Radiopharmaceutical Chemistry of Targeted Radiotherapeutics, Part 2: Radiolytic Effects of ^{211}At α-Particles Influence *N*-Succinimidyl 3-^{211}At-Astatobenzoate Synthesis

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A variety of promising targeted radiotherapeutics labeled with α-emitters have been developed. Clinical investigation of these radiopharmaceuticals requires the production of high activity levels, which can be hindered by α-particle–mediated radiolytic effects on labeling chemistry. The purpose of this study was to investigate the effects of radiation dose on the synthesis of *N*-succinimidyl 3-^{211}At-astatobenzoate (SAB), a compound used in our clinical trials for labeling antibodies with α-particle–emitting ^{211}At. **Methods:** Yields for the synthesis of SAB as a function of the radiation dose received by the reaction medium were determined. The variables studied included the radiohalogenation precursors *N*-succinimidyl 3-(tri-*n*-butylstannyl)benzoate (BuSTB) and *N*-succinimidyl 3-(trimethylstannyl)benzoate (MeSTB); the solvents chloroform, benzene, and methanol; and the addition of acetic acid and the oxidant *N*-chlorosuccinimide. The ^{211}At product spectra were determined from high-performance liquid chromatograms and then plotted against radiation dose. **Results:** SAB production declined rapidly with increasing dose, consistent with the documented radiolytic decomposition of BuSTB and MeSTB in chloroform. Even though these tin precursors were not appreciably degraded in benzene, SAB could not be produced in this solvent; instead, highly lipophilic ^{211}At-labeled species were generated in nearly quantitative yields. Although a dose-dependent decline in SAB yield also was observed in methanol, both in the presence and in the absence of an oxidant, the results were better than those obtained with the other solvents. An unexpected observation was that SAB could be obtained at a yield of greater than 30% when the reaction was run in methanol without the addition of acetic acid or an oxidant; these 2 components previously were considered essential for astatodestannylation. **Conclusion:** Radiolytic factors can play an important role in the synthesis of clinical-level activities of ^{211}At-labeled radiopharmaceuticals, necessitating the development of reaction conditions different from those that are used successfully at lower activity levels.

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The approval of ^{90}Y-ibritumomab tiuxetan (Zevalin; Biogen Idec Inc.) and ^{131}I-tositumomab (Bexxar; McKesson BioServices) as treatments for lymphoma was a critical step for targeted radionuclide therapy for several reasons. An important aspect is that the emergence of these products demonstrated the feasibility of the commercial development of molecularly targeted radiotherapeutics. From a radiopharmaceutical chemistry perspective, the fact that monoclonal antibodies (mAbs) could be labeled in reliable fashion at high radioactivity levels while maintaining appropriate biologic properties was a key accomplishment. The fact that the half-lives of the radionuclides involved ranged from 2.7 d (^{90}Y) to 8.1 d (^{131}I) presumably helped to minimize the deleterious effects of radiation deposited during the reaction on the labeling chemistry because only a small fraction of total nuclear decay occurred during the procedures.

Radionuclides decaying by the emission of α-particles are attractive for certain types of targeted radionuclide therapy because they deposit large amounts of energy in a volume equivalent to only a few cell diameters (*1–3*). This characteristic is potentially advantageous for treating compartmentally spread tumors and destroying tumor vasculature. On the other hand, it also may increase the likelihood that the high-radiation fields will result in radiation-induced problems for the labeling chemistry, particularly when the half-life of the α-particle–emitting radionuclide is relatively short. Unfortunately, this is a property of ^{213}Bi (46 min) and, to a lesser extent, ^{211}At (7.2 h); these 2 α-emitters have been investigated for targeted radiotherapy in patients (*4,5*).

Our own experience with labeling of a mAb with ^{211}At for clinical studies in brain tumor patients is a practical example of the difficulties encountered in maintaining efficient labeling with escalating radiation doses of α-particles. This

procedure involved 2 steps: the production of *N*-succinimidyl 3-^{211}At-astatobenzoate (SAB) by destannylation of *N*-succinimidyl 3-(tri-*n*-butylstannyl)benzoate (BuSTB) and then coupling of the SAB to the mAb (*6*). Although these methods were quite effective when doses of less than 370 MBq of labeled mAb were needed, thereafter, the SAB yield declined rapidly and approximately half of the ^{211}At activity added to the mAb coupling reaction could not be removed from the reaction vessel.

In order to understand better the various factors that could contribute to the adverse effects of α-particle radiation on ^{211}At labeling chemistry, we have been investigating in stepwise fashion variables in the synthesis of SAB that might be susceptible to radiolysis-mediated effects. The first article in this series demonstrated that the nature of the solvent had a profound influence on the degradation of the tin precursors used for SAB synthesis and, in the case of chloroform, on the generation of a cold by-product that could interfere with the production of SAB (*7*). In the present study, we investigated the nature of the radioactive products generated with different combinations of tin precursors and solvents as a function of radiation dose. A somewhat surprising observation was the fact that at certain radiation doses, SAB and other products could be generated without the addition of an oxidant or acid, conditions previously thought to be critical for performing astatodemetallation reactions.

