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Relative concentration of astatine-211 and iodine-125 by human fetal thyroid and carcinoma of the thyroid in nude mice

L. M. Cobb[1], A. Harrison[1], N. E. Dudley[2], T. E. F. Carr[1] and J. A. Humphreys[1]

[1]*Division of Experimental Pathology and Therapeutics, Medical Research Council Radiobiology Unit, Harwell, Didcot, Oxon OX11 0RD, U.K. and* [2]*Division of Surgery, John Radcliffe Hospital, Headington, Oxford OX3 9DU, U.K.*

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Summary

The concentrations of ^{211}At and ^{125}I were measured in various tissues in nude mice bearing xenografts of human thyroid tissue (fetal and malignant). The relative concentration of the two halogens was obtained at 4 and 24 h after injection. Samples were taken of the host blood, muscle and thyroid gland and the grafted tissues. The mouse thyroid concentrated ^{125}I more efficiently than ^{211}At but the human grafts concentrated both halogens about equally.

Introduction

Follicular and papillary carcinoma of the thyroid can be treated successfully in many patients with β-radiation from ^{131}I given orally; but there is unfortunately a percentage of patients in whom insufficient ^{131}I is accumulated in the tumour to give a useful absorbed dose. Brown et al. [2], reporting on 42 patients treated with ^{131}I, gave a 35% 5-year survival measured from the commencement of ^{131}I therapy.

The possibility that one of the α-emitting isotopes of the halogen astatine, ^{211}At ($t_{1/2}$ = 7.2 h), might be of value in the treatment of thyroid cancer is being investigated by us. Biological studies carried out shortly after the discovery of astatine [3] showed that the thyroid gland of subhuman primates and rats selectively accumulated ^{211}At. Tracer amounts of ^{211}At were at that time given to eight patients with various thyroid disorders immediately before thyroid surgery [7]. The excised thyroid tissues (normal, hyperthyroid and malignant) showed

Address for correspondence: L.M. Cobb, Division of Experimental Pathology and Therapeutics, Medical Research Council Radiobiology Unit, Harwell, Didcot, Oxon OX11 0RD, U.K.

accumulation of astatine only marginally below that predicted for radioactive iodine. In the study reported here the uptake of ^{211}At is compared with that of ^{125}I in xenografted human thyroid tumour and the likely therapeutic effect of ^{211}At in man assessed. In addition, the uptake of the two halogens is compared in xenografts of human fetal thyroid – as an example of rapidly proliferating non-malignant thyroid tissue.

Materials and methods

Radionuclides

Astatine-211 (Fig. 1) was prepared by the ^{209}Bi(α, $2n$)^{211}At reaction in the U.K.A.E.A. Variable Energy Cyclotron at Harwell and was supplied in a solution containing NaOH and Na_2SO_3 [11]. Carrier free ^{125}I ($t_{½}$ = 60 d) in a solution of NaOH was supplied by Amersham International P.L.C., Amersham, Bucks., U.K. Aliquots of the ^{211}At and ^{125}I solutions were mixed and diluted with phosphate buffered saline so that the ratio of activities ^{211}At: ^{125}I was 10:1 at the time of intraperitoneal (i.p.) injection.

Tissues for implantation

The thyroid gland was dissected from surgically aborted 18 or 20 week fetuses and either implanted within 6 h of abortion or stored at −196°C in a solution of 10% dimethylsulphoxide, 20% fetal calf serum, 70% Ham's F-12 until required. Two moderately differentiated follicular carcinomas (A 208 and A 210) and an anaplastic thyroid carcinoma (A 213) were either implanted within 4 h of surgical excision or stored at −196°C until required for implantation.

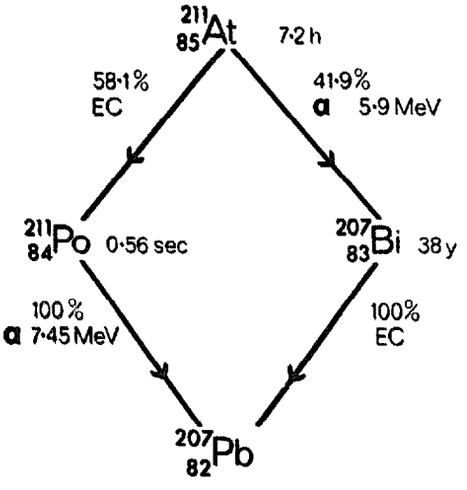

Fig. 1. Simplified decay scheme for astatine-211. For an elaboration of the scheme see Jardine [9].

Animals and implantation

The animals receiving the xenografts were adult male congenitally athymic MF1/*nu*/*nu*/Ola mice. They were fed a diet containing ~1 μg/g iodine and housed in air-filtered isolators to maintain pathogen-free status. The transplantation was carried out in a sterile environment. Pieces of tissue ~2 mm^3, were implanted subcutaneously in the left flank of each mouse under Penthrane* anaesthesia.

