Could o-Aminoquinones of Estrogen Metabolites Serve as Platforms for Redox Cycling?

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Introduction
Oxidative metabolism of estrogens can produce catechols and further oxidation lead to o-quinones, reactive electrophiles capable of binding to DNA. It is also thought that 3-OH-C19 steroidal compounds, 2-hydroxy estrogens have shown a procarcinogenic animal model while 2-OHE is not even they both possess similar redox potentials. The corresponding o-quinones however show marked differences in reactivity with DNA with the E-3,4-Q being the more reactive.

Materials and Methods
Synthesis of 1-GABA-E-3,4-Q
The quinone of 4-OHE was combined with five equivalents of NaOMe in acetonitrile. This mixture was stirred at 0°C for 10 minutes in the E-3,4-Q form. Five equivalents of 4-OHE (acid) and GABA (dissolved in 1 M acetic buffer pH 4.93) is added to the newly formed E-3,4-Q. This solution was allowed to reach a room temperature for 30-60 minutes to form the 1-amine-E-3,4-Q. Reaction can be monitored visually by the formation of the dark red product. The solution was then filtered to remove the NaOAc and the remaining solution rotary evaporated to remove the solvent. This crude product can be dried in the dry air at 55°C.

Purification of 1-GABA-E-3,4-Q
The crude product was dissolved in a minimal volume of methanol for injection in the HPLC. The 1-GABA-E-3,4-Q is purified on a column that begins at 30% water and 70% acetonitrile and reaches 100% water in 20 minutes. The HPLC was monitored at two wavelengths 220 nm and 480 nm. Percentage methanol was set in the 220 nm range and the dark red product absorbs well at both 220 nm and 480 nm. The product peak eluted at 12 minutes approximately at 85% methanol 15% water. The product peak was collected for all the injections and the solvent was removed via rotary evaporation to yield the pure product. This can be stored under vacuum at -70°C.

NMR of 1-GABA-E-3,4-Q
Approximately 8 mg of the 1-GABA-E-3,4-Q was dissolved in 99.9% deuterated methanol. A dried glass NMR tube and paper was used to try to minimize the water in the sample. Such a low mass of product was used because of the stacking that occurs at higher concentrations. The breadth of some of the NMR

Current Vs. Potential of 1-GABA-E-3,4-Q

Abstract
The metabolites of estrogen can lead to the formation of two isomeric o-quinones, o-catechol-3,4-quinone (E-3,4-Q) and o-stilbene-3,4-quinone (E-3,4-Q). The more reactive E-3,4-Q is genotoxic and can damage DNA by forming aromatic sites, whereas, E-2,3-Q does not form aromatic sites. Estrogen quinones may also be involved in nucleic acid damage by producing reactive oxygen species (ROS), which is another genotoxic pathway. What is not yet clear is why E-3,4-Q would induce nucleic acid damage while the non-carcinogenic E-2,3-Q would not. Nitroarenes react with the E-3,4-Q at the 5-position. With DNA bases as the nitro (o) nucleophile, the adducts form are cathecols. We have investigated the reaction of sodium nitro (o) nucleophiles with both E-3,4-Q and E-2,3-Q (Fig. 1). The E-3,4-Q reacts quickly with an aromatic adduct (GABA) at the 5-position to form a red, o-aromatic quinonemoduct whereas E-2,3-Q does not react with GABA to form an o-aromaticquinone. The reaction proceeds through an electron rich catechol intermediate which is oxidized by the original E-3,4-Q to produce equal amounts 1-amine-E-3,4-Q and the catechol of the E-3,4-Q. Hydroxynitro (4-OHE), the reaction is done in the presence of an oxidant (NaOAc), of OHE is not produced in the E-3,4-Q is observed. The E-3,4-E-4-Q can be reduced to a catechol with sodium dithionite to produce the 1-amine-o-GABA. This catechol is oxidized to the o-aromatic quinone when exposed to air indicating a possible nucleic acid cycling platform. Cyclic voltamogram analysis of an o-aromatic quinone, versus an Ag/AgCl reference electrode, displays reversible behavior with first and second reduction potentials of -0.945 V and -1.440 V, respectively. These values are close to 0.5 V, lower than E-3,4-Q reduction potentials. Since the oxidation potentials of 4-OHE and 2-OHE are almost identical, perhaps ROS are produced from nitroaromatics E-3,4-Q in the form of o-aromatics.

Results

Carbon NMR of the 1-GABA-E-3,4-Q

Proton NMR of the 1-GABA-E-3,4-Q

Figure 1: NMR of 1-GABA-E-3,4-Q

Figure 2: Cyclic Voltamogram of 1-GABA-E-3,4-Q

Without acid, the redox cycle is quasi reversible. The first and second reduction potentials are -0.945 V and -1.440, respectively. Once the acid is added, the reduction potential becomes more positive making that quinone more stable. In the presence of HCl, the first and second reduction potentials are 0.275 V and 0.495 V, respectively. This change in reduction potentials supports the idea to add the quinone in Figure 3: before reduction with sodium dithionite.

Refrences

Conclusion
We were successful in synthesizing the 1-GABA-E-3,4-Q by reacting o-catechol-3,4-quinone with 2-aminoarboxylic acid (GABA) in an acetonitrile/acetic acid buffer solution. The bright red colored compound was characterized by 1D and 2D NMR analysis and high resolution mass spectrometry. We demonstrated that the o-aromatic compounds could reversibly convert between o-aromatic and catechol by the reduction of sodium dithionite in acidic medium followed by oxidation back to o-quinone at higher pH. Furthermore, this redox cycling can be observed by the change in color of the compound and was monitored by UV/Vis spectrometry. Cyclic voltammetry displayed a quinone with a large negative first reduction potential. This reduction potential was made more positive in the presence of HCl consistent with the dithionite reduction.

Future work involves reaction of α-naphthylamine and β-naphthylamine nucleophiles with the estrogen quinones. In addition, the ability of these compounds to produce ROS in vivo will be examined with o-aromatics estrogen adducts can serve as platforms for nucleic acid cycling.