Project 1D

DEVELOPMENT AND THERAPEUTIC APPLICATION OF INTERNALLY EMITTING RADIOPHARMACEUTICALS

PROGRESS REPORT

for the period September 1, 1979 - February 28, 1981

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Prepared for:

U.S. Department of Energy
Contract No. DE-AC02-76EV04115

ABSTRACT

This project is concerned with developing the potential of alpha-emitting radionuclides as agents for radiotherapy. Alpha emitters seem ideally suited for this application because their high linear energy transfer and short range permit the deposition of considerable energy in a very small volume of tissue. Unlike the beta particles of iodine-131 which have a range of about 1-2 mm in tissue, 5-7 meV alpha particles would traverse only a few cell diameters. Among the available α-emitters, astatine-211 appears most promising for testing the efficacy of α-emitters for therapeutic applications because 1) it has some chemical similarities to iodine, an element that can readily be incorporated into numerous proteins and peptides, 2) it has a half life that is long enough to permit chemical manipulation yet short enough to minimize destruction of healthy cells due to degradation of the label over time, and 3) α-emission is associated with 100% of its decays with no accompanying β emission.

If appropriate biological carriers can be labeled with an alpha emitter such as ^{211}At, they could be of great utility in several areas of therapeutic medicine where elimination of specific cell populations is desired. For example, astatine-211 labeled-monoclonal antibodies or other vehicles for tumor-directed localization might be useful for selectively destroying malignant cells. Of primary importance to the application of ^{211}At-molecules as radiotherapeutic agents is the development of suitable techniques for labeling proteins, peptides and other potential biologic carriers with ^{211}At. While previous attempts to astatinate proteins using standard iodination techniques have been unsuccessful, we have been able to effectively label proteins with astatine by first synthesizing an aryl astatide and then coupling this compound to the protein via an acylation reaction.

We are currently investigating several different aryl astatide-followed by-acylation approaches including an astatinated Bolton-Hunter type reagent using Concanavalin A (ConA) and melanocyte stimulating hormone (MSH) as model compounds. The relative utility of each technique will be evaluated by comparing the *in vivo* stability, specific activity, and immunospecificity of ^{211}At-ConA and ^{211}At-MSH labeled using each method. We will determine the extent of binding and radiotoxicity of these ^{211}At-substances to certain potential target cell lines so that the feasibility of this approach for the destruction of specific cell populations can be assessed. In addition, the *in vivo* radiotherapeutic effectiveness of ^{211}At-MSH against malignant melanomas will be determined. If these results are promising, this approach will be applied to labeling monoclonal antibodies, histocompatability antigens, liposome-antibody conjugates and other potential site-directing vehicles, and then investigate their radiotherapeutic effectiveness in the appropriate *in vitro* and *in vivo* models. The development of ^{211}At-particulates as colloids, resins, microspheres, and liposomes as potential agents for *in situ* radiation therapy is also being pursued. The aforementioned ^{211}At-particulates will be synthesized and their relative *in vivo* stability assessed. The radiotherapeutic effectiveness of the most promising systems will be compared against an ascites tumor and their deleterious side effects to normal tissue will also be evaluated.

I. Mediastinal lymphoscintigraphy reflects cell kinetics of developing malignant ascites

A number of observations suggest that ascitic tumour cells are capable of occluding lymphatic channels in the diaphragm and that this occlusion leads to the development of ascites. For example, Holm-Nielsen (1953) showed that, while the absorbtion of particulate materials through the diaphragmatic surfaces of mice with carcinomatous ascites was negligible, control mice showed diffuse mottling of diaphragmatic surfaces and substernal lymphatics. In mice bearing intraperitoneal (i.p.) tumour cells, Feldman et al. (1972) found diminished egress of labelled erythrocytes from the peritoneal cavity as well as tumour cell invasion of diaphragmatic lymphatics, before the development of frank ascites.

