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# Evaluation of $^{211}$At-labelled monodisperse polymer particles *in vivo*: Comparison of different specific activities.

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## Summary

The α-particle emitter $^{211}$At was covalently coupled to 1.8 μm aminated monodisperse polymer particles (MDPP) and used to irradiate the intraperitoneal cavity in mice with disseminated tumour cells. Specific activity has previously been shown to influence the therapeutic efficacy of α-particle emitting compounds and the therapeutic efficacy of $^{211}$At-MDPP with various specific activity was therefore investigated. Groups of mice (10 animals per group) were treated with intraperitoneal injections of 100 kBq of $^{211}$At-MDPP with specific activities of 0.19, 0.55, 1.7, 5.0, 15, and 45 MBq/mg. A significantly prolonged survival was observed in the treated groups compared to the control group (from 19 to 26 days vs. 12 days, median). The difference in survival between the $^{211}$At-MDPP treated groups was not significant, but some animals with short survival were observed in

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the groups that had received the 0.19, 15 and 45 MBq/mg preparations. K13 monoclonal antibody values, which are an indicator of tumour growth, were high in some animals in the 15 and 45 MBq/mg groups (day 7 values).

**Key words:** Tumour-cell irradiation, $\alpha$-particle-emitting compound

## Introduction

Radiotherapy with $\alpha$-particle-emitting radiopharmaceuticals has been suggested as an alternative to $\beta$-emitting radiocolloids in the treatment of intraperitoneally disseminated cancer and ascites (1-3). The highly localized energy deposition, low oxygen enhancement ratio and high relative biological effectiveness (RBE) of $\alpha$-particles may be advantageous provided the tumour cells can be effectively targeted (4). High therapeutic efficacy has been demonstrated with $\alpha$-emitters in preclinical models, but because of a relatively high uptake of the radionuclides used in some normal tissues, the margins between lethal and therapeutic doses have been narrow (5,2).

Recently, we developed a procedure where $^{211}$At was covalently attached to micro-particulates by coupling [$^{211}$At]astatobenzoate to 1.8 μm aminated monodisperse polymer particles (MDPP) (6), using the intermediate N-succinimidyl-3-[$^{211}$At]astatobenzoate originally developed for protein labelling (7). *In vitro* and *in vivo* experiments confirmed a high stability of the astatine label. Autoradiography made after intraperitoneal injection in mice of $^{211}$At-MDPP showed that the radioactivity remained mainly on the surface of intraperitoneal organs (8).

Therapeutic experiments with Balb/C mice inoculated with murine tumour cells were performed (9). The K13 murine hybridoma cell line was used as a tumour model. Intraperitoneal injections of $10^6$ cells into Balb/c mice resulted in a rapid and uniform intraperitoneal superficial tumour spread and a short survival (approximately 10 days) of the animals (10). The therapeutic efficacy of $^{211}$At-MDPP was compared with that of $^{90}$Y and $^{32}$P colloids in this tumour model, and in contrast to the two radiocolloids $^{211}$At-MDPP caused cures at low doses. Treatment with unlabelled MDPP did not

improve the survival compared to untreated control. One level of specific activity (0.8 MBq $^{211}$At per mg MDPP) was used and four dose levels of $^{211}$At-MDPP (0.1, 0.5, 1.0, and 2.5 MBq/mouse) were investigated. The number of cures was similar (27-29%) for the 0.1 to 1.0 MBq groups but no cures were observed in the 2.5 MBq group (9). The lack of a dose response relationship may reflect a heterogeneous microdistribution of $^{211}$At-MDPP in the peritoneal cavity of mice.

Chemical composition, size, and specific activity are parameters governing the microdistribution of carrier-vehicles in internal therapy with radionuclides. Since the radiation range is typically less than 100 μm, treatment with α-particle emitters is expected to be particularly influenced by source microdistribution (11,12). Previous studies have indicated that specific activity is an important parameter when α-particle-emitting radioimunoconjugates are used to treat disseminated tumour cells (13-15). For $^{211}$At-MDPP the relatively large size of each particulate may limit the number of particulates that can approach remote tumour cells which may to a large extent be surrounded by normal tissue. When steric conditions only allow one or a few particulates to approach within the 55-80 μm α-particle range of a cell, the number of $^{211}$At bound per particulate would be crucial for the efficacy of the treatment.

The present study was conducted to investigate whether it was possible to optimize the therapeutic efficacy of $^{211}$At-MDPP by adjusting the specific activity of the compound. Mice with intraperitoneally disseminated K13 cells were treated with 100 kBq of $^{211}$At-MDPP with six different specific activities and compared for survival.

## Materials and Methods

### *Production of $^{211}$At*

$^{211}$At was produced at the Scanditronix MC 35 cyclotron at Oslo University by irradiation of a 0.2 mm thick bismuth layer melted onto an aluminum backing with a beam of 28 MeV α-particles (6). A beam intensity of 10-12 μA was used. $^{211}$At was separated from the Bi-target by dry distillation at 650-660 °C for 30-60 min. Argon was used as carrier-gas and the $^{211}$At was trapped in two serial ice-cooled 2 ml gas-washing bottles containing 0.5 ml of chloroform.

