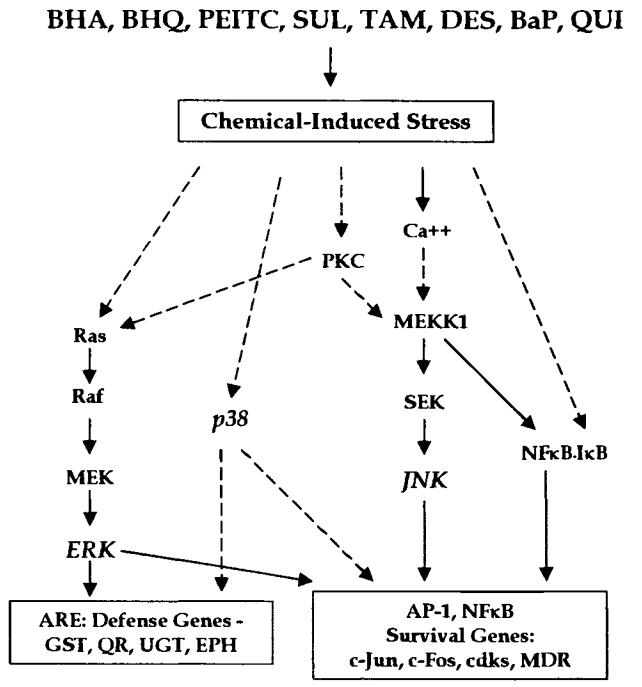

Fig. 1. Schematic representation of chemical-induced stress response leading to activation of the MAPK pathway ERK, JNK, and p38, and the NFκB pathway, which results in gene expression, and potentially cell survival.

and target nucleotide exchange factor SOS to the membrane-bound small G-protein Ras (31). Activated Ras will recruit Raf-1 (a MAPKKK) to the membrane, leading to the activation of Raf-1 (32). Once activated, Raf-1 will then phosphorylate and activate a dual specificity kinase MEK (a MAPKK), which, in turn, will activate ERK (a MAPK). In addition to the tyrosine kinase receptors, certain G-protein-coupled receptors and protein kinase C (PKC) are also able to activate the ERK cascade (33). Another MAPK group is c-Jun N-terminal kinase (JNK), which is governed by a parallel signaling module, consisting of MEKK1-MKK4 (SEK1, JNKK)-JNK (34,35). However, the JNK cascade, unlike the ERK pathway, is only modestly activated by growth factors and phorbol esters, and instead is strongly activated by stress signals including inflammatory cytokines (36), DNA damaging agents (37), protein synthesis inhibitors (38), ceramide (39), UV light (40), γ-radiation (41), and chemopreventive agents (20). Therefore, JNK is also known as the stress-activated protein kinase (SAPK). A third parallel signaling module is called the p38 MAPK. p38 MAPK was originally identified in endotoxic lipopolysaccharide (LPS)-treated murine monocytes and macrophases as a homologue of a yeast gene, the high osmolarity glycerol response-1 (Hog1). (42,43). Independently, the p38 MAPK was identified in human cells as a cytokine-supppressive anti-inflammatory drug-binding protein (CSBP) (44) or as a reactivating kinase (RK) (45,46). Recently, other new members of p38 subfamily have been cloned (47). p38 is activated by phosphorylation on threonyl and tyrosyl residues within the tripeptide motif TGY by dual-specificity kinases, MKK3 (48) and MKK6 (49), which, in turn, are regulated by the upstream MAP kinase kinase kinases (MEKKs) and small GTP-binding proteins, Rac1 and Cdc42 . Once activated, MAPKs can phosphorylate many transcription factors, such as c-Myc, p62TCF/Elk-1, c-Jun, ATF2, CHOP/GADD153, MEF2C, and SAP-1, and ultimately leading to the changes in gene expression (24,50). Given the fact that MAPKs are activated by such a wide range of factors, these signaling cascades may serve as common mechanisms and integrate with other signaling pathways to control gene expression, hemeostasis, proliferation, differentiation, cell cycle arrest, or apoptosis, in response to various extracellular stimuli, including chemopreventive agents and therapeutic drugs.

