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UPTAKE AND CYTOTOXICITY OF NOVEL NITROIMIDAZOLE–POLYAMINE CONJUGATES IN EHRLICH ASCITES TUMOUR CELLS

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Abstract—A number of tumour cells, including Ehrlich ascites tumour cells (EATC), possess a polyamine uptake system which selectively accumulates endogenous polyamines and structurally related compounds by an active energy dependent system(s). We suggest that it may be possible to utilize this uptake system to target certain cytotoxic agents to those tumour cells possessing this system. In an initial attempt to determine the feasibility of this suggestion, we have synthesized a series of 2- and 5-nitroimidazoles linked to polyamines and determined their ability to utilize the polyamine uptake system. Within the limited series of compounds synthesized, 2-nitroimidazole-polyamine conjugates were more potent inhibitors of spermidine uptake into EATC than the 5-nitroimidazole conjugates. It has been assumed partly based on the competitive nature of this inhibition, that the ability of these compounds to inhibit spermidine uptake is also a measure of their ability to be accumulated by EATC. A greater than 700-fold variation was observed in the ability of different analogues to inhibit spermidine uptake. The most potent inhibitors retained certain structural characteristics similar to those of spermidine. Those compounds linked to polyamines were much more potent inhibitors of polyamine uptake than the parent nitroimidazoles i.e. metronidazole and misonidazole. The toxicity of the parent compounds and their polyamine conjugates in control and polyamine-depleted EATC was assessed by measuring inhibition of tritiated thymidine incorporation. Polyamine depletion, by prior exposure to difluoromethylornithine, results in a compensatory increase in the uptake of polyamines and related structures which may result in an increase in toxicity. Whilst many of the novel conjugates showed only little or moderate toxicity to control cells, the toxicity of several of the conjugates but not the parent nitroimidazoles increased in the polyamine-depleted cells. A clear distinction was also observed between the ability to inhibit spermidine uptake (and hence affinity for the uptake system) and toxicity, e.g. compound 430, a dinitroimidazole–polyamine conjugate, was the best inhibitor of spermidine uptake studied but showed no toxicity. These results support the hypothesis that linking polyamines to nitroimidazoles facilitates the entry of the latter into cells, such as EATC, which possess the polyamine uptake system and may therefore have therapeutic application in the delivery of polyamine-linked cytotoxics to certain tumours.

Polyamines are ubiquitous low molecular weight organic cations, which are required for growth and differentiation [1]. Whilst the majority of mammalian cells synthesize their own polyamines, a number also possess a specific active transport system for polyamine uptake. The characteristics of polyamine transport in mammalian cells have been reviewed recently [2]. A similar uptake system is present in a number of tumour cells including rat prostatic tumour cells [3], neuroblastoma cells [4], B16 melanoma cells [5], human colonic [6] and lung tumour cell lines (unpublished results), cultured human lymphocytic leukaemic cells [7], and L1210 and Ehrlich ascites tumour cells (EATC¶) [8]. An optimal spacing of at least four carbon atoms separating two positively charged nitrogen atoms appears to be a characteristic feature of good substrates of this uptake system [9–11].

In this study, we attempt to utilize this polyamine uptake system in order to target cytotoxic compounds to EATC. We have therefore synthesized a series of polyamines conjugated to 2- and 5-nitroimidazole compounds and assessed the ability of these compounds to inhibit polyamine uptake as an indication of their own potential to be taken up by the polyamine uptake system. Their subsequent *in vitro* toxicity has also been studied. Some known antitumour agents, such as MGBG, are known to be accumulated by the polyamine uptake system [7, 12]. MGBG is accumulated because of its structural similarity to the polyamine spermidine [7]. Intracellular polyamines may be depleted by pretreatment of cells with DFMO, an irreversible inhibitor of ornithine decarboxylase, the initial rate-limiting enzyme in polyamine biosynthesis [1, 13]. In order to compensate for the depletion of intracellular polyamines, many cells compensate and try to restore normal levels by increasing the uptake

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¶ Abbreviations: EATC, Ehrlich ascites tumour cells; MGBG, methylglyoxal bis(guanylhydrazone); DFMO, difluoromethylornithine.

of extracellular polyamines as well as structurally related compounds such as MGBG resulting in intracellular concentrations over 1000-fold greater than extracellular concentrations [7]. These high intracellular concentrations of MGBG lead to cytotoxicity possibly by damage to mitochondria [12] or by effects on polyamine biosynthesis [14].

Nitroimidazoles are used clinically as antiprotozoal and antibacterial agents. Many nitroimidazoles are also radiosensitizers to hypoxic tumour cells [15]. Their mechanism of action is thought to involve reduction of the nitro group to form a metabolite which interacts with DNA [16]. We propose that conjugation of nitroimidazoles with polyamines may increase their cellular uptake and may also target the DNA, due to the polycationic character of the polyamines which gives them a high affinity for DNA [1, 17]. It may be possible to exploit therapeutically the presence of the polyamine uptake system in tumours in the design of novel anticancer agents [18, 19]. Structural modification of polyamines, such as substitution at the central nitrogen of spermidine with cytotoxic compounds, may lead to high cytotoxic concentrations in tumour cells possessing the uptake system [19].