Experimental procedures

Accumulation of ^{211}At and ^{125}I. The term accumulation as used here can be expected to be the net result of uptake, concentration, and release of the two elements by the thyroid and other tissues. The 37 mice (Table I) were injected i.p. with 370 or 740 kBq ^{211}At together with 37 or 74 kBq ^{125}I and were killed by i.p. sodium pentobarbitone 4 or 24 h later. The grafts, host thyroid and a part of the quadriceps muscle were immediately dissected, weighed and put into phosphate-buffered formalin. Approximately 1 ml of blood was withdrawn from the heart and weighed. All samples were assayed for ^{211}At and ^{125}I in an LKB Compugamma** which measured the 80 keV X-rays emitted during the electron capture decay of ^{211}At to ^{211}Po [9] and the X-and γ-rays from ^{125}I decay.

The viability of the grafts was assessed by histological examination of haematoxylin and eosin (H&E) stained sections of the formalin-fixed tissues. Only those grafts which were assessed to have more than 70% of their mass as viable fetal thyroid or tumour tissue are reported on in this study.

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* Methoxyfluorane B.P., Abbott Laboratories, Queenborough, Kent, U.K.

** L.K.B. Wallac, Turku, Finland.

TABLE I

Concentration of ^{211}At and ^{125}I in xenografts of human thyroid tissue (tumourous and fetal) and in mouse tissues at 4 and 24 h after injection.

Graft	Interval (h) injection to death	Mice (n)	% Injected activity/g tissue Mean ± (S.E.) ^{211}At				^{125}I			
			Human tissue	Mouse			Human tissue	Mouse		
				thyroid	blood	muscle		thyroid	blood	muscle
Fetal thyroid	4	5	14.9 (4.0)	575 (185)	1.3 (0.2)	0.7 (0.1)	10.4 (8.3)	2330 (756)	0.81 (0.5)	0.21 (0.1)
Fetal thyroid	24	2	9.3 (5.3)	285 (255)	0.28 (0.1)	0.18 (0.05)	3.9 (2.8)	2510 (1770)	0.05 (0.01)	0.02 (0.01)
208	4	5†	3.0 (0.3)	408 (157)	1.5 (0.3)	1.8 (0.9)	3.8 (1.1)	2043 (512)	0.77 (0.2)	0.23 (0.09)
208	24	4†	0.92 (0.30)	285 (51)	0.26 (0.1)	0.14 (0.06)	0.70 (0.4)	2400 (427)	0.02 (0.003)	0.01 (0.003)
210	4	9	*	522 (162)	1.04 (0.1)	0.49 (0.05)	*	2331 (690)	0.36 (0.07)	0.17 (0.03)
210	24	4	*	305 (97)	0.20 (0.06)	0.10 (0.03)	*	1035 (308)	0.03 (0.01)	0.01 (0.003)
213	4	5	3.4 (0.2)	410 (47)	1.1 (0.07)	0.44 (0.04)	0.37 (0.07)	2435 (199)	0.57 (0.10)	0.10 (0.02)
213	24	3	1.0 (0.5)	408 (159)	0.37 (0.1)	0.17 (0.1)	0.50 (0.36)	3026 (1412)	0.14 (0.10)	0.03 (0.01)

† Two mice from each group given 2.2 MBq ^{131}I 17 weeks before the implant in an attempt to ablate the host thyroid.
* Data excluded. Graft contained <70% viable tumour.

Autoradiography. In order to assess the micro-distribution of ^{211}At autoradiographs (ARG) were prepared of xenografts of A 208 (3 mice), A 210 (5 mice), A 213 (2 mice) and fetal thyroid (2 mice). The mice were killed by i.p. sodium pentobarbitone 4 h after an injection of 0.19 or 3.7 MBq of ^{211}At. At the point of death the implant was quickly dissected out, snap frozen in liquid nitrogen and the processes of sectioning and exposure to photographic emulsion carried out at −20°C. Sections were cut with a Frigocut freezing microtome* and transferred directly to glass slides which were previously coated with dipping emulsion** or stripping film***. Exposure was made over 4 days. Finally the sections were fixed in 70% ethanol, developed and stained with H&E. The handling at −20°C was maintained until the development of the emulsion which was carried out at room temperature.

