ASTATINATED RADIOPHARMACEUTICALS FOR TARGETED ALPHA PARTICLE RADIOTHERAPY*

G. VAIDYANATHAN, M.R. ZALUTSKY
Department of Radiology, Duke University Medical Center,
Durham, North Carolina, United States of America

Abstract. The radionuclides generally used for targeted radiotherapy such as ^{131}I and ^{90}Y, emit ∃-particles whose range in tissue is of the order of several millimeters. Alpha particles, on the other hand, traverse only a few cell diameters; thus, targeted alpha particle therapy would be ideally suited for micrometastases, tumours of circulation such as lymphoma, and compartmental-grown tumours such as cystic brain tumour, ovarian cancer and neoplastic meningitis. For example, it has been calculated that the absorbed fraction ratio for ^{211}At alpha particles, compared with ^{90}Y ∃-particles, increases from 9:1 for 1-mm diameter tumours to 33:1 for 0.2 mm diameter ones. Although a number of ∀-particle-emitting radionuclides exist, the properties of ^{211}At make it perhaps the most attractive candidate ∀-emitter for radiotherapy. Because ^{211}At is a halogen, radioiodination chemistry can be adapted for astatination. This paper describes the production of ^{211}At, as well as the preparation and evaluation of astatinated radiopharmaceuticals such as monoclonal antibodies and the 5-iododeoxyuridine analogue AUdR.

1. INTRODUCTION

An important point in selection of an appropriate therapeutic radionuclide is the spatial configuration (size and geometry) of the tumour. Iodine-131 and ^{90}Y are the most commonly used radionuclides for targeted radiotherapy. These emit high energy ∃-particles, the mean range in tissue of which is several millimeters. Alpha particles on the other hand, would traverse only a few cells. Theoretical calculations have shown that absorbed fraction ratio for ^{211}At ∀-particles, compared with ^{90}Y ∃-particles, increases from 9:1 to 33:1 as tumour diameter decreases from 1mm to 0.2 mm [1]. Thus, targeted alpha particle therapy could be ideally suited for micrometastases, tumours of circulation such as lymphoma and tumours with sheet-like geometry such as cystic brain tumours, ovarian cancer and neoplastic meningitis. Alpha particles have several radiobiological advantages such as high linear energy transfer (LET) and relative biological effectiveness (RBE), marginal dependence on dose rate, and an oxygen enhancement ratio close to unity. Although more than 100 ∀-particle emitting radionuclides exist, only ^{212}Bi, ^{213}Bi and ^{211}At have received serious attention. Astatine-211 decays by a double branched pathway resulting in the emission of one alpha particle per disintegration having an average energy of 6.8 MeV and 55–70 :m range. The LET of ^{211}At μ-particles is about 100 keV/:m at which maximum RBE occurs. Recently it has been shown that it is possible to externally image tissue distribution of ^{211}At by nuclear medicine techniques including SPECT [2]. This capability will be valuable for optimizing treatment strategies as well as determining tumour and normal tissue radiation dosimetry. Being a halogen, more often than not, radioiodination chemistry is adaptable for astatination. These facts have led to the development of a number of astatinated radiopharmaceuticals as potential agents for targeted radiotherapy [3, 4].

2. PRODUCTION OF ^{211}At

Astatine-211 is produced by the cyclotron bombardment of natural bismuth metal targets with 28 MeV α-particles using the ^{209}Bi(α,2n)^{211}At reaction. Until recently, an external target was used; however, the yields were not sufficient for clinical investigations. Using an internal target, ^{211}At could be produced in high yields and purity [5]. Production efficiency of 41 ± 7 MBq/:A≅h has been obtained routinely with this target. Up to 4 GBq of ^{211}At has been produced to date, a level that has permitted initiation of a clinical trial with ^{211}At at our institution.

