Redox Activity of Quinone-Chelator Q1 and its Chelate Complexes with Iron Ions in Cancer Cells Media. EPR Study

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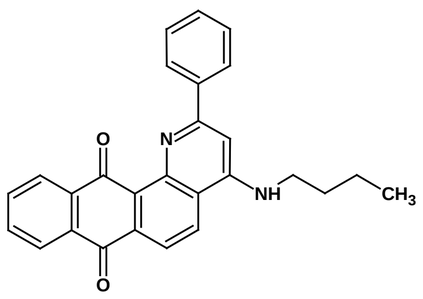
Introduction

Quinones-chelators have been used for a long time for the treatment of cancer . There are two main anticancer mechanisms of action of these drugs: inhibition of topoisomerase II; redox activity and generation of reactive oxygen species (ROS), which can disrupt the performance of cells, tissues, and organs. In this work, we studied the effect of the chelator 2-phenyl-4-(butylamino)naphtho[2,3-h]quinoline-7,12-dione (Q1) and its complexes with Fe(III) ions on the generation of ROS in cancer cell cultures. The EPR method with spin traps DEPMPO and CP-H was used in this investigation. The main purpose of this study was to investigation the ability of the penetration of quinone Q1 and its complexes with Fe(III) ions into cells and the generation of active oxygen radicals.

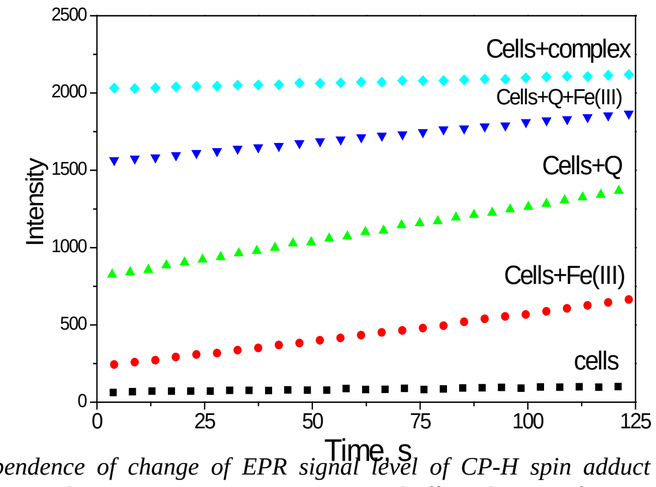


$$Q + e \rightarrow Q^{\cdot -}$$
$$Q^{\cdot -} + O_2 \underset{2H^+}{\leftrightarrows} Q + O_2^{-}$$
$$O_2^{-} + O_2^{-} \longrightarrow H_2O_2 + O_2$$
$$O_2^{-} + H_2O_2 \rightarrow O_2 + HO^{-} + HO^{\cdot}$$
$$Q^{\cdot -} + H_2O_2 \rightarrow Q + HO^{-} + HO^{\cdot}$$
$$Q^{\cdot -} + Fe(III) \rightarrow Q + Fe(II)$$
$$H_2O_2 + Fe(II) \rightarrow Fe(III) + HO^{-} + HO^{\cdot}$$

CP-H spin adducts

Another study on the redox activity of quinone Q1 and its Fe(III)-Q1 complex was the analysis of the rate of release of CP-H adducts in cell solutions in the presence of various oxidizers. Based on the figure, it can be seen that the highest rate of formation of CP-H adducts in A549 cell cultures is achieved in the case of Fe(III) and Q1 ions. In the case of the complex, the growth rate of the signal from the CP-H adduct is insignificant. Similar studies were carried out with other cancer cell cultures. The table shows that quinone is most effective in the case of A549 and MOLT-4 cell cultures. However chelate complexes act as low-efficiency pro-oxidants in all three cell cultures.

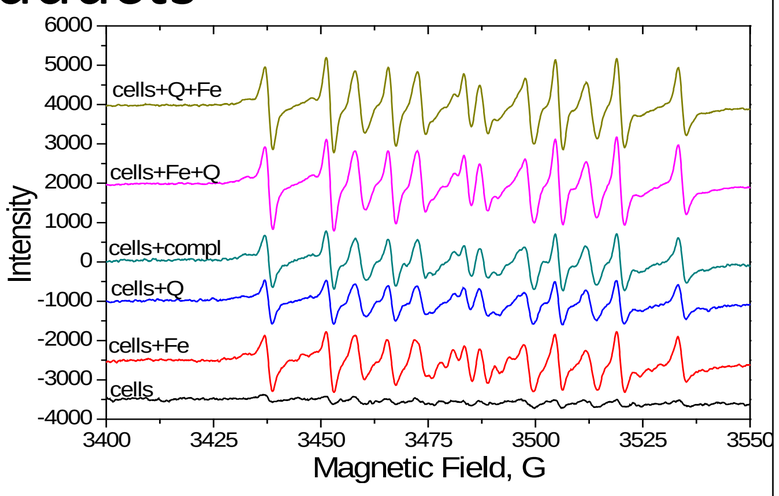
Sample	**k, s^{-1}**		
CP-H + HEPES	0.058±0.013		
CP-H + HEPES +Q	3.5±0.017		
CP-H + HEPES + Fe	6.63±0.016		
CP-H + HEPES +complex	1.51±0.025		
	MDA-MB-231	**A549**	**MOLT-4**
Pure cells	0.20±0.01	0.29±0.017	0.052±0.014
Cells +Q	1.38±0.011	33.02±0.18	8.76±0.021
Cells +complex	0.55±0.013	0.73±0.014	1.43±0.03