MATERIALS AND METHODS

General Procedures

For additional experimental details, please see the first article in this series (*7*). Briefly, studies were performed with the 2 tin precursors that have been used in the past for SAB radiosynthesis. BuSTB and *N*-succinimidyl 3-(trimethylstannyl)benzoate (MeSTB) were prepared as described previously (*8,9*), and their purities were confirmed by thin-layer chromatography before each set of experiments. All solvents were reagent grade or better and were used as purchased. High-performance liquid chromatography (HPLC) analyses were performed with a Beckman System Gold HPLC apparatus equipped with a diode array detector and a radioisotope detector. For reverse-phase chromatography, a Waters Xterra column (4.6 × 250 mm; 10 μm) was used. Normal-phase HPLC was done with an Alltech Partisil silica column (4.6 × 250 mm; 10 μm). The elution for reverse-phase chromatography was done as follows: a gradient of solvent B (acetonitrile:water:acetic acid [95:5:0.1]) in solvent A (water:0.1% acetic acid), kept at 48% solvent B for 13 min; a 48%–100% linear gradient of solvent B over 2 min; and 100% solvent B until the end of the HPLC run. The flow of 1 mL/min was increased to 1.5 mL/min at 15–15.5 min and kept there until the end of the chromatography run. The ultraviolet signals at both 220 and 254 nm and the radioactive signal were monitored. Normal-phase chromatography was carried out under isocratic conditions with a hexane:ethyl acetate:acetic acid (70:30:0.12) solvent at 1 mL/min.

Production of ^{211}At and Radiation Dose Calculations

^{211}At was produced at the Duke University Medical Center cyclotron and purified by dry distillation as described previously (*10*).

All of the α-particle and α-recoil nuclei decay energy was assumed to be deposited in the solutions in which the astatine was dissolved, because of the short range of the emissions relative to the dimensions of the reaction mixtures. Uniform distribution of the reactants in the solvents also was assumed. The absorbed dose was calculated as follows (*6*):

$$D = A_i(1 - e^{-\mu})\frac{1}{\lambda}\frac{1}{m}\Delta_i.$$

In this equation, D is expressed in grays, A_i is the initial activity (MBq), λ is the decay constant for ^{211}At (s^{-1}), t is the exposure time (s), m is the mass of the solution (g), and Δ_i is the mean energy emitted per nuclear transition. On the basis of dose contributions from α-particles and α-recoil nuclei, a Δ_i of 1.09×10^{-3} Gy·g/MBq·s was calculated (*11*). Densities of 1.49, 0.791, and 0.879 g/mL were used for chloroform, methanol, and benzene, respectively, to convert solvent volume to mass.

Radiolysis Experiments and SAB Synthesis

The majority of the experiments were performed at an acidic pH, with acetic acid at 0.67 mol/L, the conditions generally used for SAB synthesis (*6*). In all of the experiments, the final volumes were the same, and the reaction mixtures contained only ^{211}At activity, the tin precursor, and acetic acid when acidic conditions were studied. All of the experiments were conducted under atmospheric conditions and at room temperature.

To each reaction vial were added 50 μg of the tin precursor (BuSTB or MeSTB) dissolved in 100 μL of solvent (chloroform, methanol, or benzene), 20 μL of acetic acid, and ^{211}At in sufficient solvent to yield a final reaction volume of 520 μL. To study the effect of pH, we performed separate experiments in which parallel reactions were performed on the same day with acetic acid and without acetic acid. ^{211}At was used immediately after the completion of the target distillation to minimize the effects of radiolysis processes taking place while ^{211}At was in the solvent distillation trap. The activities used ranged from approximately 10 to 250 MBq and were measured with a CRC-7 dose calibrator (Capintec).

Samples for HPLC analyses were taken at various times selected on the basis of the approximate dose levels of interest in each experiment. The radiation dose deposited in the reaction vessel ranged from approximately 200 to 20,000 Gy, and the exposure periods ranged from minutes to approximately 24 h. Aliquots of 50 μL were used for HPLC analyses, except for longer exposure times (~24 h) and when the initial activity was low; in these instances, aliquots of greater than 50 μL were used. Samples were injected into the HPLC apparatus immediately after they were obtained. In all of the experiments, authenticated standards of 3-iodobenzoic acid (IBA) and *N*-succinimidyl 3-iodobenzoate (SIB) also were run for comparison. Because high levels of α-particles can have deleterious effects on HPLC columns over time, it is recommended that standards be run before each set of experiments.

When the synthesis of SAB was investigated in the presence of an oxidant, SAB was prepared by adding 50 μg of BuSTB in 50 μL of methanol, 100 μg of *N*-chlorosuccinimide (NCS) dissolved in 50 μL of methanol, and 20 μL of acetic acid to 0.4 mL of ^{211}At in methanol in a Reacti-vial (Pierce Biotechnology, Inc.). The reaction mixture was shaken for 20 min, and 50-μL aliquots were removed periodically for HPLC analyses.

HPLC analyses were performed with the normal-phase and reverse-phase systems for BuSTB and with the reverse-phase

system for MeSTB. The sample with the peak corresponding to the retention time of SAB was recovered, and its activity was measured. In addition, the areas of other astatinated species were integrated. Results were expressed as a percentage of the total activity of the sample and plotted as a function of the radiation dose in grays.