In this study, we have investigated the toxicity of various nitroimidazoles and their polyamine conjugates in normal and polyamine-depleted EATC. The potential uptake of the nitroimidazole–polyamine conjugates has also been assessed indirectly by measuring the ability of these compounds to inhibit the uptake of spermidine into EATC. The rationale for this is based partly on our previous studies with the herbicide paraquat, which possesses important structural similarities to putrescine. Paraquat is toxic to the lung partly due to its selective accumulation by the pulmonary polyamine uptake system [20, 21] which bears many similarities to the polyamine uptake system present in tumour cells [19]. Paraquat, putrescine, spermidine, spermine, MGBG and cystamine all appear to be accumulated by the lung by an identical or very similar uptake system [21, 22]. This conclusion was based partly on the similar characteristics of the saturable energy-dependent uptake of these compounds and also on their ability to inhibit each others uptake competitively [22]. In this study because of the lack of a simple assay for the nitroimidazoles and their polyamine conjugates, their uptake into cells was not measured directly. However, the ability of the nitroimidazoles or their polyamine conjugates to inhibit spermidine uptake into EATC competitively was used as an indirect measure to suggest that these compounds were also accumulated by the polyamine uptake system.

Our studies demonstrate that nitroimidazole–polyamine conjugates are much better inhibitors of polyamine uptake than the corresponding parent nitroimidazole compounds, indicating they have a higher affinity for the uptake system. The results also suggest that the conjugates enter the cells via the uptake system since polyamine depletion, which results in an increased uptake of polyamine analogues, enhanced the toxicity of a number of the conjugates but not the parent nitroimidazoles.

MATERIALS AND METHODS

Chemicals. MGBG was purchased from the Aldrich Chemical Co. (Gillingham, U.K.). DFMO was kindly provided by the Merrell Dow Research Institute (Strasbourg, France). [^{14}C]Spermidine (sp. act. 117 mCi/mmol) and [6-^{3}H]thymidine (sp. act. 25 Ci/mmol) were purchased from Amersham International (Amersham, U.K.). The various nitroimidazole–polyamine conjugates were synthesized as described in the Appendix and their structures are shown in Fig. 1.

Cell culture. All culture media and plastic ware were obtained from ICN-Flow (High Wycombe, U.K.). EATC were maintained in culture in Dulbecco's Modification of Eagle's Medium with 10% foetal calf serum at 37° in an atmosphere of 5% CO_2/95% air. Cells were harvested in the logarithmic growth phase prior to uptake and toxicity studies.

Uptake determinations. The ability of the nitroimidazole–polyamine conjugates to inhibit the uptake of [^{14}C]spermidine into EATC *in vitro* was studied. A suspension of EATC (0.5 mL of 1×10^5/mL) was aliquoted into 24-well multi-well plates and incubated for 18 hr to form a monolayer. Cells were incubated in duplicate at 37° for 30 min with 1.0, 2.0, 3.0, 4.0, 5.0 and 10 μM [^{14}C]spermidine alone, or in the presence of 1, 10, 50 or 100 μM nitroimidazole–polyamine conjugate or the parent nitroimidazole. The medium was removed and the cell monolayer washed twice with 1 mL cold 0.9% NaCl containing spermidine (1 mM) to displace non-specifically bound [^{14}C]spermidine. The cell monolayer was then digested overnight with 1 M NaOH (400 μL). Aliquots (200 μL) were transferred to scintillation vials and neutralized with 1 M HCl (200 μL). Aquasol (4 mL) was added to each vial and the radioactivity measured using a scintillation counter. Results were expressed as pmol spermidine uptake/min/5×10^4 cells. The mechanism of inhibition was determined using Lineweaver–Burk plots.

In vitro *toxicity*. The toxicity of the nitroimidazole–polyamine conjugates was assessed by inhibition of [^{3}H]thymidine incorporation into DNA in control and polyamine-depleted EATC. A suspension of EATC (0.5 mL of 5×10^3/mL) was aliquoted into multi-well plates and incubated for 18 hr when a further 1 mL of medium with or without DFMO (0.5 mM) was added and incubated for a further 72 hr in order to deplete intracellular polyamines. This level of depletion was sufficient to increase the rate of spermidine uptake 10-fold. The nitroimidazoles and their polyamine conjugates (0–100 μM) were added to the cells in a final volume of 2 mL and incubated for 24 hr when the medium was removed and fresh medium added. The cells were incubated for a further 48 hr to allow any DNA damage to be manifested as cell death. [^{3}H]-Thymidine (0.5 μCi) was added to each well for the final 2 hr of the incubation. At the end of the incubation the medium was removed and the cells washed twice with 1 mL cold 0.9% NaCl containing 1 mM thymidine to remove non-specifically bound [^{3}H]thymidine. Cold 10% trichloroacetic acid (1 mL) was added to each well for 10 min and the precipitate