PHENOLIC COMPOUNDS ACTIVATE MAPK PATHWAY AND INDUCE PHASE II GENE EXPRESSION

When mammalian cells exposed to various agents such as BHA, BHQ, phenethyl isothiocyanate (PEITC), sulfarophane, tamoxifen, or green tea polyphenols (GTP), expression of various genes including immediate early genes, *c-jun, c-fos*, or Phase II drug metabolizing enzymes such as GST, NQO, and UGT were induced (17,51–60). The signal transduction mechanisms elicited by these chemical agents leading to the induction of genes are not well understood previously. Recently the effects of various chemicals including phenolic antioxidants such as GTP (21), BHA and BHQ (22), the isothiocyanates such as PEITC (20), and sulforaphane (61), environmental pollutant (benzo[a]pyrene, BaP) (62), various chemotherapeutic agents such as adriamycin (37), tamoxifen (63,64), and microtubule agents such as paclitaxel (taxol), colchicine, vincristine, nocodazole (65), anti-malarial drug quinacrine (QUI) (66), and PKC-inhibitor chelerythrine (67) on the activation of MAPK pathway were studied. Exposure of human hepatoma HepG2 cells to GTP (10 to 100 μg/ml) activated JNK1 activity in a concentration- and time-dependent manner. Activation of JNK activity was seen at 30 min, peaked at 120 min, and sustained up to 6 h. Whereas, activation of ERK2 activity by GTP occurred only at higher concentrations and earlier kinetics (21). These results implied that activation of ERK2 and JNK1 activities by GTP may occur by separate mechanisms. One of the biological consequences of the activation of MAPK activities by external stimuli such as GTP led to the induction of the mRNA expression of immediate early genes such as *c-jun*, and *c-fos*, as well as transcriptionally activated the ARE/EpRE chloramphenicol acetyltransferase (CAT) reporter gene (21). ARE/EpRE is present in at least sixteen different genes identifiable in the GeneBank (68) including many Phase II drug metabolizing enzymes, GST, NQO, (52–54) as well as genes encoding for the transcriptional factor such as hepatocyte nuclear factor 3/fork head homolog 11 (69).

When BHA was added, it showed human cervical carcinoma HeLa cell line demonstrated rapid but transient activation of ERK2 activity whereas a more delayed and sustained JNK1 activity (similar to that induced by GTP) (22). BHQ, the metabolite of BHA, also activated ERK2 but weakly stimulated JNK1 activity. Furthermore, activation of ERK2 by BHQ was late and prolonged, showing kinetics different from that stimulated by BHA. This suggests that the signaling mechanism elicited by BHA may not be dependent on its metabolite BHQ *via* metabolic demethylation. ERK2 activation by BHA and BHQ required the involvement of an upstream signaling kinase MEK, as evidenced by the inhibitory effect of a MEK inhibitor, PD98059. Pretreatment with *N*-acetyl-L-cysteine (NAC), glutathione (GSH), and vitamin E, inhibited ERK2 activation by BHA and BHQ, but JNK1 activation was inhibited to a much lesser extent. These results again suggested that differential mechanisms were involved in the activation of ERK2 and JNK1 activities by BHA and BHQ, and that the activation of ERK2 by BHA and BHQ may involve phenoxyl radicals and/or their metabolites (22).

Our recent studies on the activation of p38 MAPK by the phenolic antioxidants BHA/BHQ showed that p38 activity was stimulated by BHA and BHQ in a dose-and time-dependent fashion, similar to the stimulation of ERK and JNK activities by these agents (70). However, interestingly, the roles of ERK2, JNK and p38 MAPK in the transcriptional activation of an ARE-luciferase (LUC) reporter gene assay showed differential effects. Using genetic cDNA mutants and biochemical inhibitor methods, ERK2 was found to activate ARE-LUC; JNK1 seemed to have no effect on ARE-LUC induced by BHQ; whereas p38 was found to negatively regulate ARE-LUC induced by BHQ (61,70). These results suggest that the coordinate modulation of MAPK cascades may be critical in the regulation of Phase II genes through the ARE/EpRE induced by various xenobiotics.

ISOTHIOCYANATES ACTIVATE MAPK PATHWAY AND INDUCE PHASE II GENE EXPRESSION

Similar to the phenolic agents described above, exposure of cells to various natural or synthetic chemopreventive isothiocyanates such as PEITC, and sulfarophane, induce expression of various Phase II drug metabolizing enzymes such as GST, NQO, and UGT (54,55,58,59). We have recently shown that

various isothiocyanates including PEITC (20) and sulfarophane (61) activated the MAPK pathway. However, there were differences in the activation of MAPK by PEITC and sulforaphane in HepG2 cells. PEITC stimulated both JNK and ERK activities, whereas sulforaphane stimulated only the ERK activity but inhibited both basal and UV-stimulated JNK activity, suggesting that chemical structure may play an important determinant in the activation of MAPK. Similar to BHQ described above, transfection of a mutant ERK2 cDNA into HepG2 cells, attenuated transcriptional activation of ARE-LUC and Phase II NQO enzyme induction induced by sulforaphane, implicating that the ERK2 pathway probably plays a pivotal role in Phase II gene induction by the isothiocyanates such as sulforaphane (61).

