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^{211}At-labelling of polymer particles for radiotherapy: Synthesis, purification and stability.

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Summary.

Cyclotron-produced ^{211}At was distilled from a Bi metal target and coupled to N-succinimidyl-3-(trimethylstannyl)benzoate. The resulting N-succinimidyl-3-(^{211}At)astatobenzoate was thereafter coupled to aminated monosized polymer particles with a diameter of 1.8 μm. The total time elapsed from the end of the cyclotron irradiation until the final product was prepared was about 2.5 hours. From 23 to 51 % of the target activity at the end of bombardment was measured in the final conjugate. Solid-liquid extraction purification of the astatinated intermediate, using Sep-pak columns (Waters), gave more reproducible yields in the final conjugation step. The ^{211}At-labelled particles were incubated with fetal calf serum, human serum and human full blood at room temperature. The ^{211}At

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activity on the particles was measured before and after three times washing at 4, 24 and 48 hours. The stability was not significantly different from 100 % for all media and for all time points. This indicates that ^{211}At-labelled particles can be stable under *in vivo* conditions, and may thereby be a promising agent for intracavitary radiotherapy on free-floating cancer cells or surface fixed cells.

Key words: Astatine-211; radiolabelled polymer-particles; α-particle emitting radiopharmaceutical; radiotherapy.

Introduction.

For many years, ^{211}At has been considered as a nuclide with a potential for radiotherapeutic use. With a half-life of 7.21 hours, this radionuclide decays along two branches to ^{207}Bi and ^{207}Pb, in either case leading to α emittance. The energies of the α-particles are 5.87 and 7.45 MeV and the ranges in unit density tissue are 55 μm and 80 μm, respectively (1). Hence, the density of ionisation in tissue is very high compared to X-rays, γ-rays and β-particles and the potential for irreparable cell damage is therefore far greater for the α-particles. If coupled to tumor-selective compounds, astatine can give a highly localized radiation dose to the malignant cells (2).

Astatine is the heaviest member of the halogens (the new Group 17 or the old Group VIIB) and many of its chemical properties are similar to those of iodine, although At has a more metallic nature (3). Organic astatine compounds are normally less stable than their iodine analogs. However, when incorporated in aromatic structures or vinyl groups, the C-At bond may be sufficiently stable for *in vivo* conditions. Some of these aromatic compounds show a stability similar to their iodinated analogs (4).

Most biological studies with astatine have been focused on the application in tumor targeting with monoclonal antibodies (5-7) or with smaller molecules with high tumor affinity (8). Although this approach has been partly successful in preclinical settings, there

are still many problems to be solved before application in humans. Another approach is to use astatine absorbed on tellurium colloid for intracavitary treatment. This strategy was succesfully exploited in a peritoneal ovarian cancer model in mice, but the margins between therapeutic and toxic doses were very narrow (9). The release of astatide from the colloid led to serious toxic effects in various tissues. ^{211}At complexed to sulphydryl groups on albumin particles was reported to have a higher *in vivo* stability than astatine absorbed on Te-colloid. However, the uptake of astatine in thyroid and stomach indicated a considerable release of free astatide (10).

The primary objective for the present investigation was to develop a new astatinated compound for selective radiation of cavitary regions. The compound had to be large enough to be trapped in the intraperitoneal cavity, and yet small enough to be able to give an efficient irradiation of free-floating and surface bound cells and micrometastases. Furthermore, the labelled compound had to be radiochemically stable at physiological conditions. In addition, the radiolabelling and purification steps should be completed within resonable time compared to the halflife of the nuclide, and the procedures should be conducted in consistence with safety rules for α-particle emitters. To meet these criteria we applied a two-step labelling procedure based on the activated tinn ester (ATE) method using N-succinimidyl-3-(trimethylstannyl)-benzoate, which had earlier been succesfully used for astatine labelling of proteins (6,11).

In this paper we describe the complete production route for production of astatine, labelling of the particles and stability testings of the final product.

Materials and methods.

Coupling-reagent and polymer particles.

N-succinimidyl-3-(trimetylstannyl)benzoate (NSTB) was prepared according to previously published procedures (12). It was purified twice by flash-chromatography, and the purity confirmed to be larger than 95% on normal phase HPLC (column, silicagel;

mobil phase, 69.9% hexane, 30% ethylacetate, 0.1% acetic acid). Monosized polymeric particles (13) (type 2731) were provided by the polymer research group at SINTEF, Trondheim, Norway. The particle size was 1.8 μm and they were aminated to a density 3.55 μmol/mg particles as described elsewhere (14).

Cyclotron irradiations.

^{211}At was produced by bombarding a 0.25 mm thick ^{209}Bi-target, fused onto a water-cooled aluminium-backing, with a 10-12 μA particle beam of 28 MeV α-particles. The irradiations lasted for 1-2 hours.

