Involvement of semiquinone radicals generated from dihydroxybenzenes in the in vitro cytotoxicity of cigarette smoke tar

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Background

- Cigarette smoke contains free radicals in both the gas and particulate (tar) phases
- Free radicals can induce damage to cells in physiological media
- Free radicals in cigarette smoke have been suggested to be responsible in part for the harmful effect of smoking
- Significant amount of dihydroxybenzenes is found in the tar of main stream cigarette smoke
- Dihydroxybenzenes can undergo autoxidation to form semiquinone radicals

Free radicals in cigarette smoke

- **Tar radicals:** very stable, suggested to be hydroquinone/semiquinone/quinone in a polymeric matrix.
- **Gas phase radicals:** Unstable alkoxy, alkyl and carboxyl radicals. Formed during a steady reaction in the gas phase with nitric oxide as precursor.
- **Semiquinone radicals:** Stable, formed from the oxidation of dihydroxybenzenes.
- **Reactive oxygen species (ROS)**: Superoxide radical anion, hydrogen peroxide, and hydroxyl radical. Generated during the oxidation of smoke constituents.

Objectives

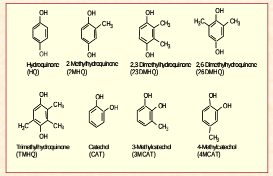
- Evaluate the cytotoxicity of pure compounds found in the tar (particulate phase) of cigarette smoke.
- Evaluate the potency of the dihydroxybenzenes to generate semiquinone radicals.
- Determine if there is a contribution of semiquinone radicals to the cytotoxicity of the dihydroxybenzenes.

Strategy

Use model compounds to :

- Determine the *in vitro* cytotoxicity of pure dihydroxybenzenes.
- Determine the potency of pure dihydroxybenzenes to autoxidize and form semiquinone radicals in solution.
- Compare semiquinone radicals formation and cytotoxicity.
- Determine the factors affecting the semiquinone radical formation and cytotoxicity.

Dihydroxybenzenes found in the tar of cigarette smoke



Neutral Red Uptake Cytotoxicity Determination

- Mouse embryo BALB/c 3T3 cells
- Cells were grown in Dulbecco's Modified Eagle Medium (DMEM) with 5% fetal calf serum (FCS)
- Test substances dissolved in dimethyl sulfoxide (DMSO) or ethanol.
- Exposure of test substance to cells for 24 h.
- Photometrical determination of viable cells which have taken up by the neutral red within 3 h.
- Calculation of the equal effect concentration (EC_{50}) from S-shaped logistic function of nonlinear concentration-response curve.

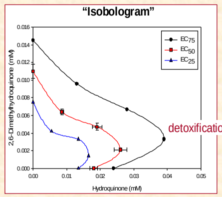
Cytotoxicity of pure dihydroxybenzenes

Dihydroxybenzenes	EC_{50} (mM) (a)	Yield in tar of cigarette smoke (b) (mg/cig.)
HQ	0.021 ± 0.004	40.9
2MHQ	0.011 ± 0.001	4.2
23DNMHQ	0.015 ± 0.005	1.9
26DMHQ	0.016 ± 0.002	0.7
TMHQ	0.026	3.3
CAT	0.33	45.1
3MCAT	0.036 ± 0.005	5.3
4MCAT	0.052 ± 0.013	4.4

(a): mean ± SD, N = 3; The EC_{50} is the concentration of the substance required to kill 50% of the cells.

(b) Tar was collected from 2R4F research cigarettes under ISO conditions.

Interactions between dihydroxybenzenes: 2,6-dimethylhydroquinone + hydroquinone

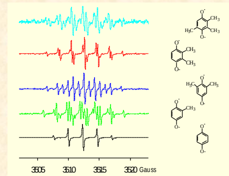


EC_{50} values ± 95% confidence limit.

EPR detection of semiquinone radicals in the presence of dihydroxybenzenes

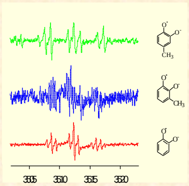
- Pure dihydroxybenzenes were dissolved in cytotoxicity media (DMEM), aged for 10 min, 1 hour and 24 hours.
- Autoxidation of dihydroxybenzenes monitored using EPR.
- Yield of semiquinone radicals determined.

