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Quinone Methide Bioactivation Pathway: Contribution to Toxicity and/or Cytoprotection?

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Abstract

The formation of quinone methides (QMs) from either direct 2-electron oxidation of 2- or 4-alkylphenols, isomerization of *o*-quinones, or elimination of a good leaving group could explain the cytotoxic/cytoprotective effects of several drugs, natural products, as well as endogenous compounds. For example, the antiretroviral drug nevirapine and the antidiabetic agent troglitazone both induce idiosyncratic hepatotoxicity through mechanisms involving quinone methide formation. The anesthetic phencyclidine induces psychological side effects potentially through quinone methide mediated covalent modification of crucial macromolecules in the brain. Selective estrogen receptor modulators (SERMs) such as tamoxifen, toremifene, and raloxifene are metabolized to quinone methides which could potentially contribute to endometrial carcinogenic properties and/or induce detoxification enzymes and enhance the chemopreventive effects of these SERMs. Endogenous estrogens and/or estrogens present in estrogen replacement formulations are also metabolized to catechols and further oxidized to *o*-quinones which can isomerize to quinone methides. Both estrogen quinoids could cause DNA damage which could enhance hormone dependent cancer risk. Natural products such as the food and flavor agent eugenol can be directly oxidized to a quinone methide which may explain the toxic effects of this natural compound. Oral toxicities associated with chewing areca quid could be the result of exposure to hydroxychavicol through initial oxidation to an *o*-quinone which isomerizes to a *p*-quinone methide. Similar *o*-quinone to *p*-quinone methide isomerization reactions have been reported for the ubiquitous flavonoid quercetin which needs to be taken into consideration when evaluating risk-benefit assessments of these natural products. The resulting reaction of these quinone methides with proteins, DNA, and/or resulting modulation of gene expression may explain the toxic and/or beneficial effects of the parent compounds.

Keywords

bioactivation; P450; quinone; quinone methide; selective estrogen receptor modulators (SERMs)

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1. Introduction

Quinone methides (QMs) are reactive metabolites of a variety of phenolic compounds containing ortho or para alkyl substituents and could be responsible for the cytotoxic/genotoxic effects of the parent compounds including hepatotoxicity and/or idiosyncratic adverse drug reactions [1–5]. They are highly electrophilic species that have been called resonance-stabilized carbocations because of the charge separated aromatic resonance structure that contributes to the overall resonance hybrid (Scheme 1) [5, 6]. Due to their reactivity they are often not detected since they rapidly react with a variety of nucleophiles including proteins and DNA via non-enzymatic Michael addition (Scheme 1, Scheme 2). However, quinone methide formation can be inferred by trapping them with reactive thiol nucleophiles such as GSH or N-acetylcysteine [7].

There are three major pathways by which these intermediates are formed in vivo (Scheme 1; A) two electron enzymatic oxidation, B) *o*-quinone isomerization, C) and elimination of a good leaving group (X) [5]. In the first case, the successive removal of two electrons (or alternatively, an electron and a hydrogen atom) from 4-alkyl-substituted phenols is usually catalyzed by cytochromes P450 or other oxidative enzymes such as peroxidases (Scheme 1, A) [2, 5]. Oxidation of butylated hydroxytoluene (BHT) and 4-allyl-2-methoxyphenol (eugenol) represent the most extensively studied and reviewed examples of quinone methide formation by the oxidative enzyme mechanism [2, 5, 8, 9]. More recently, it has been shown that cytochrome P450 and peroxidases also convert selective estrogen receptor modulators (SERMs) such as tamoxifen, toremifene, and raloxifene to quinone methides [5, 9, 10]. Alternatively, 4-alkylcatechols can be initially oxidized to *o*-quinones which, depending on the acidity of the hydrogen on the 4-alkyl carbon, spontaneously isomerize to *o*-hydroxy-*p*-quinone methides (Scheme 1, B). This oxidation-isomerization pathway occurs, for example, with hydroxychavicol [11, 12], catechol estrogens [13], and quercetin [5, 14]. Finally, quinone methides can be formed by base-catalyzed elimination of facile leaving groups such as halides and protonated alkylamines [15–17] as shown for the anesthetic phencyclidine [16] and for NO-ASA analogs [17–19].

The focus of this mini-review is on drugs and natural products which generate reactive quinone methides either directly or after enzyme catalyzed bioactivation mechanisms. The biological targets of quinone methides could result in toxic effects through covalent modification of proteins and/or DNA as well as depletion of GSH leading to an altered redox balance within cells (Scheme 2, Toxicity mechanism). Alternatively, cytoprotection could be observed from quinone methide formation through covalent modification of Keap1 leading to activation of Nrf2 and subsequent induction of detoxification enzymes such as NAD(P)H:quinone oxidoreductase (NQO1) and heme oxygenase (Scheme 2, Cytoprotective mechanism) [19–22]. Quinone methide mediated depletion of GSH could also lead to activation of stress response gene expression and synthesis of detoxification enzymes (Scheme 2, Cytoprotective mechanism) [19, 23]. Several examples of drugs whose biological effects could be attributed to quinone methide formation are discussed below.

