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Ubiquinone-0 (2,3-dimethoxy-5-methyl-1,4-benzoquinone) as Effective Catalyzer of Ascorbate and Epinephrine Oxidation and Damager of Neuroblastoma Cells

Vitaly A. Roginsky, Gernot Bruchelt†‡ and Olga Bartuli†*

*N. N. Semenov Institute of Chemical Physics, Russian Academy of Sciences, Kosygin St. 4, 117977 Moscow, Russia

†Children's Hospital, University of Tuebingen, Ruemelinstrasse 23, D-72070 Tubingen, Germany

ABSTRACT. The kinetics of ascorbate ($AscH^-$) and epinephrine (EP) oxidation in the presence of 2,3-dimethoxy-5-methyl-1,4-benzoquinone (UQ) were studied in 0.05 M phosphate buffer, pH 7.4, at 37°C by using a Clark electrode and ESR techniques. UQ at nanomolar concentrations displayed a pronounced catalytic effect on $AscH^-$ oxidation which exceeded that of all reported organic catalysts tested in this system. The process was accompanied by the intensive oxygen consumption and increase in the steady-state concentration of the ascorbyl radical Asc^-. The rate of oxygen consumption (R_{OX}) was maximal at the moment of reagent mixing ($(R_{OX})_0$) and then reduced over a few minutes until a steady-state level ($(R_{OX})_{SS}$) was achieved. $(R_{OX})_0$ was found to be proportional to $[UQ][AscH^-]$ without regard to the concentrations of the individual reagents; $(R_{OX})_{SS}$ was directly related to [UQ] at a given concentration of $AscH^-$. The difference between $(R_{OX})_0$ and $(R_{OX})_{SS}$ decreased as $[AscH^-]$ decreased. The presence of a lipid phase (sodium dodecylsulphate micelles) only moderately decreased UQ activity as a catalyst of $AscH^-$ oxidation. Adding micromolar concentrations of UQ induced the acceleration of EP autoxidation. The capability of UQ to catalyze the oxidation of EP exceeded by approximately 25 times that of adrenochrome, a quinoid product of EP oxidation. These catalytic properties of UQ allowed us to predict its pronounced cytotoxicity, especially in the presence of $AscH^-$ and to cells of the sympathetic nervous system which are rich in catecholamines. This possibility was confirmed by experiments with human neuroblastoma cells in culture. The capability of UQ to injure neuroblastoma cell line SK-N-SH exceeded that of well-known neurotoxic agents 6-hydroxydopamine and menadione. BIOCHEM PHARMACOL 55;1:85–91, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. quinones; ascorbate; epinephrine; ascorbyl radical; redox cycling; cytotoxicity

Many quinones (Q§) have cytotoxic properties which have made them useful as anticancer and antibacterial drugs [1–7]. Among several molecular mechanisms of Q toxicity suggested in the literature [1, 5–7], the mechanism associated with Q redox cycling with the participation of NADH- and NADPH-dependent reductases seems to be the most general. This enzymatic reduction of Q results in the formation of semiquinones (Q^-) followed by the generation of reactive oxygen species (oxidative stress). Additionally, non-enzymatic redox cycling may occur due to Q interaction with ubiquitous bioreductants, most notably ascorbate ($AscH^-$) [1, 8, 9]. The reduction of Q by $AscH^-$ is accompanied by the formation of two free radicals:

$$Q + AscH^- \rightarrow Q^- + Asc^- + H^+ \quad (1)$$

Under aerobic conditions, Q^- reacts with oxygen resulting in the regeneration of Q and the formation of superoxide (O_2^-):

$$Q^- + O_2 \rightarrow Q + O_2^- \quad (2)$$

Reactions (1)–(2), which provide the effective redox cycling while concomitantly producing O_2^-, may initiate a cascade of events leading to cell damage. The following reactions of the ascorbyl radical (Asc^-) yield dehydroascorbate, the oxidized form of ascorbic acid. Consequently, the process under consideration may be considered as the oxidation of $AscH^-$ catalyzed by Q.

‡ Corresponding author: Dr. G. Bruchelt, Children's Hospital, University of Tuebingen, Ruemelinstr. 23, D-72070 Tuebingen. TEL: +49(07071)2984710/2984711; FAX: +49(07071)294448; E-mail: gernot.bruchelt@uni-tuebingen.de.

§ *Abbreviations*: $AscH^-$, ascorbate; Asc^-, ascorbyl radical; EP, epinephrine; O_2^-, superoxide; Q, quinones; R_{OX}, rate of oxygen consumption; $(R_{OX})_0$, starting rate of oxygen consumption; $(R_{OX})_{SS}$, steady-state rate of oxygen consumption; 6-OHDA, 6 hydroxydopamine; SOD, superoxide dismutase; UQ, 2,3-dimethoxy-5-methyl-1,4-benzoquinone (ubiquinone-0); UQ^-, semiquinone from UQ; UQH_2, 2,3-dimethoxy-5-methyl-1,4-hydroquinone.