CYTOTOXICITY AND CELL DEATH

The biological process of cell death will ultimately lead to the cessation of all cellular and biological activities. It is generally believed that apoptosis and necrosis are two distinct and mutually exclusive modes of cell death (71,72). Apoptosis, frequently referred to as "programmed cell death", is an active and physiological mode of cell death. It was first reported by Kerr *et al.* in 1972 (73), and the pharmacodynamics of apoptosis has been reviewed recently by Au *et al.* (74). Apoptosis plays many important roles in a variety of biological processes and diseases. Many growth factors prevent apoptosis, whereas the inflammatory cytokines such as tumor necrosis factor α (TNF-α), Fas (CD95), or TRAIL (Apo2L) induce apoptosis (75). In addition to these endogenous regulators of apoptosis, many environmental stresses also cause apoptosis. Recent studies have shown that oxidative stress, calcium, ATP, and mitochondria membrane potential can induce apoptosis (reviewed in (76)). In spite of the diversity of apoptosis-inducing agents, numerous studies indicate that signals leading to the activation of a family of intracellular cysteine proteases, the caspases, may play a pivotal role in the initiation and execution of apoptosis induced by various stimuli (75–77). To date, at least fourteen different members of caspases in mammalian cells have been identified (77,78). Among the best characterized caspases is ICE, or caspase-1, which was originally identified as a cysteine protease responsible for the processing of interleukin-1β (79). Crystal structure of caspase-1 indicates that catalytically active form is a tetramer consisting of $(p20)_2/(p10)_2$, which are derived from the cleavage of a 45 kDa proenzyme (p45), presumably by autocatalysis (80,81). Overexpression of caspase-1 induces apoptosis in fibroblasts and potentiates Fas/APO-1-induced apoptosis (82). However, caspase-1-knockout mice do not show any apparent defects in apoptosis (83). Another emerging apoptotic protease is caspase-3, previously described as CPP32/Yama/Apopain, which shares even higher homology with Ced-3, the death protease of *C.elegans*, than does ICE (84–86). Analogans to caspase-1, caspase-3 is synthesized as an inactive p32 proenzyme that requires proteolytic activation. Unlike caspase-1, caspase-3 does not appear to undergo autocatalytic cleavage and/or activation. This implies that activation of caspase-3 may involve other upstream aspartate-specific protease(s). Indeed, recently, it was shown that caspase-3 can be activated by caspase-8 (via the death receptors, DRs pathway) and/or caspase-9 (through the mitochondria-cytochrome C pathway) (76,77). Once activated, caspase-3 can cleave a variety of substrates, including poly (ADP-ribose) polymerase (PARP) (85), DNA-dependent protein kinase (DNA-PK) (87), DNA fragmentation factor (DFF; (88)), 70-kDa subunit of the U1 small ribonucleoprotein (89), GDP dissociation inhibitor for the Ras-related Rho family GTPases (D4-GDI; (90)), and protein kinase C θ (91). Most recently, using gene knock-out technology, Hakem *et al.* (92) had identified at least four different apoptotic pathways present in mammalian cells; caspase-3-dependent, caspase-9 dependent, caspase-3/9-dependent, and caspase3/9-independent.

CASPASES, JNK, AND APOPTOSIS

A number of studies suggest that JNKs may be important regulators in apoptosis. Since JNKs are strongly and preferentially activated by many stress stimuli, this signaling pathway, as one of the stress response, might be functionally involved in cell survival and/or apoptosis (24). In cultured neurons, apoptosis induced by withdrawal of nerve growth factor (NGF) may require JNK activation (93). Similar results were also seen with gamma radiation and UVC (40). Overexpression of MEKK, the JNK kinase kinase, had a lethal effect on fibroblasts (94). Many studied suggested that both JNK and ICE-Ced-3 proteases are important regulators of apoptosis, the relationship between these two pathways is still not very clear. In human Jurkat cells, JNK stimulation appears to be upstream of caspaces in apoptosis induced by UV and gamma radiation (40). However, in U937 cells, JNK1 activation stimulates leads to caspace activation and apoptosis (95). However, Fas-mediated JNK activation is a downstream event of caspace activation (40,96). Most recently, it was shown that JNK cascade played a role in stress-induced apoptosis in Jurkat cells by up-regulating Fas ligand expression (97). Thus, the interaction between JNK and caspase in regulating apoptosis remains unclear, probably dependent on the type of cells and stimuli.