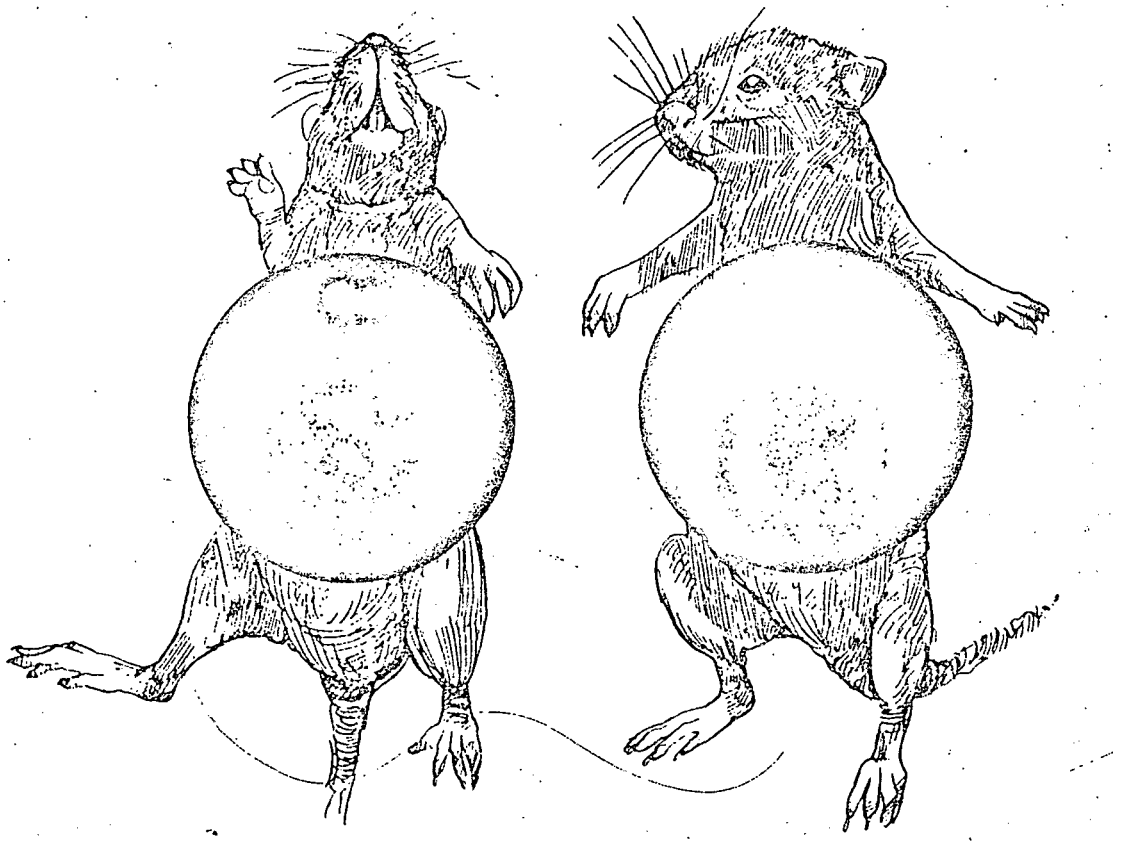

FIG. 1.

Representative mediastinal lymphoscintigrams. The animal on the left is a non-tumour bearing control; paired regions of activity are present within the mediastinum. The animal on the right had been injected with 10^6 tumour cells i.p. 1 day before study; there is no mediastinal activity. Equivocal mediastinal activity was observed only rarely.

Based on these observations, we speculated that the onset of lymphatic blockade was related to the absolute number of i/p/ tumour cells and that the rate of onset was dependent on the rate of tumour cell division.