*Detection of $^{211}At$*

During the chemical procedure $^{211}At$ was monitored using a Geiger Müller counter (Scaler Timer ST 7, Nuclear Enterprises), at two fixed distances between the counter and the preparations. Samples of lower radioactivity were measured using a multiwell LKB Multigamma 1640 counter which was adjusted for detection of the 77-92 keV X-rays accompanying the $^{211}At$ disintegration. The efficiency of these detectors was determined using a Ge(Li) detector as a primary standard.

*Carrier Compound for $^{211}At$*

Compact monodisperse polymer particles (MDPP) with a diameter of 1.8 µm, were prepared according to the activated swelling method (16) by copolymerization of glycidyl methacrylate and ethylene glycol dimethacrylate followed by treatment of the epoxy groups with ethylene diamine. The MDPP had 3.55 mmoles of $NH_2$/g polymer.

*Labelling reagent*

The labelling reagent, N-succinimidyl-3-(trimethylstannyl)benzoate (NSTMB) was used to couple $^{211}At$ to the particulates. The synthesis of NSTMB was done according to published procedures (17), the composition of the product was confirmed using nuclear magnetic resonance spectroscopy, and the purity was determined to be above 96% using high performance liquid chromatography (silicagel column, Supelcosil SPLC-si; mobil phase of hexane/ethylacetate/acetic acid 70/29.91/0.09 v/v%; detection at 254 nm).

*Astatination of MDPP*

$^{211}At$ was bound to the NSTMB using a method previously described (6) with only minor modifications. Briefly, $^{211}At$ in 0.5 ml of chloroform was added to 0.25 mg of NSTMB and 2 mg of *tert*-butylhydroperoxide and mixed strongly for 20 min. The reaction mixture was then evaporated to dryness in a stream of Ar-gas, re-dissolved in 50 µl of $CHCl_3$, and loaded onto a Sep-pak silicagel cartridge (Waters). The Sep-pak was eluted with 30 ml of hexane, 30 ml of 8% ethylacetate in hexane, and finally 10 ml of 30% ethylacetate in hexane collected in 1 ml fractions. Fractions 3-5,

which contained the major portion of the eluted product, N-succinimidyl-3-[$^{211}$At]astatobenzoate (NS[$^{211}$At]AB), were used further. The fractions were pooled and evaporated to dryness with Ar-gas.

In the final labelling step NS[$^{211}$At]AB was coupled to the amino groups on the particulate. Briefly, 100 µL to 2 ml of MDPP solution (10 mg/ml in borate buffer, pH 8.5) was added. The solutions were incubated on a mixer for about 20 min. The final product, $^{211}$At-MDPP, was washed in PBS and centrifuged and the supernatant with unconjugated $^{211}$At was removed. If the supernatant contained more than 5% of the activity the washing step was repeated. Specific $^{211}$At activities of 0.19-45 MBq/mg were used.

### *Quality Control and Stability of the Preparations*

The stability of $^{211}$At-MDPP was measured after 24 h incubation in fetal calf serum (FCS) at room temperature. After three times washing/centrifuging the radioactivity associated with the pelleted particulates was above 90% for all of the preparations.

### *Therapeutic efficacy In Vivo*

Female Balb/c mice were purchased at Charles River U.K. Ltd. (Kent, England). The animals were taken care of in accordance with the Guidelines on Care of Laboratory Animals of the Royal Society/UFAW (18). The mice weighed approximately 20 g at the start of the experiment. Approximately $10^6$ K13 cells obtained from *in vitro* cultures and suspended in 0.5 ml of DMEM were inoculated intraperitoneally in each mouse. Intraperitoneal injections of 100 kBq of $^{211}$At-MDPP with various specific activities were given 30 hours later to groups of 10 animals. Tumour progression was followed by measuring the level of K13 cell-produced monoclonal antibody (mAb) in blood samples as described (10). Bodyweight of the mice was also measured and animals were sacrificed by cervical dislocation either when showing a weight increase of more than 20%, or deteriorating condition.

## Results

Typical yields of the cyclotron irradiations were 10-14 MBq/(µA•h). The NS[$^{211}$At]AB was prepared with yields generally above 65% (recovered after purification) while the MDPP was labeled with NS[$^{211}$At]AB with yields above 90% regardless of the specific activity. The recovery of $^{211}$At from target to the final product was 30-60% (25-50% net radioactivity recovery) for $^{211}$At-MDPP. The time required in a typical production sequence for $^{211}$At-MDPP was 2 h and 15 min from the end of the cyclotron irradiations to the end of the final purification step.

Survival data of mice with K13 tumour treated with 100 kBq of $^{211}$At-MDPP with specific activity ranging from 0.19 - 45 MBq/mg show that the treatments had a significant therapeutic effect for all six groups ($P < 0.05$) when each group was individually compared to the untreated control group. The survival data of the treated animals is presented in Table 1.

