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Radioimmunotherapy of infection with ^{213}Bi -labeled antibodies

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Abstract

Bismuth-213 (^{213}Bi) (physical half-life 46 min) is a beta-emitter (97%) and an alpha-emitter (3%) which decays to short lived alpha-emitter Polonium-213 and could therefore be used as an in vivo generator of alpha particles with the energy of around 8 MeV. ^{213}Bi has been successfully used during the last decade in both clinical and pre-clinical work for radioimmunotherapy (RIT) of cancer with ^{213}Bi -labeled monoclonal antibodies (mAbs). RIT has been proposed as a novel technology for treatment of infectious diseases. ^{213}Bi -labeled mAbs have been successfully used for treatment of experimental fungal, bacterial and viral infections with transient or none hematologic toxicity. The mechanisms of RIT of infection with ^{213}Bi -labeled mAbs include “direct” killing of cells and induction of apoptosis. In vivo RIT results in decrease of inflammation in infected organs. Among the delivery vehicles for RIT of infection whole IgG1 mAbs seem to be the most suitable in terms of the highest uptake in the target organs and the lowest - in normal tissues. RIT with alpha-emitter ^{213}Bi involves the application of established technology developed for the treatment of malignancies to infectious diseases. The development of RIT for infectious diseases is potentially easier than its application to tumor therapy given antigenic and tissue perfusion differences between sites of microbial infection and tumor infiltration. Nevertheless, considerable pre-clinical and clinical development work is likely to be required to learn how to use RIT for infection optimally.

Keywords

Bismuth-213; radioimmunotherapy; fungal infection; bacterial infection; viral infection; apoptosis; radiobiological mechanisms; inflammation

INTRODUCTION

Bismuth-213 (^{213}Bi) (physical half-life 46 min) is a beta-emitter (97%) and an alpha-emitter (3%) which decays to short lived alpha-emitter Polonium-213 and could therefore be used as an in vivo generator of alpha particles with the energy of around 8 MeV. ^{213}Bi is conveniently obtained from the $^{225}\text{Ac}/^{213}\text{Bi}$ generator [1]. During the last decade there has been a lot of interest towards using ^{213}Bi in both clinical and pre-clinical work: encouraging results have been obtained with ^{213}Bi -labeled monoclonal antibodies (mAbs) in patients with myeloid leukemia and melanoma [2,3]; in pre-clinical studies ^{213}Bi -labeled mAbs proved to be effective in radiation- and chemoresistant leukemia [4], disseminated gastric cancer [5], breast cancer metastases in the neu-N transgenic mouse model [6]; and somatostatin-receptor-targeted ^{213}Bi was therapeutically effective in human pancreatic adenocarcinoma cells [7]. Very recently, the mathematical modeling and feasibility analysis of targeting cancer stem cell using ^{213}Bi have been described [8].

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There is enormous need for new approaches to therapy of infectious diseases due to increasing number of immunosuppressed individuals in whom antimicrobial therapies are not effective; growing prevalence of diseases caused by highly resistant microorganisms, some of which are not susceptible to any currently available antimicrobial agents; and a dearth of new antimicrobial drugs in the development pipeline. To further complicate matters new microbes are regularly identified and for many there are no available therapies and there is the specter of biological weapons. Several years ago we performed proof-of-principle experiments in the experimental *Cryptococcus neoformans* infection aimed at demonstrating the usefulness of radioimmunotherapy (RIT) in treatment of infection [9]. One of the therapeutic radionuclides investigated for RIT of infection was ^{213}Bi which was selected for two main reasons: powerful alpha-emission which would allow for the destruction of radiation-resistant microbial cells and short physical half-life which would allow for the delivery of cytotoxic radiation doses within the time-span which matches the doubling time of microorganisms (20 – 120 min for bacteria and fungi, respectively). Here we review the applications of ^{213}Bi -labeled antibodies in RIT of fungal, bacterial and viral infection, the choice of antibodies with different isotypes or size as delivery vehicles for ^{213}Bi , the mechanisms of action of RIT on microbes and the safety issues of RIT treatments.

