NITROPYRROLE RADIOSENSITIZERS: STRUCTURE FUNCTION RELATIONSHIPS

J. A. RAILEY,* J. D. CHAPMAN,* A. P. REUVERS,* J. E. BIAGLOW,†
R. E. DURAND‡ and A. M. RAUTH§

From the *Medical Biophysics Branch, Atomic Energy of Canada Limited, Whiteshell Nuclear Research Establishment, Pinawa, Manitoba, Canada, the †Division of Radiation Biology, Department of Radiology, Case Western Reserve, Cleveland, Ohio, U.S.A., the ‡Department of Human Oncology, Wisconsin Clinical Cancer Center, University of Wisconsin, Madison, Wisconsin, U.S.A., and the §Department of Medical Biophysics, Ontario Cancer Institute, Toronto, Ontario, Canada

Summary.—Nitropyrrole derivatives have been tested as hypoxic cell radiosensitizers in vitro and in vivo. Radiosensitizing potential generally increases with nitropyrrole electron affinity. N-hydroxyethyl substitution decreases toxicity relative to N–CH₃, N–CH₂CH₃ and N–CH₂CH₂CH₃ substitution. The most effective nitropyrrole tested in vivo is N-hydroxyethyl-2-cyano-5-nitropyrrrole (NP-1).

One approach to the improvement of radiotherapy involves a search for chemicals which radiosensitize hypoxic mammalian cells. Hypoxic cells are relatively radioresistant and modify the response of animal tumours to X-rays (Hewitt, 1966). By 1969 numerous chemicals had been shown to be effective hypoxic cell radiosensitizers (Ashwood-Smith et al., 1970). The suggestion that electron affinity was important (Adams and Dewey, 1963) led to the successful application of p-nitroacetophenone (PNAP) as a radiosensitizer of hypoxic mammalian cells (Adams et al., 1971; Chapman, Webb and Borsa, 1971).

A study of PNAP analogues showed the nitroaromatic structure to be essential for activity and that electronegative substituents (Cl, COCH₃, CONH₂, CN, NO₂ and NR₃⁺) increased nitrobenzene effectiveness (Raleigh et al., 1973).

Extension of these studies to tumours in vivo required a less toxic nitroaromatic than nitrobenzene. Nitropyrroles were chosen as they appeared to have acceptable toxicity (Benazet al., 1966), could be readily substituted with electronegative substituents and were structurally related to the 2- and 5-nitroimidazoles metronidazole and misonidazole (Ro-07-0582) (Fowler, Adams and Denekamp, 1976) respectively. Synthesis and preliminary investigation (Rauth, Kaufman and Thomson, 1975) of N-hydroxyethyl-2-cyano-5-nitopyrrrole (HCNP or NP-1) showed in vivo radiosensitization of C3H mouse KHT fibrosarcomas. On this basis, a series of nitropyrroles has been investigated in in vitro and in vivo tumour models.

The effect of the nitropyrroles on cellular respiration has also been studied. This property may have a bearing on drug toxicity and on the size of the hypoxic cell fraction in solid tumours treated with the drugs (Durand and Biaglow, 1974).

MATERIALS AND METHODS

Nitropyrroles NP-1, 2, 3, 4, 5, 6 and 10 (Table) were synthesized and characterized by standard methods (Raleigh and Whitehouse, unpublished). Nitropyrroles 7, 8 and 9 were generously supplied by Ortho Pharmaceuticals, New Jersey.

Radiosensitization studies of single cell suspensions of hypoxic V79-395A Chinese hamster cells were performed according to procedures previously described (Chapman et al., 1972). Irradiations were performed with a Westinghouse X-ray therapy unit operating at 250 kV and 15 mA. Beam filtration and nitrogen gassing procedures have been described previously (Chapman, Webb and Borsa, 1971).

Spheroids of V79 cells were grown as previously described (Sutherland and Durand,
The spheroid suspensions were irradiated in water-jacketed growth flasks by means of a Picker Nuclear $^{60}$Co-γ teletherapy unit at a dose rate of 150 rad/min. The procedure for dissociation of spheroids to single cells by trypsinization, cell counting and colony formation assay were identical to those previously described (Sutherland and Durand, 1973).