RESULTS

It is helpful to relate the radiation dose ranges evaluated here to those that would be encountered in the preparation of SAB for higher clinical doses of ^{211}At-labeled radiopharmaceuticals. As a benchmark, at a dose of 370 MBq of ^{211}At-labeled mAb, difficulties were encountered in reproducible preparation of the radiopharmaceutical. On the basis of the yields of SAB synthesis and mAb coupling and the times required for synthesis and purification, the initial ^{211}At activity would need to be greater than 1,500 MBq; at the volume used for SAB synthesis, this activity would deliver doses of 2,500 Gy in chloroform, 4,700 Gy in methanol, and 4,250 Gy in benzene because of the different densities of these solvents (*7*). For this reason, experiments were performed at dose ranges that included values both above and below these benchmarks.

Because chloroform has been used for clinical-level SAB production, this solvent was investigated initially. The tin precursors each were exposed under acidic conditions to ^{211}At, and the radioactive species present in the reaction mixture were analyzed by HPLC. A series of normal-phase chromatograms from HPLC analyses of BuSTB and ^{211}At (59–252 MBq for 2.43–3.92 h) reaction mixtures receiving doses of 644, 1,658, and 4,157 Gy are shown in Figure 1. Chromatograms from reverse-phase HPLC analyses of MeSTB and ^{211}At (54–227 MBq for 2.6–4.5 h) reaction mixtures receiving doses of 1,254, 3,252, and 6,724 Gy are shown in Figure 2. SIB and, in some instances, IBA standards also were run to aid in the identification of SAB and other radioactive products. With both tin precursors, HPLC analyses indicated that as the radiation dose in the reaction mixture increased, the size of the peak with a retention time corresponding to SIB, presumed to be SAB, decreased. Also, a clear shift toward astatinated species that were eluted at lower retention times also was observed as the dose increased.

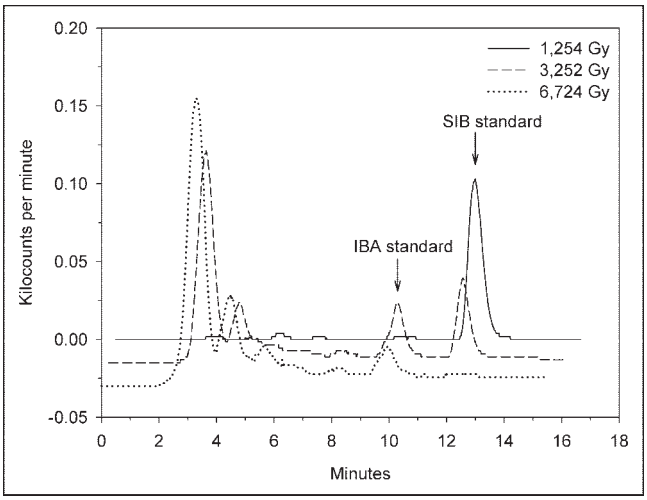

FIGURE 2. Reverse-phase HPLC profile of radioactive species present after exposure of MeSTB precursor to ^{211}At at doses of 1,254, 3,252, and 6,724 Gy and acetic acid in chloroform. Arrows indicate retention times of SIB and IBA standards. Chromatograms are offset on both axes for display purposes.

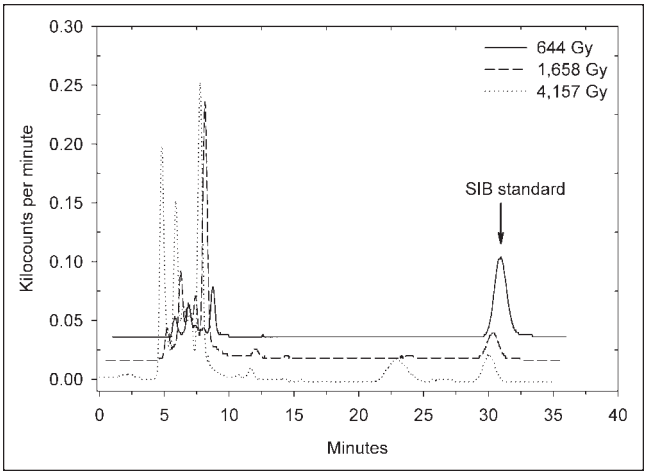

FIGURE 1. Normal-phase HPLC profile of radioactive species present after exposure of BuSTB precursor to ^{211}At at doses of 644, 1,658, and 4,157 Gy and acetic acid in chloroform. Arrow indicates retention time of SIB standard. Chromatograms are offset on both axes for display purposes.

Because of the deleterious effects of chloroform on the HPLC runs, before injection, the samples were evaporated over approximately 20 s with a stream of argon and then redissolved in methanol for reverse-phase HPLC (MeSTB experiments) or 30% ethyl acetate in hexane for normal-phase HPLC (BuSTB experiments). This evaporation step does not affect the level of SAB but, as will be explained in detail later, it does affect the concentrations of volatile species, such as free astatine. For this reason, the SAB production yield was calculated as a percentage of the total activity in the sample before evaporation and was plotted as a function of the radiation dose in grays deposited in the reaction medium.

