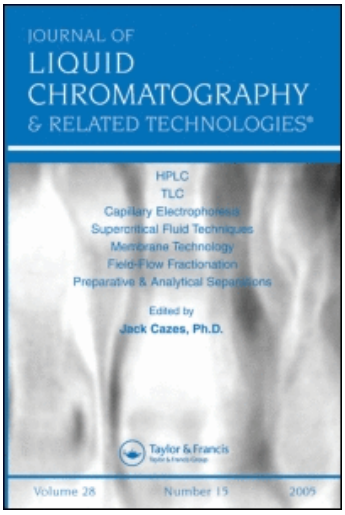

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LIQUID CHROMATOGRAPHIC ANALYSIS OF 4-NITROQUINOLINE 1-OXIDE AND METABOLITES

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ABSTRACT

High pressure liquid chromatographic (HPLC) methods for separation of the carcinogen 4-nitroquinoline 1-oxide (NQO) from its reduced metabolites, 4-hydroxyaminoquinoline 1-oxide (HAQO) and 4-aminoquinoline 1-oxide (AQO), are described. Simultaneous fluorescence and U.V. absorbance analysis, using a gradient system with reversed phase HPLC, gave good resolution of the metabolites.

INTRODUCTION

4-nitroquinoline 1-oxide (NQO) is a carcinogen that has been widely used in both *in vivo* and *in vitro* systems (4). 4-hydroxyaminoquinoline 1-oxide (HAQO), which is produced by reduction of NQO in mammalian cells (14) appears to be a more potent carcinogen than NQO on an equimolar basis (3,6,11). Reduction of HAQO yields 4-aminoquinoline 1-oxide (AQO), which has not demonstrated substantial carcinogenic activity (4).

Both NQO and HAQO have been reported to act directly on DNA *in vitro* (7,9). However, recent data indicate that the carcinogenic effects of NQO may be due, to a large extent, to HAQO

formed by the enzymatic reduction of NQO (13,14). In our studies, which use NQO or HAQO to inactivate the infectivity of cytomegalovirus (CMV) (1,2) or herpes simplex virus (HSV) (12), we have followed the reduction of NQO to HAQO and AQO in lysates of CMV- or HSV- infected cells. HPLC analysis of NQO-treated cell lysates has been an effective method for following the metabolism of NQO to HAQO and AQO.

MATERIALS AND METHODS

Chemicals

NQO and HAQO were supplied by the NCI Carcinogenesis Research Program through IIT Research Institute, Chicago, Illinois. AQO was the generous gift of Dr. Yutaka Kawazoe of the Faculty of Pharmaceutical Sciences, Nagoya City University, Nagoya, Japan. Solvents were from Burdick and Jackson (Muskegon, Michigan).

Chromatography

For TLC, Brinkmann Instruments (Westbury, New York) 20 cm x 20 cm, 0.25 mm thick Silica G plates without fluorescence indicator were used. Whatman (Clifton, New Jersey) 7.6 cm x 7.6 cm, 0.20 mm thick KC_{18} plates were used for reverse phase TLC. HPLC was performed on a Waters Associates, Inc. (Milford, Mass.) model GPC/LC-204 instrument equipped with a model 660 Solvent Programmer, two model M6000A pumps and a model U6K injector system. HPLC separation of the ethanol extracts of NQO-treated cell lysates was accomplished on a 3.9 mm x 30 cm μBondapak C_{18} reverse phase column (Waters Assoc.). A model SF-770 variable wave-length spectrophotometric detector and a model FS-970 fluorescence

detector (Schoeffel Inst. Corp., Westwood, New Jersey) were connected in series to the outlet of the column. The signals from the detectors were recorded on a Houston Instruments (Austin, Texas) model A5211-1 two pen recorder. U.V. absorbance was monitored at 360 nm. The excitation wavelength of the fluorescence detector was set at 360 nm and a KV-470 emission filter was used. In the HPLC system, a 15 min linear gradient at 1.5 ml per minute was produced from 10 or 20% methanol in water to 90 or 100% methanol.

Fractions of the HPLC eluant were collected at 1 min intervals and analyzed on an Aminco-Bowman spectrophotofluorometer equipped with a 416-992 xenon lamp, a R136 photomultiplier tube and a 1620-809 X-Y recorder. The fractions were scanned in 1 x 4 cm fused quartz cells at sensitivity 39, slit arrangement 3 and meter multiplier readings of 0.03 to 0.001.

Sample Preparation

Details of cell culture, virus propagation, carcinogen treatment and virus inactivation have been previously published (1). NQO, when used to treat virus stocks, was dissolved immediately prior to use in dimethylsulfoxide and diluted in maintenance medium (1). HAQO was dissolved in 0.50N HCl. After dilution of the HAQO solution in maintenance medium (1:20), the pH was adjusted to 7.2 by the addition of 7.5% sodium bicarbonate ($NaHCO_3$). When used as standards for TLC or HPLC, the carcinogens were dissolved in the indicated organic solvent immediately prior to use.