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3. ASTATINATED MONOCLONAL ANTIBODIES

3.1. Astatination of mAbs

Combining the tumour cell specificity of monoclonal antibodies (mAbs) with ∀-particle-emitting radionuclides is an area which has received serious consideration in targeted therapy. Unlike radioiodination, it is not possible to astatinate proteins and antibodies in a stable form by direct electrophilic substitution. The first successful attempt to astatinate mAbs was made using *para*-[^{211}At]astatobenzoic acid (PABA) which was coupled to proteins via a mixed anhydride route [6]. Although this method resulted in astatinated mAbs with improved *in vivo* stability, radiochemical yields and specific activities were less than desired; the preparation of PABA itself was cumbersome. At Duke, a method was developed a decade ago to radioiodinate mAbs with excellent *in vivo* stability [7]. This utilized a conjugation agent, *N*-succinimidyl 3-[$^{125/131}$I]iodobenzoate (SIB) which was prepared from a tin precursor. It was possible to prepare the astato analogue, *N*-succinimidyl 3-[^{211}At]astatobenzoate (SAB) starting from the same tin precursor in excellent yields [8]. Monoclonal antibodies could be astatinated with [^{211}At]SAB under very mild conditions in excellent yields and specific activities [9] (Figure 1). The resultant astatinated mAbs retained affinity and immunoreactivity and were stable *in vivo*.

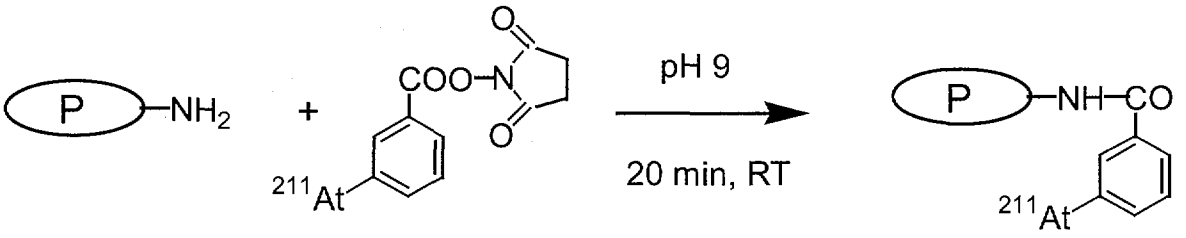

FIG. 1. Conjugation of mAbs with [^{211}At]SAB.

3.2. Tissue distribution of labelled mAbs

To investigate the potential utility of astatinated mAbs, chimeric mAb 81C6 was astatinated using [^{211}At]SAB. Murine 81C6 is an IgG_{2b} mAb which reacts with the extracellular matrix antigen tenascin that is present on gliomas and other tumors, but not on normal brain tissues. The human/mouse chimeric 81C6 mAb was constructed by linking the variable regions of murine 81C6 to human IgG_2 constant regions [10]. A paired-label tissue distribution of chimeric mAb 81C6 labelled with [^{211}At]SAB and [^{131}I]SIB was performed in athymic mice bearing D54-MG human glioma xenografts. The tumour uptake of ^{211}At increased from about 5% ID/g at 0.5 h to about 20% ID/g at 16 h, and remained constant thereafter (Figure 2). Up to 16 h, the tumour uptake of both ^{131}I and ^{211}At was similar. Tumour uptake of ^{131}I from 16 through 48 h was 10–39% higher ($p < 0.05$) than that of ^{211}At. Uptake of both nuclides in blood and other normal tissues decreased gradually. With the exception of the spleen and stomach, ^{211}At- and ^{131}I-labelled mAbs had similar uptake in all normal tissues over 48 h period. The ^{211}At/^{131}I ratios seen in this study were similar to those reported for another mAb in normal mice [11]. Taken together, these results suggest that astatinated intact mAbs behave, to a considerable degree, like their radioiodinated analogues.