As shown in Figure 3, exponentially decreasing yields of SAB synthesis were observed with increasing radiation doses for both tin precursors when the reactions were run in chloroform. Higher percentages of SAB were obtained when MeSTB was used, particularly at lower radiation doses. The data for each set of experiments were fit to exponential curves with good correlations (MeSTB, r^2 = 0.998; BuSTB, r^2 = 0.996). With these equations, at a benchmark dose of 2,500 Gy in the reaction medium, the yields calculated for SAB production were 11% and 2% for MeSTB and BuSTB, respectively. These data are consistent with the fact that at this 2,500-Gy dose, less than 3% and

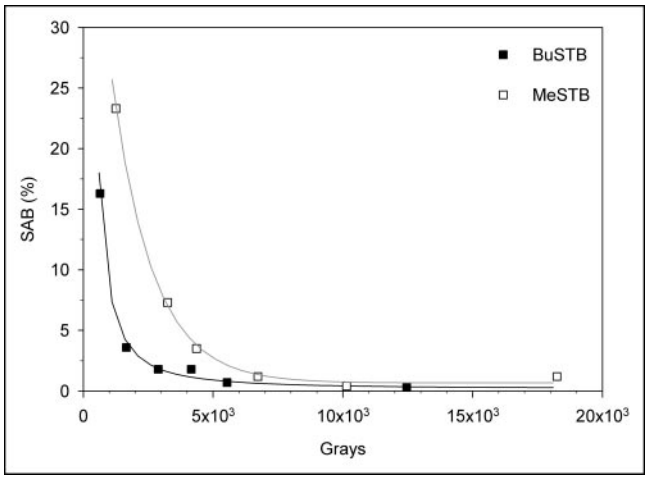

FIGURE 3. Production of SAB after incubation of BuSTB and MeSTB with chloroform, acetic acid, and ^{211}At as function of radiation dose.

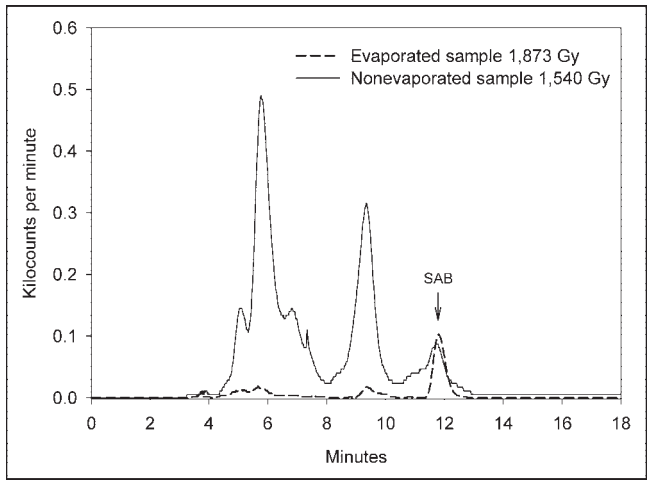

FIGURE 4. Effect of sample evaporation on HPLC profile. Shown are chromatograms from reverse-phase HPLC analyses of radioactive species in samples from reaction of MeSTB with methanol, acetic acid, and ^{211}At for reaction mixtures receiving doses of 1,873 Gy (evaporated) and 1,540 Gy (nonevaporated). Arrow indicates SAB.

1% of the MeSTB and BuSTB precursors, respectively, remained intact (*7*).

For reactions run in methanol, analyses were performed by reverse-phase HPLC. If normal-phase HPLC had been used, the methanol in the sample would have to have been evaporated because of the harmful effects of this polar solvent on a normal-phase column. However, during evaporation, volatile astatinated species could be lost, a problem that was obviated through the use of reverse-phase HPLC. To illustrate this point, the reverse-phase HPLC profiles of 2 samples taken from the same MeSTB reaction vial were compared; 1 sample had been injected without evaporation, and the other sample had been subjected to evaporation and then redissolved in methanol. The samples received doses of 1,540 and 1,873 Gy, respectively, and the volumes of the aliquots were the same for both. As shown in Figure 4, evaporation resulted in the loss of many astatinated species; however, it did not alter the level of SAB. Thus, evaporation must be avoided when analyses of other astatine molecules is required.

In experiments performed under acidic conditions with methanol as the solvent, doses in the reaction mixture ranged from 1,723 to 5,634 Gy (36.6–93.6 MBq; 2.82–5.75 h) for BuSTB and from 1,873 to 5,591 Gy (51–145 MBq; 0.57–4.48 h) for MeSTB; each experiment was performed 3 times. As shown in Figure 5, an exponentially decreasing yield of radiation-induced SAB production with increasing radiation dose was observed. As in the experiments performed with chloroform, yields observed with the MeSTB precursor were higher than those observed with the BuSTB precursor. Yields obtained for the synthesis of SAB in methanol were higher than those obtained in chloroform at the same radiation doses.