For TLC, NQO-treated virus stocks were lightly sonicated, added to an equal volume of the first TLC solvent, and 5 µl volumes were repeatedly spotted on the TLC plates. Fluorescence of the carcinogens was observed with a model B-100A longwave U.V. lamp (Ultra-Violet Products, Inc., San Gabriel, Calif.).

Samples from NQO-treated, virus-infected cell lysates were prepared for HPLC by heating in boiling water for 1 min and sedimenting the precipitated protein at 240 x G for 10 min. Ethanol (95%) was added to the supernatant to reach a 65% ethanol concentration. The specimen was then maintained on ice for 15 min and clarified by centrifugation as before.

RESULTS

In our previous data with virus inactivation by NQO, no convincing photodynamic component was evident in virus survival curves (1,12). NQO, however, has been shown to be photodynamically activated and such a component would be anticipated based on the structure of NQO (7,8). Reduction of NQO to HAQO and AQO had been observed in rat cells (13,14) and, therefore, might be expected in human cells. HAQO, in contrast to NQO, is not photodynamically activated (4) and has been shown to inactivate both transforming DNA (10,15) and bacteriophage T_4 (5). Thus, it appeared that HAQO, rather than NQO, might be responsible for the observed inactivation of virus infectivity. NQO-treated lysates of virus-infected cells were, therefore, examined for the presence of HAQO.

TLC separation of NQO, HAQO and AQO was first performed by the method of Sugimura et al. (13) using silica gel plates and a solvent

system of sec-butanol:ethyl acetate:water (1:1:1). Comigration of samples with known standards was observed, suggesting that detectable amounts of HAQO and AQO were formed in the lysates of virus-infected cells. However, variability of the Rf values of both standards and samples, as observed by Sugimura et al. (13), was noted. Silica gel plates developed first with methanol and then with ethyl acetate gave somewhat more consistent results. Reverse phase TLC with acetonitrile:water (75:25) as the solvent was used to confirm results with the above systems. Unfortunately, during TLC procedures, degradation of HAQO was noted (13) and recovery of standards and samples was poor.

HPLC separation of NQO, HAQO and AQO standards (Fig. 1) was developed to permit reproducible separation and recovery of these chemicals from cell lysates. HPLC separation of extracts of NQO-treated virus-infected cells demonstrated significant amounts of HAQO and AQO (Fig. 2). The fluorescent and U.V. peaks observed during the chromatography of the NQO-treated lysates of virus-infected cells corresponded to the retention times of the NQO, HAQO and AQO standards. Addition of standards to NQO treated lysates of virus-infected cells resulted in the expected increase in the size of the corresponding peak on the chromatogram.

The use of HPLC separation allowed for ready spectrofluorometric examination of the eluate. The maximum excitation of the HAQO containing fractions was at 360 nm and maximum emission was at 478 nm (Fig. 3). These values and the

shape of the emission and excitation scanning curves are in good agreement with the published values for HAQO (4,13,14) and data obtained using a HAQO standard on the same equipment.

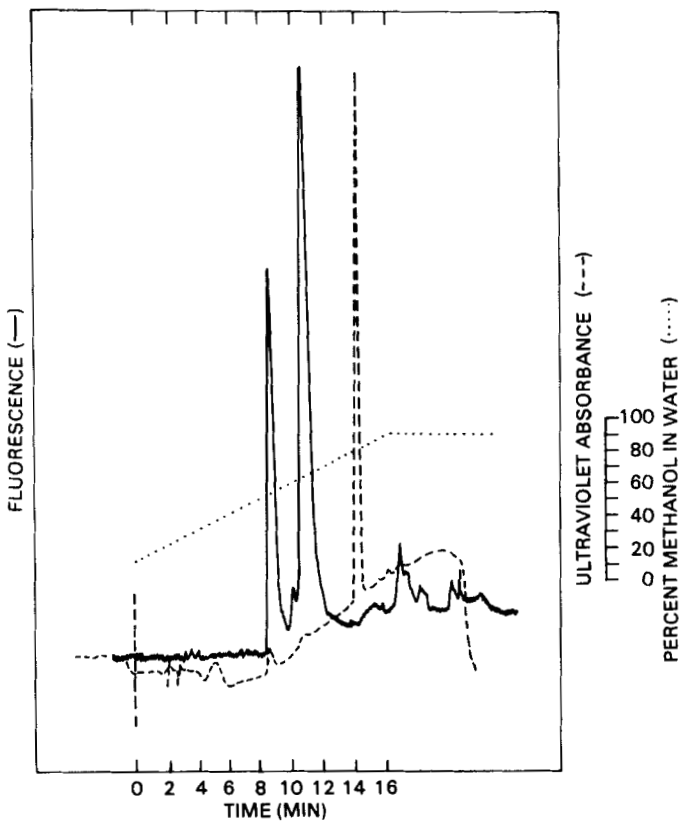

Figure 1. HPLC analysis of NQO, HAQO and AQO standards. A reverse phase column was developed with a 10 to 90% methanol gradient (····) in water. Fluorescence (——) was measured with excitation at 360 nm and emission at 470 nm. U.V. absorbance (----) was measured at 360 nm.

