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Synthesis and biodistribution of ^{111}In, ^{67}Ga and ^{153}Gd-radiolabelled conjugates of nitroimidazoles with bifunctional complexing agents: imaging agents for hypoxic tissue?@

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Conjugates of the putative hypoxia-selective agent 2-nitroimidazole with bifunctional complexing agents have been prepared and radiolabelled with ^{111}In, ^{67}Ga or ^{153}Gd. Although some evidence for tumour localisation was found control experiments revealed that the gallium and indium labelled complexes of the simple ligand 1,4,7-triazacyclononane-triacetate are themselves promising tumour-imaging agents.

It has been established that nitroimidazoles, such as misonidazole, 1, tend to localise in the cells of normal and malignant tissues which are poorly oxygenated. The selective retention of these molecules is related to the presence in the cell of reductase enzymes whose activity is enhanced in areas of low oxygen tension.[2] Misonidazole, 1, has been shown to be retained preferentially in the hypoxic (oxygen deficient) regions of certain tumours[3] or brain tissue[4] raising the possibility that by conjugating a suitable nitroimidazole to a bifunctional complexing agent[5] designed to bind an imaging isotope (e.g. the γ-emitters ^{111}In, ^{67}Ga), targeted imaging of local areas of hypoxia (e.g. poorly vascularised regions of tumours) may be possible.† If sufficiently selective targeting can be achieved, then therapeutic radioisotopes (e.g. ^{90}Y, β^-, $t^1/_2$ 64h) may in principle be used. Some related work has concentrated on the study of ^{99m}Tc-radiolabelled conjugates of nitroimidazoles for the imaging of regional hypoxia in ischemic heart disease,[5,6] following the observation that misonidazole when labelled with ^{18}F, 1, gave good contrast between normoxic and hypoxic regions of myocardial tissue in a PET imaging study (PET is positron emission tomography).[7,8]

Conjugates have been prepared of 2-nitroimidazole with bifunctional complexing agents which have been shown to form a kinetically stable complex with ^{111}In (2, 4 and 5),[9,10] ^{67}Ga (4 and 5),[10,11] and ^{153}Gd[1P] (2 and to a lesser extent 3).[12,13] In each case the ligand is tribasic so that the radiolabelled complex is charge-neutral at ambient pH. This should allow the conjugates at least to passively diffuse in and out of the target cells.

The derivatives 2 to 5 were prepared according to standard procedures.[9–12] Thus 2 was prepared following reaction of the active ester 7[12] with 1-aminopropyl-2-nitroimidazole 8 (DMSO 20°C, 73% following purification by reverse-phase HPLC [Dynamax C18]). The diamide 3 was prepared by reaction of the anhydride of diethylene-triaminepenta-acetic acid, 9 with 8 (pyridine, 90% following HPLC purification) and the conjugates of 1,4,7-triazacyclononane triacetic acid, 4 and 5 were prepared following reaction of the respective active esters

@ This work was reported at the International Symposium on Macrocyclic Chemistry, University of Sheffield, July, 1991.

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† It is generally observed with misonidazole, 1, that the optimum interval for uptake in tissues is 24 hours. Compare this to the half-lives of ^{111}In, $t^1/_2$ 2.81 days; ^{67}Ga $t^1/_2$ 3.25 days; and 6.01 hours for ^{99m}Tc.

[1P] In this work, ^{153}Gd-labelled complexes were examined (^{153}Gd, $t^1/_2$ 241 days). The related yttrium complexes will be substantially similar in their in vivo stability, (ref. 12).