Separation of ^{211}At from Bi target.

^{211}At was distilled from the bismuth target by dry distillation (15) at 650-660 °C for 30-45 min. Argon or nitrogen was used as carrier gas and the gas stream was led through refluxing chloroform in a water-cooled condensation column filled with glas spheres. The column was connected in series with a gas-washing flask, a carbon gas-mask filter and finally a water aspirator (Fig. 1).

Labelling of aminated monosized polymer particles with ^{211}At using the ATE method.

After the end of the distillation, the column was washed with about 0.2 ml chloroform. The vial was carefully disconnected and 0.5-1.0 μmol of NSTB and 20-40 μmol of *tert*-butylhydroperokside were added. The solution was stirred for approximately 30 min to give N-succinimidyl-3-(^{211}At)astatobenzoate (NS(^{211}At)AB) (Fig. 2), using a magnetic stirrer, whereafter the solution was evaporated to dryness by a stream of argon. The residue was dissolved in 50 μl of chloroform and loaded on a sep-pack silicagel column. The reaction vial was washed with additional 50 μl of chloroform which was also loaded onto the column. Elution with 30 ml of hexan, 30 ml of 8 % ethylacetate in hexane and finally 15 ml of 30% ethylacetate in hexane was performed to purify the astatinated intermediate from unconjugated astatine and the major part of the NSTB. The 30 % etylacetate fractions

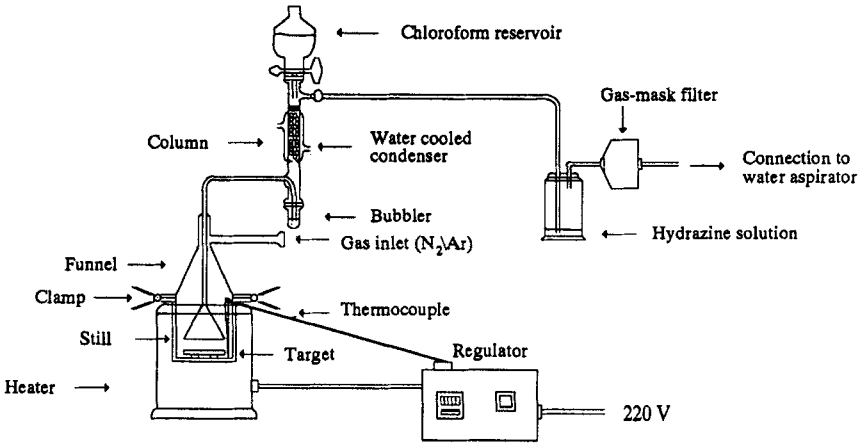

Figure 1. Schematic presentation of a set up for separation of astatine from bismuth. The *target* was placed on a quartz disc in the *still*, whose upper and lower parts were held together with *clamps*. The temperature of the *heater* (Müller, Germany) was controlled via a *thermocouple* placed between the *heater* and the *still*. The *thermocouple* was connected to a *regulator* that controlled the power supply to the *heater*. The *still* with the *funnel* was made of quartz. The outlet of the *still/funnel* was connected to a *condenser* via a glass tube. The *condenser* consisted of a bubbler in series with a water cooled *column* filled with glass spheres and with a *reservoir* for chloroform on top of it. The outlet of the *condenser* was connected to a teflon tubing which led to a *gas-washing bottle* with a 0.1 M hydrazine solution. The outlet of the *gas-washing bottle* was connected to a carbon *gas-mask filter*, which was connected to a *water aspirator*.

During distillation a gas pipe was connected to the *carrier-gas inlet* on the *still*. The carrier-gas (Ar or N_2) flow and the *water aspirator* were adjusted to give a gentle flow through the *condenser*. The *still* was heat insulated with mineral wool and aluminium foil. Evaporized astatine was retained when the carrier gas passed through the bubler and the column which contained refluxing chloroform. After discontinuing the distillation and stopping the gas flow the column was washed with chloroform from the reservoir, which was collected in the bubbler. Thereafter, the bubbler was disconnected from the rest of the condenser.

Dimensions of components: *Aluminium target backing*, 42x40 mm, thickness 3 mm and with a machined circular cave with a diameter of 25.4 mm and depth of 0.5 mm; *bismuth target*, thickness 0.25 mm fused into the cave and machined; *still*, height 20 cm, i.d. 7 cm; *condenser column*, length 5 cm, i.d. 5 mm.

containing NS(^{211}At)AB were concentrated, transferred to a glass vial and evaporated to dryness with a stream of N_2 or Ar.

