Radiosensitizers targeted to DNA using Platinum. Synthesis, Characterisation, and DNA Binding of *cis*-[$PtCl_2(NH_3)$(Nitroimidazole)]

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The preparation and characterisation of *cis*-[$PtCl_2(NH_3)$(misonidazole)] and *cis*-[$PtCl_2(NH_3)$(metronidazole)] is reported; these complexes bind to DNA and radiosensitize more efficiently than their analogues containing two nitroimidazole groups, and the results confirm the possibility of targeting radiosensitizing ligands to DNA by complexation with platinum.

The area of metal-based radiosensitizers is one of current interest. Radiosensitization refers to the enhancement of radiation-induced damage by certain drugs, especially in hypoxic (oxygen-deficient) cells.[1] The greater resistance to radiation of hypoxic cells, in comparison with normally oxygenated cells, can limit tumour cure by radiotherapy.

Considerable effort has been expended on the clinical development of nitroimidazoles as radiosensitizers such as misonidazole [1-(2-nitroimidazolyl)-3-methoxypropan-2-ol, (**1**)] and metronidazole [1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole, (**2**)]. These compounds behave by an 'electron-affinic' mechanism which implies the acceptance of an electron from a target molecule such as DNA, thus 'fixing' the lesion. The identification of DNA as the ultimate target of radiation damage prompted us[2] and others[3,4] to explore the targeting of nitroimidazoles to DNA by complexation to platinum because of the strong binding of this metal, in its complexes, to purine and pyrimidine bases and as analogues of *cis*- and *trans*-[$PtCl_2(NH_3)_2$]. Both the platinum–amine complexes potentiate cellular radiation damage.[5,6] However, the initial promise shown by the complex *cis*-[$PtCl_2$(metronidazole)$_2$] was unfortunately not sustained.[5]

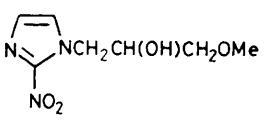
The lack of success of species such as the above and *trans*-[$PtCl_2$(misonidazole)$_2$] led us to question whether this was owing to their labile nature or whether they were indeed binding to DNA, and the necessity of two nitroimidazole (NO_2Im) groups. Since the hypoxic cytotoxicity is expected to come from the nitro group, as in free ligand, the presence of two NO_2Im groups bound to Pt may not be essential. Consequently, we have prepared mono complexes containing only one NO_2Im group, *cis*-[$PtCl_2(NH_3)(NO_2Im)$], (**1a**; NO_2Im = misonidazole) and (**2a**; NO_2Im = metronidazole), and in this communication we report their synthesis and compare their DNA binding and radiosensitization with the bis complexes [$PtCl_2(NO_2Im)_2$].

The complexes are easily prepared by reaction of 1 equiv. of nitroimidazole in aqueous solution to 1 equiv. of the monoammine complex, K[$PtCl_3(NH_3)$], equation (1). The *cis* geometry is indicated by the presence of two peaks in a broad, strong band at 338 and 332 cm^{-1} (**1a**) and 336 and 330 cm^{-1} (**2a**). There is an increase in the electrochemical reduction potential of the nitro group, as found for the bis complexes [for (**1a**), $E_{\frac{1}{2}}$ (*vs.* Ag/AgCl, phosphate buffer) increases to −0.275 V from −0.38 of the free ligand]. U.v.–visible spectra show maxima at 310 (**1a**) and 300 nm (**2a**). Molecular wt. measurements in MeOH gave values of 475 and 435 daltons for (**1a**) and (**2a**) (calc. 484 and 454 respectively). The white to light yellow complexes are soluble in H_2O, MeOH, and acetone. A generalized structure is therefore shown as (A).

$$K[PtCl_3(NH_3)] + NO_2Im \longrightarrow cis\text{-}[PtCl_2(NH_3)(NO_2Im)] + KCl \quad (1)$$

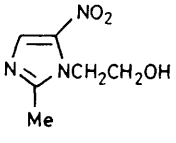
To assess their DNA binding, a plasmid assay examining for inhibition of restriction endonuclease activity was employed.[7] In this assay, the closed circular plasmid DNA, pSV2-gpt (5.2kb), is linearized using the enzyme PvuII. Cleavage by restriction endonucleases BamHI (recognition sequence CCTAG/G) or EcoR1 (recognition sequence CTTAA/G) gives 2 fragments, since purified linear plasmid is used. The binding of the complex at or near the recognition site inhibits the restriction activity.