The yields of SAB as a function of the radiation doses are shown in Figure 6 for reactions carried out with an oxidant (synthesis) and without an oxidant (radiolysis). Also shown for comparative purposes is the degradation of the tin precursor as a function of dose (*7*). All experiments were carried out with BuSTB in methanol and were performed in the presence of acetic acid at 0.67 mmol/L. These results demonstrated that in methanol, SAB can be produced by radiolysis; however, the addition of an oxidant is required to obtain higher yields. It is important that the decline in SAB yields from both processes (synthesis and radiolysis) at higher radiation doses cannot be attributed to the degradation of the tin precursor.

The effect of pH on the production of SAB in methanol as a function of the α-particle dose also was investigated. Paired experiments were performed 3 times with BuSTB on the same day in the presence and in the absence of acetic

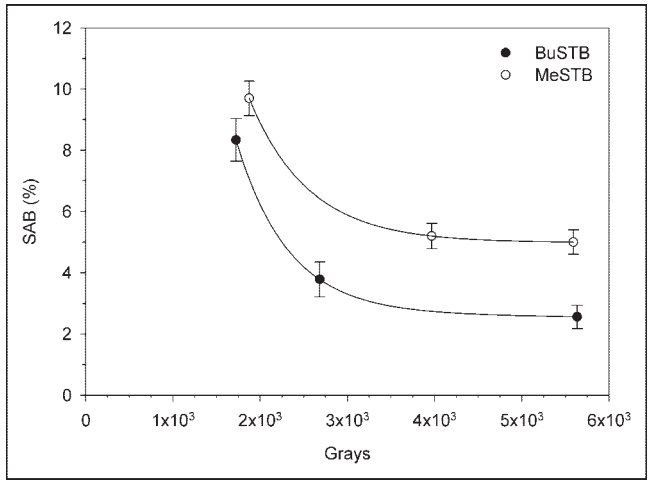

FIGURE 5. Production of SAB after incubation of BuSTB and MeSTB with methanol, acetic acid, and ^{211}At as function of radiation dose. Error bars indicate SDs.

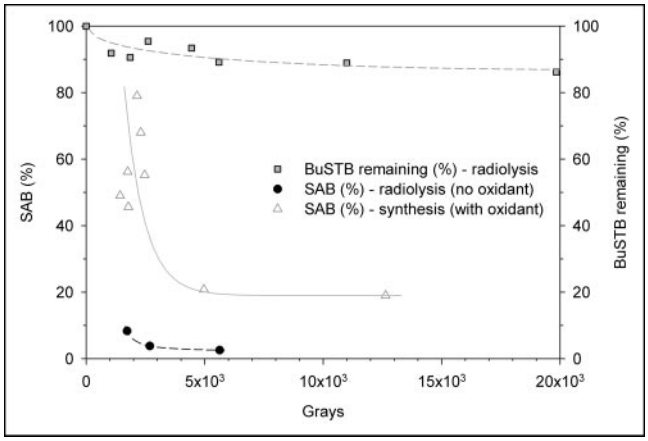

FIGURE 6. Production of SAB from reaction of BuSTB and ^{211}At with methanol and acetic acid by radiolysis (no oxidant added) and radiosynthesis (oxidant added). Decomposition of BuSTB as function of radiation dose (*1*) is shown for comparison.

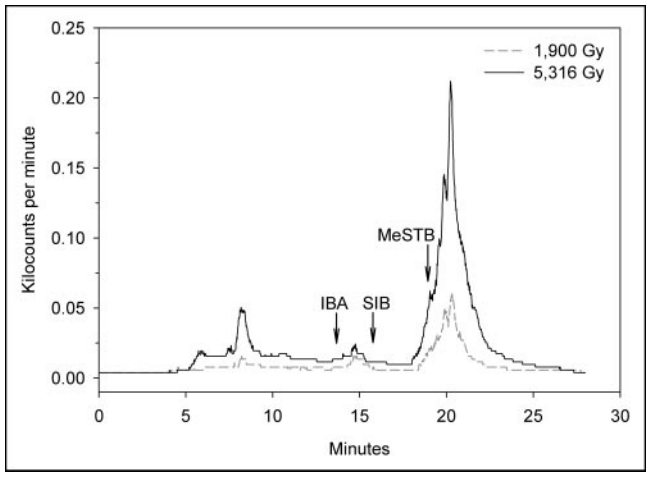

FIGURE 8. Reverse-phase HPLC profile of radioactive species present after exposure of MeSTB precursor to ^{211}At at doses of 1,900 and 5,316 Gy in benzene and acetic acid. Arrows indicate retention times of MeSTB, SIB, and IBA standards.

acid at 0.67 mol/L, a component of our standard SAB production protocol (*6*). As shown in Figure 7, SAB production attributable to radiolysis decreased with increasing radiation dose. However, in the dose range of 1,500–6,000 Gy, yields for the production of SAB were significantly higher without acetic acid, with the difference being greater at the lower end of this dose range.