ACTIVATION OF ICE/CED-3 PROTEASES (CASPASES) LEADING TO APOPTOSIS

In human hepatoma HepG2 cells, at low concentrations BHA activated both ERK2 and JNK1, whereas BHQ activated only ERK2 (22). At these concentrations, the survival of HepG2 cells was not affected by either BHA or BHQ, but at concentrations above 100 μM, both BHA and BHQ began to induce cell death (22). The inhibitory concentration (IC_{50} of cell death) for BHA was higher than BHQ in HepG2 as well as in HeLa cells. These results were similar to that reported by Choi *et al.* (51), that at low concentrations (100 μM), BHA induced *c-jun* and *c-fos* mRNA expression, and reached maximum induction at 150 μM in HepG2 cells. However, cytotoxic effects were seen at concentrations above 150 μM. Questions remain how these phenolic antioxidants, such as BHA and BHQ, induce cell death at higher concentrations, which might be associated with the toxicity observed in animals (98,99). To address this cytotoxicity issue, we have studied the activation of the cell death protease proteins caspases, particularly caspase-1 (ICE or ICE-like) and caspase-3 (CPP-32 or CPP-32-like) families, by BHA and BHQ. Treatment of HeLa cells with BHA (250 to 500 μM) activated caspase-3 activity in a time- and concentration-dependent fashion, with maximum activation of about 20 fold as compared to the control untreated cells (100). Nuclei staining of the cells with diamidino-2-phenylindole (DAPI) as described

previously (62,101), apoptotic morphologies such as chromatin condensation, and cell surface membrane blebbing were observed, indicating that most of the cells died *via* apoptosis. When BHA concentrations increased above 750 μM, extensive necrosis occurred very rapidly within 2 hr, as shown by trypan blue staining (100).

The effects of various isothiocyanates on the activation of MAPK (20) and caspase (101) pathways in HeLa cells were studied. Exposure of HeLa cells to PEITC (5–10 μM; in phenol red-free medium) potently activated JNK1 in a time- and concentration-dependent manner. (Previous studies were conducted in phenol-red medium, and higher concentrations of PEITC were required to activate JNK activity (20)). At similar concentrations, PEITC activated both JNK1, and ERK2 in Hep G2 cells in a time- and dose-dependent fashion. Increasing the concentrations of PEITC from 10 to 20 μM, strongly stimulated caspase-3-like (CPP32-like) protease activity in HeLa cells, and induced apoptosis as determined by DAPI nuclear staining (101). Pretreatment of cells with a specific inhibitor of caspase-3-like proteases, Ac-DEVD-CHO, decreased the PEITC-stimulated activity of caspase-3, and attenuated apoptosis induced by PEITC. Other structurally related isothiocyanates, phenylmethyl isothiocyanate (PMITC), phenylbutyl isothiocyanate (PBITC) and phenylhexyl isothiocyanate (PHITC) but not phenyl isothiocyanate (PITC) induced apoptosis in HeLa cells in a time-and dose-dependent fashion. These isothiocyanates, except PITC, also stimulated proteolytic activity of caspase-3, leading to the cleavage of a death substrate PARP (101). In contrast, ICE (caspase-1) activity was not stimulated by all these agents. Further increment of PEITC concentrations induced cell death predominantly *via* necrosis. These results suggest that compounds such as PEITC has a narrower therapeutic window than phenolic antioxidants BHA or BHQ as described above, in the various human and rodent cancer cell lines tested. Future studies will determine whether these concentration-dependent effects will be observed *in vivo* in animal studies.