Because the lymphatic channels of the diaphragm drain into the mediastinum, mediastinal lymph nodes (primarily those of the internal mammary chains) are normally visualized scintigraphically after the i/p/ or subcostal injection of

radiolabelled colloid; visualization does not occur, however, in the presence of overt ascites; (Atkins et al., 1970; Ege and Bronskill, 1978). Moreover, normal mediastinal lymphoscintigrams are observed in ascites of non-malignant origins (Coates et al., 1973). The mediastinal lymphoscintigram thus appeared to be a potentially sensitive indicator of incipient malignant ascites and a useful instrument for testing our hypothesis.

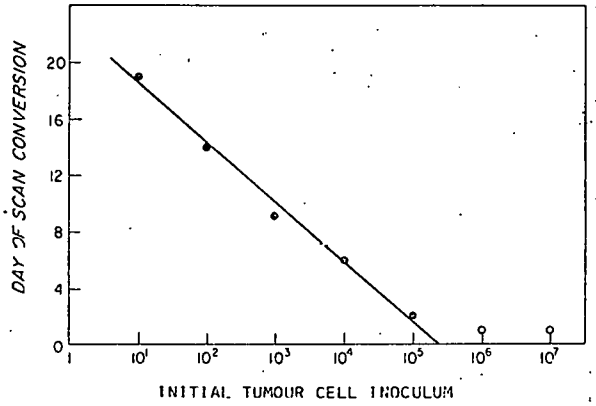

FIG. 2.

TABLE I

GENERATION TIMES AT LYMPHOSCINTIGRAM CONVERSION IN TUMOUR BEARING MICE

Initial tumour cell concentration	Day of scan conversion	Generation time* (h) at day of scan conversion
10^1	19	21
10^2	14	30
10^3	9	23
10^4	6	20
10^5	2	17

*Mean ± SD = 22 ± 5

The tumour used in these experiments arose spontaneously in the ovary of a C3H mouse and has been maintained in its ascitic form by serial i.p. transplantation in female C3HeB/FeJ mice (Jackson Laboratory). A regular relationship has been observed between the size of the tumour cell inoculum and the rate of weight gain as well as the median time to death (Bloomer and Adelstein, 1977).

Lymphoscintigraphy was performed two hours after the i/p. injection of 100-150 µCi $^{99}Tc^m$ sulphur colloid. Animals were sacrificed by cervical dislocation and scintigrams obtained in the anterior projection using a scintillation camera fitted with a pinhole collimator (Fig. 1).

The mean generation of ascitic tumour cells was calculated at the time of lymphoscintigram conversion by the repetitive labelling method (Steel, 1977). Mice were injected i.p. with 10 µCi ^{3}H-thymidine at two hour intervals over a 10-12 hour period. Two animals were sacrificed 30 minutes after each injection, tumour cells harvested, washed, fixed in methanol:acetic acid (3:1) and dropped on to slides. Autoradiography was performed with NTB-2 nuclear track emulsion (Eastman Kodak).

The median day of lymphoscintigraphic conversion from visible to non-visible mediastinal activity was recorded as a function of the initial tumour cell inoculum (Fig. 2). A linear relationship was observed between the day of scan conversion and log initial tumour cell innoculum within the range $10\text{-}10^5$ cells. The y-intercept of this line $(2.3 \pm 0.2) \times 10^5$ cells, is presumed to be the minimum number of tumour cells necessary for scan conversion to occur. Thus, when cells exceeding this number (10^6 or 10^7) are innoculated, conversion of the lymphoscintigram is observed within one day. No weight gain or ascites was detected in mice at the time of scan conversion nor was there any microscopic evidence of tumour invading diaphragmatic tissue at the time the scans changed.