3.3. Radioimmunotherapy

As mentioned above, targeted α-particle therapy should be well-suited for the treatment of neoplastic meningitis, a disease characterized by the leptomeningial spread of a variety of tumours within the cerebrospinal fluid compartment. Therapy experiments were carried out using the astatinated murine 81C6. A rat model was used for this study. Neoplastic meningitis was initiated by injecting TE-671 human rhabdomyosarcoma cells via a subarachanoid catheter [12]. Treatment was initiated 8 days after implantation of $5 \cdot 10^5$ cells, or 4 days after implantation of $6 \cdot 10^6$ cells. Three experiments — 2 eight days after and 1 four days after — were done with all reagents given via the

indwelling catheter. In the first experiment, groups of 9–10 rats were treated with saline or a single dose of 148 kBq, 259 kBq, and 481 kBq of ^{211}At-labelled 81C6. In the second experiment, animals received either saline, 444 kBq of ^{211}At-labelled nonspecific mAb, 45.6, 444 kBq or 666 kBq of ^{211}At-labelled 81C6. The third experiment, performed after 4 days of implantation, included both a saline control group and groups given 666 kBq of ^{211}At-labelled 81C6 or ^{211}At-labelled 45.6. Statistical analysis of survival data was performed using the Wilcoxon rank sum test, and $p < 0.05$ was considered to be significant.

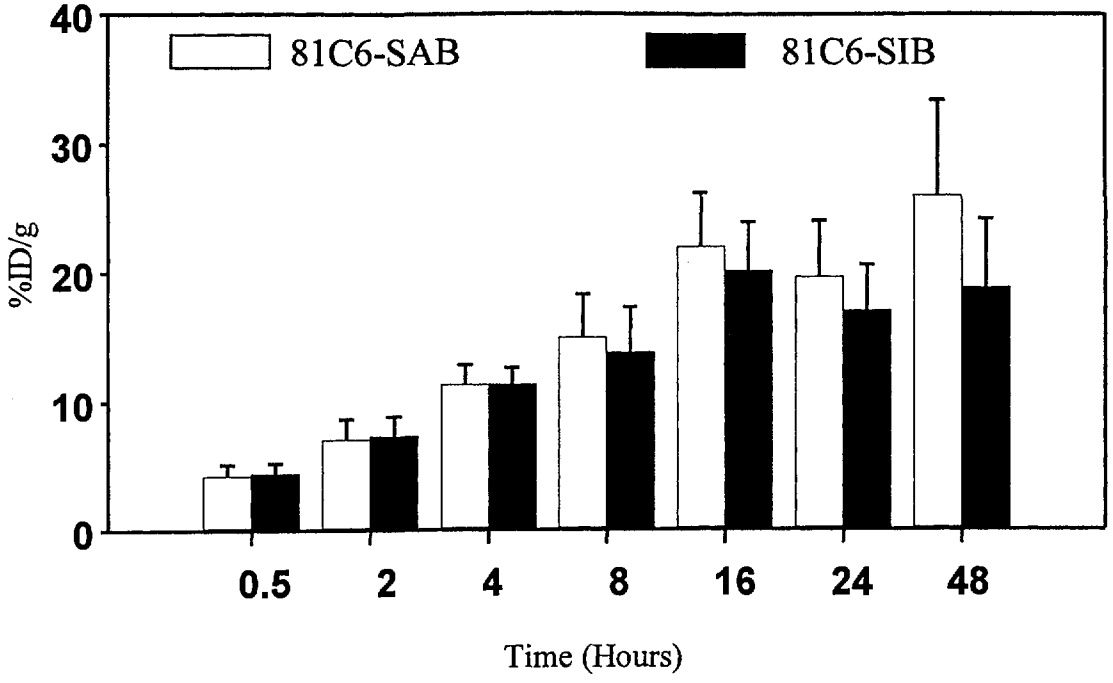

FIG. 2. Paired-label tumour uptake of chimeric 81C6 labelled with [^{131}I]SIB and [^{211}At]SAB in athymic mice hosting D-54 MG human glioma xenografts