DISCUSSION

These concentration-dependent biological or pharmacological responses elicited by BHA, BHQ, and PEITC are very intriguing indeed. Some other examples can be found in the literature. Low concentrations of amethopterin, calcium ionophore, chlorambucil, ethanol, heat, hydrogen peroxide, and UV have been shown to induce apoptosis of HL-60 cells, while necrosis occurred at high concentrations (102). Similarly, exposure of T cells to intracellular sulfhydryl-modifying agent diamide, can induce apoptosis at low concentrations, but necrosis at higher concentrations (103). In rat liver, both apoptosis and necrosis can be induced by thioacetamide (104). When RINm5F pancreatic cells were treated with increasing concentrations of redox-cycling 2,3-dimethoxy-1,4-naphthoquinone (DMNQ), it progressively resulted in cell proliferation (10 μM), apoptosis (30 μM) and necrosis (100 μM) (105). These concentration-dependent cellular responses have also been found in the immune system (71). Antigenic stimulation gave a biphasic proliferative response, in clonal populations of T cells. At low doses of mitogen, cells responded with an increasing proliferative response as a function of increasing stimulatory dose. When the antigen dose continued to rise, however, just the opposite was observed: a decreasing proliferative response as a function of continued increase in the antigen dose. The proliferative inhibition was due, in large part, to cell death (106). Hence, a stimulus that can drive proliferation can also trigger cell death. These dose-dependent phenomena were also seen in the induction of B lymphocytes cell death as a dose-dependent function of the antigen (107), and induction of neuronal cell death selectively by excitatory amino acids at high concentrations (108,109).

Figures 1 and 2 summarize our concept/hypothesis regarding the concentration-dependent effects on the signal transduction mechanisms induced by various drugs and chemicals which was first proposed previously (110). At low concentrations, these agents stimulate signaling kinases such as MAPK leading to the induction of gene expression and cell survival. However, at higher concentrations, additional signaling molecules such as the caspases will be activated leading to apoptosis and cell death, which will potentially resulting in toxicity. This idea is conceived in part from the data obtained from our studies with the isothiocyanates (PEITC, sulforaphane), the phenolic compounds (BHA, BHQ, GTP), the reactive oxygen specie (ROS) H_2O_2, and other agents described previously, as well as from data published in the literatures described above. Xenobiotics, such as PEITC or BHA, can induce some form of oxidative stress either through lowering of the intracellular glutathione (GSH) and/or modification of protein thiols (e.g., *via* oxidation of critical cysteine residues); through modification of plasma membrane with the release of ceramide and/or other phospholipid metabolites such as arachidonic acid, 4-hydroxynoneneal; or through modification of the mitochondria/endoplasmic reticulum (ER) with the release of calcium, or cytochrome C, presumably leading to the activation of the ERK, p38 and/or JNK signaling pathways, and in some cases to caspases activation and apoptosis (Figs. 1 and 2). Activation of the Ras-ERK pathway is usually rapid, but transient, potentially leading to the transcriptional activation of AP-1 and/or ARE/EpRE responsive genes, such as *c-fos, c-jun, GST, NQO*, or *MDR* (multi-drug resistance; (111)), which may protect the cell from cell death and/or may enhance cell survival. Other signaling pathways may also participate in the regulation of ARE-responsive genes *in vitro* as well as *in vivo* (57,70,112). Recently, it was shown that modulation of expression of cell survival genes such as MDR and Phase I drug metabolizing enzymes such as cytochrome P450 3A (CYP3A), by various xenobiotics may be coordinately and/or independently regulated (113,114). However, the signal transduction events leading to the regulation of gene expression of MDR and/or CYP3A by these diverse xenobiotics remain unclear but may involve diverse signaling mechanisms (115,116). Identification of the transcription factor-binding elements present in the 5′-flanking region of these genes will yield insight into their signaling and regulation.

Of the JNK and NFκB activation by these agents are usually delayed as compared to the activation of the ERK pathway. Our preliminary studies showed that the kinetics of NFκB pathway activation may be similar to that of JNK1 activation, consistent with previous report showing that MEKK1 (Fig. 1) coordinately activates the JNK pathway and NFκB (117). The activation of JNK1 activity by phenolic compound such as BHA may require intracellular calcium since blocking its intracellular release by BAPTA/AM inhibited JNK1 activation (100), and this inhibitory effect was similarly observed for taxol- or endothelin-1-induced JNK1 activation

