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

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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 (caspas) 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 caspas, 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 (homeostasis) 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 define 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).

dnone oxidoreductase or NAD(P)H: menadione reductase; NAT, N-acetyltransferase; EPH, epoxide hydrolases; ST, sulfoconjugates; 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-tetradecanoyl 13-acetate.
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., CYP1A, 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-glucuronosyltransferase (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 CYP1A, CYP2A, CYP3A, 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, diphensols/phenolic antioxidants, organic isothiocyanates, diterpene, indoles, unsaturated lactones, thiocarbamates, barbiturates, planar aromatic hydrocarbons (PAHs), phorbol esters (e.g., 12-O-tetradecanoyl-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)/electibile response element (EpRE), xenobiotic-responsive element (XRE)/aromatic hydrocarbon responsive element (AhRE), activator protein-1 (AP-1), and nuclear factor-kappa B (NF-kB) 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) which 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).

**BHA, BHQ, PEITC, SUL, TAM, DES, BaP, QUI**

![](Fig_1_Schematic_representation_of_chemical-induced_stress_response_leading_to_activation_of_the_MAPK_pathway_ERK_JNK_and_p38_and_the_NF-kB_pathway_which_results_in_gene_expression_and_potentially_cell_survival.png)

- **BHA, BHQ, PEITC, SUL, TAM, DES, BaP, QUI**
- **Chemical-Induced Stress**
- **Ca++**
- **PKC**
- **MEKK1**
- **SEK**
- **JNK**
- **Nf-kB**
- **ARE: Defense Genes - GST, QR, UGT, EPH**
- **AP-1: NF-kB Survival Genes: c-Jun, c-Fos, cdks, MDR**

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