10 and 11 with 8 (DMSO, Et_3N: 53 and 71% respectively).#

IN VITRO STUDIES

A measured number of Chinese hamster lung fibroblasts (V-79 cells) in suspension medium were incubated with the radiolabelled complexes under hypoxic (N_2/CO_2) or normoxic (air/CO_2) conditions. The ratio of activity in the cell volume to that in the incubation medium gives a measure of the extent to which the radiolabelled complex is taken up or retained by the cell. With misonidazole, labelled with tritium (prepared as in reference 1), this ratio exceeded 5 with *both* hypoxic and normoxic cells. With all of the simple radiolabelled complexes studied and with their nitroimidazole conjugates, this ratio was less than unity irrespective of the degree of oxygenation of the cells. For example with [^{67}Ga.5] under hypoxic conditions, the ratio of activity in the cells to that in the medium was 0.81:1 (after 4h). This compares to a value of 0.99:1 for [Ga6] under similar conditions and a value of 5.6:1 for tritium labelled misonidazole,[1] 1, itself.

Two main conclusions can be drawn from this limited study. Firstly, these results imply that misonidazole binds to intracellular contents under normoxic and hypoxic conditions. In addition, whilst the charge neutral complexes may be able to diffuse in and out of cells with some degree of freedom, there is no evidence for either an active uptake nor a selective retention mechanism.

BIODISTRIBUTION STUDIES

It is, of course, the behaviour of the radiolabelled conjugates in vivo that is much more important in assessing the potential of these systems for possible application. Notwithstanding the rather equivocal in vitro results, the biodistribution of the radiolabelled complexes and conjugates 2 to 6 was examined in mice.

The biodistribution of [^{153}Gd.2] was examined in normal mice in the absence and presence of misonidazole (given at 300μmol kg^{-1}, in order to avoid the premature reduction in the bloodstream of the nitroimidazole in the tracer dose of [^{153}Gd.2]-0.1μmol kg^{-1}). No significant differences in tissue content were noted for the absence/presence of 1, and there was no selective build-up or uptake in tissues which are expected to be hypoxic (e.g. eyes, oesophagus, liver).* The complex cleared quickly from the blood with less than 1% of the injected dose found in the blood 1 hour post-injection. The complex was excreted via the kidneys, and after 24 hours more than 99% of the activity had cleared from the body, consistent with the excellent in vivo stability of the conjugate.[14]

In the case of [^{153}Gd.3], the biodistribution was examined in immunocompetent mice bearing a xenograft of a KHT tumour (a sarcoma). The proportion of hypoxic tumour cells in such xenografts has been shown to be 10% in the G_1 cell cycle phase.[18] In a control experiment the extracellular, anionic complex, [^{153}Gd.DTPA]$^{2-}$, was administered at the same dose, again in the presence of a hundred-fold excess of misonidazole, 1 (ca. 100 μmol kg^{-1}). As expected the [^{153}Gd.3] conjugate was slower to clear from the blood than [^{153}Gd.DTPA]$^{2-}$, but the normalised tumour to blood ratio was comparable in both cases over the time period 4 to 24 hours. The ratio of the activities in tissue for [Gd.3] compared to [Gd.DTPA]$^{2-}$ (Table 1), reveal no significant tumour localisation for the nitroimidazole conjugate and notwithstanding the slower blood clearance of [Gd.3], the higher levels of ^{153}Gd found in the bone with [Gd.3] are simply a consequence of its inferior in vivo stability compared to [Gd.DTPA]$^{2-}$. The lower stability in vivo of charge neutral DTPA-bis amide complexes of ^{153}Gd and ^{90}Y has been noted by us previously, and is manifested by deposition of the radiolabel in the bone.[13,15,16]

A lack of selective localisation in hypoxic tissue was also found with [^{111}In 4]. The biodistribution of [^{111}In 4] was examined in normal mice (0.1μmol kg^{-1} was administered together with 500 μmol kg^{-1} of 1) and the tissue biodistribution of the label measured at 24h, in comparison to that found for [^{111}In. 6]. No clear evidence for selective localisation in hypoxic tissue was found (e.g. the activity in the eyes, oesophagus, or liver (each of which

Table 1 Biodistribution of [^{153}Gd3] Compared to [^{153}Gd.DTPA]$^{2-}$in Mice Bearing a KHT Tumour (ratio of experimental to control, % injected dose per gm tissue)

Tissue	*4h*	*24h*
Blood	7.1	20
Kidneys	1.6	1.1
Liver	1.1	1.0
Spleen	–	0.18
Femur	4.7	5.6
Tumour	1.9	1.5

New compound gave satisfactory mass spectra and NMR data in accord with the proposed structures and homogeneity was demonstrated by analytical reverse-phase HPLC.