The experimental system used for testing the nitropyroles in vivo was according to published procedures (Rauth and Kaufman, 1975). C3H mice were injected s.c. on the flank with $2 \times 10^5$ KHT tumour cells 10 days before the experiment. On the day of the experiment, the tumour volume was about 1 cm$^3$; 10–30% of the viable cells were hypoxic. Groups of 3 animals each were injected i.p. with buffer or buffer plus different concentration of drug; 15–30 min later the animals were exposed to $^{137}$Cs-γ whole body irradiation at a dose rate of 96 rad/min. The groups of animals were sacrificed, blood samples taken and suspensions made of the tumour cells. The number of viable cells was assayed by the in vivo lung colony assay of Hill and Bush (1969). The level of drug present in the plasma was measured polarographically.

For metabolic studies with the nitropyroles, Chinese hamster V79 cells were grown as a monolayer culture. The conditions for cell growth as well as the procedure for measuring inhibition of cell respiration have been described (Biaglow and Durand, 1976). Ehrlich ascites cells were grown and harvested in DCF mice as described previously (Biaglow et al., 1977).

RESULTS AND DISCUSSION

Nitropyroles are efficient radiosensitizers of hypoxic mammalian cells. NP-1 and NP-6 approach, at higher concentration, the effectiveness of oxygen (Fig. 1). NP-1, NP-6 and NP-7 compare favourably with the 5-nitroimidazoles in this regard (Fowler et al., 1976). Even though electron-negative substituents, such as NO$_2$ and CN alone, do not produce good radiosensitizing properties (e.g. NP-2, 3, 4) a general trend linking nitropyrrole electron affinity ($E_1$) to radiosensitizing ability is seen (Table). Alkyl substitution at the ring nitrogen increases electron affinity but also has an effect on toxicity. Toxicity of N-CH$_3$, N-CH$_2$CH$_3$ and N-CH$_2$CH$_2$CH$_3$ substituents prevented study of NP-7, 8 and 9 above 1 mm. On the other hand, NP-1 and NP-6, possessing similar electron affinity but with N-CH$_2$CH$_2$OH substitution, could be tolerated up to 10 mm.

It seems likely that an intramolecular hydrogen-bonded interaction between OH and NO$_2$ may exist in NP-1, NP-6 and NP-10 (Urbanski, 1959). The reduced toxicity of these compounds could be due to a diminished rate of enzyme reduction (a possible source of reactive, toxic intermediates) (Biaglow et al., 1977) because of steric hindrance or overall reduced lipid solubility of NP-1, NP-6 and NP-10. The possibility of increasing therapeutic ratios of nitroaromatic sensitizers by sterically hindering enzymic reduction while maintaining electron affinity requires further study. At 1 mm concentration where comparisons can be made, the radiosensitizing effectiveness is dependent on N-substitution in the order N-CH$_3$ > N-CH$_2$CH$_2$OH > CH$_2$CH$_3$, CH$_2$CH$_2$CH$_3$ > N-H.
The single cell suspension culture is a useful screening system for \textit{in vivo} radiosensitizers. NP-1 and NP-6 are not only most effective in the \textit{in vitro} system, but also have radiosensitizing properties \textit{in vivo} (Table). Of the 4 nitropyroles studied \textit{in vivo}, NP-1 is the most promising (Table and Fig. 2) although NP-6 may warrant further studies. The radiosensitizing effect of NP-10 plateaus at low sensitizing potential independent of increasing NP-10 concentration (Rauth and Paciga, 1977).

