ACCELERATED PAPER

p-Quinone methides are the major decomposition products of catechol estrogen o-quinones

Judy L. Bolton and Li Shen

Department of Medicinal Chemistry and Pharmacognosy (M/C 781), College of Pharmacy, University of Illinois at Chicago, 833 S. Wood St, Chicago, Illinois 60612-7231, USA

To whom correspondence should be addressed

The mechanism of catechol estrogen-induced carcinogenesis could involve alkylation of critical cellular macromolecules by electrophilic quinoids. The o-quinones formed from peroxidase/P450-catalyzed oxidation of catechol estrogens have previously been implicated as the ultimate carcinogens. In the present study, we have shown that additional reactive intermediates can be produced from isomerization of the catechol estrogen o-quinones to highly electrophilic p-quinone methides (QMs). The o-quinones of the catechol estrogens were incubated at 37°C (pH 7.4) in the absence of GSH. Aliquots were removed at various times and combined with GSH. The GSH adducts were isolated and characterized by 1H-NMR, UV, and electrospray mass spectrometry. The o-quinone of 2-hydroxyestrone isomerized to two QMs; a QM stabilized by one alkyl substituent in the B ring, 2-OHE-QM1 (3-hydroxy-1-(10),3(4),5(6)-oestratrien-2,17-dione) and one having two alkyl substituents on the methylene group in the C ring, 2-OHE-QM2 (2-hydroxy-1(2),4(5),9(10)-oestratrien-3,17-dione). Only one QM was observed from the o-quinone of 4-hydroxyestrone, 4-OHE-QM2 (4-hydroxy-1(2),4(5),9(10)-oestratrien-3,17-dione) which is analogous to the C ring analog (2-OHE-QM2) from the o-quinone of 2-hydroxyestrone. The GSH adduct of 4-OHE-QM2 decomposed at pH 7.4 to give 9(11)-dehydro-4-hydroxyestrone as the major product. Finally, the disappearance of the estrogen o-quinone GSH adducts correlated with the formation of the GSH conjugates of the QMs. These data suggest that in cells with low levels of GSH, the formation of these potent electrophiles represents the major reaction pathway for estrogen o-quinones. The implications of the o-quinone/QM pathway for the in vivo effects of catechol estrogens are not known; however, given the direct link between excessive exposure to endogenous estrogens and the enhanced risk of breast cancer, the potential for formation of additional reactive intermediates needs to be explored.

Introduction

There is a clear association between excessive exposure to synthetic and endogenous estrogens and the development of cancer in several tissues including breast, endometrium, liver and kidney (1,2). Besides heredity, the known risk factors for women developing breast cancer are all associated with longer estrogen exposure; early menses, late menopause (3), and long term estrogen replacement therapy (ERT) (4,5). Recent data indicate that as many as 30% of post-menopausal women in the US are currently receiving ERT (6). Although there are many benefits of ERT including a substantial reduction in the risk of coronary heart disease and osteoporosis, such figures show the importance of fully understanding all the deleterious effects of estrogens including their potential to initiate and/or promote the carcinogenic process.

Although the carcinogenic effects of estrogens have been mainly attributed to hormonal properties (1), estrogens could also act as chemical carcinogens by binding to cellular macromolecules. The endogenous steroids estrone and 17β-estradiol are hydroxylated by various cytochrome P450 (P450) enzymes at several positions including the 2 and 4 carbons to form the 2- and 4-catechol metabolites (7,8). The catechol metabolites are oxidized to o-quinones that undergo reoxidation cycling mediated through cytochrome P450/P450 reductase and thus could contribute to the carcinogenicity through the induction of oxidative damage to DNA (9,10). In addition to generating reactive oxygen species, o-quinones are Michael acceptors and covalent modification of DNA has been observed by the 32P-postlabeling method (11,12). Chemical or enzymatic activation of estrone or 17β-estradiol and their catechol metabolites also leads to protein alkylation (13,14).