* Supplementary data is available on request from the authors showing the biodistribution of the complex in the major organs and in the blood or skeleton, and where appropriate in the tumour xenograft.

Table 2 Biodistribution in Normal Mice 1h for [^{67}Ga5] and [^{67}Ga6] (% injected activity; standard deviations in parentheses)[a]

Tissue	*[^{67}Ga.5]*	*[^{67}Ga.6]*
Blood	0.28(0.18)	0.53(0.25)
Kidneys	0.63(0.26)	3.30(3.9)
Liver	2.24(0.53)	1.20(0.29)
Intestinal Tract	22.1(1.7)	0.80(0.35)

[a] After 24 hours; there was no difference statistically in the % of the injected activity in tissues groups of mice who had been give [^{67}Ga.5] or [^{67}Ga.6]. In each case less than 0.05% of the injected dose per gram of tissue was measured in the blood, lungs, kidney or gut, with 0.15% in the liver. No evidence for localisation in tissues which are hypoxic (eye, oesophageal lining, foot pads) was found.

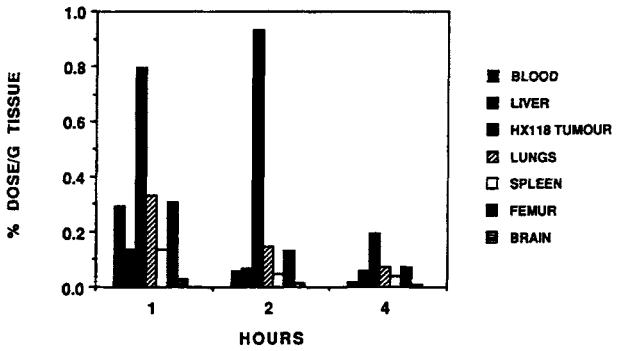


Figure 1 Biodistribution of [^{111}In.6] in Mice Bearing a Xenograft of a Human Melanotic Melanoma (HX 118).

is a somewhat hypoxic tissue) was very similar when compared to the control [^{111}In 6].*

More promising results were obtained with the ^{67}Ga-labelled complex of 5. Compared to the control, [^{67}Ga 6], the complex cleared more rapidly from the blood and was excreted via the endogenous faecal route as well as by the renal clearance pathway (Table 2). For example, 22% of the injected dose with [^{67}Ga.5] is in the gut at 1 hour. In mice bearing a xenograft of a human melanotic melanoma, HX-118, which is known to accumulate misonidazole[1-3], (Table 3), some modest tumour localisation was noted for [^{67}Ga.5]. Indeed the tumour to blood ratio was higher than for tritium labelled misonidazole,[1,17] and remained constant between 4 and 24 hours.

We have reported earlier that the simple complex [^{67}Ga.6] gave even higher tumour to blood ratios at all time points with 0.17% i.d. per gm tissue in the tumour at 2 hours, and the tumour to blood ratios at 2,4,8 and 24 hours were found to be 12,22,15 and 19:1 respectively. Even more interesting behaviour was observed with [^{111}In.6]. Although most of the complex was excreted by the kidneys, selective retention of the complex in tumour tissue was observed, with between 0.5 and 1% of the injected dose (per gram of tissue) found in the tumour at 2 hrs. At this 2 hour time point, the tumour to blood ratio was 17:1 and the tumour to liver ratio was 13:1, (Figure 1)[19].