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In this work, the detailed kinetic studies of the oxidation of two bioreductants, $AscH^-$ and epinephrine (EP) in the presence of 2,3-dimethoxy-5-methyl-1,4-benzoquinone, ubiquinone-0 (UQ), the simplest synthetic analogue of coenzyme Q, were performed for the first time. As UQ was found to be an extremely powerful catalyst for $AscH^-$ and EP oxidation, this compound was subjected to the MTT test to assess its cytotoxic capability with cell cultures of neuroblastoma, tumor cells of the sympathetic nervous system.

MATERIALS AND METHODS

Chemicals and Solutions

2,3-Dimethoxy-5-methyl-1,4-benzoquinone and epinephrine (Sigma), ascorbic acid (Fluka), Chelex-100 resin (Bio-Rad) were used as received. Sodium phosphates, Na_2HPO_4 and NaH_2PO_4, of highest grade were purchased from Merck. All the other materials were of highest available grade. Aqueous solutions were prepared with doubly distilled water. Fifty millimolar solutions of Na_2HPO_4 and NaH_2PO_4 used for the buffer preparation were purged from traces of transition metals by Chelex-100 by using a batch method [10]. Experiments were performed with 50 mM phosphate buffer, pH 7.40 ± 0.02, at 37° unless otherwise indicated. Aqueous stock solutions of substances under study were added to the phosphate buffer to prepare solutions for testing.

Measurement of Oxygen Uptake

The kinetics of oxygen consumption were studied with a Yellow Springs Instruments Co. Model 5300 Oxygen Biological Monitor with a Clark electrode as a sensor with vigorous stirring of the tested solution with a magnetic agitator. Prior to adding the active components ($AscH^-$, UQ, EP), the buffer solution and a plunger with a Clark electrode were stored in the working cell for 10 min to allow thermal, dissolved oxygen concentration and electrochemical equilibrium to be reached. The rate of oxygen consumption (R_{OX}) was calculated from a slope of $[O_2]$ traces. As R_{OX} was expected to depend on $[O_2]$ (for $AscH^-$ oxidation as was demonstrated in our previous work [11]), R_{OX} was determined over a narrow range of $[O_2]$ in solution from 100 to 80% of starting value of *ca.* 200 μM. When $[O_2]$ decreased below the above value, a plunger with the Clark electrode was removed from the tested solution for a few minutes to restore the starting concentration of O_2; it was then reimmersed into the solution and monitoring was resumed. In this case, a kinetic curve of O_2 consumption might be reconstructed by graphical integration of the plot of R_{OX} vs. time.

ESR Measurements

The steady-state concentration of $Asc^{\cdot}$ was determined with a Varian E 12 ESR spectrometer operating at 9.25 GHz and equipped with a TE_{104} dual cavity and a temperature controller. Instrument settings were as follows: modulation frequency, 12.5 kHz; microwave power, 5 mW; and modulation amplitude, 0.63 G. More details of the protocol we followed for ESR determinations have been reported elsewhere [11, 12]. The solutions tested were prepared just before a run. The recording of ESR spectra was started 2 min after inserting a sample into the cavity to allow temperatures to equilibrate. To maintain constant $[O_2]$, the solution tested was steadily bubbled in the cell with air using a thin quartz capillary with polymer coating.

Experiments with Neuroblastoma Cell Culture

The human neuroblastoma cell line SK-N-SH was obtained from ATCC. Cytotoxic effects of UQ, 6-hydroxydopamine (6-OHDA) and menadion were investigated both in the absence and presence of $AscH^-$ by the MTT test [13]: 10,000 SK-N-SH cells/200 μL cell culture medium (RPMI 1640, supplemented with 10% fetal calf serum) were seeded in 96 well plates. Twenty-four hr later different concentrations of the substances under study were added. Incubation was carried out for 1 hr at 37°. Thereafter, the incubation medium was removed from the adherent cell layer. After addition of 200 μL cell culture medium, incubation was carried out for 3 days. For the determination of cell vitality, the MTT test was carried out as described [13].

The values given in the table and plotted are means of 2 to 5 individual determinations. The variance was not more than ±10% for either R_{OX} or $[Asc^{\cdot}]$ and ±15% for experiments with cell culture.