In the first experiment, the median survival for the saline control group was 22.5 days compared with 30, 29, and 34 days for 148, 259, and 481 kBq, respectively. The prolongation in median survival for all doses was statistically significant ($p = 0.004$–0.02) compared to saline control; but difference between 148 and 259 kBq was not. Two rats from 481 kBq group and one from 259 kBq group was alive at 190 days with no evidence of disease. Results from the second study showed that, although compared with saline treatment there was a 33% (32 days vs 23.5 days) increase in the median survival for control mAb, the difference was not statistically significant. In contrast, treatment with the same amount of ^{211}At-labelled 81C6 increased median survival by 113%, which was significant compared to both saline and nonspecific mAb. With 666 kBq of ^{211}At-labelled 81C6, the median survival was 84 days, a 357% survival prolongation. In comparison, even with 11.1 MBq of ^{131}I-labelled Mel-14 F(ab')$_2$ fragment (another tumour reactive mAb), only 12% survival prolongation was noticed in this model [13]. There were 1 of 10, 0 of 9, 3 of 9, and 5 of 10 long-term survivors in the saline, 444 kBq 45.6, 444 kBq 81C6, and 666 kBq 81C6 groups, respectively, when the experiment was terminated on day 295. In the experiment with higher tumour burden, the median survival was 15 days for the saline control and 19 and 23 days for 666 kBq or 45.6 and 81C6, respectively. The increase in median survival observed with 81C6 was statistically significant ($p < 0.001$) compared with both the saline and nonspecific mAb controls. Taken together, these results

indicate that the therapeutic benefit of ^{211}At-labelled 81C6 is specific and is considerably more effective.

4. META-[^{211}At]ASTATOBENZYLGUANIDINE (MABG)

Radioiodinated *meta*-iodobenzylguanidine (MIBG), an analogue of the neurotransmitter norepinephrine, has been used for the detection and therapy of neuroendocrine tumours such as neuroblastoma. Since micrometastases are often associated with neuroblastoma, an astatinated analogue of MIBG should be advantageous for the treatment of such diseases. We have developed a synthetic method for the preparation of MABG and have evaluated its potential as a therapeutic agent. Details can be found in an accompanying paper in these proceedings.

5. 5-[^{211}AT]ASTATO-2'-DEOXYURIDINE

Being a thymidine analogue, 5-iodo-2'-deoxyuridine (IUdR) can be incorporated into the DNA of rapidly dividing cells. When labelled with very short range, high LET Auger electron-emitting ^{123}I or ^{125}I, IUdR is extremely cytotoxic to cells undergoing division. However, the strength of [^{125}I]IUdR as a radiotherapeutic agent, its specificity for rapidly dividing cells, is also its most severe limitation: those tumour cells not undergoing DNA synthesis are not subjected to its cytotoxic effects. It is thus desirable to have an agent which could act with high LET effects not only on the cells incorporating the agent into DNA, but also to those adjacent ones not in S-phase.

As a consequence of its α-particle emission, ^{211}At also emits another type of high LET radiation, short-range α-particle recoil nuclei. The mean range of these is 0.092 :m, and their mean LET is about 8 times higher than that of their α-particles. It was hypothesized that an astatinated IUdR analogue might be lethal not only to cells undergoing DNA synthesis, but also to those not in S-phase due to bystander killing. It has been predicted that the D_0 level of cell kill with an astatinated analogue of IUdR would be achieved with as low as 1 decay/cell [14]. To investigate this, an efficient method for the synthesis of 5-[^{211}At]astatodeoxyuridine (AUdR) was first developed and then the cytotoxicity of this potential therapeutic agent was studied.

5.1. Synthesis of AUdR

Two methods have been reported for the synthesis of AUdR. In the first, AUdR was prepared via a diazonium intermediate [15]. The radiochemical yield was unacceptably low. Although higher yields were obtained starting with a mercury precursor, the preparation involved iodine carrier [16]. Recently, radioiodinated IUdR has been prepared from a tin precursor [17]. It was possible to convert the same tin precursor to AUdR in 85–90% radiochemical yield by sonicating it for less than a minute with hydrogen peroxide/acetic acid mixture (Figure 3). AUdR was isolated by reverse-phase HPLC in high chemical and radiochemical purity.