While the single cell suspension screening system seems adequate, studies with the more complex multicellular spheroid tumour model, which incorporates both oxic and hypoxic cells in tumour-like array, has shown the need to take cognisance of drug metabolism and its possible effect on the size of the hypoxic cell fraction in solid tumours (Biaglow and Durand, 1976). Inhibition of cellular respiration in the peripheral oxic cells by the drugs leads to oxygenation of the hypoxic cell fraction and, therefore, radiosensitivity. The effect of the nitropyroles on cellular respiration of V79 Chinese hamster cells and Ehrlich ascites tumour cells is shown in the Table. NP-2, 4, 5, 6, 8 and 9 inhibit respiration while NP-1, 7, 10 have little effect on V79 cells. The nitropyroles inhibit respiration in EAT cells more effectively than in V79 cells. The nitropyroles have a range of effects on the survival of the hypoxic cell fraction in multi-cellular spheroids (Table and Fig. 3). NP-1, 4, 6, 7, 10 sensitize (NP-6 maximally) while NP-2 and NP-5, both of which lack N-substituents, protect.

An explanation for these types of effect presented by Biaglow and his collaborators

\begin{table}
\centering
\begin{tabular}{|c|c|c|c|c|c|}
\hline
\textbf{Pyrrole} & \textbf{E}_1^{1/2} & \textbf{V97 EAT} & \textbf{V79 Single cell} & \textbf{Spheroid} & \\
\hline
NP-7 & -0.35 & 22 & 79 & 2.5 & 1.5 \\
NP-1 & -0.35 & 6 & 74 & 1.9 & 2.6 \\
NP-9 & -0.35 & 72 & 89 & 1.8 & 1.6 \\
NP-10 & -0.36 & 28 & 77 & - & 1.6 \\
NP-8 & -0.37 & 38 & 87 & 1.8 & 1.2-1.4 \\
NP-6 & -0.375 & 44 & 76 & 1.9 & 2.5 \\
NP-5 & -0.42 & 53 & 90 & 1.6 & 2.5 \\
NP-4 & -0.50 & 59 & 75 & 1.4 & 1.4 <1.0 \\
NP-2 & -0.51 & 50 & - & 1.4 & 1.0 \\
NP-3 & -0.53 & - & - & 1.5 & 1.7 \\
\hline
\end{tabular}
\caption{Nitropyrole Electron Affinity, Inhibition of Cellular Respiration and Radiosensitization of Model Tumour Systems}
\end{table}
(1978) for other types of nitroaromatic radiosensitizers are probably valid for the nitropyroles as well. NP-6 is an exceptionally effective sensitizer in the spheroid system (E.R. \( \geq 2.6 \)). It is possible that this compound is acting as an electron-affinic type radiosensitizer and, at the same time, inhibiting cellular respiration in the spheroid periphery and thereby promoting oxygenation of the hypoxic cell fraction. In terms of in vivo radiosensitizer selection, nitrogen heterocycles lacking N-substituents are unlikely candidates as they might protect solid tumours. On the other hand, nitroaromatics which strongly inhibit oxic cellular respiration, though effective radiosensitizers of spheroids, may prove too toxic for in vivo use. Nitroaromatics which have little effect on respiration, such as NP-1 and NP-10 would seem to be good compounds for further study.

In summary, some nitropyroles are very effective radiosensitizers of in vitro tumour models. Radiosensitization increases with increasing electron affinity (\( E_\beta \)). For a fixed nitropyrole electron affinity, N-substitution affects efficacy in the order N-CH\(_3\) \( > \) N-CH\(_2\)CH\(_2\)OH \( > \) N-CH\(_2\)CH\(_3\), N-CH\(_2\)-CH\(_2\)CH\(_3\). N-hydroxyethyl substitution diminishes drug toxicity relative to N-CH\(_3\), N-CH\(_2\)CH\(_3\) and N-CH\(_2\)CH\(_2\)CH\(_3\). A specific hydrogen-bonded interaction between the OH group of the N-hydroxyethyl substituent and the ring NO\(_2\) could be involved and might be exploited to increase the therapeutic ratio of heterocyclic nitroaromatic radiosensitizers. Drugs which in-
hibit cellular respiration are, in general, effective radiosensitizers of multicellular spheroids. Compounds which strongly inhibit respiration may be too toxic for in vivo application. Nitropyroles without N-substitution protect the spheroid system and are not likely to be useful in vivo. Compounds such as NP-1 and NP-10 which are neutral with respect to respiration would seem the best candidates for further study in vivo.

REFERENCES