**Table 1. Survival of mice inoculated intraperitoneally with $10^6$ K13 cells and treated 30 h later with $^{211}$At-MDPP of different specific activity.**

| Specific activity[a] (MBq/mg) | Survival[b] (days) | |
|---|---|---|
| | Median | Range |
| 0.19 | 21 | 14-30 |
| 0.55 | 26 | 19-120[c] |
| 1.7 | 23 | 19-52 |
| 5.0 | 19 | 16-21 |
| 15 | 21 | 14-42 |
| 45 | 19 | 12-120[c] |
| Control | 12 | 9-14 |

[a]Each mouse was injected with approximately 100 kBq of $^{211}$At-MDPP.
[b]Each group consisted of 10 animals.
[c]Surviving animals were sacrificed at day 120. No signs of tumour growth were observed at necropsy.

The control group had a survival of 12 days median. No significant differences in median survival were observed among the $^{211}$At-MDPP groups. However, some animals with short survival were

found in the groups given 0.19, 15, and 45 MBq/mg preparations. Long term survivors (no signs of tumour development within 120 days) were observed in the 0.55 MBq/mg and 45 MBq/mg groups (one animal in each group).

**Table 2. K13 mAb level in blood of mice with intraperitoneal K13 tumour treated with $^{211}$At-MDPP of different specific activity.**

| Specific activity[a] (MBq/mg) | K13 level[b] (mg/L) | |
|---|---|---|
| | Median | Range |
| 0.19 | 4.0 | 0.2-11 |
| 0.55 | 4.5 | 0.2-13 |
| 1.7 | 6.5 | 0.2-11 |
| 5.0 | 6.5 | 3.0-12 |
| 15 | 6.0 | 0.2-50 |
| 45 | 7.0 | 0.2-60 |
| Control | 55 | 9.0-110 |

[a]Each mouse was injected with approximately 100 kBq of $^{211}$At-MDPP.

[b]At day 7 after tumour cell inoculation. Each group consisted of 10 animals.

The measured K13 mAb level in blood of mice with K13 tumour is presented in Table 2. The data represent the values at day seven after cell inoculation. The median K13 mAb levels were similar in the $^{211}$At-MDPP treated groups and on average approximately 1/10 of the level of the control group. A larger spread in values was seen in the 15 and 45 MBq/mg groups compared to the other $^{211}$At-MDPP groups.

## Discussion

Particulates as colloids of sizes in the micrometer range have for a long time been applied as carrier-vehicles for radionuclides in cancer treatment. One of the main advantages with particulates is the ability to trap the radionuclide within the region treated. This is different from radiolabeled

monoclonal antibodies and smaller radiolabelled molecules which are distributed all over the body giving significant doses to tissue distant from the tumour area. The use of a physiologically stable $^{211}$At-MDPP conjugate makes it possible to irradiate body cavities selectively and, due to the short ranges of the $\alpha$-particles emitted, without delivering harmful radiation doses to the deeper regions of adjacent tissues. Preliminary toxicologic data indicates that the maximum tolerable radioactivity of $^{211}$At-MDPP is more than ten times higher than the maximum tolerable radioactivity of $^{211}$At-labelled antibodies when both types of compounds are injected intraperitonelly (9,18). This is because $^{211}$At-MDPP injected intraperitoneally is highly retained and mainly localizes on the surface of intraperitoneal organs, as shown previous with autoradiography (8), while antibodies gradually diffuse out of the intraperitoneal cavity and into the blood giving higher hematological toxicity.

The results of the present investigation indicate that the number of $^{211}$At atoms per particulate does not significantly influence the response of intraperitoneally disseminated tumour cells to $^{211}$At-MDPP treatment. No significant difference was found even though the $^{211}$At/particle ratio varied from 19 to 4400 and the number of injected particles varied from $8.4 \cdot 10^5$ to $2.0 \cdot 10^8$ (vs. $10^6$ K13 cells injected). Although short survival was observed for some animals in the 0.19, 15, and 45 MBq/mg groups, the average survival was not significantly different compared to the intermediate groups. Comparison of K13 mAb blood-level values showed a high spread of values in the 15 and 45 MBq/mg groups while the 0.19 MBq/mg was similar to the intermediate groups. This suggests that a specific activity below 15 MBq/mg may give a more reproducible response to treatment. The treatment responses were generally consistent with our previous findings (9,19), although the percent long-term survivors was higher in the first study.

Since the results in the present study indicate that the specific activity alone cannot account for the lack of response to dose increases at higher doses, other possible causes should be investigated. Further studies using particulates as carrier for $\alpha$-particle emitting radionuclides should therefore be undertaken with compounds of smaller size since these are less likely to be sterically hindered from approaching tumour cells. Also a possible development would be to make particulates tumour specific by coupling monoclonal antibodies to the surface of the particulate (20).

In conclusion significant and similar therapeutic effects were obtained in the treatment of mice with intraperitoneal tumours for a wide range of specific activities of $^{211}$At-MDPP.

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