The reactions were also run in benzene in the presence of acetic acid and with ^{211}At activity levels and reaction times such that doses in the reaction mixtures were 6,988–14,582 Gy (173 MBq; 6.07–27.03 h) for BuSTB and 1,900–12,464 Gy (63–153 MBq; 4.12–23.32 h) for MeSTB. Samples were analyzed by reverse-phase HPLC. A new reverse-phase column was used in these experiments; because longer retention times are expected with a new column, SIB, IBA, BuSTB, and MeSTB standards were run. Even though in benzene, as in methanol, only low levels of precursor radiolytic decomposition are observed (*7*), the results obtained for SAB production in the 2 solvents were quite different. No SAB was detected in the radiolysis experiments performed with benzene; instead, most of the ^{211}At activity was present as a very lipophilic species. For example, Figure 8 depicts the HPLC profiles for the reaction of MeSTB at 2 different radiation doses; most of the activity was seen at retention times longer than those of the tin precursor. Similar HPLC profiles were seen after the reaction of BuSTB with ^{211}At in benzene. The percentages of the total activity eluted in the major lipophilic peak(s) at different radiation doses are summarized in Table 1 and ranged from 89% to 95% for MeSTB and from 82% to 97% for BuSTB.

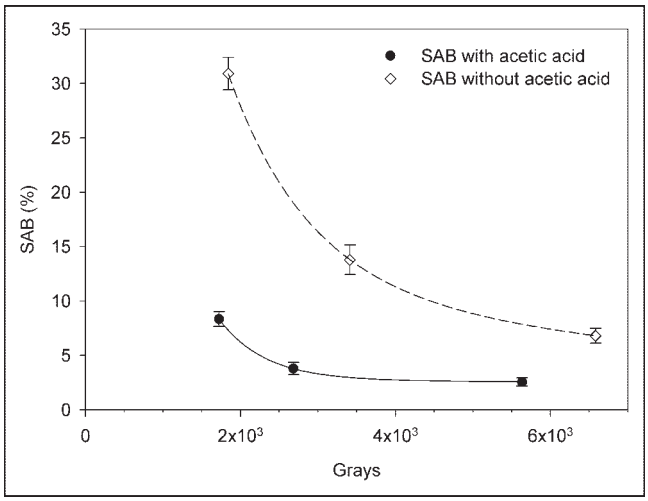

FIGURE 7. Effect of addition of acetic acid on SAB synthesis from BuSTB in methanol as function of radiation dose. Error bars indicate SDs.

DISCUSSION

Because of their high energy and short path length, α-particles such as those emitted by ^{211}At represent high linear energy transfer (LET) radiation. This property is desirable for efficient killing of tumor cells because the cytotoxicity of α-particles is nearly independent of oxygen concentration, dose rate, and cell cycle status (*12*). On the other hand,

TABLE 1
Percentages of Activity Eluted at 18–23 Minutes in Benzene

Precursor	Dose (Gy)	% Activity
MeSTB	1,900	95
	5,125	91
	5,316	91
	12,464	89
BuSTB	6,988	82
	14,582	97

the highly focal nature of α-particle energy deposition can present a major challenge to the radiochemist because this property also can create an environment in which radiolytic effects have a significant impact on labeling chemistry. The energy deposition per unit volume for ^{211}At α-particles is 2 orders of magnitude greater than that for the highly energetic β-particles of ^{90}Y (*13*), suggesting that the challenge for overcoming radiolysis-induced problems will be much greater for high-activity ^{211}At labeling.

Astatodemetallation is a versatile strategy for ^{211}At labeling, and SAB is the most commonly used molecule synthesized by this approach (*1,14*). SAB was used for the production of clinical-level batches of ^{211}At-labeled mAbs (*6*); however, as radiation dose escalation increased, the chemistry became intractable, suggesting that radiolysis was in some way interfering with the labeling process. Radiation-induced ionization is a complex process, producing electrons, free radicals, and molecules after interaction with the medium. With regard to the conditions of SAB labeling, the interaction of α-particles will occur primarily with the organic solvent; however, the effects of and on the other components of the reaction mixture—the tin precursor, an oxidant, acetic acid, and ^{211}At species—also need to be considered. As discussed below, some of these reagents previously thought to be critical for astatodestannylation reactions may not be required and in fact may interfere when the reactions are run at high activity levels.

With regard to solvent-mediated radiolytic effects, our previous study (*7*) documented 2 problems with using chloroform, our original solvent for SAB synthesis, at high radiation doses. Not only was extensive radiolytic decomposition of the tin precursor observed, but also a radiolytically induced by-product was generated. This species, most likely *N*-succinimidyl 3-chlorobenzoate (SCB), could account for the low SAB yield, mAb coupling efficiency, and immunoreactivity seen at high ^{211}At activity levels (*6*). Nonetheless, our results demonstrate that it is possible to synthesize SAB in chloroform in the absence of an oxidant, and at doses on the order of 1,000 Gy, the yield is only approximately a factor of 2 lower than that obtained at these doses in the presence of an oxidant (*6*).

Unfortunately, the radiolysis-mediated synthesis of SAB decreased rapidly with increasing radiation dose, contraindicating the optimization of this mechanism as a means for SAB production at high levels of ^{211}At. The exposure of chloroform to radiation is known to result in the production of free radicals, including $^{\bullet}CCl_3$ and Cl (*15*). It is possible that these radicals could degrade either the tin precursor or the SAB; moreover, the increasing production of SCB with increasing radiation dose indicates that the Cl atoms can compete effectively with ^{211}At for reaction with the tin precursor.