2. Bioactivation of drugs to quinone methides

Bioactivation of alkylphenols to quinone methides followed by covalent modification of endogenous macromolecules is one mechanism leading to drug-induced hepatotoxicity and/or idiosyncratic adverse drug reactions [1, 24]. For example, nevirapine is an antiretroviral drug with reported idiosyncratic hepatotoxicity and adverse skin reactions [25–27]. Two-electron oxidation of nevirapine generating an α -quinone methide could contribute to both mechanisms of toxicity [26, 28, 29] (Scheme 3). Similarly, the oral antidiabetic agent troglitazone (rezulin) was withdrawn from the U.S. market due to reports of severe hepatotoxicity and drug-induced liver failure [30]. α -Quinone methide formation as shown in Scheme 4 could contribute to the hepatotoxic effects of troglitazone [7, 31]. The anesthetic phencyclidine induces idiosyncratic psychosis potentially through quinone methide mediated covalent modification of crucial macromolecules in the brain [16, 32] (Scheme 5). Phencyclidine has also been shown to be a mechanism-based inactivator of P4502B6 and quinone methide formation likely explains the inhibition mechanism [33]. Unlike nevirapine and troglitazone where direct two-electron oxidation by P450 or peroxidases likely explains the mechanism of quinone methide formation (Scheme 1, A), P450-catalyzed aromatic hydroxylation followed by base-catalyzed elimination of the piperidine leaving group (Scheme 1, C) gives the quinone methide shown in Scheme 5.

Several selective estrogen receptor modulators (SERMs) can be converted to quinone methides which may represent a bioactivation mechanism contributing to toxicity of the parent SERM [10]. For example, quinone methide formation from the prototypical SERM tamoxifen may contribute to the increased risk of endometrial cancer in women taking tamoxifen either for breast cancer treatment or prevention [34, 35]. The mechanism likely involves aromatic hydroxylation of tamoxifen at the 4-position catalyzed primarily by CYP2D6, giving the potent antiestrogen 4-hydroxytamoxifen [36]. 4-Hydroxytamoxifen may undergo direct P450-mediated two-electron oxidation to form a *para*-quinone methide (Scheme 6) [37]. Simple *para*-quinone methides are transient and normally rapidly react by non-enzymatic 1,6-Michael addition in biological systems, generating benzylic adducts; however, tamoxifen quinone methide possesses extended conjugation with two phenyl rings and a vinyl group and as a result is unusually stable for a quinone methide ($t_{1/2} = 3$ h) [38]. Tamoxifen quinone methide has been reported to form stable adducts with the exocyclic amine of deoxyguanosine *in vitro* via 1,8-Michael addition [37, 39]. However, the only DNA adducts detected in women taking tamoxifen likely result from carbocation formation at the α -carbon instead of quinone methide formation [40, 41]. Similar to phencyclidine [33], it has also been reported that tamoxifen is a mechanism-based inactivator of CYP2B6 and covalent modification of the P4502B6 apoprotein by the tamoxifen quinone methide may contribute to the inhibition mechanism [42, 43]. Finally, 4-hydroxytamoxifen has been shown to induce NQO1 and activate ARE in HepG2 cells potentially through a quinone methide mediated mechanism [44, 45].

Toremifene, which has a β -chloro substituent, forms substantially less DNA adducts compared to tamoxifen and does not cause hepatic carcinogenesis in rats [38, 46, 47]. The bulky, electron-withdrawing chlorine group at the β -position of toremifene probably decreases the potential of quinone methide formation from toremifene [10, 38].