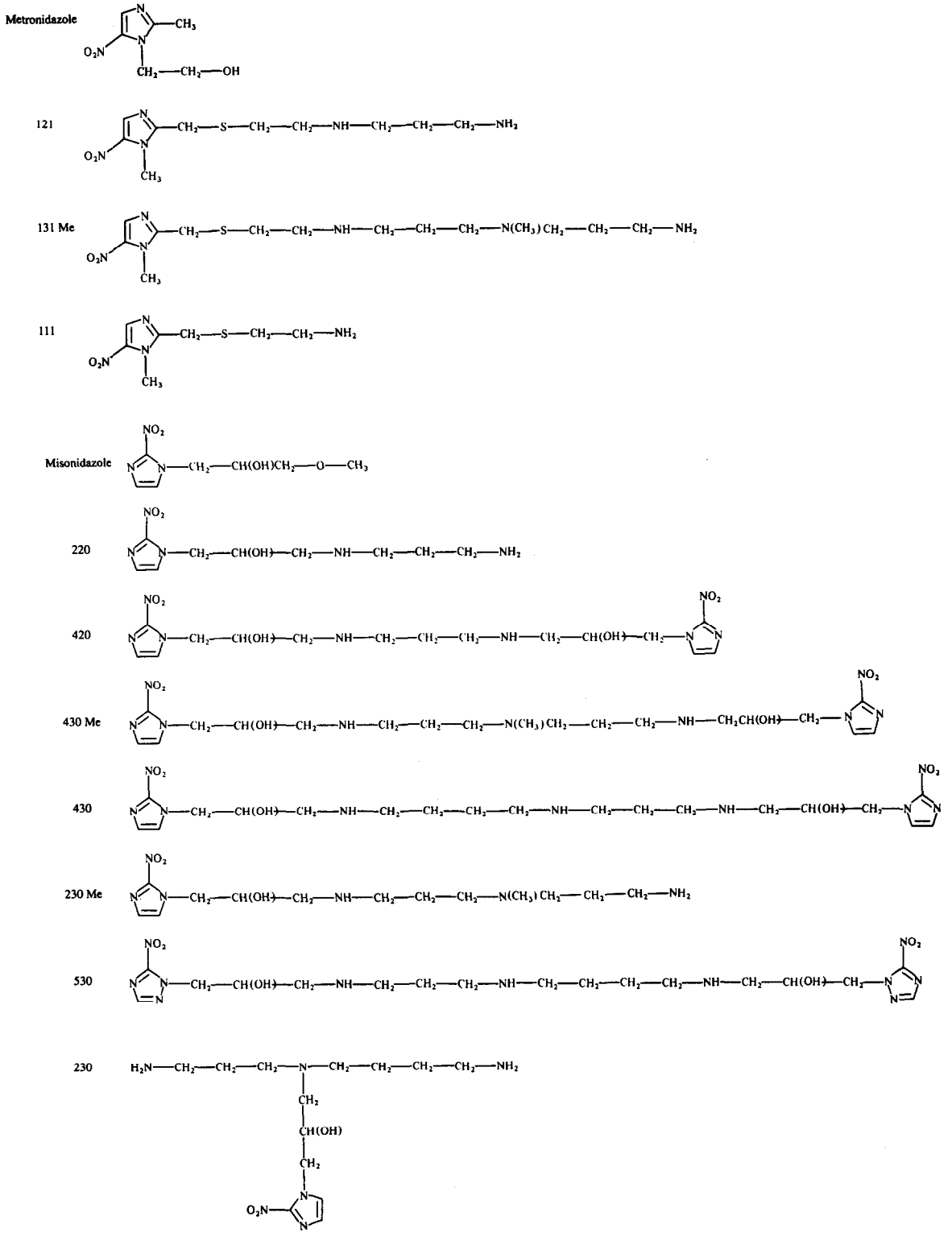

Fig. 1. Structures of nitroimidazole–polyamine conjugates used in this study. The three-figure code used to label these structures is related to the functionality present in the molecules. The first digit represents the class of heterocycle where 1 is equivalent to the 5-nitroimidazoles, 2 to the 2-nitroimidazoles, 4 to the twin 2-nitroimidazoles and 5 to the twin 5-nitrotriazole. The second digit represents the number of polyamine nitrogens and the third digit the number of sulphur atoms present in the molecule. The additional Me represents the substitution of one methyl group on the central nitrogen.

Table 1. Inhibitory effects of nitroimidazole–polyamine conjugates on spermidine uptake and their toxicity to control and polyamine depleted EATC by DFMO

Compound*	K_i (μM)	IC_{50} (μM) Control	DFMO
Metronidazole	No inhibition	>100	>100
121	174 ± 49.8 (4)	80	15 (3)
131Me	184 ± 70 (3)	>100	71 (2)
111	400 (1)	>100	>100 (1)
Misonidzole	No inhibition	>100	>100 (2)
220	40 ± 7.1 (3)	88.5	25.5 (2)
420	329 (2)	>100	60 (2)
430Me	19.3 ± 2.65 (3)	>100	>100 (2)
430	0.63 ± 0.14 (3)	>100	>100 (2)
230Me	4.66 ± 1.50 (3)	>100	93 (2)
530	0.66 (2)	>100	>100 (2)
230	1.45 (2)	42.7	9.0 (2)
MGBG	29.8 ± 7.9 (4)	12.7 ± 4.2 (4)	0.8 ± 0.14 (4)

EATC (5×10^4) were incubated at 37° for 30 min with [^{14}C]spermidine (1–10 μM) either in the absence or the presence of the compound under study (1, 10, 50 or 100 μM). The accumulation of ^{14}C-label into the cells was measured, the K_i values determined from Lineweaver–Burk plots and the mean values shown.

