Uptake and Therapeutic Effectiveness of $^{125}$I- and $^{211}$At-Methylene Blue for Pigmented Melanoma in an Animal Model System

Eva M. Link, Ian Brown, Robert N. Carpenter, and Joseph S. Mitchell

Department of Chemical Pathology, Academic Unit, University College and Middlesex School of Medicine, Cleveland Street, London W1P 6DB, United Kingdom.

Abstract

The investigations concerning a targeted radiotherapy for pigmented melanoma with a radiolabeled phenothiazine derivative, 3,7-[(dimethylamino)phenazathionium chloride [methylene blue (MTB)], were continued using melanotic and amelanotic sublines of B16 melanoma. Two radio-isotopes, $^{125}$I and $^{211}$At, emitting Auger electrons and a particles, respectively, replaced $^{35}$S previously studied since their biological effectiveness is significantly higher. In vitro autoradiography revealed a selective accumulation of methylene blue labeled with either of the radioisotopes in pigmented melanoma cells but its absence in nonpigmented cells. Treatments with $^{125}$I-MTB and $^{211}$At-MTB were performed both in vitro and in vivo, with their effectiveness determined by lung clonogenic assay. $^{125}$I-MTB proved to be relatively ineffective when incorporated into melanosomes distributed in the cytoplasm, i.e., too far away from the genome. Conspicuous therapeutic effects were achieved with $^{211}$At-MTB for pigmented melanoma only. $^{211}$At itself did not affect either of the investigated sublines of B16 melanoma confirming once again the high affinity of methylene blue to melanin. Calculations of cumulative radiation doses from $^{125}$I-MTB deposited in melanotic melanoma tumors and pigmented normal organs which would be at a particular risk led to the conclusion that $^{211}$At-MTB could be used for a highly selective and very efficient targeted radiotherapy of pigmented melanomas without damaging normal tissues.

Introduction

Melanoma is one of the most malignant neoplasms and, although it accounts for a relatively small proportion of annual cancer presentations, a distressing rise has been noted in its overall incidence over recent years. Melanoma arises most often in the skin, usually as a highly pigmented tumor, and metastasizes widely at its early stages. Since the effectiveness of the presently available therapy is limited, the investigations of melanoma-specific compounds as potential diagnostic and therapeutic agents have been initiated. The abundance of melanin in pigmented melanoma cells suggested the use of compounds with a high binding affinity to this natural polymer such as thioamides (1), chloroquine (2), or phenothiazine and its derivatives (3, 4). These compounds used as a carrier for radioactive isotopes would be expected to accumulate in pigmented tissues including melanotic melanomas in proportion to their melanin content giving a selectively localized source of radiation. Investigations concerning in vivo distribution of $^{35}$S Chlorpromazine revealed the highest level of this phenothiazine derivative in pigmented melanomas (5). However, since the carrier for a radioisotope should be characterized not only by the possibility highest affinity to melanin but also by a very low general toxicity, another phenothiazine derivative exhibiting both these features, namely 3,7-[(dimethylamino)phenazathionium chloride (methylene blue), was chosen for further studies (6–8). Its radiolabeled derivative, $^{35}$S-methylene blue, was incorporated 6 times more efficiently by cultured pigmented melanoma cells than by those of its nonpigmented variety and revealed exceptionally high and stable level in pigmented melanoma when administered in vivo (6–8). Its presence in the melanotic tumor caused a significant delay in the growth of the neoplasm, whereas it did not affect nonpigmented tumors (8).

Since these observations revealed a significant therapeutic advantage, the investigations were continued using methylene blue labeled with either $^{125}$I or $^{211}$At. $^{211}$At is an a particle emitter characterized by a high LET* optimal for an endoradiotherapy (98.84 keV/μm), a short physical half-life of 7.21 h, and a mean range in a tissue of 60–65 μm (9). Auger electrons emitted by $^{125}$I exhibit features similar to a high LET radiation if the radionuclide is placed in a close proximity to DNA (10).

The data presented in this paper confirm conspicuous effects of such targeted radiotherapy for pigmented melanomas and demonstrate that $^{211}$At is the most suitable radioisotope from those already investigated.

Materials and Methods

Cell Lines

Experiments were carried out in vitro and in vivo using amelanotic and melanotic B16 mouse melanoma. A primary B16 melanoma cell line was obtained by courtesy of Professor S. Bleehen of the Department of Dermatology, Royal Hallamshire Hospital, University of Sheffield. Melanotic and amelanotic sublines were isolated from the primary line by a subsequent s.c. implantation into male C57BL/6 mice of small tumor fragments characterized by either a strong pigmentation or a low melanin content. A single-cell suspension from both types of B16 melanoma was obtained by dispersion of excised tumors with 0.25% trypsin in PBS.