RESULTS

Ascorbate Oxidation in the Presence of UQ

In the absence of UQ, $AscH^-$ was oxidized rather slowly. Adding UQ caused a visible increase in R_{OX} (Fig. 1). The maximum value of R_{OX} was observed immediately after mixing reagents ($(R_{OX})_0$), then R_{OX} decreased progressively with time and within a few minutes reached a steady-state level ($(R_{OX})_{SS}$) that was constant for a rather long period (Fig. 1, trace 2). The duration of the period when oxygen uptake occurred was limited only by $AscH^-$ concentration. The concentration of oxygen consumed and thus that of $AscH^-$ oxidized [11] typically exceeded many times the starting concentration of UQ. This observation suggests that the oxidation of $AscH^-$ in the presence of UQ is truly a chain, catalytic process with UQ serving as a catalyst. Catalase and superoxide dismutase (SOD) somewhat retarded oxygen consumption (traces 3 and 4 in Fig. 1). This is evidence for the formation of hydrogen peroxide and the involvement of $O_2^{\cdot}$ in the process under study.

As Fig. 2A suggests, both $(R_{OX})_O$ and $(R_{OX})_{SS}$ increased at $[AscH^-] = 1$ mM directly with [UQ]. The determination of $(R_{OX})_O$ at various concentrations of $AscH^-$ showed that $(R_{OX})_O$ was proportional to the [UQ] $[AscH^-]$ product without regard to concentrations of the individual reagents

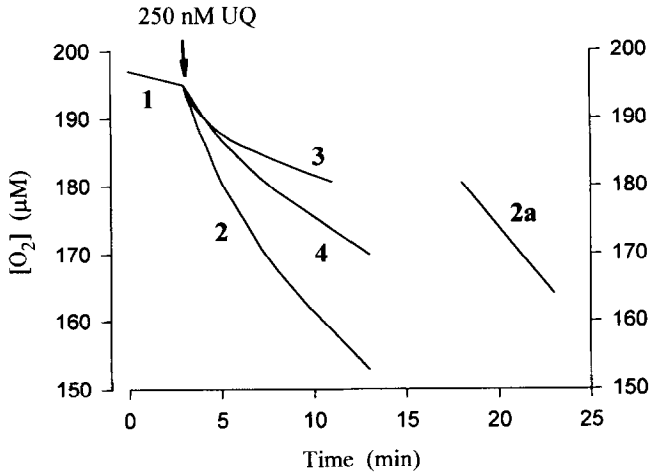

FIG. 1. Kinetics of oxygen consumption during the oxidation of 1 mM $AscH^-$ alone (trace 1) and in the presence of 250 nM UQ in 0.05 M phosphate buffer, pH 7.40, at 37° containing no enzyme (traces 2 and 2a), 100 U/mL SOD (trace 3) and 200 U/mL catalase (trace 4). Trace 2a was recorded after 5 min of restoring oxygen concentration as indicated in "Materials and Methods." Arrow shows the moment when UQ was added.

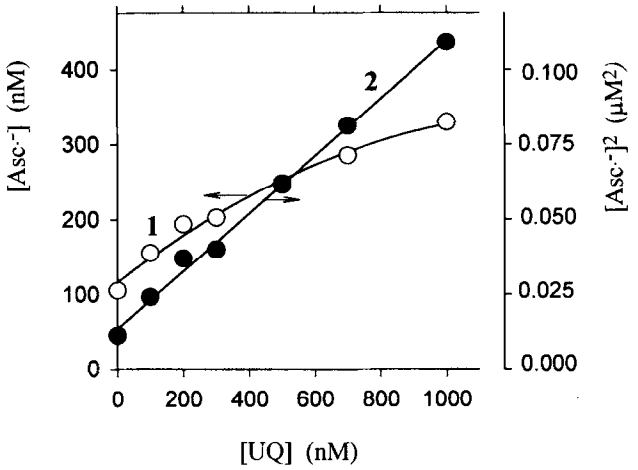

FIG. 3. Effect of [UQ] on steady-state concentration of $Asc^{\cdot}$ (plot 1) and $[Asc^{\cdot}]^2$ (plot 2) during the oxidation of 1 mM $AscH^-$ in 0.05 M phosphate buffer, pH 7.40, at 37°.