Additional studies were undertaken to confirm the observation that the conversion of mediastinal lymphoscintigrams was related to the presence of a critical number of tumour cells. First, silica particles in the range of 5-20 μ were injected i.p. and lymphoscintigraphy performed one and two days later. There was no conversion of lymphoscintigrams with silica doses of 10-60 mg despite the fact that toxicity was observed when the amount of silica injected exceeded 30 mg. Second, because this tumour is antigenic and appears to elicit an intense inflammatory response in diaphragmatic tissue, mice were treated with 5 mg hydrocortisone i.p. twice daily for 1-4 days before injection of 10^6 tumour cells. This anti-inflammatory treatment failed to prevent scan conversion 24 hours later and is consistent with our hypothesis that scintigraphic conversion is dependent on tumour cell burden.

If scan conversion takes place when tumour cells exceed a critical number, the mean population doubling time (30 ± 1 h) can be estimated from the slope of the straight line in Fig. 2, i.e. from the time it takes a given tumour cell inoculum to reach this critical size. The compare this value with that obtained by a more traditional method, we performed repetitive labelling experiements (Steel, 1977) on cells (Table I). The mean generation time obtained in this manner is 22 ± 5 h. The fraction of labelled cells reached a plateau at 0·8-0·9, suggesting the presence of non-cycling or quiescent cells and/or heterogeneity of tumour cells, some of which have very long cell cycle times. Although not generally considered an important factor in early ascites, cell loss may also be present. Both quiescent cells and cell loss would be expected to lengthen the population doubling time observed by lymphoscintigraphy.

Although it is surprising that a binary all-or-none visualization of mediastinal lymphatics should yield reliable quantitative estimates, these experiements demonstrate that mediastinal lymphoscintigraphy is a very sensitive and possible specific indicator for small numbers of i.p. tumour cells. Moreover, it appears that scan conversion can be used to predict in vivo the cell kinetics of developing malignant ascites. Thus, it is possible that lymphoscintigraphy may have special significance in the clinical evaluation of patients with ovarian cancer not only as a diagnostic test for early peritoneal dissemination (Ege and Bronskill, 1978) but also as a marker for assessing the kinetics of tumour cell division and the response to treatment.

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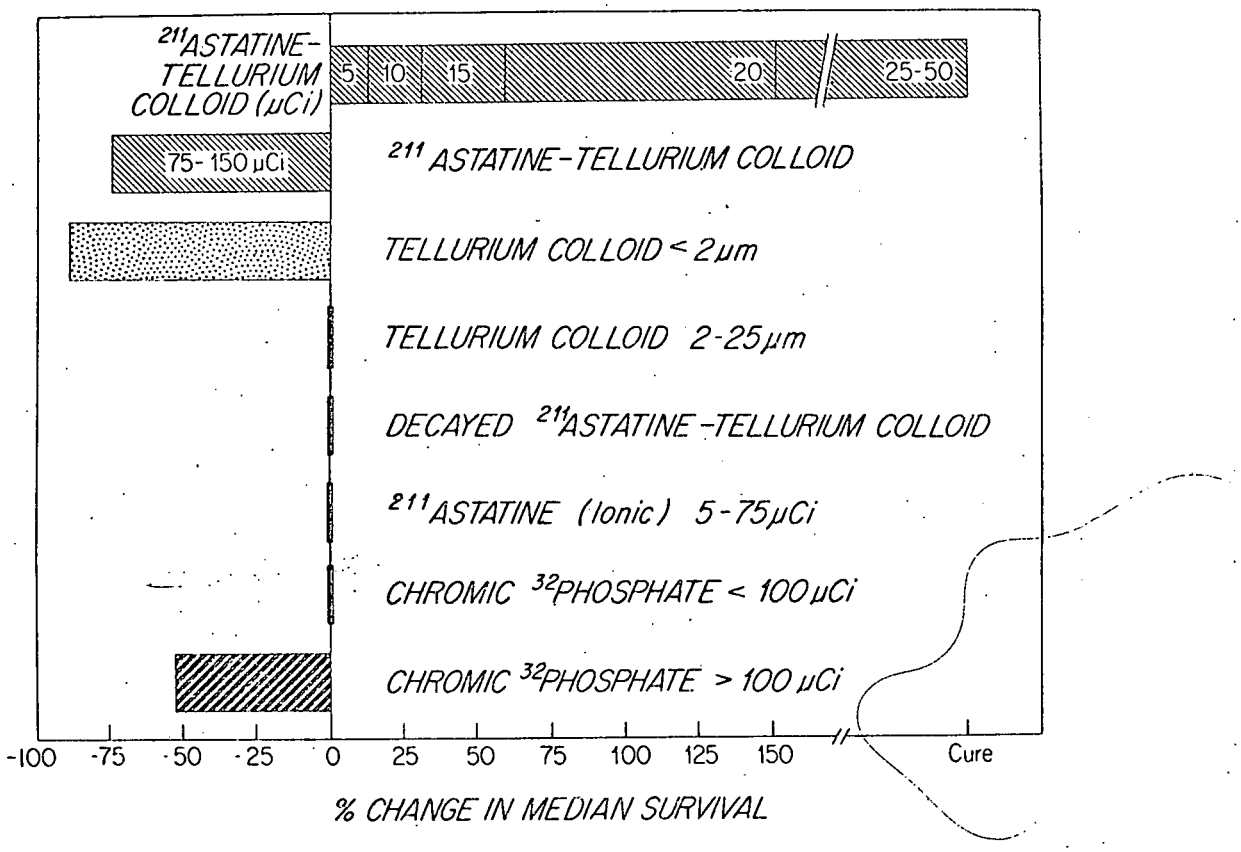
II. ^{211}At Radiotoxicity