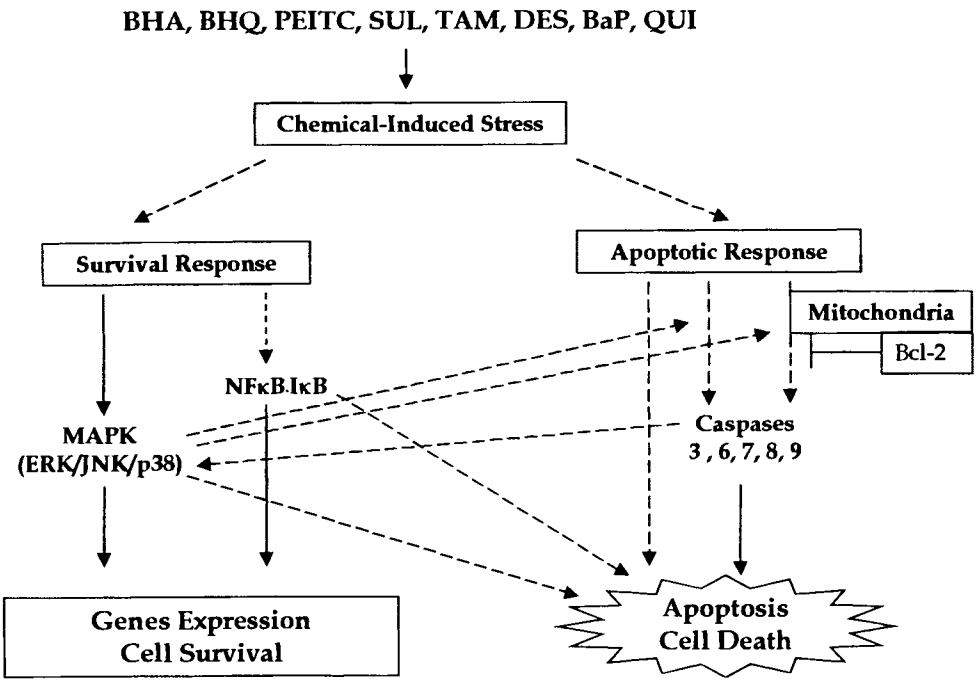

Fig. 2. Schematic representation of chemical-induced stress response leading to activation of the MAPK pathway ERK, JNK, and p38, and the NFκB pathway (signaling in cell survival), and the activation of the ICE/Ced-3 (caspase) pathway (signaling in cell death and apoptosis).

(65,118). Future studies will elucidate how intracellular calcium can be induced by various chemicals such as BHA, and what are the potential targets in the activation of JNK pathway. It is not clear as to whether activation of the JNK pathway can lead to protection from cell death, such as induction of survival genes (119), or can lead to apoptosis (40,41,94,97). From our GTP and BHA studies, at low concentrations, both GTP and BHA strongly activated JNK, but little cell death occurred (21,22), whereas adriamycin potently activated JNK1, and strongly induced cell death through apoptosis (37). Therefore, whether the activation of JNK activity leads to cell survival or cell death, may depend on the activation of other signaling events such as activation of the ERK and/or NFκB pathways simultaneously may protect the cells from dying, whereas activation of the caspase pathway simultaneously will probably lead to apoptosis, as for adriamycin (37,120). Future studies will show how caspases are activated by PEITC, BHA, or other chemicals.

In conclusion, our studies with various xenobiotics such as phenolic antioxidants (BHA, BHQ), flavonoids (GTP), structurally related isothiocyanates (PEITC, SUL), environmental pollutant (e.g., BaP), chemopreventive drug (TAM), and chemotherapeutic drugs (adriamycin, paclitaxel, colchicine, nocodazole, vincristine, TAM, diethylstilbestrol; DES), provided important insights into the signal transduction mechanisms induced by these agents. At low concentrations, these compounds may activate the MAPKs pathway (ERK2, p38 and/or JNK1) which may lead to the induction of gene expression such as *c-fos, c-jun, GST,* or *NQO,* resulting in protection and/or survival mechanisms. Increasing the concentrations of these compounds may also activate the MAPK pathway, but the caspase pathway will be activated concomitantly, which may lead to apoptosis and cell death. Further increase of concentrations to suprapharmacological concentrations may lead to nonspecific cell death predominantly occurring *via* necrosis. Future *in vivo* studies may shed light on whether this concentration-dependent cellular response phenomenon explains in part the beneficial pharmacological effects observed in animals after administration of low doses of BHA, whereas its undesirable toxicological effects after high doses (121). Many drugs (e.g., acetaminophen, rifampin, isoniazid, erythromycin, ketoconazole, azathioprine) (122) induce liver toxicity and some of these agents also induce drug metabolizing enzymes. Future studies on the signaling mechanisms would shed light on the mechanisms of induction of drug metabolizing enzymes versus liver toxicity. Furthermore, studies with *in vitro* cell lines and *in vivo* animals on the signal transduction mechanisms may offer insights into the pharmacodynamics (beneficial pharmacological responses at lower therapeutic concentrations), and the toxicodynamics (adverse toxicological effects at higher drug concentrations) of drug action. Thus, the study of signaling events in cell survival (homeostasis) and cell death (cytotoxicity) may have practical application during pharmaceutical drug development.

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