In vitro experiments were carried out using MEM supplemented with 200 mM L-glutamine (1 ml/100 ml MEM), 7.5% NaHCO$_3$ (0.4 ml/100 ml MEM), penicillin (10,000 units/100 ml MEM), streptomycin (10 mg/100 ml MEM), and 10% fetal bovine serum unless otherwise stated.

$^{211}$At Production and Synthesis of Radiolabeled Derivatives of Methylene Blue

$^{211}$At was produced by the $^{209}$Bi(α,2n)$^{211}$At nuclear reaction using a 28-MeV α particle external beam from the Nuffield 1.52-m cyclotron located in the Department of Physics, University of Birmingham (11). A preparation of $^{125}$Iiodoacetamide blue and $^{211}$Atastomatene blue has been conducted by thermal isotopic halogen exchange in the

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2 To whom requests for reprints should be addressed, at Department of Chemical Pathology, Academic Unit, University College and Middlesex School of Medicine, Cleveland Street, London W1P 6DB, United Kingdom.


4 The abbreviations used are: LET, linear energy transfer; PBS, phosphate-buffered saline; MEM, modified Eagle's minimal essential medium.
presence of 18-crown-6 ether according to the method described by Brown et al. (11).

Uptake of $^{125}$I-Iodo-MTB and Na$^{25}$I into Amelanotic and Melanotic B16 Melanoma Cells

Kinetics of an in Vitro Uptake of $^{125}$I-Iodo-MTB, $^{131}$I-MTB or Na$^{25}$I as a control were added to serum-free MEM to give a final radioactivity of 7.4 kBq/ml. Subsequently, amelanotic or melanotic cells were added to this medium to a density of $3.3 \times 10^6$ cells/ml and 1.5-ml aliquots of the cell suspension immediately distributed into 10-ml test tubes were incubated at 37°C. At 30-min intervals, 2-3 test tubes were taken at random from both experimental groups. The cells were centrifuged, washed three times with PBS, dried, suspended in 150 μl 1 N NaOH, and left at 37°C for 24 h. Afterwards, cells from each test tube were mixed with 10 ml of scintillation liquid and the radioactivity of $^{125}$I accumulated by the cells was determined using LS 1800 Beckman scintillation counter.

Intracellular Distribution of the Label. Melanotic and amelanotic cells obtained from B16 melanoma tumors were cultured on microscopic slides for 4-5 days. Afterwards, the medium was changed for a fresh one containing 3.8 kBq of $^{125}$I-MTB, $^{131}$I-MTB, Na$^{25}$I or Na$^{131}$I per ml MEM. Cells on each slide were covered by 5 ml of such medium and incubated for 2 h at 37°C. Subsequently, the slides were washed 5-7 times with PBS, fixed with 2.5% glutaraldehyde, and washed with distilled water and afterwards with 70, 90, 95%, and absolute ethyl alcohol. Dry slides were coated with K2 IIford emulsion in gel form (with a diameter of silver grains 2-3 times smaller than that of melanosomes present in pigmented cells) and left in a refrigerator (4°C) in the dark for 6 days. The slides were then developed with a high contrast developer (Ilford Contrast FF) and fixed with ammonium thiosulfate (Hypam Fixer; Ilford), stained with Giemsa R66 stain, a high contrast developer (Ilford Contrast FF) and fixed with ammonium thiosulfate (Hypam Fixer; Ilford), stained with Giemsa R66 stain, and washed with distilled water, 70%, 95%, and absolute ethyl alcohol. Dry slides were coated with K2 IIford emulsion in gel form (with a diameter of silver grains 2-3 times smaller than that of melanosomes present in pigmented cells) and left in a refrigerator (4°C) in the dark for 6 days. The slides were then developed with a high contrast developer (Ilford Contrast FF) and fixed with ammonium thiosulfate (Hypam Fixer; Ilford), stained with Giemsa R66 stain, and washed with distilled water, 70%, 95%, and absolute ethyl alcohol. Finally, slides were washed with xylene, dried, and mounted with DePex mounting medium (BDH Chemicals Ltd., Poole, England) for microscopic examination.

