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Joint toxic effects of the type-2 alkene electrophiles

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ABSTRACT

Human populations are exposed to complex environmental mixtures of acrolein, methylvinyl ketone (MVK) and other type-2 alkenes. Many members of this chemical class are electrophiles that possess a common molecular mechanism of toxicity; i.e., protein inactivation via formation of stable cysteine adducts. Therefore, acute or chronic exposure to type-2 alkene mixtures could represent a health risk due to additive or synergistic interactions among component chemicals. Despite this risk, there is little experimental information regarding the joint effects of type-2 alkenes. In the present study we used sum of toxic units ($TU_{sum} = \sum TU_i$) to assess the relative toxicity of different type-2 alkene mixtures. These studies involved well characterized environmental type-2 alkene toxicants and included amide (acrylamide; ACR), ketone (methyl vinyl ketone; MVK), aldehyde (2-ethylacrolein; EA) and ester (methyl acrylate; MA) derivatives. *In chemico* analyses revealed that both binary and ternary mixtures could deplete thiol groups according to an additive joint effect at equitoxic and non-equitoxic ratios; i.e., $TU_{sum} = 1.0 \pm 0.20$. In contrast, analyses of joint effects in SNB19 cell cultures indicated that different permutations of type-2 alkene mixtures produced mostly synergistic joint effects with respect to cell lethality; i.e., $TU_{sum} < 0.80$. A mixture of ACR and MA was shown to produce joint toxicity in a rat model. This mixture accelerated the onset and development of neurotoxicity relative to the effects of the individual toxicants. Synergistic effects in biological models might occur when different cellular proteomes are targeted, whereas additive effects develop when the mixtures encompasses a similar proteome.

1. Introduction

Type-2 alkenes such as acrolein, acrylamide (ACR) and methyl vinyl ketone (MVK) have extensive industrial applications and, consequently, human occupational exposure is significant. In addition, these chemicals are recognized as environmental pollutants and dietary contaminants; e.g., acrolein from the combustion of petrochemical fuels and ACR in foods prepared at high temperatures. Type-2 alkenes are also prevalent components of cigarette smoke; e.g., acrolein, ACR and acrylonitrile. Acute or chronic exposure to these type-2 alkenes has been linked to major organ system toxicity and to possible carcinogenicity in humans and laboratory animals [11,19,29,32,33]. In addition to environmental intoxication, acrolein, 4-hydroxy-2-nonenal (HNE) and certain other type-2 alkene derivatives are highly toxic by-products of membrane lipid peroxidation associated with cellular oxidative stress. Growing evidence indicates that these endogenous type-2 alkenes play a pathogenic role in diseases that have oxidative stress as a common molecular etiology; e.g., atherosclerosis, Alzheimer's disease, [2,9,20,21]. The type-2 alkenes are, therefore, an important class of chemicals with respect to environmentally acquired toxicity and endogenous disease pathogenesis.

Type-2 alkene derivatives are characterized by a conjugated α,β-unsaturated structure formed when an electron-withdrawing group (e.g., a carbonyl) is linked to an alkene (see Table 1). The electrons in these conjugated systems are polarizable and thus, such compounds are defined as "soft" electrophiles by the hard and soft, acids and bases theory (HSAB; [31]; reviewed in Ref. [23].

Abbreviations: MVK, methylvinyl ketone; EA, 2-ethylacrolein; MA, methyl acrylate; ACR, acrylamide; HNE, 4-Hydroxy-2-nonenal; NAC, N-acetylcysteine; GSH, glutathione; HSAB, Hard and Soft, Acids and Bases; E_{LUMO}, Lowest Unoccupied Molecular Orbital energy; E_{HOMO}, Highest Occupied Molecular Orbital energy; PBS, phosphate buffered saline; DMSO, dimethyl sulfoxide; PA, propionaldehyde; DTNB, 5,5′-dithiobis (2-nitrobenzoic acid); FBS, fetal bovine serum; RPMI, Roswell Park Memorial Institute; EC_{50}, concentration producing 50% of total effect.

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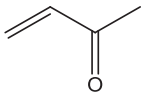
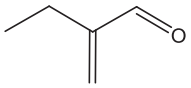
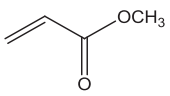
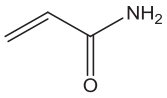
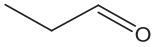
Table 1
In Chemico study: Concentration-response and HSAB parameters for individual toxicants.

