# MAMMALIAN CELL TOXICITY OF NITRO COMPOUNDS:DEPENDENCE UPON REDUCTION POTENTIAL

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Received August 3,1976

## SUMMARY

The toxicity of several classes of nitro-aromatic and -heterocyclic compounds towards V79 mammalian cells in vitro has been determined. Cells with varying concentrations of drugs were incubated in air at 37°C for up to 14 days in order to form colonies. It was found that the concentration of nitro compound required to reduce cell colony-forming ability by 50% was a function of the one-electron reduction potential of the compound, more cytotoxic compounds having more positive potentials.

## INTRODUCTION

Some nitrofurans and nitroimidazoles are used in medicine as anti-fungal and anti-bacterial agents (1), the compounds often showing a selective toxicity towards anaerobes. These and other nitro-aromatic compounds have been shown to sensitize hypoxic cells to the lethal effects of ionizing radiation (2-6) and their potential use as adjuncts in radiotherapy has been investigated (7). Redox properties have been shown to be important in determining the efficiency of several classes of nitro compounds as radiosensitizers (8) and we report here a similar correlation between reduction potential and aerobic cytotoxicity for a variety of nitro compounds.

## METHODS

Chronic aerobic cytotoxicity was estimated by measuring the inhibition of colony-forming ability by varying concentrations of each nitro compound. Chinese Hamster V79-379A cells were grown in spinner culture (8) and plated in 6 cm plastic petri dishes containing 3 ml Eagles' Minimal Essential Medium supplemented with 15% foetal calf serum. Cells were allowed to attach for 2 hr before the medium was removed by aspiration and replaced with fresh medium containing the nitro compound. The cells were incubated at 37°C for 7-14 days in 95% air/5% $CO_2$ and colonies of 50 or more cells were scored to determine plating efficiency.

One-electron reduction potentials were measured using the method described by Meisel and Neta (9,10).

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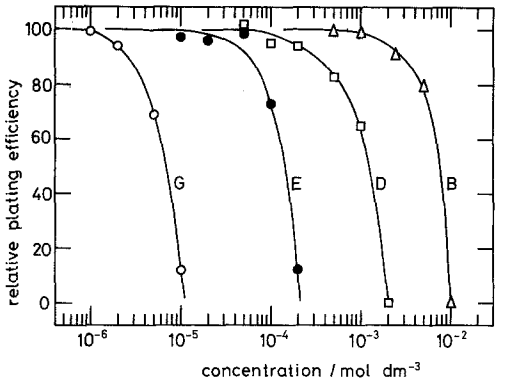


Fig.1 - Relative plating efficiencies of V79-379A cells, incubated for 7-14 days in the presence of varying concentrations of nitro compounds. Curves are labelled using the codes listed in Table 1.

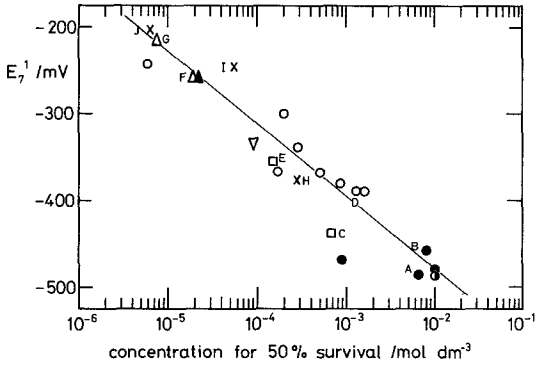


Fig.2 - Relationship between one-electron reduction potentials and chronic aerobic cytotoxicities of nitroaromatic compounds and quinones. (○): 2-nitroimidazoles; (●) 5-nitroimidazoles; (□) nitrobenzenes; (△) 5-nitrofurans; (▽) a 3-nitropyridinium compound; (X) quinones. Some of the points are labelled using the codes given in Table 1. Data from Mohindra and Rauth (12) are shown for comparison: (▲) nitrofurazone (F) and (◑) metronidazole (A).

## RESULTS

Figure 1 shows typical data for the effect of drug concentration on cell colony-forming ability. The concentration of compound resulting in a 50% decrease in relative plating efficiency is taken as a measure of chronic aerobic cytotoxicity and included in Table 1 are data for compounds of current interest in medicine or radiobiology. Full details of all the measurements

Table 1: Chronic aerobic cytotoxicity and one-electron reduction potentials of some of the compounds studied

| Figure code | Compound | Concentration for 50% survival /mmol $dm^{-3}$ | $E_7^1$/mV |
|---|---|---|---|
| A | metronidazole[a] | 6.5 | -486[b] |
| B | nitrimidazine[c] | 8 | -457[b] |
| C | m-nitroacetophenone | 0.66 | -437[d] |
| D | Ro-07-0582[e] | 1.3 | -389[d,b] |
| E | p-nitroacetophenone | 0.15 | -355[d] |
| F | nitrofurazone[f] | 0.019 | -257[g] |
| G | nifurpipone[h] | 0.0075 | -214[g] |
| H | 9,10-anthraquinone-2-sulfonate | 0.28 | -375[d,b] |
| I | duroquinone | 0.056 | -244[i,j,k,b] |
| J | 2-methyl-1,4-naphthaquinone[l] | o.0065 | -203[k] |