Among the currently available alpha emitting radionuclides, astatine-211 appears most promising. Astatine (from the Greek astatos meaning unstable) was discovered in 1940 by Corson et al. when bismuth was bombarded by alpha particles in the 60 in. Berkeley cyclotron. The name given to this element with an atomic number of 85 indicates that instability of all its isoptopes to radioactive decay (Corson et al., 1947); the longest lived isotope, ^{210}At, appears in the Periodic Table. Despite its being the fifth halogen element, astatine has considerably different chemical properties from iodine, its nearest halogen neighbor; nonetheless, ionic astatine is concentrated by thyroid tissue, although less avidly than iodine (Lavrukhina and Pozdnyakov, 1970). Cyclotron produced ^{211}At decays by a complex doubly branched pathway to lead-207 directly by alpha emission (42%, E_α=5.9 MeV) and indirectly through electron capture (58%) to polonium-211 which almost spontaneously decays by alpha emission (E_α=7.5 MeV). The physical half life is 7.2 hours. The average alpha particle energy is 6.8 MeV and the range in water 60 µm; the linear energy transfer is 113 keV per µm averaged over a 60 µm path length. Clonogenic survival assays in V-79 Chinese hamster cells using ionic ^{211}At yield a linear dose response relationship with no shoulder in the low dose region; furthermore, the oxygen enhancement ratio of ~1.5 is close to that of other densely ionizing particles (Harris et al., 1978).

We have investigated the therapeutic efficacy of an ^{211}At colloid in an experimental malignant ascites tumour model and report that the radiocolloid can be curative with minimal normal tissue toxicity. Under these circumstances, the therapeutic ratio should be highly favorable because the decay characteristics of ^{211}At are appropriate and the colloid vehicle represents a form of directed administration that permits physical separation and hence protection of the sensitive mucosal lining of the intestine from the emitted radiations.