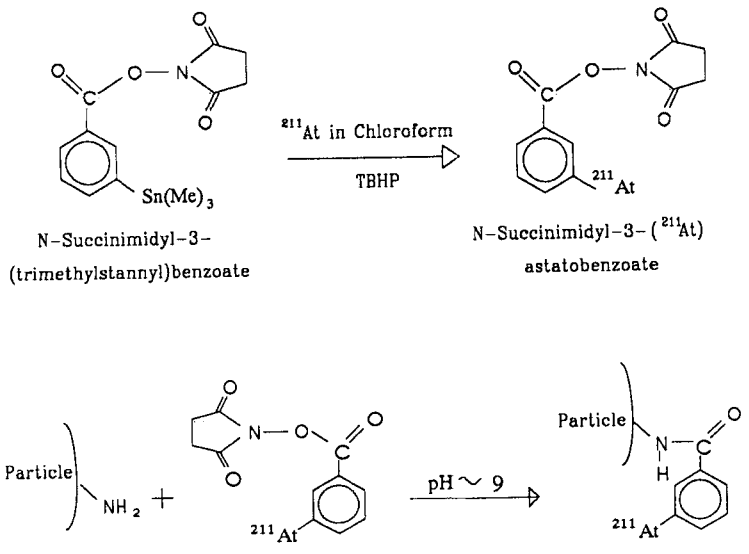

Fig. 2. Labelling of aminated monosized polymer particles with ^{211}At using the activated tinn ester (ATE) method. TBHP: *tert*-butylhydroperoxide.

A dispersion of 5-10 mg monosized aminated particles in 0.5-1.0 ml of water was added borax to adjust pH to about 9. The solution was then transferred to the vial containing NS(^{211}At)AB and mixed on a rotary shaker for 20 min. Then the astatinated particles were added PBS-buffer (0.1 M phosphate, 0.15 M saline, pH 7.4) to dilute the reaction mixture to 1/4 of its original concentration. The particles were finally centrifuged and washed three times with PBS.

Stability testings of the final conjugate.

Volumes of 35 μl of a dispersion of 5 mg/ml ^{211}At-labelled particles in PBS, with a specific activity of 2 MBq/mg particles, were added to test tubes with 150 μl of either fetal calf serum, human serum or human full-blood. The stability of the astatinated particles was analysed by incubating at room temperature with an incubating shaker for either 4, 24 or 48 hours. After an incubation period, triplicates of each type of suspension were measured for radioactivity in a LKB Wallac 1260 Multigamma II counter, before washing in PBS and

centrifugation. After repeating the washing step two times, the particles were again suspended in 185 μl PBS to adjust the detection geometry and the radioactivity measured once more. The decay corrected activity measured after the washing procedure was divided by the original activity to find the fraction astatine stably bound to the particles.

Results.

The routine irradiation of bismuth at the cyclotron gave a production yield of 8-12 MBq/μA h. By distillation, 40-70 % of the activity was collected in chloroform after one hour. 70-90 % of the activity was incorporated into NS(^{211}At)AB in 30 min.

The conjugation of NS(^{211}At)AB to the particles gave a reaction yield of 80 to 95 % after 20 min. reaction. The total preparation time from the end of bombardment at the cyclotron to the end of washing of the final product, was normally about 2.5 hours. Approximately one third of the astatine initially on the target was recovered on the final labelled microspheres (Table 1).

The stability measurements (mean ± SD for three paralleles per point) are presented in Table 2. The stability was not significantly different from 100 % at any point.

Experiments were performed without the sep pak purification to see if it was possible to save time. Although the total number of particle-bound amino groups exceeded the total number of NSTB molecules by a factor of 10 or more, the quality of the final preparations were varying. There was an increase in absorption of activity on the walls in the reaction vial probably caused by the lipophilic NSTB which has a relatively low solubility in water. To secure the reproducibillity of the experiments all preparations used for stability tests were therefore purified using the sep-pak procedure.

Discussion.

When developing radiopharmaceuticals for therapy with shortlived nuclides like ^{211}At, short production time, high purity and high stability of the final compound are critical. In

Table 1 Recovery of ^{211}At during preparation

Procedure	Chemical yield	Time spent
Separation of ^{211}At from Bi target	40-70 %	60 min.
Labelling of NSTB + purification	80-95 %	40 min
Conjugation to particles + purification	90-95 %	35 min
Decay correction	29-63 % × 0.81[a]	Total: 135 min
Total recovery of ^{211}At	23-51 %	

[a]Fraction of ^{211}At activity left after 135 minutes decay.