The new complexes do indeed inhibit endonuclease activity whereas the bis complexes *cis*-[$PtCl_2$(metronidazole)$_2$] and *trans*-[$PtCl_2$(misonidazole)$_2$] do not inhibit at equivalent concentrations. Thus, the approximate concentration for 10% inhibition of BamHI is 55 μM for (**1a**) and 60 μM for (**2a**) in comparison with 12 μM for *cis*-[$PtCl_2(NH_3)_2$]. The *trans* isomer of [$PtCl_2(NH_3)_2$] inhibits to the same extent as the *cis*, in accord with previous results.[8] Both (**1a**) and (**2a**) inhibit BamHI to a greater extent than EcoR1. The preference for the guanine-rich BamHI over EcoR1 has also been noted previously for platinum–amine complexes, in accord with the preferred binding of these complexes to G-rich areas of DNA.[8–10] The presence of a bound amine group in (**1a**) and (**2a**) may enhance the DNA-binding affinity of these complexes; simple imidazole complexes such as [$PtCl_2$(*N*-MeIm)$_2$] are not active chemotherapeutic agents and do not bind to DNA, properties attributed to the absence of an NH group bound to the metal.[11] The importance of at least one amine bound to platinum has been demonstrated in recent structural and theoretical studies on adducts of *cis*-[$PtCl_2(NH_3)_2$] with di- and tri-nucleotides where the presence of hydrogen bonding between the amine and the phosphate oxygen has been substantiated;[12–14] this requirement is emphasised in the present case. The presence of only one nitroimidazole ligand, plus the presence of the primary amine, facilitates the binding. For the bis(nitroimidazole) complexes it is possible that the presence of two planar rings at some dihedral angle to the co-ordination square plane[2–4] may also be sterically hindering



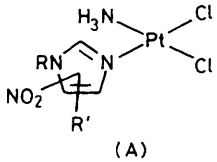
Misonidazole

(**1**)



Metronidazole

(**2**)



(A)

for an approach to DNA. The complex $[Pt(bipy)py_2]^{2+}$ (bipy = bipyridine, py = pyridine) does not intercalate into DNA because of the steric hindrance of the pyridine ligands.[15]

As radiosensitizers, both mono complexes (**1a**) and (**2a**) are more effective than the bis complexes, especially when normalised per mole of radiosensitizer ligand.[16] It is tempting to attribute the enhanced radiosensitization to greater DNA binding, or more specific DNA binding because of the change in structure. The initial results presented here show that there is indeed validity in the idea of targeting nitroimidazoles to DNA by platinum complexation. Complexes which bind to DNA (as assessed by the inhibition of restriction endonuclease activity), are better radiosensitizers than those close analogues which do not bind.

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References

1 G. E. Adams in 'Cancer. A Comprehensive Treatise,' ed. F. F. Becker, Plenum Press, New York, 1977, vol. 6, pp. 181—224.
2 N. Farrell, T. M. Gomes Carneiro, F. W. B. Einstein, T. Jones, and K. A. Skov, *Inorg. Chim. Acta*, 1984, **92**, 61.
3 J. R. Bales, C. J. Coulson, D. W. Gilmour, M. A. Mazid, S. Neidle, R. Kuroda, B. J. Peart, C. A. Ramsden, and P. J. Sadler, *J. Chem. Soc., Chem. Commun.*, 1983, 432.
4 J. R. Bales, M. A. Mazid, P. J. Sadler, A. Aggarwal, R. Kuroda, S. Neidle, D. W. Gilmour, B. J. Peart, and C. A. Ramsden, *J. Chem. Soc., Dalton Trans.*, 1985, 795.
5 A. H. W. Nias, *Int. J. Radiat. Biol.*, 1985, **48**, 297.
6 E. B. Douple, *Platinum Metals Rev.*, 1985, **29**, 118.
7 K. A. Skov, H. Adomat, D. C. Konway, and N. P. Farrell, *Chem. Biol. Inter.*, 1987, in the press.
8 W. M. Scovell and L. R. Kroos, *Biochem. Biophys. Res. Commun.*, 1982, **104**, 1597.
9 A. L. Pinto and S. J. Lippard, *Biochim. Biophys. Acta*, 1985, **780**, 167.
10 A. T. M. Marcelis and J. Reedijk, *Recl. Trav. Chim. Pays-Bas*, 1983, **102**, 121.
11 J. Reedijk, C. H. van Krallingen, and J. K. de Ridder: *Inorg. Chim. Acta*, 1979, **36**, 69.
12 S. Sherman, D. Gibson, A. H. J. Wang, and S. J. Lippard, *Science*, 1985, **230**, 412.
13 G. Admiraal, J. L. van der Veer, R. A. G. de Graaff, J. H. J. den Hartog, and J. Reedijk, *J. Am. Chem. Soc.*, 1987, **109**, 592.
14 J. Kozelka, G. A. Petsko, S. J. Lippard, and G. J. Quigley, *J. Am. Chem. Soc.*, 1985, **107**, 4079.
15 S. J. Lippard, *Acc. Chem. Res.*, 1978, **11**, 211.
16 K. A. Skov, N. P. Farrell, and H. Adomat, *Radiat. Res.*, 1987, in the press.