We have recently explored factors which influence the rate of P450-catalyzed formation of the o-quinones from 2-hydroxyestrone (2-OHE-Q) and 4-hydroxyestrone (4-OHE-Q) as well as from estrone in rat liver microsomes (15). The initially formed o-quinones were trapped as their GSH conjugates which were separated and characterized by HPLC with electrospray-MS detection. Two A ring GSH conjugates were observed from the reaction of GSH at the C1 and C2 positions of 2-OHE-Q. In addition, a conjugate in which GSH had added twice to the A ring producing a di-GSH conjugate was detected. 4-OHE-Q gave only the C2 A-ring GSH adduct as well as a di-GSH adduct. Preliminary data also suggested that both o-quinones could isomerize to additional reactive intermediates; o- and p-quinone methides (QMs) (Figure 1). In the present investigation, we have confirmed QM formation from both 2-OHE-Q and 4-OHE-Q through the isolation and characterization of QM-GSH conjugates. These results suggest that the

© Oxford University Press
Materials and methods

All chemicals were purchased from Aldrich (Milwaukee, WI), Fisher Scientific (Itasca, IL) or Sigma (St Louis, MO) unless stated otherwise. 2-OHE and 4-OHE were either purchased from Sigma (St Louis, MO) or synthesized as described previously (16). The o-quinone GSH conjugates and 9(11)-dehydro-4-OHE were previously synthesized and characterized (15). The products of o-quinone isomerization were prepared and characterized as follows. The o-quinones of the catechol estrogens were generated by 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) oxidation in acetonitrile as described previously (15). Briefly, the catechol estrogens (20 mg) was dissolved in 20 ml of acetonitrile containing 16 mg of DDQ and stirred at 25°C for 10 min. The acetonitrile solution was added in aliquots (2X10 ml) to 100 ml of potassium phosphate buffer (pH 7.4). The CH3CN was removed after each addition under vacuum. After 30 min incubation at 37°C, 2.5 ml of 0.2 M GSH was added followed by 5 ml perchloric acid. The products were isolated from the aqueous phase on C-18 extraction cartridges (3X6 ml J.T.Baker) and eluted with methanol. The eluates were concentrated in situ and subjected to semipreparative HPLC with an Ultrasphere ODS column (Beckman) on a Shimadzu LC-10A gradient HPLC system. The quadrupole analyzer was maintained at 120°C. and unit resolution was used for all measurements. Nitrogen at a pressure of 80 p.s.i. was used for nebulization of the HPLC effluent, and nitrogen bath gas at 350°C and a flow rate of 50 ml/min was used for evaporation of solvent from the electrospray. The range m/z 200-900 was scanned over ~2 s during LC-MS. The mass spectrometer was interfaced to the above mentioned Hewlett-Packard gradient HPLC system. The quadrupole analyzer was maintained at 120°C, and unit resolution was used for all measurements. Nitrogen at a pressure of 80 p.s.i. was used for nebulization of the HPLC effluent, and nitrogen bath gas at 350°C and a flow rate of 50 ml/min was used for evaporation of solvent from the electrospray. The range m/z 200-900 was scanned over ~2 s during LC-MS.

Results and discussion

Previous work has shown that enzymatic oxidation of catechol estrogens generates o-quinones which are trapped in situ by

\[ \text{o-quinone/QM isomerization pathway may make a major contribution to the carcinogenic effects of catechol estrogens.} \]
GSH (15). The trapping reaction is very efficient due to the high concentration of GSH in the medium (1.0 mM), and the relatively fast rate of addition of thiols to o-quinones relative to amino or hydroxyl groups (17,18). In addition, QM derived GSH adducts were not observed under these experimental conditions as the rate of trapping of the o-quinone by GSH is much faster than the rate of isomerization of the o-quinone to the QM (19,20). In the absence of GSH, a time-dependent decrease in o-quinone GSH adducts was observed (Figure 3). Preliminary data implied that the o-quinones of both catechol estrogens isomerize to QMs shown in Figure 1. 2-OHE-o-quinone has the potential to form two QMs, a QM stabilized by two alkyl substituents on the methylene group in the C-ring and a QM with only one alkyl substituent in the B-ring. In contrast, 4-OHE-o-quinone can isomerize to the potentially more stable C-ring p-QM as well as the B-ring o-QM. From the pseudo-first order rate of disappearance of the o-quinone di-GSH adducts the half-lives of the o-quinones were determined (Table I). The o-quinone from 2-OHE was found to be 17-fold more reactive in aqueous solution compared to the 4-OHE-o-quinone. Exogenous 4-OHE, although not 2-OHE, is carcinogenic in hamsters (21), possibly because of the slower rate of isomerization of 4-OHE-Q to highly electrophilic QMs.