The lower SAB yield obtained with BuSTB than with MeSTB could reflect the lower degree of steric interference in the astatodestannylation reaction caused by the less bulky trimethyl precursor (*16*). It also could reflect the higher yield of the cold by-product, presumed to be SCB, obtained with the BuSTB precursor. The reaction of Cl is a free-radical substitution, and the difference in yields observed between tin precursors could reflect the effect of the leaving group on reactivity (*17*).

From the perspective of the stability of the tin precursor at high radiation doses, the best of the 3 solvents investigated was benzene. The fact that SAB could not be synthesized in benzene at any radiation dose either in the presence or in the absence of an oxidant suggests that other factors in addition to the availability of the precursor must be considered. Although the radical production yields for benzene are considerably lower than those for aliphatic compounds, recent studies have shown that benzene is not radiation inert. LaVerne and Aranos (*18*) reported that 0.72 radical per 100 eV was produced in benzene irradiated with ^{4}H ions having an LET nearly identical to that of ^{211}At α-particles. Interestingly, the primary species generated, phenyl radicals (0.37 radical per 100 eV), were scavenged very efficiently by iodine to produce iodobenzene. Another noted characteristic of benzene radiolysis was the formation of multimeric C_6 species.

In the present study, reaction of ^{211}At with benzene at high radiation doses resulted in the formation of very lipophilic species at yields of generally greater than 90%. We speculate that the product(s) could be ^{211}At-astatobenzene and possibly other ^{211}At-labeled multiring compounds. If this were indeed the case, then radiolysis-induced synthesis might be worth investigating as a labeling strategy. Previously, hydrogen substitution by astatine in benzene and its derivatives could be accomplished only by dissolving astatine in concentrated acetic acid in the presence of potent oxidizing agents ($H_2Cr_2O_7$ or $HClO_4$) at elevated temperatures (*19*).

Our studies with benzene suggested that in the presence of high α-particle doses, efficient chemical transformation of ^{211}At is possible without the addition of an oxidant. The yield for the synthesis of SAB attributable to radiolysis in methanol was higher than that in chloroform, a result that could have been partly attributable to the lack of competition of Cl atoms for the tin precursor in methanol. The addition of an oxidant to reactions performed with methanol increased SAB yields considerably; however, SAB production efficiency decreased rapidly with increasing radiation dose. At the benchmark dose of 4,700 Gy, SAB yields decreased from about 80% at approximately 1,500 Gy to only 20%, making these reaction conditions not ideal for performing ^{211}At labeling at the high doses needed for targeted radionuclide therapy. When the fact that even at radiation doses exceeding 5,000 Gy greater than 90% of the BuSTB remained intact is considered, precursor degradation cannot account for the rapidly decreasing SAB yield with increasing radiation dose. A possible explanation is a radiation-induced change in the chemical form of astatine from At^{+}, the species generally presumed to be required for efficient electrophilic astatodestannylation reactions. We

hypothesize that this scenario could occur because of the generation of reducing species during the radiolysis of methanol by high-LET ^{211}At α-particles.

With higher LET radiation, the yield of molecular products is increased while the yield of radicals that can be scavenged is decreased, reflecting an increase in intraspur reactions at the expense of radical escape from the spur (*15b,20*). In methanol, alkoxy radicals ($CH_3O^{\bullet}$) predominate during the brief spur lifetime. Because of the high probability of an interaction with a solvent, 1 pathway will involve quenching of alkoxy radicals by methanol molecules and conversion to $^{\bullet}CH_2OH$. In addition, interradical reactions occurring in the spur can yield hydrogen and formaldehyde. Thus, as the radiation dose increases, the production of these reducing species also may increase.

To explore the possibility that reducing species generated by the radiolysis of methanol by ^{211}At interfere with electrophilic astatination, we calculated the amounts of hydrogen and formaldehyde produced by using a value of 6.786×10^6 eV per decay for ^{211}At and G values of 5.4 ± 0.1 (mean ± SD) and 2.0 ± 0.1 for H_2 and HCHO, respectively (*20b*). These G values are the preferred values recommended by the National Bureau of Standards for γ-radiolysis; G values for α-particles are not available. In Figure 9, the calculated micromoles of both reducing species produced as a function of the radiation dose delivered during the reaction (20 min, 520 μL) are compared with the micromoles of NCS used for SAB radiosynthesis. At radiation doses at which SAB yields decreased precipitously, the micromoles of the reducing species approached the micromoles of NCS added to the reaction; at radiation doses exceeding approximately 3,450 Gy, they surpassed this amount. Particularly when the fact that these G values are increased by the addition of acid (*20*) is considered, the reducing species created during methanol radiolysis may account for the decrease in SAB yield with increasing radiation dose.