RADIOIMMUNOTHERAPY OF VIRAL, BACTERIAL AND VIRAL INFECTIONS

RIT of *Cryptococcus neoformans* fungal infection and biofilms

We explored the potential efficacy of RIT against an experimental fungal infection using *C. neoformans* [9]. CN is a major fungal pathogen that causes life-threatening meningoencephalitis in 6–8% of patients with AIDS. Cryptococcal infections in immunocompromised patients are often incurable because antifungal drugs do not eradicate the infection in the setting of severe immune dysfunction [10,11]. *C. neoformans* provides a good system to study the potential usefulness of RIT because there are excellent animal models available, well characterized mAbs to *C. neoformans* antigens exist, and immunotherapy of *C. neoformans* infection with capsule polysaccharide-binding antibody 18B7 is already in clinical evaluation [12]. The therapeutic studies with ^{213}Bi -18B7 mAb were performed in A/JCr mice infected systemically with *C. neoformans*. Mice treated with ^{213}Bi -18B7 mAb lived significantly longer than mice given irrelevant labeled IgG1 or PBS (Fig. 1a). We used a labeled irrelevant mAb ^{213}Bi -MOPC21 to control for the possibility that Fc receptor binding by the radiolabeled IgG to phagocytes at the site of infection might result in non-specific killing of *C. neoformans* cells. Remarkably, on day 75 post-therapy, 60% of mice in ^{213}Bi group were alive after treatment with 100 μCi (3.7 MBq) ^{213}Bi -18B7 ($P < 0.05$). The survival of A/JCr mice was dose dependent: while 50 μCi ^{213}Bi -18B7 produced no therapeutic effect, both the 100 and 200 μCi doses prolonged animal survival (Fig. 1a). Interestingly, the 200 μCi ^{213}Bi -18B7 dose was less efficient, possibly due to the fact that it may have approached the MTA (maximum tolerated activity) for this particular combination of antibody and radioisotope.

We have subsequently extended the RIT of *C. neoformans* to fungal biofilms [13]. The use of indwelling medical devices—pacemakers, prosthetic joints, catheters—is rapidly growing and is often complicated by infections with biofilm-forming microbes that are resistant to antimicrobial agents and host defense mechanisms. We investigated the use of polysaccharide-specific mAbs as delivery vehicles for targeting biofilms with ^{213}Bi . ^{213}Bi -18B7 mAb (IgG1) penetrated cryptococcal biofilms, as shown by confocal microscopy and caused a 50% reduction in biofilm metabolic activity (Table 1). In contrast, when the IgM mAb 13F1 labeled with ^{213}Bi was used - there was no penetration of the fungal biofilm and no damage. Unlabeled 18B7, ^{213}Bi -labeled non-specific mAbs, and gamma and beta types of radiation did not have an effect on biofilms. The lack of efficacy of gamma and beta radiation probably reflects the radioprotective properties of polysaccharide biofilm matrix. Our results indicate that *C.*

neoformans biofilms are susceptible to treatment with antibody-targeted alpha radiation, suggesting a novel option for the prevention or treatment of microbial biofilms on indwelling medical devices.

RIT of *Streptococcus pneumoniae*

As a model of bacterial disease for RIT we have chosen *S. pneumoniae* which is an important cause of community-acquired pneumonia, meningitis, and bacteremia. The problem of pneumococcal disease is exacerbated by increasing drug resistance. Furthermore, patients with impaired immunity are at high risk for invasive pneumococcal infections. Our earlier encouraging results with RIT of *C. neoformans* provided the impetus for applying RIT to an experimental bacterial infection. However, in contrast to fungal infections which are usually chronic, disseminated bacterial infections can progress rapidly and bacteria replicate much faster than fungi. For example, the replication rates of *S. pneumoniae* and *C. neoformans* are 20 and 120 min, respectively. Cryptococci have average diameters that are approximately 20 times larger than pneumococci [14] and, consequently, present significantly larger targets for RIT than bacterial cells. It remained to be seen if the sensitivity of a microorganism towards particulate radiation depended on the amount of DNA per volume matter. In addition, these two microbes elicit different inflammatory responses with neutrophils predominating in pneumococcal infections and macrophages predominating in cryptococcal infections. Consequently, the success of RIT against experimental cryptococcal infection could not necessarily be extrapolated to bacteria from the prior fungal study.

We investigated the feasibility of applying RIT approach to the treatment of *S. pneumoniae* infection by evaluating the susceptibility of *S. pneumoniae* to ^{213}Bi -labeled antibody *in vitro* and in an animal infection model [15]. For the specific antibody carrier we utilized a human monoclonal antibody D11, which binds to pneumococcal capsular polysaccharide 8 (PPS 8). The experiment showed that a greater percentage of mice survived in the ^{213}Bi -D11-treated group relative to the untreated group ($P < 0.01$) (Fig. 1b). In contrast, administration of unlabeled D11 (5 μg) did not prolong survival in comparison to untreated mice ($P > 0.05$). Radiolabeled control IgM also did not have any therapeutic effect ($P > 0.05$). Mice in control groups succumbed to bacteremia on Day 1–3, while mice treated with 80 μCi ^{213}Bi -D11 mAb demonstrated 87–100% survival. Furthermore, mice treated with ^{213}Bi -D11 were not bacteremic at 3, 6 and 10 h post-treatment as measured by colony forming units (CFUs) in their blood as well as on days 3 and 14. Thus, this study established the feasibility of RIT for the treatment of bacterial infections.