The benzothiophene SERM, raloxifene has also been shown to be bioactivated to an electrophilic di-quinone methide capable of reacting with cellular nucleophiles [48–51] (Scheme 7); however, no known toxicities have been linked with di-quinone methide formation since the drug's initial approval in 1997 for the treatment of postmenopausal osteoporosis [52]. The raloxifene di-quinone methide has a relatively short half-life (< 1 second) and the transient nature of this intermediate may result in indiscriminate reactions with solvent molecules, GSH, or non-critical proteins [53]. Suicide inactivation of P4503A4 and 3A5 has been reported for raloxifene and the di-quinone methide has implicated in the inhibition mechanism [51, 54–56]. Di-quinone methide formation has also been observed for the major metabolite of the SERM arzoxifene, desmethylarzoxifene [57, 58] (Scheme 8). It has been shown that the formation of this di-quinone methide can be effectively eliminated while maintaining effective ER binding, through substitution of the 4'-hydroxyl group with a fluorine atom (i.e., 4'-F-desmethylarzoxifene, Scheme 8) [57]. These studies suggest that it should be possible to modify the structure of drugs to prevent toxicity while still maintaining efficacy if quinone methide formation is identified as the ultimate cytotoxic mechanism. Alternatively, desmethylarzoxifene was found to be more effective at inducing NQO1 compared to other SERMs including raloxifene and 4-hydroxytamoxifen which may represent a chemopreventive mechanism for the desmethylarzoxifene di-quinone methide [44].

Quinone methides have also been reported from metabolism of endogenous estrogens and equine estrogens in hormone replacement therapy (HRT) formulations and could contribute to the chemical mechanism of estrogen carcinogenesis [5]. Cytochrome P450 1B1 and 1A1 catalyze the formation of catechol estrogens from these estrogens in HRT which could be further oxidized enzymatically or by metal ion catalysis giving *o*-quinones [13, 59–61]. In the absence of nucleophilic trapping agents such as GSH, these *o*-quinones can isomerize to QMs (Scheme 1, B) (Scheme 9). The relative importance and resulting biological targets of these electrophilic estrogen intermediates have not been explored in detail [62–65]. It has been shown that estrogen quinoids can directly alkylate and oxidize cellular DNA leading to genotoxic effects [5, 60, 66–71]. Cavalieri's group has shown that the major DNA adducts produced from 4-hydroxyestradiol-*o*-quinone are depurinating N7-guanine and N3-adenine adducts resulting from 1,4-Michael addition both *in vitro* and *in vivo* [5, 60, 64, 70, 72–74]. In contrast, the considerably more rapid isomerization of the 2-hydroxyestradiol-*o*-quinone to the corresponding QMs results in 1,6-Michael addition products with the exocyclic amino groups of guanine and adenine [5][64, 75]. Unlike the N7 and N3 purine DNA adducts formed from the *o*-quinones, the QM DNA adducts are stable which may alter their repair efficiency and relative mutagenicity *in vivo*. These stable bulky estrogen QM DNA adducts have been detected by ³²P-postlabeling in Syrian hamster embryo cells treated with estradiol and its catechol metabolites [76]. The rank order of DNA adduct formation which correlated with cellular transformation was 4-OHE₂ > 2-OHE₂ > estradiol. In human breast tumor tissue, stable guanine DNA adducts have been measured consistent with alkylation of 4-hydroxyestrone and 4-OHE₂ QMs [77]. The mutagenic properties of the 2-hydroxyestrone QM N2-dG and N6-dA derived stable DNA adducts have been evaluated using oligonucleotides containing these site specific adducts transfected into simian kidney (COS-7) cells where G → T and A → T mutations were observed [78]. These data suggest

that the relative importance of labile *o*-quinone depurinating adducts versus stable quinone methide DNA adducts in catechol estrogen carcinogenesis remains unclear [5]. Finally, estrogen quinoid covalent modification of KEAP1, activation of Nrf2, and induction of heme oxygenase has been reported [79, 80] which may indicate that estrogen quinoid formation could represent a cytoprotective mechanism depending on the dose, time of exposure, and target tissue.

3. Bioactivation of natural products to quinone methides

QM formation from the food and flavor agent eugenol has been extensively studied and reviewed (Scheme 10) [2, 5, 9]. Human exposure to eugenol occurs through its use as an analgesic, flavoring and fragrance additive, and in clove cigarettes [81]. Eugenol has been reported to have both pro- and antioxidant activities and both effects are probably due to quinone methide formation [82–84]. For example, eugenol likely through oxidation to an electrophilic quinone methide, induces the expression and activity of NQO1 probably through a KEAP1 Nrf2 mechanism [83]. Induction of these detoxification enzymes in addition to eugenol's antioxidant effects could contribute to the chemopreventive properties of eugenol [85]. Cytotoxic effects resulting from quinone methide mediated covalent modification of DNA could contribute to the mechanism of pulmonary toxicities reported for eugenol [81, 86].

Capsaicin is the major pungent principle in chilies and red peppers. Like eugenol, bioactivation of capsaicin to a quinone methide may explain the biological effects of capsaicinoids (Scheme 11) [87–89]. Both carcinogenic and anticarcinogenic properties have been reported for capsaicin likely due to pro- and antioxidant effects depending on the biological system [85, 90, 91]. Cytoprotective effects of capsaicin have been shown to involve induction of Nrf2 activation and heme oxygenase expression through a quinone methide mediated mechanism [92]. Conversely, capsaicin has been reported to be a potential mutagen, carcinogen, and tumor promotor [93]. Oxidative DNA damage and mechanism-based inhibition of P450s have been reported which could be due to quinone methide formation from capsaicin leading to mutagenic effects [94, 95].