In both GSH trapping studies, new GSH conjugates appear over the course of the experiment which may have been QM-GSH adducts; however, the adducts were unstable and isolation and spectral characterization were not successful.

In the present study, we unequivocally establish that both catechol estrogen o-quinones isomerize to QMs by isolating and characterizing the QM-SG conjugates. In contrast to the o-quinone GSH adducts, the QM-SG adducts were very unstable at neutral pH with essentially complete decomposition occurring within 2–3 h. The stability of these adducts was increased sufficiently to allow spectral characterization by the addition of perchloric acid in the isolation procedure. The 1H-NMR spectra of the diastereoisomers produced from reaction of GSH with 2-OHE-QM1 (Figure 2) showed intact aromatic resonances corresponding to two protons. o-Quinones give A-ring substituted GSH conjugates which would show only one

**Fig. 2.** 1H-NMR spectrum (300 MHz) of 2-OHE-QM1 GSH conjugate in CD3COD.
Finally, kinetic experiments have established that the formation of QMs correlates with the disappearance of the estrogen o-quinones (Table 1, Figure 3). The rate of formation of 2-OHE-QM2 is 50-fold slower than 2-OHE-QM1 suggesting that removal of H₂ is more facile than H₄ elimination perhaps for steric reasons. These data show that estrogen o-quinones do not decompose to give Diels-Alder products or other dimeric compounds under physiological conditions. It is quite possible however, that in aprotic organic solvents these reactions predominate since the isomerization mechanism requires catalysis by water or basic residues to remove H₂ or H₄ (23).

The relative importance of QM formation to the biological effects of catechol estrogens is not known; however, QMs have vastly different chemistry compared to o-quinones and their formation could alter the effects of catechol estrogens in vivo. In particular, o-quinones are redox active compounds as well as alkylating agents whereas QMs are considerably more electrophilic than o-quinones (19) with very little capacity for redox chemistry (24). This suggests that the o-quinones are responsible for catechol estrogen mediated induction of oxidative damage to DNA, whereas QMs could only covalently modify DNA. o-Quinones can also alkylate DNA, but their site(s) of alklylation on DNA might be quite different, leading to alterations in the relative efficiency of DNA repair. For example, a recent report has shown that 4-OHE-Q modifies adenine at the C₈ position on the purine ring under reductive conditions (25). In contrast, QMs formed from bioreductive alkylating agents tend to react with the exocyclic amino groups of deoxyguanosine and deoxyadenosine (26,27).

In summary, data have been presented on the isomerization of catechol estrogen o-quinones to highly electrophilic QMs. These data suggest that in cells with low levels of GSH, this is the major reaction pathway for these o-quinones. The implications of the o-quinone/QM pathway for the in vivo effects of catechol estrogens are not known; however, given the direct link between excessive exposure to endogenous estrogens and the enhanced risk of breast cancer, the potential for formation of additional reactive intermediates needs to be explored.

Acknowledgements

This research was supported by NIH grant ES06216 and the University of Illinois at Chicago. We thank Dr Zaiwen Huang for the synthesis of 4-OHE and 2-OHE. The electrospray-MS expertise provided by Dr Richard B van Bremen (Liquid Chromatography-Mass Spectrometry Laboratory, University of Illinois at Chicago) is gratefully appreciated.

References

Quinone methides from catechol estrogen o-quinones


Received on January 31, 1996; revised on February 20, 1996; accepted on February 20, 1996