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Reduction of 4-Nitroquinoline 1-Oxide to 4-Hydroxyaminoquinoline 1-Oxide in Lysates of Cytomegalovirus-infected Cells

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SUMMARY

The rates of virus inactivation by 4-nitroquinoline 1-oxide (NQO) and 4-hydroxyaminoquinoline 1-oxide (HAQO) were compared and samples of cytomegalovirus (CMV)-infected cell lysates to which NQO had been added were examined for the presence of HAQO. These experiments demonstrated that (i) CMV inactivation by HAQO was more rapid than with NQO, (ii) virus inactivation by either NQO or HAQO failed to demonstrate a photodynamic component, and (iii) NQO-treated stocks contained HAQO, indicating reduction of NQO to HAQO. The results support the concept that metabolism of NQO to HAQO enhances the genotoxic effect of NQO.

Previous work with 4-nitroquinoline 1-oxide (NQO) has shown that addition of this carcinogen to stocks of cytomegalovirus (CMV) (Albrecht et al., 1979) or herpes simplex virus type 1 (HSV-1) or type 2 (HSV-2) (Speelman et al., 1981) results in inactivation of virus infectivity. The rate of loss of infectivity with either virus was directly dependent on the concentration of NQO and the duration of exposure to NQO. NQO has been shown to react with DNA (Malkin & Zahalsky, 1966; Nagata et al., 1966) and thus it might be anticipated that the direct reaction of NQO with virus DNA results in inactivation of virus infectivity. However, in other studies NQO was not observed to inactivate transforming DNA (Ono, 1964) or T4 phage (Ishizawa & Endo, 1967), but inactivation was observed when 4-hydroxyaminoquinoline 1-oxide (HAQO) was substituted for NQO in these systems. The present study was undertaken to determine the possible role of HAQO in the loss of CMV infectivity from NQO-treated virus stocks. The data obtained support the concept that NQO is reduced to HAQO in CMV-infected cells and that the genotoxic activity of NQO is mediated by HAQO.

Human embryo lung (LU), thyroid (THY) and skin muscle (SM) cells used in these studies were propagated as previously described (Albrecht & Weller, 1980; Albrecht et al., 1980a). Stocks of the Davis strain (Weller et al., 1957) of human CMV were prepared in THY cells using diluted virus to prevent enrichment of defective particles as previously described (Albrecht & Rapp, 1973). Stocks of CMV had infectivities of 1 x 10⁶ to 4 x 10⁶ p.f.u./ml as determined by the modified (Albrecht & Rapp, 1973) plaque assay of Wentworth & French (1970).

NQO and HAQO were supplied by the NCI Carcinogenesis Research Program through IIT Research Institute, Chicago, Ill., U.S.A. AQO (4-aminoquinoline 1-oxide) was generously provided by Dr Y. Kawazoe of the Faculty of Pharmaceutical Sciences, Nagoya City University, Nagoya, Japan. NQO and AQO were dissolved immediately prior to use in dimethyl sulphoxide (DMSO) (Albrecht et al., 1979). HAQO was dissolved in 0·5 M-HCl. After dilution (1:20) of the HAQO solution in maintenance medium (Eagle's minimum essential medium with
Earle's salts, 5% γ-irradiated foetal calf serum, and 0.15% NaHCO₃), the pH was adjusted to 7.2 by the addition of 7.5% NaHCO₃.

Virus inactivation was done as described in an earlier report (Albrecht et al., 1979). To examine the influence of white light on the rates of CMV inactivation, experiments were done in a dark room indirectly illuminated with a Kodak safelight with an OC filter and 15 W incandescent bulb at a distance of about 1.2 m (dark inactivation). NQO-treated and control virus in 35 × 10 mm tissue culture dishes (1.5 ml/dish) were placed on a Plexiglass diffusion screen and exposed to various doses of white light from a General Electric FC12T10 fluorescent bulb about 13 cm below the Plexiglass surface.

A Waters Associates (Milford, Mass., U.S.A.) model GPC/LC-204 instrument was used for high-pressure liquid chromatography (HPLC). All samples analysed in this report were prepared by filtration through a 0.22 μm Millipore GS filter. Chromatography of samples of NQO-treated cell lysates was accomplished on a 3.9 mm × 30 cm Bondapak C₁₈ reverse phase column (Waters Associates) (Lund et al., 1981). Fractions from HPLC were scanned in 1 × 4 cm fused quartz cells in an Aminco-Bowman spectrophotofluorometer.

NQO is capable of forming two types of free radicals (Kawazoe, 1971; Nagata, 1971) and thus its interactions with DNA are enhanced by light as shown by Nagata et al. (1967). If the inactivation of virus infectivity is directly mediated by NQO, then the rate of inactivation might be expected to be enhanced by white light. To examine this possibility, CMV stocks were treated in the dark with 100 μg/ml NQO at 37 °C. At 2 h intervals, NQO-treated and control virus stock were exposed to various doses of white light and the surviving CMV infectivity was determined in the dark. A logarithmic loss of virus infectivity occurred in the presence of NQO and the absence of light, resulting in survival of 0.2% of the initial infectivity after 6 h. Loss of infectivity as a result of thermal inactivation was one sixty-fifth of the level observed with NQO (Fig. 1 a). As shown in Fig. 1 (b), when these results were plotted as a function of the dose of white light a family of horizontal lines of decreasing intercept was obtained, indicating that the inactivation of CMV was independent of white light. Similar results were also obtained with other CMV stocks and with HSV-1 and HSV-2 (data not shown). No indication of a photodynamic component is evident, since even if the inactivation kinetics contained both photodynamic and light-independent components the family of curves would have had both decreasing slopes and intercepts.