[a] 1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole; [b] Ref.(10); [c] 4-[2-(5-nitro-imidazol-1-yl)ethyl]-morpholine (naxogin, nimorazole); [d] Ref.(9); [e] 1-(2-hydroxy-3-methoxy-propyl)-2-nitroimidazole; [f] 5-nitro-2-furaldehyde semicarbazone (furacin); [g] Ref.(11); [h] 5-nitro-2-furfaldehyde-$N^1$-methyl-N-piperazino-acet-hydrazone (compound tested was dihydrochloride salt, Ro-10-7722); [i] Ref.(23); [j] Ref.(24); [k] Ref.(25); [l] menadione.

will be reported later. In Figure 2 we have plotted the logarithm of this concentration against the standard potentials (vs.NHE) for one-electron reduction, i.e. $E_7^1(RNO_2/RNO_2^-)$, which have been reported previously (9-11) or measured in the present work.

## DISCUSSION

Mohindra and Rauth (12) measured the aerobic cytotoxicity of metronidazole

and nitrofurazone towards 3 cell lines (CHO, HeLa and human bone marrow), and we have added their data to Figure 2. The present results with V79 cells support their suggestion (12) that this measure of aerobic cytotoxicity is generally independent of cell line.

Whilst our measure of cytotoxicity does not differentiate between cytostatic and cytocidal properties of the compounds, the correlation of colony-forming ability with a redox property is likely to be of value in predicting the cytotoxic properties of other nitro compounds. Raleigh et al.(4) correlated cytotoxicity of substituted nitrobenzenes with the Hammett $\sigma$ constants of the substituent. The use of such parameters is in practice restricted to benzenoid compounds and measurements of one-electron reduction potentials (9-11) enable different classes of aromatic nitro compounds to be compared. (Polarographic half-wave potentials in water at pH 7 often involve irreversible conditions and are only an approximate guide to redox properties).

The concentration of drug within the cell will be dependent upon other factors such as the lipophilicity of the compounds. However, measurements of the octanol:water partition coefficients of most of the compounds studied fail to demonstrate a significant correlation with cytotoxicity. The influence of partition properties is clearly much less than that of reduction potential in determining mammalian cell toxicity. Correlations have been made between lipophilicity and anti-trichomonal activity for a series of 5-nitroimidazoles which cannot differ significantly in redox properties (13), suggesting that the relative importance of redox and partition parameters may be different in mammalian cells and micro-organisms.

It has been suggested (14,15) that the toxicity of nitrofurans and nitroimidazoles against micro-organisms results from reduction of the nitro group to form, e.g.hydroxylamines or amines. The reduction potential $E_7^1$ ($RNO_2/RNO_2^-$) reflects the free energy change involved in the first step of reduction and the first reduction product, $RNO_2^-$, has been identified in

microsomal incubations (16,17). The increased toxicity of nitro compounds towards anaerobic compared to aerobic cells (18-21), and the effects of these drugs on the respiration of mammalian cells (22), may reflect differences in the rates or positions of equilibria of the electron-transfer reactions involved in production or repair of reductive damage (11). It may therefore be possible to distinguish between toxicity arising from the formation of toxic reduction products or a mechanism involving bypassing or inhibition of electron-transport chains, by comparing the toxicity of nitro compounds with that observed with other, non-nitro compounds of similar redox properties.

The one-electron reduction potentials of quinones may be calculated from dissociation constants or measured directly (9,10,23-25), and preliminary data for the aerobic cytotoxicity of 3 quinones are shown in Figure 2. These quinones have $E_7^1(Q/Q^-) < E_7^1(O_2/O_2^-)$ (9,23-25), and whilst they share a common feature with the nitro compounds in the relative stability of the one-electron reduction product and its reaction with oxygen to form the superoxide ion, $O_2^-$, (11, 24-26), the cytotoxicity of quinones is likely to arise through involvement in electron-transport processes rather than toxic reduction products (27). Our preliminary data suggests that the aerobic cytotoxicity of quinones is similar to that of nitro compounds of similar $E_7^1$. This may be evidence that the formation of toxic reduction products is not the major mechanism of the chronic aerobic toxicity of nitroaromatic compounds towards mammalian cells.

ACKNOWLEDGEMENTS

This work was supported by the Cancer Research Campaign. We thank Dr. I. R. Flockhart (Gray Laboratory) and Dr. J. Parrick (Brunel University) for helpful discussions and Carlo-Erba Research Institute, Gruppo Lepetit S.p.A., May and Baker Ltd., Pfizer Inc. and Roche Products Ltd. for the supply of compounds.

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