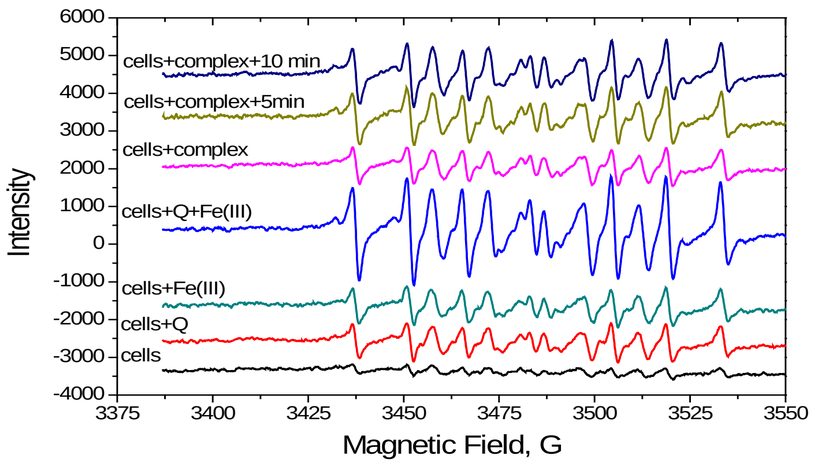
Dependence of change of EPR signal level of CP-H spin adduct in various solution on time in various HEPES buffer solutions of A549 cell culture (5 mil/ml) with NaCl (150 mM) (pH 7.1) and 1% DMSO. Q1 (50 mcM); $Fe(NO_3)_3$ (50 mcM) Fe(III)-Q1 (40 mcM).

DEPMPO spin adducts

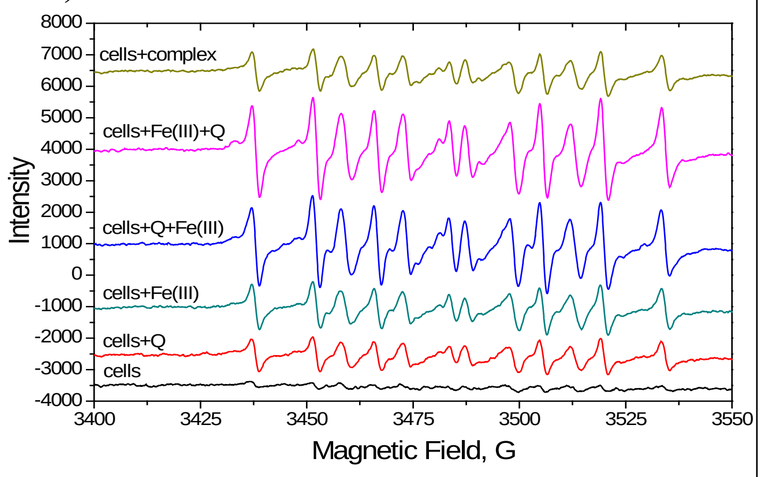
To study the redox activity of Q1 and its complex with the Fe(III) ion, EPR experiments were carried out with a DEPMPO spin trap. Based on the figures, it can be seen that the yield of free radicals is insignificant in the absence of oxidizing agents. The signal increases significantly under addition of quinone Q1 to the solution. A similar signal is observed upon addition of the Fe(III) salt. Sequential incubation of cells with Fe(III) and Q1 salts results in an additive yield of DEPMPO spin adducts. This phenomenon does not depend on the order in which substances are added to the solution. However, in the case of the Q1-Fe(III) complex, the level of the signal from spin adducts is similar to the signal from quinone but increases with time. Thus, the rate of penetration of quinone into cells is lower than that of pure quinone or Fe(III) ions.



EPR spectra of solutions of sells MOLT-4 (1 mil/ml) with spin trap DEPMPO (10^{-1} M) in buffer HEPES (50мM) and NaCl (150 mM) (pH 7.1) with 1% DMSO; Q1 (50 mcM); $Fe(NO_3)_3$ (50 mcM); $[Fe(Q1)_3]$ (40 mcM).