(Fig. 2B). Previously this was observed during the oxidation of $AscH^-$ catalyzed by ubiquinone-1 [8]. Consequently, the relationship between R_{OX} and concentrations of $AscH^-$ and UQ may be expressed by the equations:

$$(R_{OX})_0 = (k_{eff})_0 [AscH^-] [UQ] \quad (1)$$

$$(R_{OX})_{SS} = (k_{eff})_{SS} [AscH^-] [UQ] \quad (1a)$$

where $(k_{eff})_0$ and $(k_{eff})_{SS}$ are effective bimolecular rate constants characterizing the capability of UQ to catalyze $AscH^-$ oxidation. The value of $(k_{eff})_0$ calculated from the slope of the plot given in Fig. 2B was found to be 480 ± 35 M^{-1} sec^{-1}. $(k_{eff})_{SS}$ at 1 mM $AscH^-$ was found from Fig. 2A to be as much as 185 ± 10 M^{-1} sec^{-1}. Unlike Eq. 1, Eq. 1a is not universal, being valid only for a given concentration of $AscH^-$. The difference between $(k_{eff})_0$ and $(k_{eff})_{SS}$ decreased as $[AscH^-]$ decreased. When $[AscH^-]$ was less than ~200 μM, $(k_{eff})_0$ and $(k_{eff})_{SS}$ became almost indistinguishable (not shown).

$AscH^-$ oxidation in the presence of UQ was accompanied by the increase in the steady-state concentration of $Asc^{\cdot}$ (Fig. 3). In our previous work [11], $[Asc^{\cdot}]$ was reported to be related to the rate of $AscH^-$ oxidation in 50 mM phosphate buffer, pH 7.4, at 37° by the following simple equation

$$[Asc^{\cdot}]^2 = 5 \times 10^{-5} R_{OX} \quad (2)$$

where $[Asc^{\cdot}]$ is given in M and R_{OX} in M/sec independent of the nature and concentration of a catalyst. Combining Eqs. 1b and 2, the relation between $[Asc^{\cdot}]$ and concentrations of reagents may be obtained

$$[Asc^{\cdot}]^2 = [Asc^{\cdot}]_0^2 + 5 \times 10^{-5} (k_{eff})_{SS} [AscH^-] [UQ] \quad (3)$$

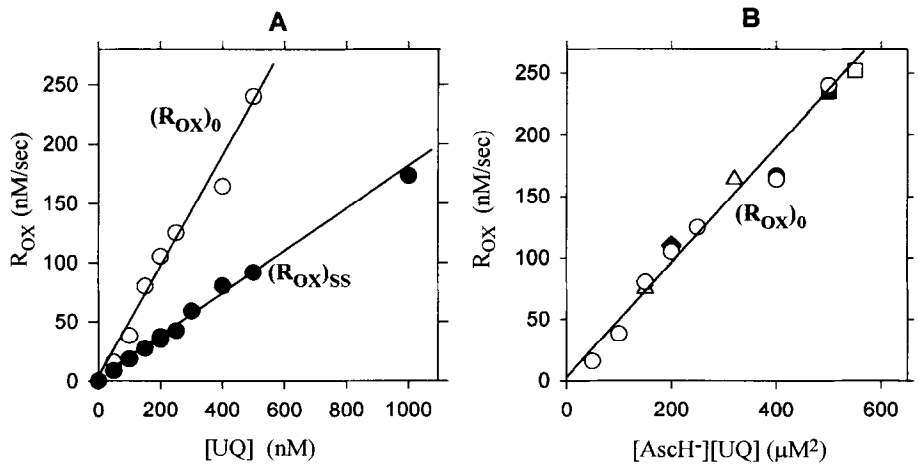

FIG. 2. Effect of reagent concentrations on R_{OX} during the oxidation of ascorbate in the presence of UQ in 0.05 M phosphate buffer, pH 7.40, at 37°. A) Plots of $(R_{OX})_0$ and $(R_{OX})_{SS}$ vs. [UQ] at 1mM $AscH^-$. B) Plot of $(R_{OX})_0$ against the product of $[UQ][AscH^-]$ at different concentrations of $AscH^-$: 50 μM (◆); 80 μM (△); 100 μM (●); 0.5 mM (□); 1 mM (○); 2 mM (■). Both $(R_{OX})_0$ and $(R_{OX})_{SS}$ were calculated as the difference between R_{OX} in the complete reaction mixture and that without UQ.

where $[Asc^{\cdot}]_0$ is a concentration of $Asc^{\cdot}$ in the absence of UQ. As is seen in Fig. 3, $[Asc^{\cdot}]^2$ is a linear function of [UQ] as predicted by Eq. 3. The $[Asc^{\cdot}]^2$–[UQ] plot provides the second way to determine $(k_{eff})_{SS}$. The value of $(k_{eff})_{SS}$ calculated from the slope of this plot (Fig. 3) was found to be as much as 215 ± 10 M^{-1} sec^{-1}, in close agreement with that of 185 ± M^{-1} sec^{-1} determined above from the $(R_{OX})_{SS}$–$[AscH^-][UQ]$ plot (Fig. 2B).