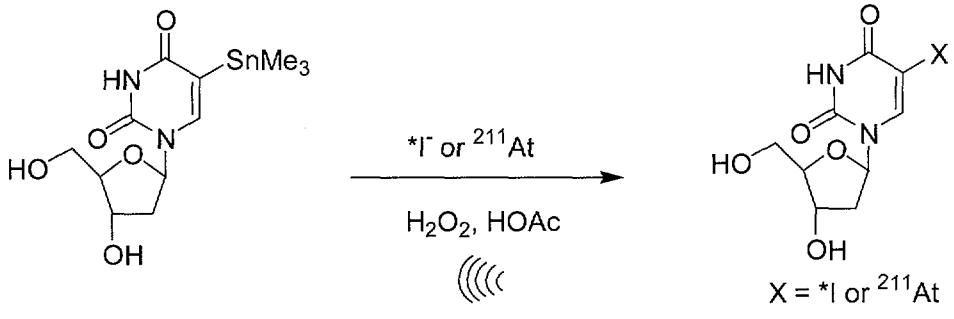

*FIG. 3. No-carrier-added synthesis of [*I]IUdR and AudR.*

5.2. In vitro stability of AUdR

The lack of stability of astatinated radiopharmaceuticals is a major concern. With the exception of ^{211}At-labelled intact mAbs, most astatinated compounds exhibit less than ideal stability in tissue culture and animal models. The intrinsic bond strength of carbon-astatine bond is the least of all carbon-halogen bonds. In addition, due to the high LET nature of ∀-particles, radiolytic degradation is another problem to be reckoned with. IUdR itself is not very stable *in vivo*, with half-life of the order of minutes. This is as a result of the cleavage of its *N*-glycosidic bond by nucleoside phosphorylases. We compared the *in vitro* stability of AUdR with that of [^{125}I]IUdR in tissue culture medium at 37°C as a function of time. Purity at various time points was determined using HPLC. The per centage of ^{125}I and ^{211}At present as intact tracer declined with time. Catabolism appeared to be exclusively due to direct dehalogenation as no 5-halouracil was detected. AUdR was less stable than [^{125}I]IUdR. For example, when normalized to initial purity, 94% and 87% of [^{125}I]IUdR and AUdR, respectively, was present as intact tracer after a 6 h incubation. A similar trend was seen in serum. At 24 h after incubation, 91% of [^{125}I]IUdR was present as intact tracer; in comparison, the amount of AUdR present in intact form at this time was 87%. In this case, 5-halouracil was detected as a catabolite. Although AUdR appears to be slightly less stable than IUdR *in vitro*, the difference in stability between the two was not high.

5.3. Cellular uptake and DNA incorporation

IUdR and its bromo analogue, 5-bromo-2'-deoxyuridine (BUdR) are derivatives of thymidine obtained by replacing the methyl group of thymidine by iodine and bromine, respectively. Both IUdR and BUdR behave remarkably like thymidine. This is probably because the van der Waal's radii, and thus the size, of iodine and bromine are similar to that of the methyl group (2.15 Å and 1.95Å, respectively versus 2.0 Å) that they have replaced. The van der Waal radius of astatine has been estimated to be 2.3. This suggests that ^{211}At for methyl group substitution may alter the molecular properties in AUdR. To investigate this, the cellular uptake and DNA incorporation of IUdR and AUdR was determined *in vitro* in a paired-label format as a function of radioactivity concentration. Because the treatment of brain tumours is one of the most likely applications of AUdR, a human glioma cell line, D-247 MG, was used for these studies. Briefly, exponentially growing cells were incubated with varying activity concentrations of both [^{131}I]IUdR and AUdR for a period of 24 h. To determine the nonspecific uptake, a parallel assay was performed wherein the cells were co-incubated with 10 :M unlabelled IUdR. The cell-associated activity was determined at the end of the incubation period. Simultaneously, the per centage of cell-associated activity incorporated into DNA was determined following a literature protocol [18].