Toxicant	Structure	Electrophilicity (ω; ev)	EC_{50} (mM)	Solubility (Mol/L)
Methyl vinyl ketone (MVK)		3.38	0.108 (0.105–0.112)	5.0
2-ethylacrolein (EA)		3.62	2.21 (1.97–2.47)	0.1
Methyl acrylate (MA)		3.22	12.9 (12.1–13.6)	0.6
Acrylamide (ACR)		2.62	419 (404–436)	9.0
Propionaldehyde		2.32	nd	3.5

Electrophilicity values were calculated for the s-*cis* conformation as shown in each case. $EC_{50\text{-}toxicant}$ values for individual type-2 alkenes were calculated based on concentration-response curves in Fig. 1 (values in parentheses are 95% confidence intervals).

According to this theory, soft electrophiles of the type-2 alkene chemical class will cause toxicity via a common molecular mechanism involving the formation of covalent adducts with soft nucleophiles, which in biological systems are functionally critical thiolate sulfhydryl groups on protein cysteine residues [23,24]. Substantial experimental evidence now supports this proposed mutual mechanism (e.g., see Refs. [3,4,17,18,22,26,27]; reviewed in Refs. [23,24].

As a consequence of both natural and anthropogenic production, type-2 alkenes have a significant environmental presence (e.g., see Refs. [5,10,23,28]. Human populations are, therefore, exposed to complex mixtures of type-2 alkenes, where the chemical compositions of these mixtures depend upon geographical location, occupation and personnel habits. Although individual environmental type-2 alkene pollutants likely exist at very low concentrations, exposures to mixtures of these chemicals might nonetheless represent a significant human health risk. Specifically, mixtures of type-2 alkenes could exhibit additive or even synergistic toxic effects due to the corresponding common molecular mechanism of action [1,13]. Therefore, as an initial investigation of type-2 alkene mixture toxicity, we determined the relative abilities of amide (ACR), ketone (MVK), aldehyde (2-ethylacrolein) and ester (methyl acrylate) derivatives to interact. These chemicals exhibit a range of electrophilic reactivity that is quantitatively indicated by the ω values shown in Table 1. In the absence of steric effects, these values are directly related to the toxic potencies of the electrophiles (reviewed in LoPachin and Gavin, 2012, [24].

The sum of toxic units ($TU_{sum} = \sum TU_i$; see equations (1) and (2)) has been widely used to describe the mixture toxicological effects at both equitoxic and non-equitoxic ratios [6,34,35]. Therefore, our studies employed this method to characterize the potential joint toxicity of the type-2 alkenes. Our experimental design was based on a tiered approach that began with a simplified *in chemico* model to determine interactions at the acellular chemical level. In the next series of studies we defined mixture effects in a simplified biological model - cultured SNB19 glioblastoma cells. Our analyses suggested that the different type-2 alkenes could interact synergistically to produce joint toxic effects. Finally, we determined mixture effects in a mammalian model of subchronic intoxication. This system represents the penultimate model of biological complexity and provided toxicologically relevant evidence for the interactions of environmental chemical toxicants.

2. Materials and methods

2.1. Chemicals and materials

All reagents were high-performance liquid chromatography grade or better and water was double distilled and deionized. Dimethyl sulfoxide (DMSO), propionaldehyde (PA), acrylamide (ACR), methyl acrylate (MA), 2-ethylacrolein (EA), methyl vinyl ketone (MVK), N-acetyl-L-cysteine (NAC), 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) were purchased from Sigma/Aldrich Chemical Company (Bellefonte, PA). Fetal bovine serum (FBS) and Roswell Park Memorial Institute (RPMI) medium and PrestoBlue® Cell Viability Reagent were purchased from Invitrogen (Grand Island, NY).

2.2. Calculations of HSAB quantum mechanical parameters

To calculate Hard and Soft, Acids and Bases (HSAB) parameters for the electrophiles involved in this study, energy values for the Highest Occupied Molecular Orbital (E_{HOMO}) and the Lowest Unoccupied Molecular Orbital (E_{LUMO}) were obtained using Spartan '14 (version 1.1.8) software (Wavefunction Inc., Irvine CA). For each chemical structure, ground state equilibrium geometries were calculated with Density Functional B3LYP 6-31G* in water starting from 6 to 31G* geometries. Global (whole molecule) hardness (η) was calculated as $\eta = (E_{LUMO}\text{-}E_{HOMO})/2$ and the index of electrophilicity (ω) was calculated as $\omega = \mu^2/2\eta$, where μ is chemical potential of the electrophile and $\mu = (E_{LUMO} + E_{HOMO})/2$.

2.3. Determination of individual toxicant EC_{50} values: in chemico analyses

A simplified *in chemico* model (i.e., chemical reactions conducted in a buffer) was used to evaluate the joint effects of type-2 alkenes. This large class of electrophiles has a common mechanism of action involving the formation of adducts with protein cysteine residues (see Introduction). Therefore, we measured N-