Therapeutic Effects of 4-$^{131}$At-Astato-MTB for B16 Melanoma

The therapeutic effectiveness of $^{131}$At-MTB was investigated for both sublines of B16 melanoma in vitro as well as in vivo using the lung colony assay.

Experiments in vitro-in Vivo. Melanotic and amelanotic cells obtained from melanoma tumors were added to medium supplemented with $^{131}$At-MTB or Na$^{25}$I (11 kBq/1 × 10⁶ cells). Aliquots (2.5 ml) of the suspension each containing $7 \times 10^6$ cells were distributed into 10-ml test tubes and incubated at 37°C for 1.5 h. Afterwards, the cells were centrifuged and suspended in 0.15 ml PBS, and 0.1 ml of such cell suspension immediately distributed into 10-ml test tubes and incubated at 37°C for 24 h. Afterwards, cells from each test tube were mixed with 10 ml of scintillation liquid and the radioactivity of $^{125}$I accumulated by the cells was determined using LS 1800 Beckman scintillation counter.

Intracellular Distribution of the Label. Autoradiographic studies have been carried out in order to determine the intracellular distribution of radio-labeled MTB and the corresponding radiisotope as a control within both types of melanoma cells.

The experiments revealed a highly selective accumulation of $^{125}$I-MTB and $^{131}$At-MTB by pigmented melanoma with a very characteristic distribution of the radiolabeled compound within these cells (Figs. 2A and 4A). A large number of silver grains were present overlying the pigmented melanoma cells and their localization was mirroring that of melanosomes in both the body of the cell and its dendrites. $^{125}$I-MTB and $^{131}$At-MTB were absent in amelanotic cells; none or a few grains were found overlying these cells (Figs. 3A and 5A).

Na$^{25}$I and Na$^{131}$I were not accumulated efficiently by either of the investigated melanoma sublines; very few silver grains distributed randomly were observed overlying melanotic and amelanotic cells (Figs. 2B, 3B, 4B, and 5B).

These data confirmed that the significant uptake of methylene blue into pigmented melanoma cells was due to a high melanin content of these cells.

Therapeutic Effects of 4-$^{131}$At-Astato-MTB for B16 Melanoma

Experiments in vitro-in Vivo. $^{131}$At-MTB at the radioactivity used exhibited a very significant but selective therapeutic effect 5 times greater than that by amelanotic cells (Fig. 1). The net accumulation of $^{125}$I-MTB in cells of both melanoma sublines was initially rapid with the radioactivity reaching a maximum for nonpigmented cells at about 20 min and for pigmented cells after 1 h (Fig. 1). In the latter case the maximum uptake of the radioisotope was 0.23% of the total $^{125}$I-MTB available in the medium and it remained almost constant during the following incubation period (Fig. 1). The amount of $^{125}$I-MTB accumulated by amelanotic cells within the first 20 min of incubation (5.0 × 10⁻²%) of the total $^{125}$I-MTB available) decreased gradually thereafter (Fig. 1).

The accumulation of Na$^{131}$I by melanotic and amelanotic melanoma cells was quantitatively comparable in both sublines and approximately 10 times less than that observed with $^{131}$At-MTB for pigmented melanoma cells (Fig. 1).

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Therapeutic Effects of 4-$^{131}$At-Astato-MTB for B16 Melanoma

Experiments in vitro-in Vivo. $^{131}$At-MTB at the radioactivity used exhibited a very significant but selective therapeutic effect
"I- AND !["I-METHYLENE BLUE AND PIGMENTED MELANOMAS

A.

Fig. 2. Autoradiographs of melanotic B16 melanoma cells exposed to (4) !["I-]MTB or (B) Na\(^{125}\)I.

for B16 melanoma (Fig. 6). Only melanotic tumor cells were affected by !["I-]MTB and the total number of colonies found in mouse lungs decreased to an average of 40/mouse. Since the mean value for respective control metastases counted on the lung surface only because of their high density within the organ amounted to 1039, the surviving fraction for melanotic cells exposed to !["I-]MTB was less than 3.8%. There was no such effect for amelanotic cells; Fig. 6 shows a lack of a significant difference between the number or size of control metastases and those growing from !["I-]MTB-treated cells of the non-pigmented B16 melanoma. It is also obvious that the observed specific efficacy of !["I-]MTB for melanotic tumor cells was not due to a particular affinity of !["I-] for itself to the pigmented cells since Na\(^{211}\)I, used for in vitro incubation instead of !["I-]MTB, did not affect these cells and resulted in the average of 1167 colonies growing on the lung surface yielding the surviving fraction of 112.3% (see Fig. 6). The data, therefore, argue for methylene blue as a carrier for !["I-]MTB thus enabling the radioisotope to be selectively incorporated into pigmented melanoma cells; they also confirm the previous observations concerning the selective uptake of methylene blue into melanotic cells [Figs. 1–5]. In addition, the results demonstrate that the effects found are not due to radiation absorbed by the cells from their surroundings, i.e., !["I-]MTB or Na\(^{211}\)I present in the incubation medium. If this were the case, Na\(^{211}\)I would be as effective as !["I-]MTB and the survival of amelanotic cells would decrease in a manner comparable to that of melanotic ones (amelanotic melanoma is well known to be much more sensitive to ionizing radiation than pigmented melanoma).