EATC (2.5×10^3) were also incubated in the presence or absence of DFMO (0.5 mM) for 72 hr. The compound under study (0–100 μM) was added for 24 hr, the medium replaced and the cells incubated for a further 48 hr. [^{3}H]Thymidine was added for the final 2 hr of the incubation and incorporation into DNA measured. The concentration of compound inhibiting [^{3}H]thymidine incorporation into DNA by 50% (IC_{50}) was determined.

* Structures of compounds are given in Fig. 1.

formed dissolved in 400 μL 1 M NaOH. Aliquots (200 μL) were neutralized as before and processed for scintillation counting. For each conjugate concentration, [^{3}H]thymidine incorporation was expressed as percentage control (i.e. no conjugate) incorporation. The concentration required to inhibit [^{3}H]thymidine incorporation by 50% was calculated (IC_{50}) for each compound in the presence or absence of DFMO. DFMO alone gave approximately a 50% reduction in [^{3}H]thymidine incorporation in control (untreated) cells. Relevant DFMO controls were therefore used when calculating IC_{50} values.

For both the uptake and toxicity studies, MGBG was used as a positive control as it has been shown to utilize the polyamine uptake system [7, 22] and its uptake and toxicity are increased by the prior depletion of polyamines with DFMO [7, 12, 14].

RESULTS

Inhibition of spermidine uptake

The ability of the nitroimidazoles and their polyamine conjugates to inhibit [^{14}C]spermidine uptake into EATC was assessed as described in Materials and Methods by determining their K_i values. The lower the K_i the more potent the compound was as an inhibitor of uptake. A greater than 700-fold variation was observed in the ability of the various compounds shown in Fig. 1 to inhibit the uptake of [^{14}C]spermidine, ranging from very potent inhibitors such as the 2-nitroimidazoles 430 and 230 (K_i = 0.63 and 1.45 μM, respectively) and the triazole 530 (K_i = 0.66 μM) (Table 1) to metronidazole and misonidazole which showed no inhibition within the range of concentrations tested (1–100 μM).

In all cases, where examined kinetically, the compounds inhibited spermidine uptake competitively. A representative Lineweaver–Burk plot is shown for MGBG, a control compound known to utilize the polyamine uptake system, as well as for a potent and a more moderate inhibitor, compounds 430 and 220, respectively (Fig. 2). Metronidazole and misonidazole were the non-conjugated control compounds for the 5- and 2-nitroimidazole series of compounds, respectively. Neither of the parent molecules inhibited spermidine uptake *per se* (Table 1) until conjugated with various polyamines. With the range of compounds tested, 2-nitroimidazole polyamine conjugates were, in general, better inhibitors of spermidine uptake than 5-nitroimidazole conjugates (Table 1).

Toxicity of nitroimidazole–polyamine conjugates

The toxicity of the nitroimidazoles and their polyamine conjugates was assessed by their ability to inhibit [^{3}H]thymidine incorporation into DNA (IC_{50}) in control and polyamine-depleted EATC as described in Materials and Methods. The lower the IC_{50} the more toxic the compound. The toxicity of MGBG was greatly enhanced by pretreating the cells with DFMO (Table 1) which was compatible with the markedly elevated uptake of MGBG in polyamine-depleted cells. Neither metronidazole nor misonidazole was toxic in control or DFMO-pretreated cells (Table 1). The toxicity of several analogues, such as 121, 220 and 230, was enhanced markedly in polyamine-depleted cells (Table 1).

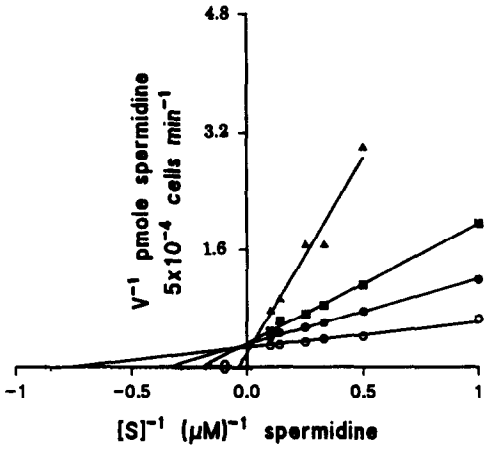

Fig. 2. Kinetics of inhibition of spermidine uptake by MGBG and nitroimidazole–polyamine conjugates. The uptake of [^{14}C]spermidine (1–10 μM) in EATC (5×10^4) was measured at 37° either alone (○) or in the presence of MGBG (50 μM) (▲), compound 220 (50 μM) (■) or 430 (1 μM) (●).

DISCUSSION

Inhibition of spermidine uptake

None of the 5-nitroimidazole compounds were particularly good inhibitors of spermidine uptake (Table 1). Both 121 (a diamine) and 131Me (a triamine) were poor inhibitors although better than the monoamine 111. This was due possibly to the fact that only three carbon atoms separate the nitrogen atoms in both the diamine and triamine, and a separation of at least four carbon atoms is required for a compound to be a good inhibitor of the polyamine uptake system, at least in lung slices [11]. In the latter system, monoamines were also poor inhibitors [11].