Subsequently we extended the RIT of bacterial infection to *Bacillus anthracis* that is a powerful agent for bioterrorism and biological warfare underscoring the need for additional effective therapies for anthrax. Administration of ^{213}Bi -labeled mAbs to anthrax toxins, namely, protective antigen and lethal factor, prolonged survival of A/JCr mice infected with *B. anthracis* germinated bacterial cells [Rivera J. et. al., unpublished observations].

RIT of HIV-1 infection

The HIV-1 epidemic continues to present a major threat to global health. Highly active antiretroviral therapy (HAART), a combination of drugs that inhibits enzymes essential for HIV-1 replication, can reduce the viremia and the on-set of opportunistic infections in most patients and prolong survival. However, HAART regimens are complicated and have significant toxicity. Replication-competent virus that persists in infected cells provides a source of virus that emerges rapidly after the cessation of HAART. A modality that targets and kills HIV-1 infected cells combined with HAART would have a major impact on the treatment of acute exposure and elimination of persistent reservoirs of infected cells. We hypothesized that it is possible to apply RIT to viral diseases by targeting infected host cells which express viral

antigens on their surface. Such strategy would eliminate the reservoirs of infected cells and viral cellular factories. This approach could be particularly useful for the treatment of drug-resistant HIV strains which present an ever-increasing problem [16].

We examined the efficacy of RIT for treatment of HIV infection *in vivo* with a radiolabeled HIV envelope-specific human anti-gp41 mAb 246-D radiolabeled with ^{213}Bi or ^{188}Re [17]. For these studies human peripheral blood mononuclear cells (PBMCs) infected with HIV-1_{JR-CSF} were injected into the spleens of SCID mice and the mice were treated IP with radiolabeled mAbs 1 hr post-infection. The mice were evaluated 72 hr later for the presence of residual HIV-1-infected cells by quantitative co-culture [18]. Treatment of mice with ^{213}Bi -246-D mAb 246-D administered after intrasplenic injection with HIV-1_{JR-CSF}-infected human PBMCs dramatically reduced the number of HIV-1-infected cells as subsequently determined by quantitative co-culture (Fig. 1c). In contrast, the administration of equivalent amounts of “cold” mAb 246-D or of a radioisotope-coupled irrelevant control mAb did not reduce the average number of infected cells detected in the SCID mouse spleens. These results establish that RIT can effectively target and kill HIV-1-infected human PBMCs *in vivo*. The demonstration of efficacy of RIT against HIV provides a proof-of-principle for the concept of treating viral infections by targeting viral-infected cells and this approach could potentially be applied to other chronic viral diseases like hepatitis C [19].

CHOICE OF DELIVERY VEHICLES FOR RIT OF INFECTION

To identify the best delivery vehicle for RIT of *C. neoformans* we have performed comparative evaluation of capsular polysaccharide-specific antibody 18B7 and its F(ab)2 and Fab fragments which were radiolabeled with ^{111}In as a surrogate for ^{213}Bi . For this purpose the biodistribution of ^{111}In -labeled 18B7 and its F(ab)2 and Fab fragments was done in AJ/Cr mice systemically infected with *C. neoformans* [20]. Whole 18B7 demonstrated 5% ID/g uptake in the lungs at 24 hr, its F(ab)2 - around 1%, while the uptake of ^{111}In -Fab in the lungs was very low at 0.1% ID/g (Fig. 2a). In cancer RIT, Fab fragments show significantly lower percent injected dose per gram tumor (%ID/g) than intact mAb despite having a smaller molecular weight, as a consequence of lower affinity resulting from losing the contribution of polyvalence to avidity. The elevated kidney uptake of F(ab)2 and Fab, which is always observed in cancer RIT due to the lower molecular weight of the fragments in comparison with the whole mAb, also manifested itself in our study. We concluded from the study that whole 18B7 mAb seems to be better delivery vehicle for delivery of ^{213}Bi to the sites of infection than its F(ab)2 and Fab fragments.