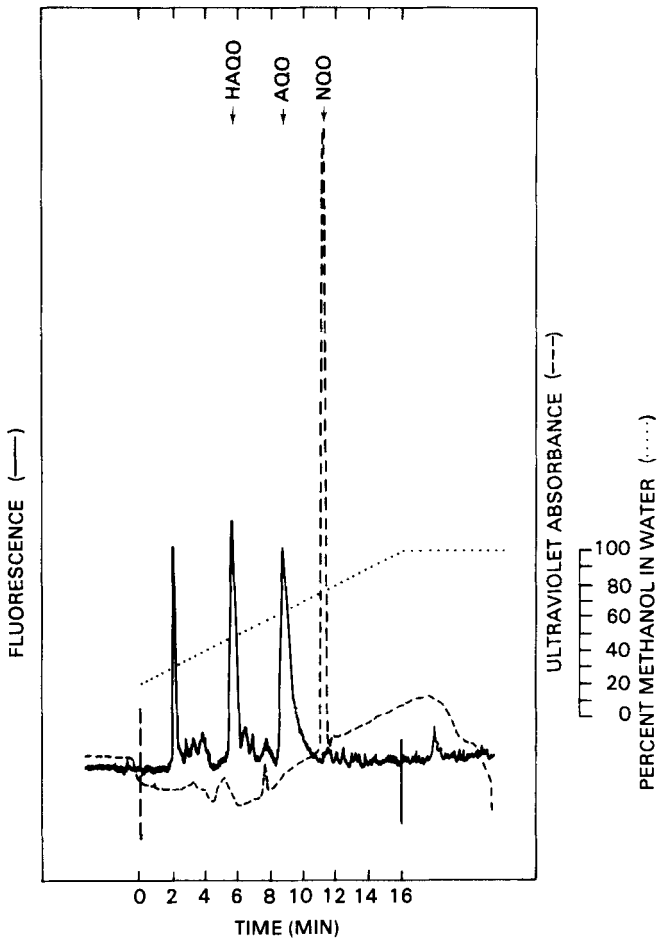

Figure 2. HPLC analysis of an ethanol extract of a NQO-treated (50 μg/ml) cytomegalovirus (strain AD-169) stock. A reverse phase column was developed with a 20 to 100% methanol gradient (····) in water. Fluorescence (——) was measured with excitation at 360 nm and emission at 470 nm. U.V. absorbance (----) was measured at 360 nm. The retention times of NQO, HAQO, and AQO standards are indicated ( ).

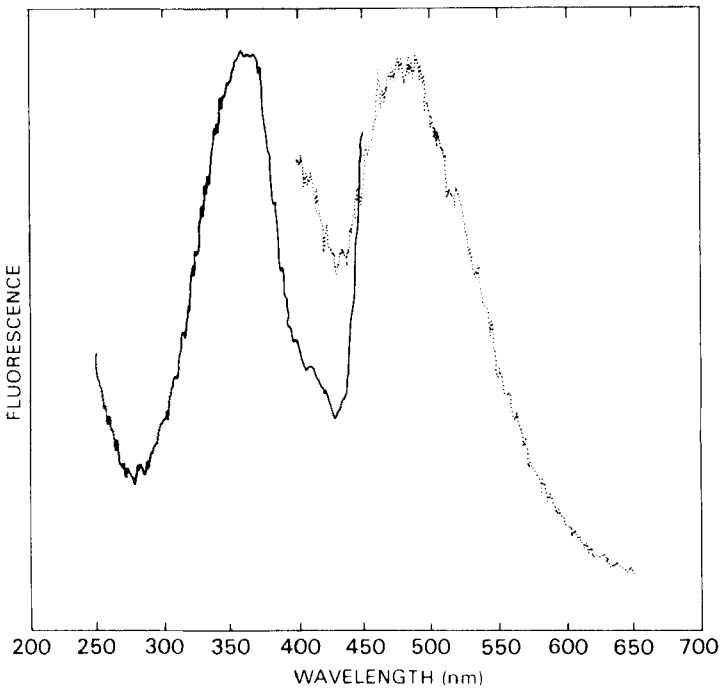

Figure 3. The fluorescence excitation (——) and emission spectra (····) of the fraction from Fig. 2 with the retention time of HAQO. For the excitation spectra, the emission was measured at 478 nm. For the emission spectra, the excitation was at 360 nm.