Results

Accumulation of ^{211}At and ^{125}I

Histological observations. The grafts grew in most animals. The grafts of A 208 (mean weight 8.5 ± 1.5 mg) and A 213 (mean weight 66.0 ± 18.0 mg)

* Reichert-Jung, Slough, Berks, U.K.
** Emulsions K2 and K5 for ^{125}I and ^{211}At respectively Ilford Ltd., Knutsford, Cheshire, U.K.
*** Kodak Ltd., Hemel Hempstead, Herts, U.K.

had a histological pattern similar to that of the patient tumour. The A 208 graft was moderately well differentiated, with numerous distinct follicles containing colloid. It also infiltrated subcutaneous fat. The A 213 graft, an anaplastic carcinoma, was sarcomatous with no clear follicular cells. In some mice bearing A 213, the abdominal wall muscle was infiltrated. By contrast, the A 210 xenografts (mean weight 6.5 ± 1.0 mg) used for the accumulation studies were accompanied by a major fibrous reaction to the extent that less than 70% of the graft was viable tumour and therefore the ^{211}At and ^{125}I data for the host tissues are included but not those for the graft.

The fetal thyroid graft (mean weight 10.1 ± 1.6 mg) continued to develop as though still in utero, so that grafts from 18 week fetuses, where follicles at that stage are primitive, showed well developed follicles with colloid after growing in the mouse for 10 weeks. One reason for the wide variation in uptake of both ^{211}At and ^{125}I by the fetal thyroids was because of the wide variation in thyroid differentiation at the time of radionuclide injection — due to the use of thyroid from different aged fetuses and the varying periods of residence under the skin (Table I).

^{211}At and ^{125}I in grafts. The concentrations of ^{211}At and ^{125}I in the human grafts and mouse tissues are expressed as a percentage of the injected activity per gram of tissue and the mean values (± S.E.) are listed in Table I. The difference between graft and blood levels of astatine showed an accumulation by the tumour and more so by the human fetal thyroid.

TABLE II

Ratio of concentration ^{211}At/^{125}I in tissue.

Graft	Interval (h) injection to death	Mice (n)	Ratio ^{211}At/^{125}I % Injected activity/g tissue			
			Human tissue	Mouse		
				thyroid	blood	muscle
Fetal thyroid	4	5	† 10.9 (9.3)	0.25 (0.02)	3.9 (1.8)	6.3 (2.3)
Fetal thyroid	24	2	2.5 (0.5)	0.10 (0.03)	6.2 (2.4)	8.6 (1.0)
208	4	5	1.1 (0.4)	0.20 (0.06)	2.5 (0.65)	12.5 (6.6)
208	24	4	2.4 (0.8)	0.14 (0.05)	12.2 (4.8)	14.0 (4.2)
210	4	9	*	0.28 (0.05)	3.6 (0.5)	3.8 (0.6)
210	24	4	*	0.29 (0.04)	6.6 (2.1)	10.7 (7.0)
213	4	5	12.5 (4.3)	0.18 (0.02)	2.5 (0.6)	5.3 (1.5)
213	24	3	2.6 (1.1)	0.15 (0.06)	3.9 (2.3)	6.8 (2.0)

† Ratio ^{211}At/^{125}I for one graft = 44.

* Data excluded. Graft contained < 70% viable tumour.

However, the host (mouse) thyroid showed by far the greatest ability to concentrate astatine. The ratio of the concentrations of ^{211}At:^{125}I was calculated for individual tissues in each mouse and the means of these values are listed in Table II. For the follicular carcinoma A 208 the concentration of ^{211}At was similar to that of ^{125}I ~3.0% per g at 4 h and ~0.9% per g at 24 h. This result seemed to indicate that the cells of the follicular carcinoma were not distinguishing between ^{211}At and ^{125}I atoms. This was not so with the anaplastic carcinoma (A 213) where at 4 h the uptake of ^{211}At, 3.4% per g, was similar to the follicular carcinoma A 208 but the ^{125}I concentration was low, 0.37% per g, producing a ^{211}At:^{125}I ratio of 12.5 (4.3). At 24 h the ratio was 2.6 (1.1).

^{211}At and ^{125}I in normal mouse tissues. The mouse thyroid gland at 4 h, and to a greater extent at 24 h, discriminated positively in favour of ^{125}I. The ratios ^{211}At:^{125}I were ~0.23 and 0.17 at 4 and 24 h respectively. The thyroid uptake of ^{211}At and ^{125}I was significantly above muscle and blood, confirming the ability of this organ to concentrate both halogens. The greater concentration of ^{211}At over ^{125}I in muscle and blood at 24 h indicated that ^{211}At might be cleared from the body more slowly than ^{125}I.

Autoradiography

The ARG of the follicular carcinomas A 208 and A 210 contained large groups of tracks over the malignant follicles strongly suggestive of an accumulation of astatine in colloid (Fig. 2). An approximately adjacent H&E stained section, which did not have ARG emulsion, gave confirmation of the presence of colloid in the areas of clustered tracks.

The selective retention of ^{211}At by fetal thyroid was illustrated by clustering of tracks over the developing follicles.