Hydroxychavicol (Scheme 12) is a major component of the Indian plant *Piper betel* leaf, which is consumed by millions of people every year [5, 96]. Although *Piper betel* leaf extract has been reported to have chemopreventive properties [96], chewing areca quid which contains betel leaf has been implicated as a major risk factor for the development of oral squamous-cell carcinoma [97]. Hydroxychavicol is also a major metabolite of the hepatocarcinogen safrole [98] as well a minor metabolite of eugenol [5, 99]. The major genotoxic pathway for safrole involves P450-catalyzed hydroxylation at the benzylic carbon, conjugation with sulfate catalyzed by sulfotransferase, and loss of the sulfate ester generating a highly electrophilic carbocation which reacts with DNA [100, 101]. Although this probably represents the major carcinogenic pathway for areca quid, hydroxychavicol could also be enzymatically oxidized forming a relatively stable *o*-quinone that is readily trapped by thiol nucleophiles including GSH [5, 11]. However, in the absence of thiol trapping agents, hydroxychavicol isomerizes to the *para*-quinone methide which could then be trapped with added GSH as shown by the full characterization of the quinone methide

GSH conjugates [11]. These data suggest that toxic effects from hydroxychavicol exposure could result from formation of both redox active quinones as well as electrophilic quinone methide alkylating agents. Alternatively, both of these quinoids could contribute to the reported chemopreventive effects of hydroxychavicol [96, 102] through mechanisms involving induction of detoxification enzymes such as glutathione S-transferase for example [102].

Quercetin is a naturally occurring flavonoid with both antioxidant and pro-oxidant activity (Scheme 13) [5, 103]. Several studies have shown in a variety of bacterial and mammalian mutagenicity experiments that quercetin has mutagenic properties that could be related to quinoid formation [5, 104, 105]. Quercetin is initially oxidized to an α -quinone which rapidly isomerizes to quinone methides and all of these quinoids could be responsible for beneficial gene expression through ERE-mediated pathways [14, 103, 106–108]. These quinoids can be trapped with GSH although the GSH conjugates are unstable and equilibrate over time producing isomeric mixtures of quinoid GSH conjugates. Protein and DNA adducts have also been observed in Caco-2 and HepG2 cells exposed to ^{14}C -labelled quercetin although these adducts were also unstable [109]. The transient nature of the quercetin quinoid conjugates as well as the rapid phase II conjugation and excretion of quercetin itself prior to quinoid formation, may have significant consequences for extrapolating quercetin genotoxicity to carcinogenicity *in vivo* [103, 110].

Celastrol is an example of a triterpenoid stable quinone methide isolated from Thunder of God vine (*Tripterygium wilfordi*) (Scheme 14) [111]. It has numerous biological effects including antioxidant, anti-inflammatory, and anticancer properties [111–113]. Celastrol also has cytoprotective effects through Nrf2 activation and upregulation of heme oxygenase [114].

4. Conclusions and future directions

The above are several examples of both structurally-simple and complex phenols for which data strongly implicate quinone methide intermediates as mediators of toxicity and/or cytoprotection for a variety of drugs and natural products [5]. These electrophiles could be considerably more important to the metabolism and biological properties of synthetic and naturally occurring phenols than is currently recognized. Quinone methides are formed both enzymatically and non-enzymatically, but the details of these processes and relationships to the structures of phenolic compounds are just beginning to emerge. As Michael acceptors, quinone methides are unique because of a stabilized ionic resonance form. Variations in the contributions of this form modulate quinone methide reactivity over a wide range, suggesting substantial differences in the biological targets and intracellular effects of quinone methides. It is clear that covalent modification of both proteins and DNA competes with detoxification mechanisms such as reaction with GSH, and that quinone methides are capable of inducing cytotoxic and possibly cytoprotective responses. Future studies will seek to clarify relationships between reactivities and biological actions of these electrophiles and to gain insight into the mechanisms involved in cellular damage/cytoprotection. These data obtained will assist in clarifying the complex biological properties of a number of phenolic drugs and natural products and provide new information on intracellular targets as

a function of electrophile reactivity which may be applicable to other types of electrophilic intermediates. Developing a better understanding of factors affecting phase II conjugation and rapid elimination of the parent phenols versus formation of quinone methides, their reactivities, and biological targets will allow advances in the drug discovery process to either enhance or prevent this pathway in vivo.

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