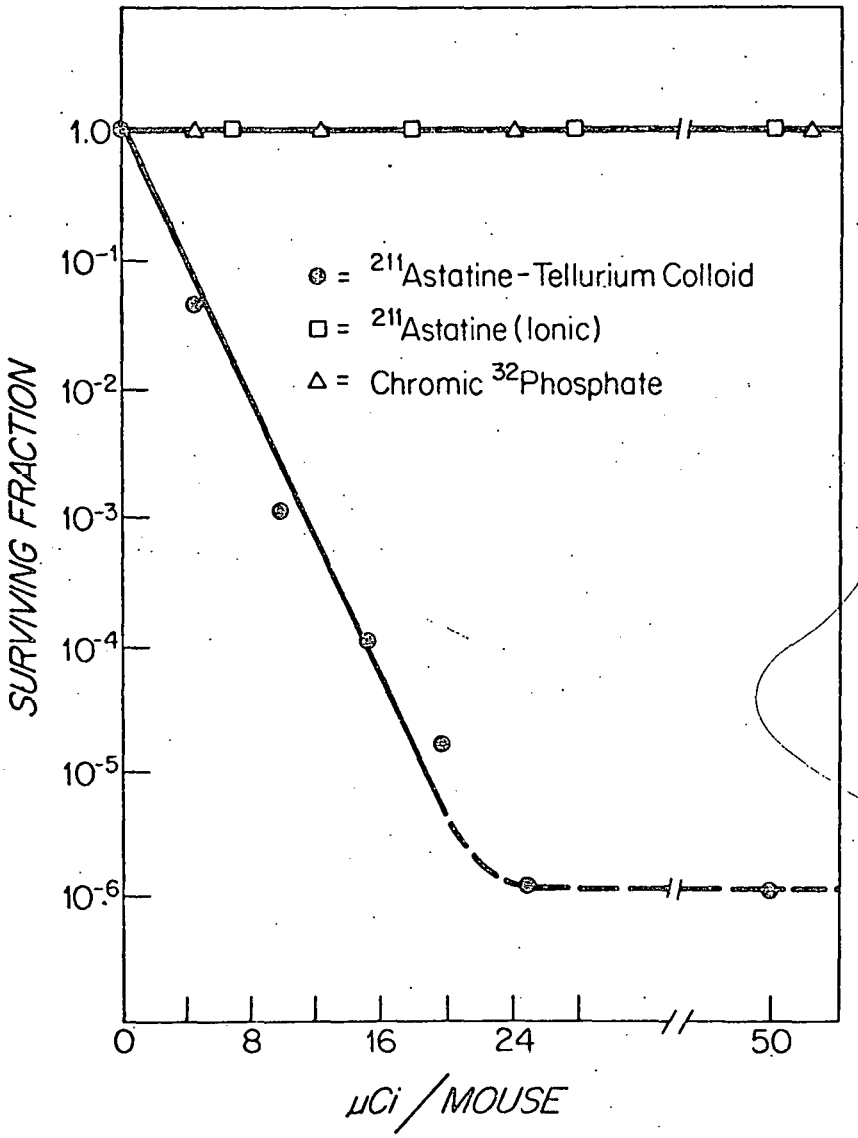
^{211}At was produced in the 60 in. cyclotron of the Brookhaven National Laboratory by (α, 2n) reaction of 28 MeV alpha particles with bismuth-209. Upon completion of a run, ^{211}At was isolated from the target by distillation at 700°C and collected in a sodium hydroxide trap. Preparations were determined to be chemically pure and carrier-free by elemental analysis as well as gamma and alpha spectrometry. Finely ground tellurium was chosen as the colloidal material because of its strong avidity for astatine at neutral and acidic pH. Tellurium particle sizing was performed by first grinding elemental tellurium to a fine powder. The powder was suspended in distilled water, shaken and sedimented by gravity for 5 minutes. The sediment contained particles ≥40 µm in size and was discarded. The supernatant was again shaken and sedimented by gravity for 10 minutes; the supernatant contained particles <0.1 µm and was discarded. The sediment thus contained particles between 0.1 and 40 µm and was filtered through a 25 µm pore screen. The filtrate was collected and washed. The final colloidal material was obtained as a pellet by centrifugation at 700 rpm for 5 minutes; the final particle size ranged between 2 and 25 µm. Acidified with nitric acid, ^{211}At was added to the tellurium colloid and shaken for 1 minute at room temperature. The ^{211}At colloid was washed in distilled water, centrifuged and resuspended in saline. Supernatants from the washes contained <0.5% free ^{211}At.