The rate of the process under study increased with temperature. With 1 mM $AscH^-$ and 500 nM UQ, the following values of $(R_{OX})_0$ were determined (in nM/sec): 54 (17°), 94 (25°), 187 (30°) and 324 (37°). The activation energy calculated from these data was as much as 16.7 ± 0.8 kcal/mol.

The study of pH effect on R_{OX} was undertaken because of the observation reported in [14] that the rate of the reduction of UQ by ascorbic acid under anaerobic conditions was pH-dependent with a maximum at pH 5.1. In contrast, we found that $(R_{OX})_0$ increased progressively with pH and did not display any extremum throughout the pH range studied (from 4.5 to 8.5). For instance, at [UQ] = 250 nM and $[AscH^-]$ = 1 mM, $(R_{OX})_0$ (in nM/sec) changed with pH as follows: ~0 at pH 4.5 and 5.0; 20 at pH 6.0; 94 at pH 7.0; 150 at pH 7.4; 1600 at pH 8.5. The reason why our observations are in conflict with those reported in Ref. 14 remains unclear.

Redox interaction of UQ with $AscH^-$ is likely accompanied by the formation of 2,3-dimethoxy-5-methyl-1,4-hydroquinone (UQH_2), a reduced form of UQ [8, 14]. It is not excluded, in principle, that the autoxidation of UQH_2 makes a certain contribution to the oxygen uptake in the system under consideration [1]. To estimate the role of UQH_2, the oxidation of this compound was studied by using a Clark electrode. Similar to a structural analogue of UQH_2, the reduced form of ubiquinone-1 [8], UQH_2, showed only a moderate capability to consume oxygen in pH 7.4 buffer even at concentrations which exceeded many times the concentrations of UQ typical of this study (commonly < 1 μM). For instance, with 100 μM UQH_2, R_{OX} was only 14 ± 2 nM/sec. This suggests that the role of UQH_2 in oxygen consumption during the oxidation of $AscH^-$ catalyzed by UQ is negligible.

Figure 4 demonstrates the effect of SDS micelles on the kinetics of $AscH^-$ oxidation catalyzed by UQ. These experiments aimed to imitate the behavior of the system under study in biological environments. R_{OX} decreased with SDS but only to a certain limit that was nearly half the R_{OX} determined in the absence of SDS (plot 1). The decrease in R_{OX} following the addition of SDS was accompanied by a corresponding decrease in $[Asc^{\cdot}]$ (not shown). The decrease in R_{OX} and $[Asc^{\cdot}]$ is most likely caused by a phase separation of the reactants. The solubility of UQ in organic phase is undoubtedly higher than that in water. Thus, when SDS increases progressively, the fraction of UQ transferred into the micellar phase increases. At the same time, $AscH^-$, being almost insoluble in organic phase, remains in water. The space separation of reactants may

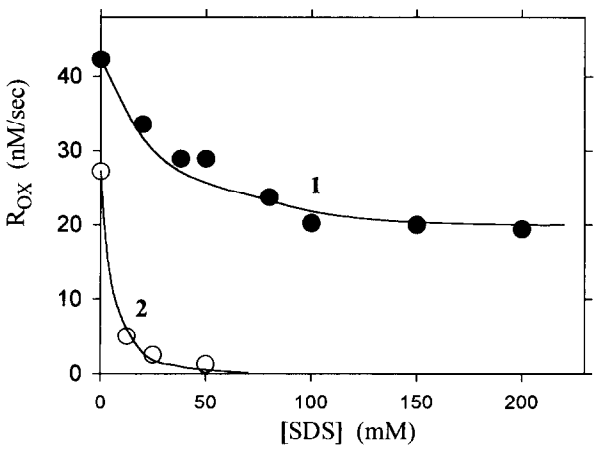

FIG. 4. SDS effect on the starting rate of the oxidation of 1 mM $AscH^-$ in the presence of 500 nM UQ (plot 1) and 5 μM ubiquinone-1 (data taken from ref. 8) (plot 2). Conditions were 0.05 M phosphate buffer, pH 7.40, 37°.

hinder their redox interaction, similar to that observed during the oxidation of $AscH^-$ in SDS micellar solution in the presence of methylene blue [11] and ubiquinone-1 [8] (see also plot 2 in Fig. 4). The observation that the value of R_{OX} in the UQ–$AscH^-$–SDS system did not show any tendency to go to zero even at very high SDS concentrations may signify that the reaction between UQ and $AscH^-$ proceeds at a reduced but measurable rate even when the reactants are separated, in contrast to that of ubiquinone-1.