Experiments in Vivo. The results obtained confirmed the therapeutic effect of !["I-]MTB for melanotic melanoma observed in in vitro-in vivo experiments. !["I-]MTB injected i.v. into tumor-bearing mice caused almost complete abolition of pigmented lung colonies; however, it was ineffective in reducing the yield of lung metastases growing from amelanotic cells (Fig. 7; compare with Fig. 6). The number of pigmented colonies treated with !["I-]MTB did not exceed 0.03% of the total number of cells injected. Since the average number of control pigmented colonies growing at the lung surface only (taking no account of those inside the organ) was 705.5, the surviving fraction for pigmented melanoma after treatment with !["I-]MTB was estimated to be below 2.1%. The corresponding value for the amelanotic form amounted to 91% (the average numbers of control and treated metastases visible at the lung surface were 1078.5 and 977.5, respectively).

In addition, the animals themselves, when observed during the period of the experiments, did not show any symptoms suggesting toxic effects caused by the radioisotope. Present long-term investigations lasting for 1 year confirm this finding: !["I-]MTB used in the doses described does not affect either survival or body weight of treated animals. Sight disturbances were also not observed.

Comparison of the Therapeutic Efficacy of !["I-]MTB and !["I-]MTB

The investigations in vitro were repeated using !["I-]MTB to compare the therapeutic efficiency of the two radioisotopes. !["I-] is an Auger electron emitter. Experimental data revealed high
therapeutic effectiveness of such electrons with an efficiency comparable to that observed for α particles, i.e., radiation of a high LET value, if their emitter was present close to the cell nucleus (within a sphere with a radius not exceeding 50 nm) (10). Since in the pigmented melanoma cells [125I]MTB is bound to melanin within melanosomes, the cytoplasmic organelles many of which are situated in close proximity to the nuclear membrane, it was thought possible that high LET effects on the genome might be achieved.

The experiments were carried out as described for experiments with [211At]MTB. Melanotic and amelanotic cells were incubated with [125I]MTB for 2 h. The [125I]MTB radioactivities used were 119.5 and 619 kBq/1 x 10^5 cells. After the incubation, 1 x 10^5 cells suspended in 0.1 ml PBS were injected i.v. into mice.

The results obtained revealed again the selectivity of the therapeutic effect of méthylène blue labeled with the radioisotope; only pigmented melanoma cells were affected by [125I]-MTB. However, the degree of cell damage by [125I]MTB was significantly less than that with [211At]MTB. A dose of 619 kBq of the radioisotope (i.e., 56 times more than the radioactivity of [211At]MTB used in previous experiments) decreased the number of lung colonies to about 30/mouse (Fig. 8). Since the control value amounted to 1291, the surviving fraction was 2.3%. The corresponding value for [211At]MTB treatment with 11 kBq was less than 3.8%.

DISCUSSION

Results described in this paper confirmed our own previous observations concerning a high toxicity of radiolabeled méthylène blue for pigmented melanomas (7, 8).

The uptake and therapeutic effects of the compound were significantly dependent on tumor pigmentation and the radioisotope incorporated into méthylène blue. Therefore, it was important that a chosen radionuclide is stably bound to méthylène blue without changing its high affinity to melanin. Both [125I] and 211At fulfill this requirement: their introduction into position 4 of the aromatic ring of méthylène blue does not affect either the affinity or binding stability of the compound...
to intracellular melanin. Furthermore, both radioisotopes are characterized by a low range of radiation emitted and a short half-life which are required in a targeted radiotherapy to protect normal tissues surrounding the tumor with the incorporated radioisotope and to limit the total dose in pigmented organs, since methylene blue is accumulated also by normal cells containing melanin (the liver, eyes, brain).