The uptake of both tracers increased linearly with increasing initial activity concentration. Furthermore, the presence of unlabelled IUdR decreased their uptake considerably, indicating competitive uptake has occurred. These results are qualitatively similar to those reported previously for [$^{123/125}$I]IUdR in V79 cells [19]; the magnitude of uptake, however, was less than reported for V79 cells. This discrepancy could be due to a number of factors. Since these tracers are taken up by cells in the S-phase, the differences in properties such as doubling time of the two cell lines (20 h vs 9 h) are critical. The assay conditions were also different (monolayer vs single-cell suspension). The level of DNA incorporation of [^{131}I]IUdR and AUdR was found to be similar. For example, at an activity concentration of 7.5 kBq/ml, the per centage of cell-associated activity incorporated into DNA was 55 $\pm$ 21% and 55 $\pm$ 13% for [^{131}I]IUdR and AUdR, respectively. These results indicate that ^{211}At for iodine substitution did not result in a significant alteration in cell uptake and DNA incorporation.

5.4. In vitro cytotoxicity

Cytotoxicity experiments were carried out by treating exponentially growing D-247 MG glioma and SK-MEL-28 melanoma cells (5×10^6 cells per flask) in 25-cm^2 flasks with varying concentrations of AUdR, [^{211}At]astatide or [^{125}I]IUdR for 2 or 20 h [20, 21]. At the end of incubation, cells were

trypsinized and washed and plated in triplicates. After 10 days, colony formation was determined. Colonies with more than 50 cells were scored as survival. Clonogenic survival was plotted as a function of the activity concentration present in the medium at the beginning of the incubation. Regression fits and 95% confidence intervals were determined using the Sigma Plot computer program.

The D_0 values calculated for the treatment of D-247 MG glioma and SK-MEL-28 melanoma cells for 2 and 20 h with both free [^{211}At]astatide and AUdR are shown in Table 1. From 20 h treatment it was shown that for both cell lines, the D_0 was about 2-fold higher for [^{211}At]astatide than for AUdR. For the 20 h treatment with AUdR, the D_0 calculated for D-247 MG cells was significantly lower than that for SK-MEL-28 cells. The number of cell-associated ^{211}At atoms needed for reduction in survival to 37% for both cell lines was about 2, which is equivalent to about one DNA-incorporated ^{211}At atom per cell.

TABLE I. D_0 VALUES CALCULATED FROM THE TREATMENT OF D-247 MG GLIOMA AND SK-MEL-28 MELANOMA CELLS WITH AUdR AND [^{211}At]ASTATIDE FOR 2 AND 24 h

Tracer	D_0 (kBq/ml)[a]			
	D-247 MG		SK-MEL-28	
	2 h	20 h	2 h	20 h
AUdR	33 (28–43)	15 (13–16)	132 (109–176)	17 (16–19)
^{211}At$^-$	132 (109–76)	28 (26–29)	125 (83–191)	29 (22–26)

[a]Mean with 95% confidence interval.

Since the majority of ^{211}At decay occurred during the incubation period and the range of its ∀-particles is more than a cell diameter, cytotoxicity related to the α-particles should be similar for [^{211}At]astatide and AUdR. Thus, the enhanced cytotoxicity of AUdR compared with [^{211}At]astatide may be related in part to sub-cellular range ∀-particle recoil radiations hitting the cell nucleus as a result of DNA-incorporation of AUdR. For the 2 h treatment, the data indicate that there is a significant difference in survival after treatment with AUdR compared with [^{211}At]astatide for the D-247 MG but not for the SK-MEL-28 cell line.