As the results failed to demonstrate a photodynamic component and to support the direct interaction of NQO with virus DNA, an alternative explanation was sought. The reduction of NQO to the non-photodynamic metabolite HAQO (Nagata, 1971) has been demonstrated in mammalian cells (Sugimura et al., 1965, 1966). Thus, one might expect to find a similar reaction in lysates of virus-infected human cells. Substitution of HAQO for NQO in the inactivation protocols (Albrecht et al., 1979) resulted in a biphasic loss of infectivity which was directly dependent on both the HAQO concentration and the time of exposure (Fig. 2). Compared to the results with NQO, which have been described in detail previously (Albrecht et al., 1979), the initial rate of the loss of infectivity (first component) was faster with HAQO.

These data suggest that NQO might have been reduced to HAQO in the infected-cell lysates used as virus stock and that HAQO, rather than NQO, inactivated the virus infectivity. Therefore, NQO-treated virus stocks were examined for the presence of HAQO. Separation of NQO, HAQO, and AQO, a reduced product of HAQO, was first achieved with thin-layer chromatography (TLC) (Sugimura et al., 1965). Substances co-migrating with the three standards and other components (unidentified) were observed in the NQO-treated stocks, but the R₆ values demonstrated some variability, as Sugimura et al. (1965) had observed. More consistent results were obtained by developing silica gel plates with methanol and then with ethyl acetate. Confirming results were obtained with reverse-phase TLC plates, demonstrating the presence of substances co-migrating with HAQO and AQO (data not shown).

Recovery of HAQO, AQO and NQO from TLC plates was poor, making quantification difficult; therefore techniques for the separation of NQO, HAQO and AQO were developed using HPLC. Separation of NQO-treated virus stocks demonstrated the presence of HAQO and AQO (Fig. 3). For example, about 4 μg/ml HAQO, 1 μg/ml AQO and 87 μg/ml NQO were
Fig. 1. Effect of white light on survival of human cytomegalovirus treated in the dark with NQO. NQO-treated virus stock (b) and virus stock mixed in equal volumes with MEM containing 5% DMSO (a) were incubated at 37 °C in the dark. At the indicated times (●, 0 h; ▲, 2 h; ■, 4 h; ◆, 6 h), 1-5 ml samples were removed and exposed to white light for various intervals. Surviving infectivity was determined by plaque assay in monolayers of human embryo skin muscle cells under an agarose overlay.

Fig. 2. Rate of inactivation of cytomegalovirus by HAQO. Each member of the family of curves is derived from assays of virus stock treated with various doses of HAQO for set periods of time (▲, 0 h; ◆, 2 h; □, 4 h; △, 6 h; ○, 8 h). Data were normalized to the control data points for virus stock incubated with solvent for each period of incubation.

detected after 24 h incubation of a CMV stock treated with 100 μg/ml of NQO. The addition of HAQO, NQO or AQO standards to the samples of NQO-treated CMV stocks immediately prior to HPLC analysis resulted in the expected increase in the size of the corresponding peak (data not shown).

Fractions collected during the HPLC of NQO-treated virus stock were examined spectrofluorometrically. Maximum excitation and emission of the fraction containing the presumed HAQO peak were observed at 360 and 478 nm, respectively. The fluorescence spectra and observed maxima are in good agreement with published data for HAQO (Nagata, 1971) and with data obtained with our HAQO standard. Similar results were also obtained on analysis of the fraction containing the presumed AQO peak.

On balance, considering that little, if any, effect of light was detected on the rate of inactivation of NQO-treated CMV, the similar CMV-inactivation curves for NQO (Albrecht et al., 1979) and HAQO (Fig. 2), and the significant amounts of HAQO and AQO detected in NQO-treated CMV stocks, HAQO may be responsible for much, if not all, of the observed loss of CMV infectivity. Apparently, our lysates of virus-infected human cells (CMV and HSV stocks) contain components capable of reducing NQO to HAQO and subsequently to AQO, which were lacking in the preparations of transforming DNA and bacteriophage (Ono, 1964; Ishizawa & Endo, 1967). Our observations support the concept that mutagenic and carcinogenic effects of NQO result following its metabolism to HAQO (Sugimura et al., 1965, 1966).

CMV forms life-long persistent infections of man (Weller, 1971) and often is found in association with tissues demonstrating elevated cancer frequencies. Recent results suggest that CMV persistently infected cells are more susceptible to transformation by chemicals than are non-infected controls (Li & Albrecht, 1982). The observed reduction of NQO to HAQO suggests a co-carcinogenic mechanism by which viruses and chemicals might interact to yield an
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Fig. 3. Analysis of NQO-treated CMV stock by HPLC. A reverse phase column was developed with a methanol gradient (---) in water. Ultraviolet absorbance (-----) was measured at 360 nm. Fluorescence (——) was measured with excitation at 360 nm and emission at 470 nm.

increased frequency of transformation. CMV, in contrast to some other herpesviruses (e.g. HSV; Sydiskis & Roizman, 1967), does not quickly shut down important cell functions, but results in stimulation of cell DNA (St. Jeor et al., 1974), RNA (Tanaka et al., 1975), and protein (Stinski, 1977) synthesis. Since these observations are apparently also valid in persistently infected cells (Albrecht et al., 1976; De Marchi & Kaplan, 1977; Mocarski & Stinski, 1979; Albrecht et al., 1980b), the intracellular metabolism of carcinogens may be influenced by expression of CMV gene products. It is interesting to note in this regard that our data suggest that CMV may induce such effects in infected cells.

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