EPR spectra of semiquinone radicals



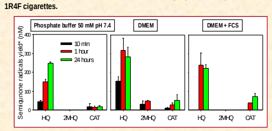
Pure dihydroxybenzenes, 1mM in DMEM

EPR spectra of semiquinone radicals



Effect of neutral red uptake assay media on semiquinone radicals formation

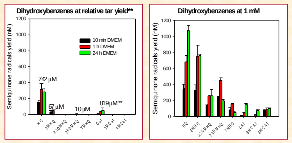
Individual components in solution at concentration proportional to yield in tar of 1R4F cigarettes.



* mean ± SD, N = 3 DMEM = Dulbecco's Modified Eagle Medium FCS = Fetal Calf Serum

- The media have some effect on semiquinone radical formation.
- HQ has the highest potency for autoxidation and semiquinone radical generation.

Effect of concentration on semiquinone radicals formation in DMEM



* mean ± SD, N = 3; ** Relative yield of the pure dihydroxybenzenes from 10 1R4F cigarettes.

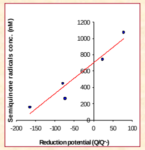
- Hydroquinone (HQ) is the most potent generator of semiquinone radicals among all the dihydroxybenzenes tested.

Reduction potential and semiquinone radical formation

Dihydroxybenzene	E(Q/Q·⁻)* mV at pH 7	Semiquinone Radicals (nM)
HQ	78	1076
2MHQ	23	743
26DMHQ	-80	448
23DMHQ	-74	261
TMHQ	-165	157
CAT	/	144
4MCAT	/	98
3MCAT	/	76

* Wardman (1989)
/ no data available

Dihydroxybenzenes 1mM in DMEM solution



- The reduction potential is a key factor governing dihydroxybenzene oxidation and semiquinone radical formation.

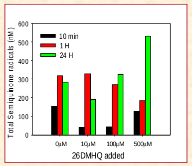
Comparison between cytotoxicity and semiquinone radical

Dihydroxybenzene	EC_{50} (mM)	Semiquinone Radicals (nM)*
HQ	0.0247	1076
2MHQ	0.0116	743
26DMHQ	0.0150	448
23DMHQ	0.0100	261
TMHQ	0.0256	157
CAT	0.3000	144
4MCAT	0.0393	98
3MCAT	0.0331	76

The concentration of radicals in 1mM DMEM solutions of dihydroxybenzenes indicates their relative potency to form semiquinone radicals.

- The EC_{50} of the dihydroxybenzenes does not depend upon the potency of the dihydroxybenzene to generate semiquinone radicals.

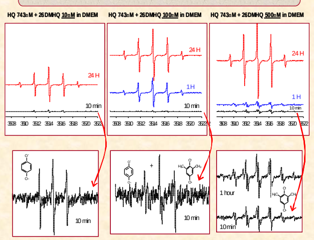
Semiquinone radicals from HQ interaction with 26DMHQ in DMEM



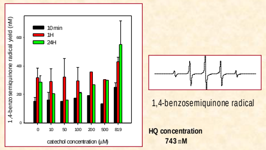
HQ concentration 743 nM

- Two different semiquinone radicals are observed depending on the concentration of 26DMHQ and reaction time.

HQ interaction with 26DMHQ



HQ interaction with CAT in DMEM



HQ concentration 743 nM

- No significant effect of CAT except at high concentration (819 mM).
- Only 1,4-benzosemiquinone radical was observed.

Summary

For the model compounds tested, we have found that:

- Hydroquinone is the most potent radical generator studied. It is abundant in the tar of 2R4F research cigarettes, but it is not the most cytotoxic dihydroxybenzene isolated from the tar.
- Methyl substituted dihydroxybenzenes are more cytotoxic than unsubstituted ones.
- Dihydroxybenzenes autoxidize and generate semiquinone radicals in the cell culture media.
- The reduction potential of the quinone/semiquinone is a key factor in dihydroxybenzene autoxidation.
- The interaction of hydroquinone with other dihydroxybenzenes is dependent on concentration and reduction potential and affects the cytotoxicity.
- No correlation was observed between the potency of dihydroxybenzenes to form semiquinone radicals and their cytotoxicity.

References

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