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Formation of the depurinating N3adenine and N7guanine adducts by reaction of DNA with hexestrol-3′,4′-quinone or enzyme-activated 3′-hydroxyhexestrol
Implications for a unifying mechanism of tumor initiation by natural and synthetic estrogens

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Abstract

The nonsteroidal synthetic estrogen hexestrol (HES), which is diethylstilbestrol hydrogenated at the C-3–C-4 double bond, is carcinogenic. Its major metabolite is the catechol, 3′-OH-HES, which can be metabolically converted to the catechol quinone, HES-3′,4′-Q. Study of HES was undertaken with the scope to substantiate evidence that natural catechol estrogen-3,4-quinones are endogenous carcinogenic metabolites. HES-3′,4′-Q was previously shown to react with deoxyguanosine to form the depurinating adduct 3′-OH-HES-6′-N7Gua by 1,4-Michael addition [Jan S-T, Devanesan PD, Stack DE, Ramanathan R, Byun J, Gross ML, et al. Metabolic activation and formation of DNAadducts of hexestrol,a synthetic nonsteroidal carcinogenic estrogen. Chem Res Toxicol 1998;11:412–9.]. We report here formation of the depurinating adduct 3′-OH-HES-6′-N3Ade by reaction of HES-3′,4′-Q with Ade by 1,4-Michael addition. The structure of the N3Ade adduct was established by NMR and MS. We also report here formation of the depurinating 3′-OH-HES-6′-N7Gua and 3′-OH-HES-6′-N3Ade adducts by reaction of HES-3′,4′-Q with DNA or by activation of 3′-OH-HES by tyrosinase, lactoperoxidase, prostaglandin H synthase or 3-methylcholanthrene-induced rat liver microsomes in the presence of DNA. The N3Ade adduct was released instantaneously from DNA, whereas the N7Gua adduct was released with a half-life of approximately 3 h. Much lower (<1%) levels of unidentified stable adducts were detected in the DNA from these reactions. These results are similar to those obtained by reaction of endogenous catechol estrogen-3,4-quinones with DNA. The similarities extend to the instantaneously-depurinating N3Ade adducts and relatively slowly-depurinating N7Gua adducts. The endogenous estrogens, estrone and estradiol, their 4-catechol estrogens and HES are carcinogenic in the kidney of Syrian golden hamsters. These results suggest that estrone (estradiol)-3,4-quinones and HES-3′,4′-Q are the ultimate carcinogenic metabolites of the natural and synthetic estrogens, respectively. Reaction of the electrophilic quinones by 1,4-Michael addition with DNA at the nucleophilic N-3 of Ade and N-7 of Gua is suggested to be the major critical step in tumor initiation by these compounds.

Keywords: Depurinating N3adenine adduct; Depurinating N7guanine adduct; Nonsteroidal synthetic estrogen; Catechol quinones

Abbreviations: Ade, adenine; dA, deoxyadenosine; DES, diethylstilbestrol; dG, deoxyguanosine; DMF, dimethylformamide; ESI, electrospray ionization; FAB-MS, fast atom bombardment mass spectrometry; Gua, guanine; HES, hexestrol; HES-3′,4′-Q, hexestrol-3′,4′-quinone; MS/MS, tandem mass spectrometry; 3′-OH-HES, 3′-hydroxyhexestrol; TFA, trifluoroacetic acid

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1. Introduction

Compelling evidence has been obtained on the role of estrogens as weak tumor initiators [1–3]. The reaction of electrophilic metabolites, predominantly catechol estrogen-3,4-quinones, with DNA can generate the critical mutations that initiate breast and other human cancers [1–3].

The critical step in the multistage process leading to tumor initiation involves metabolic formation from estradiol and estrone of their 4-catechol estrogens and oxidation to the corresponding quinones. These metabolically formed electrophilic quinones react with the N-3 of adenine (Ade) and N-7 of guanine (Gua) to form depurinating DNA adducts [1–4]. These adducts are lost from DNA by destabilization of the glycosyl bond. The resulting apurinic sites can generate mutations by error-prone repair [5].

Study of the nonsteroidal synthetic estrogen hexestrol (HES) was undertaken with the scope to substantiate evidence that natural catechol estrogen-3,4-quinones are endogenous carcinogenic metabolites. Diethylstilbestrol (DES) and HES, which is DES hydrogenated at the C-3–C-4 double bond, are carcinogenic in the kidney of Syrian golden hamsters [6,7]. The major metabolites of DES and HES are their catechols (Fig. 1) [7–10]. These catechols can be metabolically converted to catechol quinones (Fig. 1).