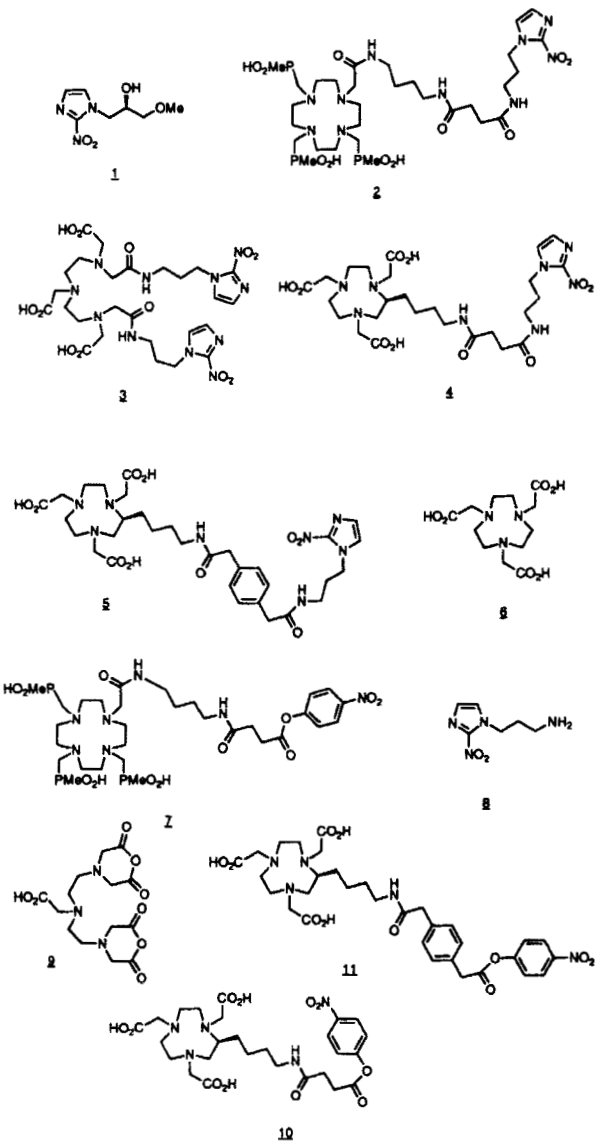
CONCLUSIONS

The ^{111}In complex of 1,4,7-triazacyclononane -triacetate or even the ^{68}Ga [^{68}Ga, B^+, $t_{1/2}$ = 68 mins.] labelled complex of 6 merit further study as simple complexes for the imaging of certain tumours, (such as a melanoma). The selective targeting of hypoxic tissues by the macrocyclic radiolabelled conjugates of 2-nitroimidazole 2 to 5 studied herein, has not been unequivocally established in the

Table 3 Biodistribution in Tumour-Bearing Mice of [^{67}Ga.5] and [^{67}Ga.6][a,b] (% injected dose per gm tissue in mice bearing an HX 118 xenograft [a melanotic melanoma])

Tissues	*[^{67}Ga.6]*			*[^{67}Ga.5]*			*[^{3}H.1]*
	Time (Hours)						
	4	*8*	*24*	*4*	*8*	*24*	*24*
Blood	0.006	0.008	0.0022	0.008	0.005	0.0016	0.0018
	(0.0015)	(0.004)	(0.0005)	(0.005)	(0.004)	(0.0004)	
HX118 (tumour)	0.127	0.097	0.042	0.085	0.075	0.022	0.014
	(0.05)	(0.02)	(0.004)	(0.03)	(0.02)	(0.001)	
Tumour / Blood	21.6	14.8	19	14.0	15.8	14.0	7.70

a) mean values of at least 3 are given with standard deviations in parentheses; for further experimental details, see reference 17.
b) for [^{67}Ga.6] the % injected dose per gm. tissue exceeded that of all other tissues (1h $\rightarrow$ 24h) except for the liver and kidney (further details are published in reference 17). Similar tumour localisation with [^{67}Ga.6] was observed in animals bearing a human colorectal tumour, (HT29)[19].



given animal models. Their relatively poor ability to localise in regions of hypoxia, may be related to their relatively high molecular weight that may preclude cellular uptake, or may be due to the fact that, for the in vivo work, these complexes were given at a relatively low dose, even though misonidazole was co-administered.

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