DISCUSSION

HPLC provided several advantages for examination of the metabolism of NQO in lysates of virus-infected cells. The separation of NQO, HAQO and AQO was readily achieved. The fluorometric assay of the HPLC eluate was highly sensitive. The retention times, peak areas and elution profiles of these compounds were reproducible. Preparation for HPLC did not appear to cause degradation of the standards as was noted with TLC.

HPLC also allowed for the simple recovery of the separated compounds for further analysis by spectrofluorometry, and thus obviated the problems encountered in the recovery of compounds from TLC plates.

The HPLC separation of these compounds for analysis and quantification should facilitate the study of these carcinogenic compounds in established models of carcinogenesis and cocarcinogenesis.

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REFERENCES

1. Albrecht, T., Speelman, D.J., and Li, J.L.H. Inactivation of human cytomegalovirus by the chemical carcinogen 4-nitroquinoline 1-oxide. J. Gen. Virol. 45, 231, 1979.

2. Albrecht, T., Speelman, D.J., Ramanujam, V.M.S., Lund, H., Legator, M.S., and Trieff, N.M. Correlation of the chemical structure of 4-nitroquinolines inactivating human cytomegalovirus and established in vivo carcinogenicity tests. Teratogenesis, Carcinogenesis and Mutagenesis. (In press, 1980).

3. Endo, H. and Kume, F. Induction of sarcoma in rats by subcutaneous injection of 4-hydroxylaminoquinoline 1-oxide. Naturwissenschaften 50, 525, 1963.

4. Endo, H., Ono, T., and Sugimura, T., editors. Recent Results in Cancer Research: Chemistry and biological actions of 4-nitroquinoline 1-oxide, volume 34. New York, Heidelberg, Berlin: Springer-Verlag, 1971.

5. Ishizawa, M. and Endo, H. On the mode of action of a potent carcinogen, 4-hydroxyaminoquinoline 1-oxide on bacteriophage T_4. Biochem. Pharm. 16, 637, 1967.

6. Kawazoe, Y., Tachibana, M., Aoki, K., and Nakahara, W. The structure-carcinogenicity relationship among derivatives of 4-nitro and 4-hydroxyaminoquinoline 1-oxides. Biochem. Pharm. 16, 631, 1967.

7. Malkin, M.F. and Zahalsky, A.C. Interaction of the water-soluble carcinogen 4-nitroquinoline N-oxide with DNA. Science 154, 1665, 1966.

8. Nagata, C., Fugii, K., and Epstein, S.S. Photodynamic activity of 4-nitroquinoline 1-oxide and related compounds. Nature 215, 972, 1967.

9. Nagata, C., Kodama, M., Tagashira, Y., and Imamura, A. Interaction of polynuclear aromatic hydrocarbons, 4-nitroquinoline 1-oxides, and various dyes with DNA. Bipolymers 4, 409, 1966.

10. Ono, T. The effect of carcinogens on the biological activity of nucleic acids (in Japanese). Tampakushitsu Kakusan Koso. 9, 1122, 1964.

11. Shirasu, Y. Comparative carcinogenicity of 4-nitroquinoline 1-oxide and 4-hydroxyaminoquinoline 1-oxide in 3 strains of mice. Proc. Soc. Exp. Biol. Med. 118, 812, 1965.

12. Speelman, D.J., Li, J.-L., Ramanujam, V.-M.S., Legator, M.S., and Albrecht, T. Herpesvirus inactivation by chemical carcinogens: Differential inactivation of herpes simplex viruses by 4-nitroquinoline 1-oxide and related compounds. (Submitted for publication.)

13. Sugimura, T., Okabe, K., and Endo, H. The metabolism of 4-nitroquinoline 1-oxide. I. Conversion of 4-nitroquinoline 1-oxide to 4-aminoquinoline 1-oxide by rat liver enzymes. Gann 56, 489, 1965.

14. Sugimura, T., Okabe, K., and Nagao, M. The metabolism of 4-nitroquinoline 1-oxide. III. An enzyme catalyzing the conversion of 4-nitroquinoline 1-oxide to 4-hydroxyaminoquinoline-1-oxide in rat liver and hepatomas. Cancer Res. 26, 1717, 1966.

15. Tanooka, H., Kawazoe, Y., and Araki, M. Inactivation mechanism of the transforming activity of DNA (in Japanese). Proc. Jap. Cancer. Ass. 27th Gen. Mtg. Tokyo, p. 35, 1968.