The 2-nitroimidazole–polyamine conjugates were more effective inhibitors of spermidine uptake than the 5-nitroimidazole conjugates (Table 1), even though in a number of these compounds the two nitrogen atoms of the polyamine moiety were separated by only three carbon atoms. This may be related to the nature of the linkage of the polyamine to the nitroimidazole. In the 5-nitroimidazoles, the polyamine is joined to C^2 of the nitroimidazole ring via a sulphur linkage whereas the 2-nitroimidazole compounds are conjugated to the polyamine via N^1 of the imidazole ring, without a sulphur linkage. Thus in the 2-nitroimidazole conjugates, there is effectively one additional nitrogen atom forming part of the polyamine chain. Additionally, this nitrogen atom is more positively charged due to the electron-withdrawing potential of the nitro group attached to the adjacent carbon atom on the ring. Similarly, the triazole 530 which was also a good inhibitor of spermidine uptake (Table 1) shares this structural characteristic.

Some preliminary studies were carried out on the effect of the nitroimidazole moieties on the ability of the conjugate to inhibit spermidine uptake. Substitution of the two nitroimidazole groups of compound 430 (the most effective inhibitor of uptake) with a 3,4-dihydroxyphenyl group caused a 10-fold decrease in inhibitory potential. Similarly, a styrene oxide adduct was 30-fold less effective at inhibiting spermidine uptake (unpublished results). These results suggest the importance of the nitroimidazole group in conferring inhibitory potential on the compound.

Two of the best inhibitors of spermidine uptake were compounds 430 (K_i = 0.63 μM) and 230 (K_i = 1.45 μM). Both these compounds contain a similar, and possibly optimal, spacing between the nitrogen atoms of the polyamine moiety to that found in spermidine. Compound 230 is also substituted on the N4 of spermidine, which has been suggested by Porter *et al.* [10] to be the optimal position for substitution. In addition to being a good inhibitor of spermidine uptake, compound 230 was also cytotoxic. Cytotoxicity was enhanced by polyamine depletion with DFMO. In contrast, compounds 430Me and 230Me were less effective inhibitors of spermidine uptake but the nitrogens of the polyamine backbone were separated by only three carbon atoms (Table 1). The differences in inhibitory potency may have been due to the differences in spacing of the nitrogen atoms of the polyamine but the presence of the methyl group on the central nitrogen atom in these compounds may also have reduced the cationic nature of the nitrogen atom. This may also explain partially the poor inhibitory potency of the 5-nitroimidazole conjugate 131Me which has a methyl group on the central nitrogen.

Owing to the lack of a simple assay for the nitroimidazoles or their polyamine conjugates, we have utilized their ability to inhibit spermidine uptake into EATC competitively to suggest that they may also be taken up into the cells by either the same or very similar carrier systems. Support for this comes from previous studies from a number of laboratories [2, 21, 23]. However, recent studies using B16 melanoma cells [5] have shown that although paraquat can inhibit competitively the uptake of putrescine, neither putrescine, spermidine nor spermine inhibited paraquat uptake suggesting that paraquat and polyamines are not taken up by the same uptake system. Thus, whilst studies demonstrating the competitive inhibition of polyamine uptake into cells suggest uptake of the inhibitors, they are not definitive.

In vitro *toxicity*

A distinction was observed between the toxicities of the 2- and 5-nitroimidazole–polyamine conjugates and their ability to inhibit spermidine uptake. With some compounds (e.g. 121 and 220) considerable toxicity to polyamine-depleted cells was shown, although they were poor inhibitors of spermidine uptake (Table 1). This suggests that significant uptake of these compounds was occurring and it was enhanced by polyamine depletion. This may have been the case because the uptake studies were carried out over a 30-min time period whereas the toxicity studies involved 24 hr exposure, which may have allowed accumulation of the compound despite its low affinity for the uptake system.

In addition, the presence of a diamine backbone in compounds 121 and 220 rather than the more favourable triamine backbone may have been responsible for their poor ability to inhibit spermidine uptake. These conjugates may have entered the cells via an uptake system for diamines such as putrescine rather than utilizing an uptake system for spermidine. It has been recognized recently that in some cells there is more than one uptake system for polyamines which although bearing several similarities also exhibit distinct differences [24].

In addition to this, polyamine depletion is known to cause conformational changes in the DNA, reducing its stability and altering its susceptibility to DNA damaging agents [25]. Polyamine depletion has also been shown to lead to an accumulation of EATC in the G2 and S phases of the cell cycle [1]. It is possible that the nitroimidazole compounds may exhibit some cell cycle specificity and polyamine depletion renders the cells more susceptible to their toxicity. However, if this was the case one may also have expected the parent compounds to have an enhanced toxicity in polyamine-depleted cells, which was not the case.