Epinephrine Oxidation in the Presence of UQ

EP oxidized with a pronounced self-acceleration (Fig. 5), suggesting that products of EP oxidation, most likely Qs, induce a catalytic effect [15]. This possibility was confirmed by the visible accelerating effect of adrenochrome, a prin-

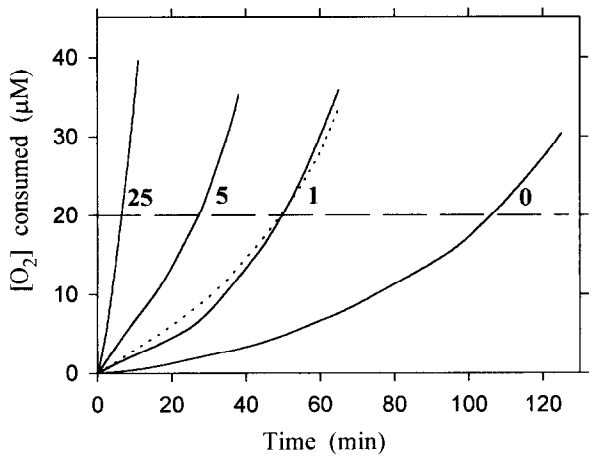

FIG. 5. Kinetics of oxygen consumption during the oxidation of 2 mM EP in the presence of concentrations of UQ indicated in μM; dotted line shows $[O_2]$ trace during the oxidation of 2 mM EP in the presence of 25 μM adrenochrome. Conditions were 0.05 M phosphate buffer, pH 7.40, 37°. Horizontal dash line shows the level of 20 μM oxygen consumption.

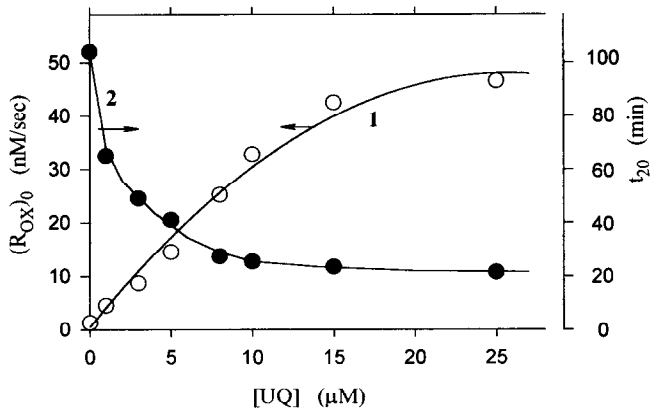

FIG. 6. Effects of [UQ] on $(R_{OX})_0$ (plot 1) and t_{20} (plot 2) during the oxidation of 2 mM EP in 0.05 M phosphate buffer, pH 7.40, at 37°.

ciple product of EP oxidation [16] (Fig. 5). Therefore, it is not a surprise that UQ exerted a well-pronounced, concentration-dependent catalytic effect on EP oxidation (Fig. 5). It should be taken into account that in addition to UQ, "aboriginal" quinoid products, e.g. adrenochrome, accumulated in the course of EP oxidation, play the role of catalyst. This may explain why EP oxidation at moderate concentrations of UQ occurred with visible self-acceleration. As evident from Fig. 5, the catalytic activity of UQ exceeds *ca.* 25-fold that of adrenochrome. At the same time, it should be noted that the minimum UQ concentration which caused a measurable catalytic effect on EP oxidation was approximately one and a half orders of magnitude higher than that of $AscH^-$ oxidation (see Figs. 1 and 5 for comparison).

To quantify the catalytic effect of UQ on EP oxidation, two values were used: the starting value of R_{OX} that was observed immediately after adding UQ ($(R_{OX})_0$) and the time it took for the uptake of 20 μM O_2 (t_{20}). Figure 6

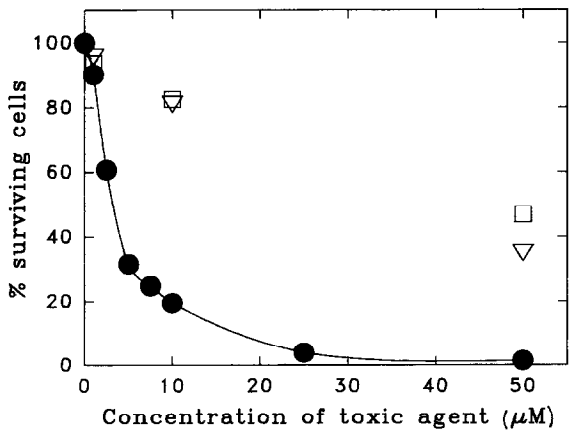

FIG. 7. Concentration-dependent cytotoxicity of UQ (●), 6-hydroxydopamine (▽) and menadion (□) on the neuroblastoma cell line SK-H-SH determined by the MTT test as indicated in "Materials and Methods."