The clonogenic survival of D-247 MG and SK-MEL-28 cell lines after a 20-h treatment with [^{125}I]IUdR resulted in biphasic curves with little increase in cell kill with >150–200 kBq/ml. The D_0 values determined were 115 and 310 kBq/ml for D-247 MG and SK-MEL-28 cell lines, respectively, corresponding to about 3700 and 5000 ^{125}I bound atoms per cell. No significant reduction in survival was seen when the two cell lines were exposed to up to 200 kBq/ml of Na[^{125}I]I suggesting low cytotoxicity of extracellularly distributed ^{125}I activity. These results suggest that AUdR is more cytotoxic *in vitro* to these two cell lines than [^{125}I]IUdR.

AUdR was also found to be extremely cytotoxic to Chinese hamster V79 cells [22]. The extracellular concentration of AUdR (30 min exposure) causing reduction in survival to 37% was 7.3 kBq/ml. Since exposure of these cells to similar concentrations of ^{211}At$^-$ did not lead to any significant reduction in survival, the decrease in survival as a result of exposure to AUdR can be ascribed solely to the effects of DNA-incorporated activity. The cytotoxicity of AUdR in V79 cells was accompanied by considerable DNA damage as demonstrated by measurement of DNA double strand breaks (DSBs). Approximately 10-fold more DNA DSBs are produced per decay when cells are labelled with ^{211}At than when they are labelled with ^{125}I, further demonstrating the exquisite cytotoxicity of AUdR.

5.5. Tissue distribution of AUdR and [^{125}I]IUdR

It is known that following i.v. administration, [^{125}I]IUdR is rapidly degraded, mainly to free iodide, limiting its usefulness for applications where compartmental or intratumoural delivery is possible [23]. To determine whether AUdR, like [^{125}I]IUdR, deiodinated rapidly *in vivo*, paired-label

tissue distribution of [^{131}I]IUdR and AUdR was determined in normal mice. The uptake of ^{131}I and ^{211}At over a period of 24 h for selected tissues is shown in Table 2. The retention of ^{211}At was significantly ($p < 0.05$) higher in most tissues except intestines and thyroid. This tissue distribution pattern was qualitatively similar to that seen for free [^{211}At]astatide and [^{131}I]iodide [11] suggesting that both halouridines dehalogenated extensively *in vivo*. This behaviour, in combination with the short physical half-life of ^{211}At, emphasizes the need for confining the use of AUdR to therapeutic applications where rapid tumour uptake and limited exposure to normal tissues can be achieved, *i.e.*, with intratumoural or compartmental delivery.

6. CONCLUSION

It is possible to produce ^{211}At in sufficient quantities and acceptable purity for clinical applications. Generally, using conditions of radioiodinations, it is possible to introduce ^{211}At onto various radiopharmaceuticals. For certain applications, targeted α-particle therapy using astatinated radiopharmaceuticals may be advantageous. Clinical trials using ^{211}At-labeled 81C6 are under way at Duke for the treatment of surgically created glioma resection cavities. The outcome will help elucidate the potential usefulness of astatinated mAbs and other agents for the therapy of otherwise untreatable neoplasms.

REFERENCES

[1] HUMM, J.L., A microdosimetric model of astatine-211 labeled antibodies for radioimmunotherapy. Int. J. Radiat. Oncol. Biol. Phys. 13 (1987) 1767–1773.

[2] JOHNSON, E.L., et al., Quantitation of ^{211}At in small volumes for evaluation of targeted radiotherapy in animal models. Nucl. Med. Biol. 22 (1995) 45–54.

[3] VAIDYANATHAN, G., ZALUTSKY M.R., Targeted therapy using alpha emitters. Phys. Med. Biol. 41 (1996) 1915–1931.

[4] BROWN, I., Astatine-211: Its possible applications in cancer therapy. Appl. Radiat. Isot. 37 (1986) 789–798.

[5] LARSEN, R.H. et al., Evaluation of an internal cyclotron target for the production of astatine-211 via the ^{209}Bi(∀, 2n)^{211}At reaction. Appl. Radiat. Isot. 47 (1996) 135–143.