In summary, these studies have demonstrated that conjugation of nitroimidazoles to polyamine carriers may help to target the compounds to cells possessing the polyamine uptake system. In general, 2-nitroimidazole conjugates had a higher affinity for the polyamine uptake system than 5-nitroimidazoles. In several cases, polyamine depletion increased markedly the toxicity of nitroimidazole–polyamine conjugates but not the parent nitroimidazoles, suggesting an increased uptake of the compounds via the polyamine uptake system and for targeting to DNA. However, compound 430 despite its high affinity for the system was not toxic. It may itself, however, be of value as a high affinity carrier molecule to which cytotoxic drugs may be linked.

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REFERENCES

1. Pegg AE and McCann PP, Polyamine Metabolism and Function. *Am J Physiol* **243**: C212–C221, 1982.
2. Seiler N and Dezeure F, Polyamine transport in mammalian cells. *Int J Biochem* **22**: 211–218, 1990.
3. Heston WDW, Kadmon D, Covey DF and Fair WR, Differential effect of α-difluoromethylornithine on the *in vivo* uptake of ^{14}C-labelled polyamines and methylglyoxal bis(guanylhydrazone) by a rat prostate-derived tumor. *Cancer Res* **44**: 1034–1040, 1984.
4. Rinehart CA and Chen KY, Characterization of the polyamine transport system in mouse neuroblastoma cells. Effects of sodium and system A amino acids. *J Biol Chem* **259**: 4750–4756, 1984.
5. Minchin RF, Martin RL and Ilett KF, Paraquat is not accumulated in B16 tumor cells by the polyamine transport system. *Life Sci* **45**: 63–69, 1989.
6. McCormack SA and Johnson LR, Putrescine uptake and release by colon cancer cells. *Am J Physiol* **256**: G868–G877, 1989.
7. Seppanen P, Alhonen-Hongisto L and Janne J, Polyamine deprivation-induced enhanced uptake of methylglyoxal-bis(guanylhydrazone) by tumour cells. *Biochim Biophys Acta* **674**: 169–177, 1981.
8. Seppanen P, Alhonen-Hongisto L and Janne J, Relation of the antiproliferative action of methylglyoxal-bis(guanylhydrazone) to the natural polyamines. *Eur J Biochem* **110**: 7–12, 1980.
9. Field M, Block JB, Oliverio VT and Rall DP, Cellular accumulation of methylglyoxal-bis-guanylhydrazone *in vitro*, 1. General characteristics of cellular uptake. *Cancer Res* **24**: 1939–1946, 1964.
10. Porter CW, Miller J and Bergeron RJ, Aliphatic chain length specificity of the polyamine transport system in ascites L1210 leukemia cells. *Cancer Res* **44**: 126–128, 1984.
11. Gordonsmith, RH, Brooke-Taylor S, Smith LL and Cohen GM, Structural requirements of compounds to inhibit pulmonary diamine accumulation. *Biochem Pharmacol* **32**: 3701–3709, 1985.
12. Dave C, Pathak SN and Porter CW, Studies in the mechanism of cytotoxicity of methylglyoxal bis-guanylhydrazone) in cultured leukemia L1210 cells. *Adv Polyamine Res* **1**: 153–171, 1978.
13. Mamont PS, Duchesne, M-C, Grove J and Bey P, Anti-proliferative properties of DL-α-difluoromethyl ornithine in cultured cells. A consequence of the irreversible inhibition of ornithine decarboxylase. *Biochem Biophys Res Commun* **81**: 58–66, 1978.
14. Williams-Ashman HG and Seidenfeld J, Aspects of the biochemical pharmacology of methyl glyoxal bis(guanylhydrazone). *Biochem Pharmacol* **35**: 1217–1225, 1986.
15. Fowler JF, Adams GE and Denekamp J, Radiosensitizers of hypoxic cells in solid tumours. *Cancer Treat Rev* **3**: 227–256, 1976.
16. Mason RP, Free-radical intermediates in the metabolism of toxic chemicals. In: *Free Radicals in Biology*, Vol. 5, pp. 161–222. Academic Press, New York, 1982.
17. Cullis PM, Symons MCR, Cohen GM and Wardman P, A general method for efficient drug delivery to DNA. *Med Sci Res* **18**: 87–88, 1990.
18. Pegg AE, Polyamine metabolism and its importance in neoplastic growth and as a target for chemotherapy. *Cancer Res* **48**: 759–774, 1988.
19. Cohen GM and Smith LL, Potential therapeutic exploitation of the pulmonary polyamine uptake system. *Biochem Soc Trans* **18**: 743–745, 1990.
20. Smith LL and Wyatt I, The accumulation of putrescine into slices of the rat lung and brain and its relationship to the accumulation of paraquat. *Biochem Pharmacol* **30**: 1053–1058, 1981.
21. Smith LL, Lewis CPL, Wyatt I and Cohen GM, The importance of epithelial uptake systems in lung toxicity. *Environ Health Perspect* **85**: 25–30, 1990.
22. Gordonsmith RH, Smith LL and Cohen GM, Pulmonary accumulation of methylglyoxalbis(guanylhydrazone) by the oligoamine uptake system. *Biochem Pharmacol* **34**: 1809–1816, 1985.
23. Byers TL and Pegg AE, Properties and physiological function of the polyamine transport system. *Am J Physiol* **257**: C545–C553, 1989.
24. Byers TL, Wechter R, Nuttall ME and Pegg AE, Expression of human gene for polyamine transport in Chinese-hamster ovary cells. *Biochem J* **263**: 745–752, 1989.
25. Seidenfeld J, Konar KA, Navjohan MF and Block AL, Effects of DFMO-induced polyamine depletion on human tumour cell sensitivity to antineoplastic DNA-crosslinking drugs. *Cancer Chemother Pharmacol* **17**: 16–20, 1986.
26. Hazeldine CE, Pyman FL and Winchester J, The tautomerism of amidines Part IV. The methylation of 4(or 5)-nitroglyoxaline and 4(or 5)-phenylglyoxaline. *J Chem Soc* **125**: 1431–1441, 1924.