In vitro studies showed that the radioactivity of $^{125}$I-MTB needed to obtain cytotoxic effects, considered as valuable therapeutically, was too high to be achieved in vivo. Therefore, Auger electrons emitted by $^{125}$I and with effects similar to those of a high LET appeared to be inefficient in this particular case; $^{125}$I-MTB incorporated in melanosomes distributed in the cytoplasm was presumably too far away from the genome. In contrast, $^{211}$At-MTB did exhibit significant therapeutic efficacy in in vivo system. The radiolabeled compound diminished the number of lung colonies to well below 3.8% of the control values when the treatment of pigmented melanoma was performed either in vitro or in vivo. The radioactivity of $^{211}$At-MTB required in vivo was only 2 orders of magnitude higher than that used in in vitro system to achieve a comparable effect. A similar exposure to $^{211}$At-MTB of nonpigmented melanoma appeared to be entirely ineffective in both in vitro and in vivo systems confirming a very high affinity of the compound to melanotic melanoma cells and excluding the possibility of contribution of the external irradiation; since in in vitro system cells of both sublines were incubated with $^{211}$At-MTB using identical experimental conditions, it is obvious that the difference in their survival was not due to the effects of an external irradiation during the period of exposure but was caused by the differences in the cellular uptake of $^{211}$At-MTB and its biological stability in melanotic and amelanotic melanoma cells.

The data presented in this paper allow calculations of lethal radiation doses from $^{211}$At-MTB deposited in melanotic melanoma tumors and corresponding doses for pigmented organs which could be at a particular risk of developing lesions caused by the incorporated radioisotope. Results presented in Fig. 1 enable an estimation of uptake of $^{[211]}$At-MTB and Na$^{211}$At by melanoma cells exposed to the radioisotope in the experimental conditions used for the investigations with $^{[125]}$I-MTB. Table 1 shows that $2.5 \times 10^{-5}$ Bq was accumulated by a single melanotic cell during 1.5 h of incubation with $^{[211]}$At-MTB. The incubation of amelanotic cells with $^{[211]}$At-MTB resulted in the radioisotope uptake almost 1 order of magnitude lower ($5.5 \times 10^{-6}$ Bq/cell) than that observed for pigmented melanoma cells and similar to the uptake achieved when Na$^{211}$At was used instead of $^{[211]}$At-MTB (2-3 $\times 10^{-5}$ Bq/cell, either pigmented or nonpigmented).

The uptakes of Na$^{211}$At by melanoma cells of both sublines and of $^{[211]}$At-MTB by amelanotic cells were comparable with the accumulation of $^{211}$At in V79 lung fibroblasts observed by Kassis et al. (9).

Melanosomes containing melanin in melanocytes are cytoplasmic organelles. $^{211}$At-MTB bound to melanin is, therefore, distributed in the cytoplasm as well. Since the shape of melanocytes is ellipsoid with mean length, width, and height of 12.5, 7.9, and 0.4 $\mu$m, respectively (12), the mean volume of a single melanocyte is $128.4 \times 10^{-12}$ cm$^3$. Taking into account that approximately 40% of tumor tissue consists of cells, 1 cm$^3$ of a melanoma tumor contains $3.2 \times 10^6$ cells. Since water is the main cell constituent (about 80%), it can be assumed that the weight of 1 cm$^3$ tissue is equal to 1 g.

The maximum uptake of $^{[211]}$At-MTB occurred after 1–1.5 h and a half-life of $^{211}$At is 7.2 h. Therefore, the actual radioactivity of $^{[211]}$At-MTB accumulated by each pigmented melanoma cell during 1.5 h incubation is $2.3 \times 10^{-4}$ Bq. This corresponds to $7.22 \times 10^3$ Bq/g tissue. Since the mean range of $\alpha$ particles from $^{211}$At is 60–65 $\mu$m, practically all energy will be deposited in the tumor. Assuming that the level of $^{[211]}$At-MTB present in cells is stable and the number of cells per g is unchanged, the number of decays per unit volume in 60 min is equal to $2.5 \times 10^5$ disintegrations/g (1 Bq = 60 dpm). Since the average energy per decay is 6.78 MeV, 1 MeV = $1.602 \times 10^{13}$ J and 1 Gy = $10^9$ disintegrations/g.