An estimate of the absorbed dose to a tumour xenograft can be obtained if the following assumptions are made, (a) the shape of the retention curve for ^{211}At in the mouse thyroid [8] and in the graft are the same, which means that the accumulated activity is proportional to the ratio of the concentrations

Fig. 2. Autoradiograph of a xenograft of the follicular carcinoma A 210. The clusters of α-tracks indicate the accumulation of ^{211}At in the tumour follicles 4 h after an i.p. injection of 3.7 MBq ^{211}At. The bar represents 50 μm.

of ^{211}At in the mouse thyroid and graft at the time of measurement, e.g. 4 h and (b) the distribution of ^{211}At within the graft is homogeneous. The retention of ^{211}At in the mouse thyroid at various times after injection has been measured previously [8] and the absorbed dose calculated to be 9.2×10^{-3} Gy per Bq ^{211}At injected per g body mass. In respect of (b) the only xenografts showing uniform distribution of ^{211}At were of the anaplastic thyroid A 213 and in the mice bearing these grafts the relative concentration of ^{211}At in the mouse thyroid and A 213 at 4 h was 120:1 (410:3.4).

On this basis, the absorbed dose to grafts of the anaplastic carcinoma (A 213) from an injection of 1 Bq ^{211}At/g body mass would be $\sim 7.7 \times 10^{-5}$ Gy. The absorbed dose required to sterilise the tumour cells with α-particles is not known but the maximum dose that could be achieved in grafts of A 213 would be ~4.7 Gy because much greater doses would require the administration of amounts of ^{211}At that would kill the mouse in a few days. Attempts to arrest tumour growth by administering 61 kBq ^{211}At/g body mass (the amount required to give an estimated dose of 4.7 Gy to the graft) have not yet been made.

Discussion

Early research into the uptake and retention of ^{211}At indicated that in rodents it was retained at high levels, but less efficiently than ^{131}I [6]. In man and subhuman primates however ^{211}At and ^{131}I were incorporated into the thyroid gland with equal efficiency [6,7]. The present work supports those findings. The normal mouse thyroid showed a relatively high incorporation of ^{125}I compared with ^{211}At, while in the grafts of human fetal and malignant thyroid the uptake of ^{211}At and ^{125}I are approximately the same.

The fetal thyroid was included in the study to provide information on the ratio of ^{211}At to ^{125}I retained by proliferating, non-malignant human thyroid tissue. Although successful grafts of both human malignant and hyperthyroid tissue have previously been reported [5,12–14] xenografts of fetal thyroid have not. As might be expected the fetal tissue behaved like the human tumour tissues and did not differentiate significantly in uptake between ^{211}At and ^{125}I.

In a review of the results of ^{131}I therapy, Brown et al. [2] reported a 35% 5-year survival estimated from the time of the first ^{131}I injection. The patients had been judged suitable for treatment on the basis of an adequate uptake of a test dose of radioactive iodine. Benefit from the use of ^{211}At in thyroid carcinoma is likely to arise because α-particles have a high linear energy transfer (LET) (80–100 keV/μm tissue) and hence can be expected to sterilize tumour cells more efficiently than the low LET β-particles ($\sim$0.2 keV/μm tissue) produced by ^{131}I decay. The relative effectiveness of the ^{211}At α- over ^{131}I β-particles for sterilizing thyroid tumour cells is not known but it can be expected to be at least 3 [1].

Other factors to be taken into account are the much shorter path length of α-particles, $\simeq$ 65 μm for ^{211} At (in this distance 95% of the energy is absorbed) compared with the longer range of the β-particles emitted by ^{131}I (90% of the energy deposition occurs within a distance of $\sim$820 μm from the source). This means that for ^{211}At a more uniform distribution of the radionuclide would be required to obtain significant tumour control.

On the discount side is our relative ignorance of the radiotoxicity of ^{211}At. The chemical toxicity would be unimportant because of the high specific activity (74 GBq ^{211}At/μg). Hamilton et al. [6] carried out acute radiotoxicity studies with ^{211}At in rats and observed depression of lymphocytes as the first sign of toxicity. The long-term effect of a single injection of ^{211}At was thyroid ablation which has been confirmed in this laboratory in mice.

In man, thyroid tumours, even if differentiated, are usually non-functional and take up little or no ^{131}I if a significant amount of normal thyroid is present [10]. When the functioning thyroid tissue has been ablated and an abnormally high thyroid-stimulating hormone level attained the optimum conditions exist for tumour uptake of ^{131}I [4]. In the mouse we were unable to obtain these optimum conditions because effective surgical or radiation ablation of the thyroid and subsequent balanced hormone replacement therapy are very difficult to achieve. Despite these deficiencies, implanted human thyroid tissue showed preferential uptake of both ^{125}I and ^{211}At relative to blood.

In conclusion, the present study points to ^{211}At as a possible candidate for the treatment of those patients with carcinoma of the thyroid in whom other forms of therapy have proved or are likely to prove ineffective.

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