The tumour used in these experiments arose spontaneously in the ovary of a C3H mouse and has been maintained in its ascitic form by serial intraperitoneal (i.p.) transplantation in female C3HeB/FeJ mice. In addition to being expressed as the percent change in median survival, therapeutic efficacy can be expressed as a cellular surviving fraction because of the regular relationship observed between median survival and initial tumour cell inoculum (Bloomer and Adelstein, 1977). Such a calculation implies little or no repair of radiation damage, an assumption that is substantiated by previous in vitro studies (Harris et al., 1978).



Single graded doses of ^{211}At colloid were administered by i.p. injection 24 hours after the i.p. injection of 10^6 tumour cells. Mice treated with <50 μCi of ^{211}At colloid demonstrated a dramatic increase in median survival that was proportional to dose. Doses of 25 and 50 μCi were curative in all animals; although some acute morbidity at these doses was manifested by weight loss and change in fur nap, there were no acute deaths. Doses >75 μCi of ^{211}At colloid were highly toxic and resulted in death in 5 to 7 days, presumably the result of gastrointestinal injury. The following control experiments were performed. Non-radioactive tessurium colloid 2 μm in size was lethal within 3 days, presumably the result of pulmonary insufficiency. Tellurium particles between 2 and 25 μm, ionic ^{211}At in doses up to 100 μCi, and decayed ^{211}At colloid were non-toxic and

did not prolong median survival. The therapeutic efficacy of ^{211}At colloid was compared with that of ^{32}P as chromic phosphate. This radionuclide decays exclusively by beta-minus emission, has a physical half-life of 14 days and a maximum depth of penetration of 8 mm. Furthermore, it is used clinically as adjuvant therapy in many cases of early stage ovarian cancer. In this tumor model, chromic phosphate had no therapeutic efficacy - at doses of 5 to 100 µCi, there was no prolongation of median survival, and at doses >100 µCi, mice generally died within 7 days of treatment, presumably due to gastrointestinal toxicity.



When the therapeutic efficacy of ^{211}At colloid is expressed as a cellular surviving fraction, the dose response curve decreases linearly to a survivng fraction of 10^{-6} at 25 µCi, beyond which a plateau exists. This plateau represents the lower limit of this *in vivo* assay system and does not necessarily represent the maximal cell killing to be obtained with 25 or 50 µCi ^{211}At colloid. Ionic astatine and ^{32}P chromic phosphate show no cytotoxicity in the range of doses where ^{211}At colloid is therapeutic. The D_{37} for ^{211}At colloid *in vivo* is 1.6 µCi compared to 1.0 µCi *in vitro*. Tumour-bearing animals cured by ^{211}At colloid therapy have been followed for at least 200 days. Long term survivors are sleek, agile and alert. Histological sections of major body organs show no evidence of tumour and are unremarkable except for some interstitial fibrosis and particulate foreign bodies in the lung secondary to escape of ^{211}At colloid from the peritoneal cavity.

In summary, ^{211}At colloid is higly effective in the treatment of experimental malignant ascites. The most compelling explanation for the increased therapeutic efficacy of ^{211}At in comparison with ^{32}P is the direct and densely ionizing character of alpha particle decay. In comparison with ^{32}P, the ratio of mean energy deposition per unit path length is 11.3. Despite a path length that is more than a hundred-fold longer, the sparce ionization of ^{32}P appears to be insufficient to cause cell death in this tumour model. Because one day old ascites tumour cells are generally well oxygenated, the increased radiobiological effectiveness of ^{211}At is probably not related to an oxygen effect. These experiments form the basis for further investigation and development of alpha emitters for radiation therapy.

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