### Table 1 Uptake of $^{211}$At and $^{[125]}$I-MTB by B16 melanoma and V79 cells

<table>
<thead>
<tr>
<th>Cells</th>
<th>Radioisotope used</th>
<th>Time of incubation (h)</th>
<th>Radioactivity (Bq/10$^6$ cells)</th>
<th>Radioactivity accumulated (Bq/cell)</th>
</tr>
</thead>
<tbody>
<tr>
<td>V79*</td>
<td>$^{211}$At</td>
<td>4.0</td>
<td>3.0 $\times 10^3$</td>
<td>2.5 $\times 10^4$</td>
</tr>
<tr>
<td>V79*</td>
<td>$^{211}$At</td>
<td>4.0</td>
<td>1.1 $\times 10^3$</td>
<td>9.2 $\times 10^3$</td>
</tr>
<tr>
<td>Melanotic</td>
<td>$^{[125]}$I-MTB</td>
<td>1.5</td>
<td>1.1 $\times 10^3$</td>
<td>2.5 $\times 10^4$</td>
</tr>
<tr>
<td>Amelanotic</td>
<td>$^{[125]}$I-MTB</td>
<td>1.5</td>
<td>1.1 $\times 10^3$</td>
<td>5.5 $\times 10^3$</td>
</tr>
<tr>
<td>Melanotic</td>
<td>$^{211}$At</td>
<td>1.5</td>
<td>1.1 $\times 10^3$</td>
<td>2.2 $\times 10^3$</td>
</tr>
<tr>
<td>Amelanotic</td>
<td>$^{211}$At</td>
<td>1.5</td>
<td>1.1 $\times 10^3$</td>
<td>3.0 $\times 10^3$</td>
</tr>
</tbody>
</table>

* Kassis et al. (9).
$1 \times 10^{-3}$ J/g, the dose given during the first h amounts to $D = 2.7$ Gy, whereas the total dose does not exceed 30 Gy (see Fig. 9). The corresponding dose for amelanotic melanoma tumors would not exceed 6 Gy.

Previous studies (6) showed that most organs including the brain revealed a negligible level of radiolabeled methylene blue accumulation. Only the liver and eyes accumulated significant amounts of the compound. Therefore, both these organs could be at a potential risk from the deposited $^{[211]At}$MTB and radiation doses corresponding to those for pigmented melanoma have been calculated. The dose for a pigmented part of the eye (the choroid) in which the maximum uptake of MTB was determined to amount to 59% of that in melanotic melanoma, and taking no account of the varying level of the radiisotope present in the tissue over the time, is 18 Gy (Fig. 9). This dose corresponds to 0.4 MBq/g of the choroid and should be well tolerated by the eyes, since the most radiosensitive part of this organ is the lens and it was shown that single doses of up to 0.9 MBq of Na$^{[211]At}$ injected into the anterior chamber of the eye did not lead to any adverse effects either clinically or histologically (13). Therefore, $^{[211]At}$MTB localized selectively in the choroid and with a mean range of $\alpha$ particles in a tissue of 60 $\mu$m would not be expected to produce any pathological changes in the eye. [Therapeutic doses from $^{60}$Co used for a treatment of choroidal melanoma are in range of 120–180 Gy (14, 15).]

The liver would be exposed to a total dose of 1.1 Gy (Fig. 9). To calculate the dose it was necessary to take into account a biological half-life ($T_B$) of methylene blue in this tissue (= 2.3 days) and substitute the physical half-life of $^{211}$At ($T_P$) with the effective half-life

$$T_E = \frac{T_P \times T_B}{T_P + T_B}$$

which, for $^{[211]At}$MTB in the liver, is 6.37 h.

It should be pointed out that the above calculations do not take into account the effects of cross-firing which, in a solid tumor, will significantly increase the efficiency of cell killing by the deposited radioisotope. They also do not include additional estimations concerning a mean diameter of macroscopic tumor which exceeds the range of $\alpha$ particles emitted by $^{211}$At. Therefore, the actual total dose required to diminish cell survival to the level observed in the in vitro experiment, i.e., below 3.8%, will be much lower than the doses calculated.

The above considerations suggest that methylene blue labeled with a properly chosen radioisotope could be used for a highly selective and very efficient targeted radiotherapy of pigmented melanomas without damaging normal tissues either surrounding the tumor or accumulating the compound themselves. A high LET radionuclides with a short half-lives are of particular interest with respect to both their exceptional therapeutic effectiveness and protection of normal organs. Since most $\alpha$ particle emitters with more suitable half-lives than that of $^{211}$At possess very radiotoxic daughter elements, $^{211}$At with its almost optimal LET for endoradiotherapeutic effects of 98.84 keV/$\mu$m unit density tissue and easy binding to methylene blue is being used in further investigations concerning human melanomas.

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