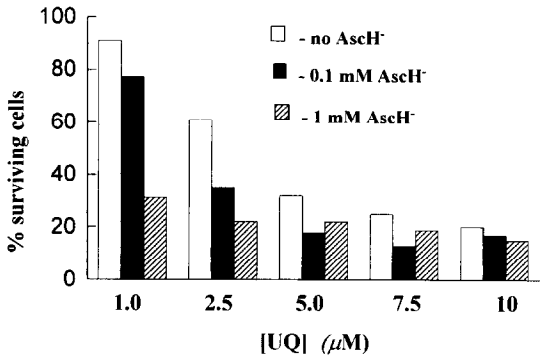

FIG. 8. Influence of $AscH^-$ on the cytotoxicity of various concentrations of UQ on neuroblastoma cell line SK-H-SH determined by the MTT test as indicated in "Materials and Methods."

demonstrates that both $(R_{OX})_0$ and t_{20} arrived at limiting levels when UQ concentration was as much as 10–15 μM.

Cytotoxic Effect of UQ on Neuroblastoma Cells

In Fig. 7 the cytotoxic action of UQ on human neuroblastoma cells is compared to that of menadione, a widely used cytotoxic quinone [1], and 6-hydroxydopamine, a well-known neurotoxin (reported as the most effective killing agent for human neuroblastoma cells [17]). UQ cytotoxicity was far in excess of the cytotoxicity of both menadione and 6-hydroxydopamine. Even at micromolar concentrations, UQ displayed a pronounced ability to kill neuroblastoma cells. The cytotoxicity of UQ was markedly enhanced in the presence of $AscH^-$ (Fig. 8). The effect of $AscH^-$ was most pronounced at the lowest UQ concentrations studied.

DISCUSSION

UQ proved to be a very active catalyst for $AscH^-$ oxidation, displaying a catalytic action even at nanomolar

TABLE 1. The values of k_{eff} characterizing catalytic activity of various oxidizing agents as a catalyst of ascorbate oxidation in 0.05 M phosphate buffer, pH 7.40, at 37° except as otherwise noted; k_{eff} was calculated from R_{OX} by Eq. (1) by replacing by that of the corresponding catalyst

Oxidizing agent	k_{eff} (M^{-1} sec^{-1})	Reference
Fe^{3+}	2.8	10
Fe^{3+} + EDTA	43	10
Cu^{2+}	880	3*
Methylene blue	4.7	10
Dipyridinium chloride	1.4	20†
Menadione	3.5	‡
Quinone from 6-OHDA	4.8	‡
Ubiquinone-1	28	9
UQ	480	this work

* pH 7.0, 20°
† pH 7.3
‡ Unpublished data of authors

concentrations. In Table 1 UQ catalytic activity is compared to that of some other catalytic agents. It can be seen that UQ reactivity substantially exceeds that of all known catalysts, both organic and transition metals, with the sole exception of Cu^{2+}. In contrast to $AscH^-$, the related data for EP oxidation are not available in the literature.

As Fig. 5 suggests, the activity of UQ as a catalyst for EP oxidation exceeds by nearly twenty five times that of adrenochrome, a "natural" catalyst of EP oxidation.

Because of the shortage of kinetic and mechanistic information, we experienced considerable difficulties in suggesting a detailed and well-substantiated kinetic scheme for the process under study. The consequence of reactions (1) and (2)

$$UQ + AscH^- \Leftrightarrow UQ^{\bullet-} + Asc^{\bullet-} + H^+ \quad (1)$$

$$UQ^{\bullet-} + O_2 \Leftrightarrow UQ + O_2^{\bullet-} \quad (2)$$

is the simplest one to provide the catalytic oxidation of $AscH^-$ and effective UQ recycling. The trigger stage of the process under consideration, the forward reaction (1), is thermodynamically unfavorable. The change of redox potential in the forward reaction (1), $\Delta E(1)$, may be calculated as the difference

$$\Delta E(1) = E(UQ/UQ^{\bullet-}) - E(Asc^{\bullet-}/AscH^-)$$

The value for $E(Asc^{\bullet-}/AscH^-)$ is as much as +280 mV [19]. $E(UQ/UQ^{\bullet-})$ has not been reported but is likely not far from that of coenzyme Q (from −110 to −150 mV [18, 19]). Thus, $\Delta E(1)$ is expected to be close to −400 mV. Consequently, the forward reaction (1) is very endothermic. This reasonably correlates with the rather high activation energy of the net process determined in this study. Equilibrium (1) can be substantially shifted to the right thanks to fast reaction (2) of $UQ^{\bullet-}$ with oxygen. As may be expected on the basis of the correlations reported in [5], the rate constant for reaction (2) is high enough to move equilibrium (2) to the right.