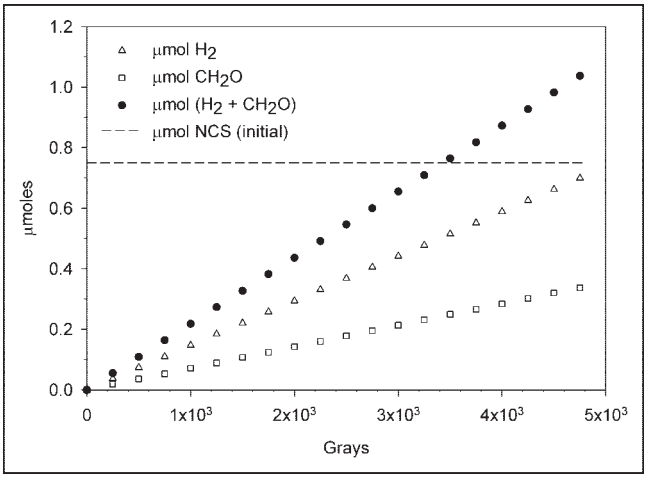

FIGURE 9. Calculated micromoles of hydrogen and formaldehyde produced in methanol by radiolysis during 20-min reaction period as function of radiation dose. For comparison, broken line shows micromoles of oxidant (NCS) added to SAB radiosynthesis reaction.

The effect of pH on SAB production at elevated radiation doses is important because electrophilic astatodestannylation reactions normally are performed in the presence of acetic acid at pHs 5.0–5.5 (*21*). Our results indicated that with methanol, yields for the synthesis of SAB at elevated radiation doses actually were considerably higher when acetic acid was excluded from the reaction mixture. This behavior cannot be explained by differences in precursor degradation because the fraction of BuSTB remaining after exposure to high doses of radiation from ^{211}At actually was lower at a neutral pH (*7*).

It seems likely that the observed acid-induced changes in SAB production reflect variations in the concentrations of the species generated by methanol radiolysis that could influence the oxidation state of astatine in the reaction mixture. Polar, protic solvents such as methanol can stabilize ions such as astatine at different oxidation states because of their relatively high dielectric constants and their capacity for solvating ions. Polar, protic solvents also contain relatively mobile protons, such as those bonded to oxygen, facilitating the exchange of protons and thereby making a different solvent effect depending on the pH more likely (*22,23*). For example, in water, changing the pH has a strong effect on the yields of the primary radiolysis products, with a pronounced decrease in $G_{e^-_{aq}}$ and an increase in G_H at pHs of less than 5 (*15c*). When methanol is acidified, the probability that solvated electrons will be converted into H is increased (*20*). Thus, potential consequences of adding acetic acid to methanol are higher G_H and therefore higher concentrations of reducing species. An increased yield of formaldehyde G_{CH_2O}, another reducing species, in methanol at an acidic pH also has been observed (*20*).

In the preparation of ^{211}At-labeled radiopharmaceuticals with high activity levels by demetallation reactions, high LET radiation and an acidic pH are both conditions that can lead to increased scavenging of primary radiolysis products and the production of higher levels of $H^{\bullet}$, H_2, and HCHO. We speculate that higher levels of reducing species generated from methanol radiolysis can contribute to the reduction of SAB production at high radiation doses through a reduction in the amounts of electrophilic astatine molecules required for the reaction. This scenario may occur either by depletion of an oxidant or by reaction with At^+ already produced by the oxidation of astatine dissolved in methanol by NCS.

It is important that the radiolysis of methanol is more complex than was indicated in the above discussion, which was focused on processes that can contribute to a decrease in labeling yield with an increase in radiation dose. However, other radiolytically induced reactions also can occur. Because these reactions were run in air, the formation of oxidizing species such as peroxide also is possible (*15d*).

Another process that could be relevant at high radiation doses is spur acidification, which can take place in solvents

such as water and methanol. Several very rapid processes can occur before the spurs and tracks expand and dissipate through the normal process of diffusion (*24*). These processes include ion–molecule reactions between the primary products and the solvent. For water, such reactions render the spur more acidic than the surrounding medium (*15*). For methanol, a similar reaction is possible between 1 of the primary products and solvent molecules, as follows:

$$CH_3OH^+ + CH_3OH \rightarrow CH_3OH_2^+ + {}^{\cdot}CH_2OH.$$

This reaction would make the spur in methanol more acidic. Because the track density increases with increasing radiation dose, this process may generate an acidic environment, generally thought to be conducive to the formation of electrophilic astatinated species, without the addition of acetic acid.

On the basis of the results presented here and the results of our previous study (*7*), methanol is the best of the 3 solvents tested for the production of SAB at the activity levels needed for clinical studies. Because of declining yields at higher radiation doses, it is recommended that researchers avoid doses of greater than 1,000–1,500 Gy by working with parallel reaction vessels so that the dose received in each vessel is less than 1,500 Gy. These conditions are equivalent to a reaction of SAB in methanol for 20 min in a 500-μL reaction volume with approximately 370–480 MBq of ^{211}At per vessel. For example, with this approach, we produced 900 MBq of SAB from 1,375 MBq of ^{211}At, equally distributed in 4 reaction vessels, each containing 50 μg of BuSTB and 60 μg of NCS.

CONCLUSION

At the high activity levels required for clinically targeted radiotherapy, the radiation dose delivered by ^{211}At α-particles can influence adversely the labeling chemistry. Depending on the solvent, potential problems include loss of the tin precursor through reaction with species generated by radiolysis (chloroform); reaction of ^{211}At with molecules generated from the solvent; and, in methanol, generation of reducing species that could alter the oxidation state of the ^{211}At present in the reaction mixture.

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