27. Wheelhouse R, Ph.D. Thesis, Leicester University, 1990.

APPENDIX

General procedure for the preparation of sulphur-linked-5-nitroimidazoles. 1-Methyl-2-chloromethyl-5-nitroimidazole [26] (0.50 g, 2.85 mmol), amino thiol hydrochloride (2.85 mmol) and triethylamine (0.4 mL) were stirred overnight in methanol (20 mL) at room temperature. The solvent was evaporated at low pressure and the residue purified by crystallization from ethyl acetate/petroleum ether (for 111) or by ion exchange chromatography on Dowex 50 W (47 mL) eluting with a gradient of hydrochloric acid (0.5 up to 2.5 M, 1000 mL total vol.). The fractions containing the product were identified by UV (for 121 and 131Me) and the hydrochloric acid removed at low pressure to yield the product as a foam.

Compound 111, Yield 0.57 g (79%). ^{1}H NMR (90 MHz) $\delta(D_2O)$: 8.08 (1H, s, imidazole ring), 4.07 (2H, s, SCH_2), 4.03 (3H, s, NMe), 3.28 (2H, t, J = 7 Hz, CH_2NH_2), 2.94 (2H, 5, J = 7 Hz, SCH_2); UV $\varepsilon = 6500$ at 325 nm. Compound 121, Yield 0.55 g (51%). ^{1}H NMR (90 MHz) $\delta(D_2O)$: 8.50 (1H, s, imidazole ring, protonated), 4.30 (2H, s), 4.12 (3H, s, NMe), 3.6–2.8 (8H, m, CH_2NH_2), 2.14 (2H, q, J = 7 Hz, $CH_2CH_2CH_2$); UV $\varepsilon = 6450$ at 325 nm. Compound 131Me, Yield 0.86 g (62%). ^{1}H NMR (90 MHz) $\delta(D_2O)$: 8.50 (1H, s), 4.31 (2H, s), 4.12 (3H, s) 3.6–2.8 (10H, m), 2.80 (3H, s, NMe), 2.40–1.82 (4H, m); UV $\varepsilon = 6490$ at 325 nm.

The amine thiols were obtained as follows. N^1-Mercaptoethanol-1,3-diaminopropane [27]. A solution of 1,3-diaminopropane (0.26 g, 3.5 mmol) in benzene (50 mL) was dried by azeotropic distillation in a Dean-Stark apparatus under a nitrogen atmosphere.

A solution of ethylene sulphide (1 g, 16.7 mmol) in sodium-dried benzene (8 mL) was added over 30 min and the resulting mixture refluxed for 2 hr. After evaporating the solvent under reduced pressure, the excess diaminopropane was removed by vacuum distillation and the semi-solid residue distilled to give the product as a white solid (0.74 g, 33%). The hydrochloride salt was prepared by the addition of excess dilute HCl to an aqueous solution of the amino thiol and evaporation to dryness. The salt was recystallized from ethanol/water.

Free base. ^{1}H NMR (300 MHz) $\delta(CDCl_3)$: 2.96 (2H, t, J = 6.9 Hz, H1′), 2.82 (2H, t, J = 6.1 Hz, H2′), 2.76 (2H, t, J = 6.8 Hz, H3), 2.69 (2H, t, J = 7.0 Hz, H1) 1.69 (4H, br s, NH, NH_2 and SH), 1.64 (2H, m, H2).

δ^{13}C (75 MHz, $CDCl_3$, DEPT) CH_2 47.55, 46.81, 39.85, 38.29, 33.15.

HCl salt. ^{1}H NMR (300 MHz) $\delta(D_2O)$: 3.28 (2H, t, J = 7.08 Hz, H1′), 3.20 (2H, t, J = 7.82 Hz, H3), 3.12 (2H, t, J = 7.63 Hz, H1), 2.89 (2H, t, J = 7.08 Hz, H2′), 2.22–2.11 (2H, m, H2).

δ^{13}C (75 MHz, D_2O) CH_2 51.80, 46.06, 38.16, 25.42, 21.25.

N^1-Mercaptoethyl N^4-methylnorspermidine [27]. This compound was prepared by an analogous method to that above using N^4-methylnorspermidine (20 g, 138 mmol) in benzene (100 mL) and ethylene sulphide (1 g, 16.6 mmol) in benzene (20 mL). The excess triamine was distilled out at 50°, 0.2 mmHg, 83% recovery. The residue was then distilled at much lower pressure (62°, 1.3×10^{-6} mmHg) to give 2.62 g (77%) of product. The hydrochloride was prepared as above and recrystallized from 95% ethanol (m.p. 105–106°).