[6] FRIEDMAN, A.M. et al., Preparation of a biologically stable and immunogenically competent astatinated protein. Int. J. Nucl. Med. Biol. 4 (1977) 219–224.

[7] ZALUTSKY, M.R., NARULA, A.S., A method for the radiohalogenation of proteins resulting in decreased thyroid uptake of radioiodine. Appl. Radiat. Isot. 38 (1987) 1051–1055.

[8] ZALUTSKY, M.R., NARULA, A.S., Astatination of proteins using an N-succinimidyl tri-n-butylstannyl benzoate intermediate. Appl. Radiat. Isot. 39 (1988) 227–232.

[9] ZALUTSKY, M.R. et al., Labeling monoclonal antibodies and F(ab')$_2$ fragments with the ∀-particle-emitting nuclide astatine-211: preservation of immunoreactivity and *in vivo* localizing capacity. Proc. Natl. Acad. Sci. USA. 86 (1989) 7149–7153.

[10] HE, X. et al., Generation and characterization of a mouse/human chimeric antibody directed against extracellular matrix protein tenascin. J. Neuroimmunol. 52 (1994) 127–137.

[11] GARG, P.K. et al., Comparative tissue distribution of the alpha emitter ^{211}At and ^{131}I as labels of a monoclonal antibody and F(ab')$_2$ fragment. Cancer Res. 50 (1990) 3514–3520.

[12] ZALUTSKY, M.R. et al., Radioimmunotherapy of neoplastic meningitis in rats using an ∀-particle-emitting immunoconjugate. Cancer Res. 54 (1994) 4719–4725.

[13] ZALUTSKY, M.R. et al., Two approaches for enhancing radioimmunotherapy: ∀-emitters and hyperthermia. Recent Results Cancer Res. 141 (1996) 101–122.

[14] HUMM, J.L. et al., Tissue dose estimates following the selective uptake of ^{125}IUdR and other radiolabelled thymidine precursors in resistant tumours. Br. J. Radiol. 64 (1991) 45–49.

[15] R_SSLER, K. et al., Labelling and animal distribution of 5-astatouracil and 5-astatodeoxyuridine (^{211}At). J. Labelled Compd. Radiopharm. 13 (1977) 271.

[16] VISSER, G.W.M. et al., The preparation of aromatic astatine compounds through aromatic mercury compounds part II: astatination of pyrimidine and steroids. J. Labelled Compd. Radiopharm. 18 (1981) 799–807.
[17] BARANOWSKA-KORTYLEWICZ, J. et al., Radiolabeling kit/generators for 5-radiohalogenated uridines. J. Labelled Compd. Radiopharm. 34 (1994) 513–521.
[18] AZURE, M.T. et al., Biological effect of lead-212 localized in the nucleus of mammalian cells: role of recoil energy in the radiotoxicity of internal α-particle emitters. Radiat. Res. 140 (1994) 276–283.
[19] KASSIS, A.I. et al., Radiotoxicity of ^{125}I in mammalian cells. Radiat. Res. 111 (1987) 305–318.
[20] VAIDYANATHAN, G. et al., 5[^{211}At]Astato-2'-deoxyuridine, an ∀-particle emitting endoradiotherapeutic agent undergoing DNA incorporation. Cancer Res. 56 (1996) 1204–1209.
[21] LARSEN, R.H. et al., The cytotoxicity of α-particle emitting 5-[^{211}At]astato-2'-deoxyuridine in human cancer cells. Int. J. Radiat. Biol. 72 (1997) 79–90.
[22] WALICAKA, M. A. et al., Survival and DNA damage in V79 cells exposed to DNA-incorporated alpha particle emitter astatine-211. Radiation Research 150 (1998) 263–268.
[23] MARIANI, G. et al., Tumour targeting *in vivo* and metabolic fate of 5-[iodine-125]iodo-2'-deoxyuridine following intratumoural injection in patients with colorectal cancer. J. Nucl. Med. 34 (1993) 1175–1183.