EPR spectra of solutions of sells MDA-MB-231 (1 mil/ml) with spin trap DEPMPO (10^{-1} M) in buffer HEPES (50мM) and NaCl (150 mM) (pH 7.1) with 1% DMSO; Q1 (50 mcM); $Fe(NO_3)_3$ (50 mcM); $[Fe(Q1)_3]$ (40 mcM).



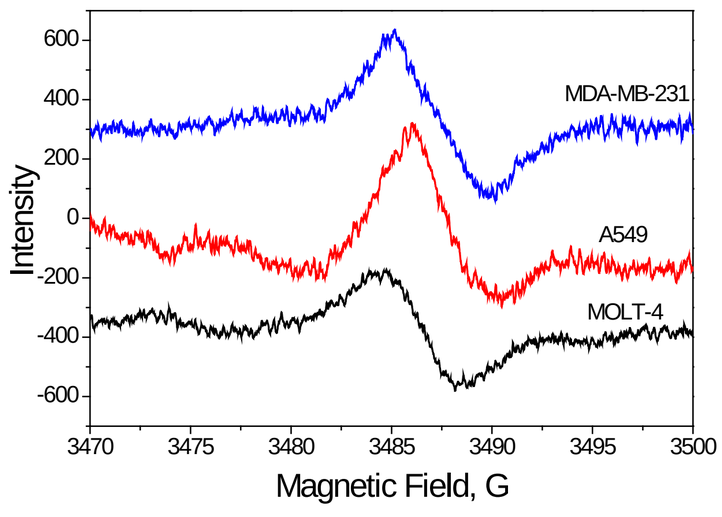
EPR spectra of solutions of sells A549 (1 mil/ml) with spin trap DEPMPO (10^{-1} M) in buffer HEPES (50мM) and NaCl (150 mM) (pH 7.1) with 1% DMSO; Q1 (50 mcM); $Fe(NO_3)_3$ (50 mcM); $[Fe(Q1)_3]$ (40 mcM).

Semiquinone radical

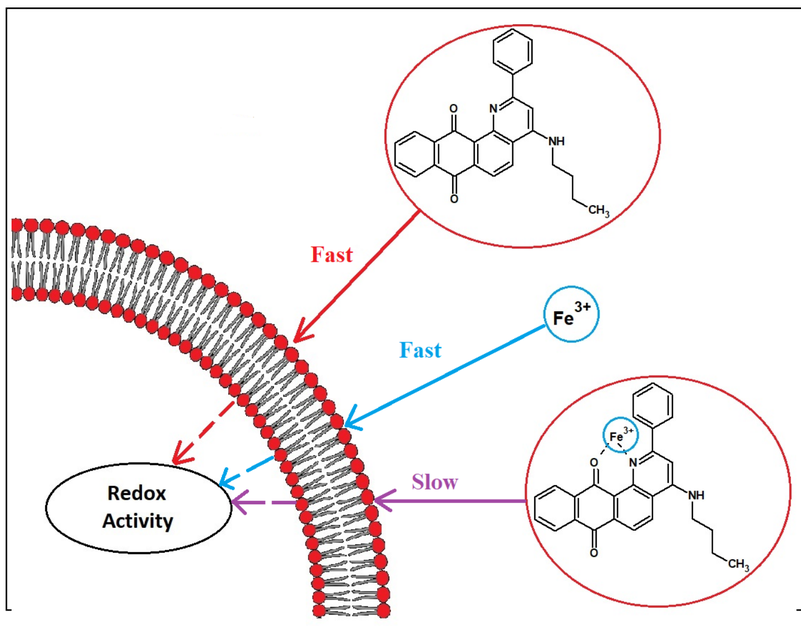
To study the penetration of quinone Q1 into cells, a series of EPR experiments were performed to detect the semiquine radical Q1 in a various cell culture solutions.

To register the semiquine radical, oxygen was preliminarily removed from the cell solution by adding glucose (10 mM) and glucose oxidase (10 arbitrary units of activity, a.u.a.) and incubating for 10 min.

Based on the EPR spectra, the signal from the semiquine is recorded, which confirms the penetration of quinone into the cells.



EPR spectra of cell cultures MOLT-4 (black) , A549 (red), MDA-MB-231 (blue) (1 mil/ml) in HEPES buffer (50 mM) with NaCl (150 mM) (pH 7.1) with 1% DMSO in presence Q1 (50 mcmol/L).



Conclusions

As a result of this study, the following results were obtained:

1. Quinone Q1 is able to penetrate into A549, MOLT-4, and MDA-MB-231 cancer cell cultures and participate in redox reactions.
2. Quinone Q1 is able to generate active oxygen radicals inside cell cultures, which confirms the increase in the yield of spin adducts of DEPMPO and CP-H traps. Quinone demonstrated the highest efficiency in A549 and MOLT-4 cell cultures.
3. The Fe(III)-Q1 chelate complex has a low membranotropic activity.

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