The electrophilic nature of HES-3′,4′-quinone (HES-3′,4′-Q) has been established by its reaction with deoxyguanosine (dG) to form the depurinating adduct 3′-OH-HES-6′-N7Gua by 1,4-Michael addition [11]. Reaction of HES-3′,4′-Q with deoxyadenosine (dA) formed by 1,4-Michael addition only the adduct at the exocyclic amino group of dA and C-6′ of HES-3′,4′-Q [11]. The catechol product obtained was further oxidized by HES-3′,4′-Q to yield the quinone adduct, HES-3′,4′-Q-6′-N^6dA.

As reported in this article, the depurinating adduct 3′-OH-HES-6′-N3Ade is formed only by reaction of HES-3′,4′-Q with Ade by 1,4-Michael addition. This adduct is obtained with Ade and not with dA because the deoxyribose moiety at the adjacent N-9 in dA hinders the approach of the electrophile HES-3′,4′-Q to the N-3 of dA. This was also observed in the synthesis of the analogous adduct of the natural catechol estrogen-3,4-quinones [4]. In reactions with DNA, however, the N3Ade adducts of the natural estrogen-3,4-quinones are formed and are rapidly lost from DNA by depurination [4]. We report here formation of the depurinating 3′-OH-HES-6′-N7Gua and 3′-OH-HES-6′-N3Ade adducts by reaction of HES-3′,4′-Q with DNA or by activation of 3′-OH-HES by tyrosinase, lactoperoxidase, prostaglandin H synthase or rat liver microsomes in the presence of DNA (Fig. 1).

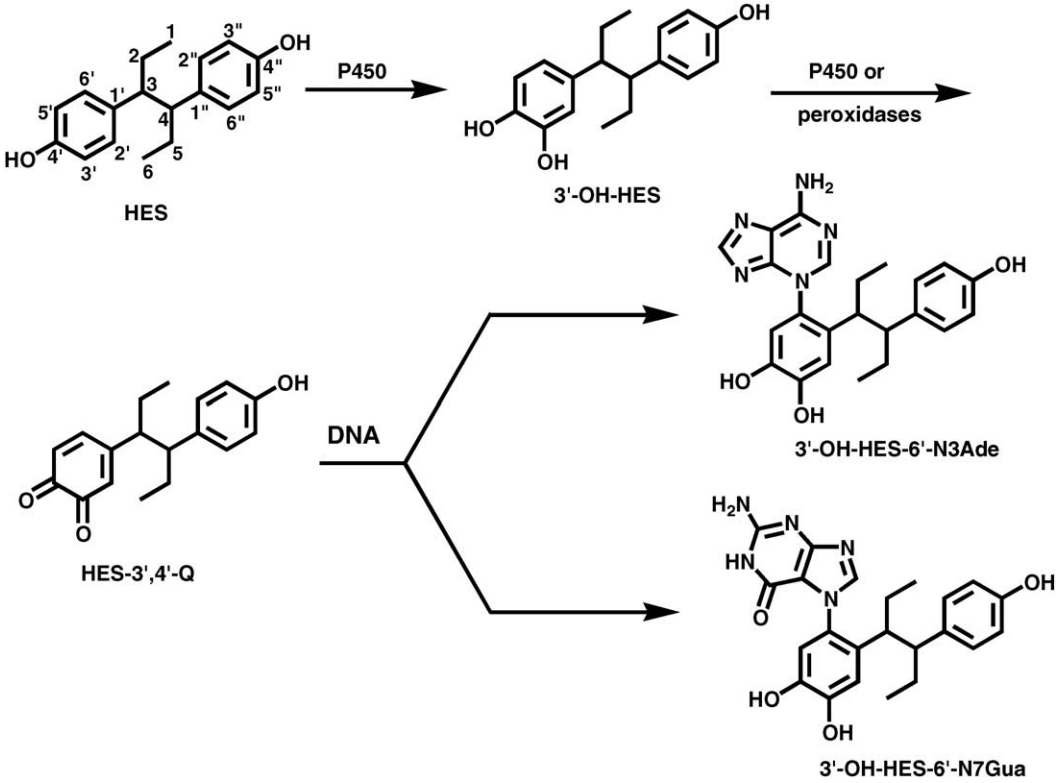

Fig. 1. Metabolic activation of HES to HES-3′,4′-Q and its reaction with DNA to form the depurinating N3Ade and N7Gua adducts.