To consider the kinetic regularities of the process under study, the simplest kinetic scheme consisting of reactions (1) and (2) should be supplemented by the following reactions:

$$Asc^{\bullet-} + Asc^{\bullet-} \rightarrow AscH^- + \text{dehydroascorbate} \quad (3)$$

$$UQ^{\bullet-} + UQ^{\bullet-} + 2\,H^+ \Leftrightarrow UQ + UQH_2 \quad (4)$$

$$UQ^{\bullet-} + O_2^{\bullet-} + 2H^+ \rightarrow UQ + H_2O_2 \quad (5)$$

$$UQH_2 + O_2^{\bullet-} \rightarrow UQ^{\bullet-} + H_2O_2 \quad (6)$$

$$AscH^- + O_2^{\bullet-} \rightarrow Asc^{\bullet-} + H_2O_2 \quad (7)$$

Reactions (4) and (5) reduce the steady-state concentration of $UQ^{\bullet-}$ and compete with reaction (2). In the course of the process, some portion of the original UQ converts into UQH_2 which, as expected, reduces the rate of catalytic oxidation of $AscH^-$. Reaction (6) followed by (2) and (4) results in the reverse transformation of UQH_2 into UQ. The reduction of R_{OX} from $(R_{OX})_O$ to $(R_{OX})_{SS}$ (Fig. 1) with time reflects, most likely, a decrease in UQ concentration due to its partial transformation into UQH_2. If so, the steady-state ratio of [UQ] to $[UQH_2]$ can be estimated from the relation

$$\frac{(R_{OX})_{SS}}{(R_{OX})_O} = \frac{[UQ]_O - [UQH_2]}{[UQ]_O} = 1 - \frac{[UQH_2]}{[UQ]_O}$$

where $[UQ]_0$ is a starting concentration of UQ. The closer the ratio $(k_{eff})_{SS}/(k_{eff})_0$ to unity, the less the steady-state concentration of UQH_2 and thus the higher recycling capability (reversibility) of the catalytic system. The competition between reactions (6) and (7) may explain why $(k_{eff})_{SS}/(k_{eff})_0$ increases when $[AscH^-]$ decreases. The less the $[AscH^-]$, the higher the probability of UQH_2 to be transformed into $UQ^{\bullet-}$ via reaction (6).

The following features of UQ make it possible to predict its high cytotoxicity, especially in the presence of an elevated concentration of $AscH^-$:

- The profound catalytic activity of UQ on $AscH^-$ oxidation, a process which results in the formation of reactive oxygen species and thus may contribute to oxidative stress. As the process under consideration is accompanied by vigorous oxygen uptake, this may further result in pronounced hypoxia in target cells;
- The very high reversibility of the catalytic processes, which may provide a long-lasting cytotoxic action likely limited only by the available amount of reductants, e.g. $AscH^-$, EP etc;
- The fact that catalytic activity of UQ only moderately decreases in the presence of the lipid phase, common antioxidative enzymes SOD and catalase suggests that UQ will maintain its action in going from the very simple chemical models studied in the current work under *in vivo* conditions;
- For the cells of the sympathetic nerve system including neuroblastoma cells which are known to be rich in catecholamines, there is possibly one more pathway of UQ cytotoxicity. This is related to its catalytic activity in the oxidation of EP observed in the current work and likely to that of other catecholamines.

As was shown in Fig. 7, UQ displays the profound capability to kill neuroblastoma cells in culture. This is not the only example of very high toxicity of methoxy-substituted quinones to tumour cells. 2,3 and 2,6-dimethoxybenzoquinones were reported to be very effective antitumor drugs in Ehrlich ascites-bearing mice but only in the presence of elevated concentrations of $AscH^-$ [9].

Curiously, the endogeneous analogs of UQ, coenzymes Q, widely distributed in living systems [21], do not display any cytotoxicity, in contrast to UQ. The presence of a

long-chain substituent with 40–50 carbon atoms in a coenzyme Q molecule provides a high lipophility. Its resultant separation from $AscH^-$ and other water-soluble reductants is most likely responsible for the dramatic difference in the toxicity observed UQ and coenzymes Q. The lack of redox interaction between $AscH^-$ and ubiquinone in the presence of lipid phase was previously demonstrated by the example of ubiquinone-1 having only 5 carbon atoms in its side-chain [8].

In conclusion, UQ and related compounds may be considered, in combination with $AscH^-$, as a potential "binary" antitumor drug whose action is based on the production of reactive oxygen species and vigorous oxygen consumption in target cells. The kinetic model developed in the current work seems to be very suitable for prebiological express-discovery of the cytotoxic potential of "binary" compositions with similar mechanisms of toxicity. Some of these compositions may be potential antitumor drugs.

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