Free base. ^{1}H NMR (300 MHz) $\delta(CDCl_3)$: 2.92 (2H, t, J = 5.26 Hz, H2′), 2.82 (2H, t, J = 5.26, H1′), 2.72 (2H, t, J = 6.87 Hz, H7), 2.65 (2H, t, J = 6.97 Hz, H1), 2.379 (2H, t, J = 7.17 Hz) and 2.38 (2H, t, J = 7.27 Hz) (H5, H3), 2.20 (3H, s, Me), 1.9–1.8 (4H, br m, NH, NH_2 and SH), 1.71–1.56 (4H, m, H2, H6).

^{13}C NMR (75 MHz) $\delta(CDCl_3$, DEPT): CH_2 55.70, 55.23, 47.72, 40.26, 38.42, 30.68, 27.20; CH_3 41.89.

m/e (M + H): observed 206.1707; $C_9H_{24}N_3S$ requires 206.1691; 172 M-SH, 158, 98, 84, 72, 58 (100%) ($CH_2CH_2NH_2$).

V_{max} 3280 m (SH, NH), 2940s, 2800, 1580w 1460m.

Procedure for the preparation of epoxide-linked compounds. 1′-(1,2-epoxyprop-3-yl)-2′-nitroimidazole. 2-Nitroimidazole (1 g, 8.8 mmol) was dissolved in methanol (15 mL), water (15 mL) and epichlorohydrin (2 mL) containing potassium hydroxide (0.5 g, 9 mmol) and stirred for 12 hr at room temperature. The solvents were removed under reduced pressure and water (5 mL) and ethyl acetate (20 mL) were added. The organic layer was separated and the water extracted with two further portions of ethyl acetate. The combined organic layers were dried ($MgSO_4$), evaporated and the product purified by column chromatography (Et_2O/EtOAc on silica) to give 1.21 g (81%) as an oil which eventually crystallized.

^{1}H NMR (90 MHz) $\delta(CDCl_3)$: 7.28 (1H, br s), 7.14 (1H, br s), 5.02 (1H, dd, J = 15 Hz and 2 Hz), 4.28 (1H, dd, J = 15 Hz and 6 Hz), 3.27 (1H, m), 2.90 (1H, t, J = 4 Hz), 2.57 (1H, dd, J = 4 Hz and 2 Hz).

Synthesis of compound 230. N^1,N^8-bis-(*t*-butoxycarbonyl)spermidine [27] (1.02 g, 2.96 mmol) and 1′-(1,2-epoxyprop-3-yl) 2′-nitroimidazole (0.5 g, 2.96 mmol) were dissolved in methanol (5 mL) and stirred for 24 hr. The solvent was evaporated and the product was purified on a silica column using ethyl acetate and methanol as eluents. The product was dissolved in trifluoroacetic acid (5 mL) and left for 1 hr. Distilled water (100 mL) was added and the solution was applied to the top of a Dowex 50 W (60 mL) and eluted with a hydrochloric acid gradient (0.5–3.0 M, 1200 mL total). The compound was detected by UV and the hydrochloric acid was evaporated off the relevant fractions to give the hydrochloride of the product as a yellowish foam (0.6 g, 44%).

^{1}H NMR (300 MHz) $\delta(D_2O)$: 7.49 (1H, s, imidazole ring), 7.21 (1H, s, imidazole ring), 4.51 (1H, br t, J = 9 Hz), 4.37 (1H, dd, J = 14 Hz and 9 Hz), 3.55 (1H, br d, J = 14 Hz), 3.45–3.25 (3H, m), 3.20–2.95 (7H, m), 2.25–2.03 (2H, m), 1.90–1.67 (4H, m).

Synthesis of compound 430. N^1,N^8-bis-(*t*-butoxycarbonyl)spermidine [27] (1.0 g, 2.9 mmol) was dissolved in dry acetonitrile (20 mL) and ethyl trifluoroacetate (1.96 g, 13.8 mmol) and refluxed for 16 hr. The solvents were removed under low pressure and replaced by methanol (20 mL) containing trifluoroacetic acid (0.66 g, 5.8 mmol) which was stirred overnight. Potassium hydroxide (0.32 g, 5.8 mmol) and water (5 mL) were added and when these had dissolved 1′-(1,2-epoxyprop-3-yl)-2′-nitroimidazole (0.98 g, 5.8 mmol) was added and stirred overnight again. A further equivalent of potassium hydroxide (0.16 g, 2.9 mmol) was added and the solution was refluxed for 30 min. The product was diluted with water (100 mL) and purified on Dowex 50 W as described above to give a yellowish foam (0.8 g, 42%).

^{1}H NMR (300 MHz) $\delta(D_2O)$: 7.49 (2H, two overlapping doublets, J = 1.2 Hz), 7.25 (1H, d, J = 1.2 Hz), 7.23 (1H, d, J = 1.2 Hz), 4.52 (1H, m), 4.38 (3H, br d, J = 7 Hz, overlapping m), 3.52–3.30 (6H, m), 3.25–3.00 (8H, m), 2.35–2.15 (2H, m), 1.95–1.75 (4H, m).