Table 2 *In vitro* stability (% astatine bound to the microspheres)

Medium	Incubation period (hours)		
	4	24	48
FCS	99 ± 7	99 ± 6	103 ± 9
HS	99 ± 1	100 ± 4	101 ± 3
HFB	101 ± 3	99 ± 2	100 ± 5

The *in vitro* stability (± SD for triplicates) of the ^{211}At-labelled polymer particles after incubation and three times centrifugation and washing. FCS: Fetal calf serum; HS: Human serum; HFB: Human full blood.

this study we describe the complete route for production of a new compound for selective radiation of cavitary regions, like the intraperitoneal cavity, which fulfils these criteria.

At the distillation step, the temperature was the most critical parameter. The most reproducible results were obtained when the distillations were performed at a temperature just below the melting point of the aluminium target-backing (660 °C).

There was no difference in yields between nitrogen and argon as carrier gas. Less than 5 % of the volatile astatine activity escaped the condensing column. During and after the distillation no activity was measured after the gas-mask filter.

NS(^{211}At)AB was synthesized without using any type of carrier from the precursor NSTB. The astatodestannylation step attached astatine covalently to an aromatic carbon atom. The final amide bonding was expected to be stable at physiological conditions and this was confirmed by our *in vitro* stability tests and has also been confirmed by *in vivo* experiments which have been presented elsewhere (14).

In summary, we have demonstrated that aminated polymer particles can be labelled with ^{211}At in high yield and that the final product has a very high stability within the time-frame of several ^{211}At half-lives. The stability found for the astatine-particle linkage supports earlier findings that aromatically incorporated astatine can be stable under physiological conditions. Even for the complex enzymatic environment in human blood, the stability was convincing. By using the compound described in this paper it seems possible to selectively irradiate the intraperitonal cavity and surface with a high radiation dose with negligible simultaneous irradiation of the deeper areas of abdominal organs like spleen, liver, kidneys and intestines. Experiments performed with tumor bearing mice indicate that therapeutic responses can be achieved at very low dose levels compared to the toxic dose-levels (16). This may be possible due to the short range of the α-particles emitted from the decay of ^{211}At, and the high chemical stability of the astatinated compound.

Acknowledgement

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References.

1. Brown I.-Appl. Radiat. Isot. 37: 789 (1986)

2. Vaughan A.T.M., Bateman W. and Cowan J.-J. Radioanal. Chem. 64: 33 (1981)

3. Appelman E.H.-J. Am. Chem. Soc. 83: 805 (1961)

4. Berei K. and Vasáros L.-Gmelin Handbook of Inorganic Chemistry, Astatine, atomic number 85, (Pietsch E.H.E., Kotowski A. and Becke-Goering M., eds.) Springer-Verlag, Berlin, 1985

5. Harrison A. and Royle L.-NCI Monogr. 3: 157 (1987)

6. Zalutsky M.R., Garg P.K., Friedman H. and Bigner D.D.-Proc. Natl. Acad. Sci. USA 86: 7149 (1989)

7. Hadley S.W., Wilbur D.S., Gray M.A. and Atcher R. W.-Bioconjugate. Chem. 2, 171 (1991)

8. Link E.M. and Carpenter R.N.-Cancer Res. 52: 4385 (1992)

9. Bloomer W.D., McLaughlin W.H., Lambrecht R.M., Atcher R.W., Mirzadeh S., Madara J.L., Milius R.A., Zalutsky M.R., Adelstein S.J. and Wolf A.P.-Int. J. Radiation Oncology Biol. Phys. 10: 341 (1984)

10. Franke W.G., Doberenz W., Wunderlich G., Doberanz I., Bredow J., Nelz P., Fisher S., Dreyer R., Heidelbach J.G., Nitzsche H., Hliscs R., Beyer R., Kessler L., Reiman T., Reiman M., Kaltschmidt A. and Grunwald P.-Z. Klin. Med. 44: 2285 (1989)

11. Garg P.K., Harrison G.L. and Zalutsky M.R.-Cancer Res. 50: 3514 (1990)

12. Garg P.K., Archer G.E. Jr., Bigner D.D. and Zalutsky M.R.-Appl. Rad. Isot. 38: 1051 (1989)

13. Ugelstad J., Mork P.C., Kaggerud K.H., Ellingsen T. and Berge A.-Adv. Colloid Interface Sci. USA 13: 101 (1980)

14. Vergote I., Larsen R. H., De Vos L., Winderen M., Ellingsen T., Bjørgum J., Hoff P., Aas M., Tropé C. and Nustad K.-Gynecol. Oncol. 47: 358 (1992)

15. Lambrecht R.M. and Mirzadeh S.-Int. J. Appl. Radiat. Isot. 36: 443 (1985)

16. Vergote I., Larsen R.H., De Vos L., Nesland J., Bruland Ø., Bjørgum J., Alstad J., Tropé C. and Nustad K.-Gynecol. Oncol. 47: 366 (1992)