MECHANISMS OF RIT WITH ^{213}Bi -LABELED MABS

Given that RIT of infectious diseases is a relatively young field, the mechanisms by which RIT is effective against microbes are uncertain. Even in oncology where the anti-neoplastic effects of RIT have been investigated for more than 25 years the mechanisms are still debated. The major radiobiological mechanisms of cancer RIT are considered to be “direct hit” and “cross-fire” effects, both of which can promote apoptosis and cell cycle redistribution [21]. We have recently evaluated clonogenic survival, membrane permeability with propidium iodide (PI), metabolic activity with a 2,3-bis(2-mety-4-nitro-5-sulfophenyl)-5-[(phenylamino) carbonyl]-2H-tetrazolium hydroxide (XTT) reduction assay, and induction of apoptosis with fluorochrome labeled inhibitor of caspase FLICA binding assay of *C. neoformans* cells subjected to alpha-particles delivered to fungal cells by ^{213}Bi -18B7 [22]. We found that ^{213}Bi -18B7 mAb did not have much effect on the cell membrane (Fig. 3a) and promoted apoptosis to some degree (Fig. 3b). ^{213}Bi -18B7 mAb delivered “directly” profoundly decreased the metabolic activity of fungal cells (Fig. 3c). We concluded that clonogenic survival proved to be the most practical measure of assessing RIT efficacy, by virtue of reflecting a combination

of multiple mechanisms leading to fungal cell death. Cells which are alive post-RIT treatment, but not replicating, may or may not contribute to the disease. We also performed microdosimetry calculations to estimate the doses which are being delivered to fungal cells by radiolabeled mAbs. For ^{188}Re -labeled mAb 18B7 the dose of 50 cGy eliminated ninety percent of *C. neoformans* cells while for ^{213}Bi -18B7 mAb this dose was two-fold lower - 25 cGy [23]. Knowledge of the radiobiological mechanisms of RIT will allow creation of more effective protocols for RIT of opportunistic fungal infections.

As mAb-mediated protection in the setting of *C. neoformans* infection may be in part due to changes in cytokine expression [24,25], we also investigated whether RIT affected IL-2, IL-4, IL-10, TNF- α , and IFN- γ expression in mice treated with ^{213}Bi -18B7 mAb [26]. These cytokines are representative of Th1- and Th2-related cytokines important in the effective immune response to CN infection [24,27,28]. RIT was associated with changes in lung and brain cytokine expression (Fig. 4a,b). There was a significant ($P<0.05$) reduction in the expression of TNF- α , INF- γ , IL-10 (brains and lungs), IL-4 (lungs), and IL-2 (brains) in RIT-treated mice relative to the untreated controls. There was a trend toward increased IL-2 in the lungs of RIT-treated mice ($P=0.06$); also unlabeled mAb 18B7 caused some increase in IL-4 in the brains of the mice. It is conceivable that local radiation could have affected inflammatory cell number or location thus resulting in an alteration in cytokine expression. Given the hypothesis that death in mice with CN infection can be caused by host-inflammatory damage [27], the decrease in the levels of cytokines in RIT-treated animals raises the tantalizing possibility of benefit from reduced inflammatory damage.

SAFETY ISSUES FOR RIT OF INFECTION

Based on the data accumulated in clinical RIT of cancer, the primary toxicity of RIT of infection is likely to be bone marrow suppression. We note that RIT of non-Hodgkin lymphoma (NHL) is effective in patients who have received several unsuccessful courses of chemotherapy and consequently, have depleted bone marrow reserves and weakened immunity not unlike immunocompromised patients susceptible to infections. Nevertheless, the application of RIT to infectious diseases will require optimization of the dose to ascertain and minimize toxic effects.

In our studies of RIT of fungal, bacterial and viral infections we evaluated the hematologic toxicity of radiolabeled antibodies in mice by platelet counts [9,15,17,29]. The platelet counts nadir usually occurs 1 week after radiolabeled antibody administration to tumor-bearing animals [30,31]. For example, in C57BL/6 mice infected systemically with *S. pneumoniae* and treated with 80 μCi ^{213}Bi -D11 mAb only a transient drop in platelet count was noted on day 7 post-treatment with counts returning to normal by day 15 [15]. Also, in the RIT of HIV study we did not observe any changes in platelet counts in mice treated with 100 μCi ^{213}Bi -246-D mAb on days 4, 8 and 15 post-treatment in comparison to the infected non-treated controls, with platelet counts being stable at $(1.5\pm0.2) \times 10^9$ platelet/mL blood. This lack of hematologic toxicity can be explained by the very specific targeting of radiolabeled antibodies to the microbes/infected cells. In fact, one of the advantages of using RIT against infections as opposed to cancer is that, in contrast to tumor cells, cells expressing microbial antigens are antigenically very different from host tissues and thus provide the potential for exquisite specificity and low cross-reactivity. It should be noted that in all our studies the radiolabeled mAbs were administered IP, and IP administration of the radiolabeled mAbs was reported to be better tolerated than IV route [32].

As a safety issue specific for *C. neoformans* infection which often affects the lungs – we evaluated long-term pulmonary toxicity of treatment with ^{213}Bi -18B7 mAb [29]. BALB/c mice with chronic lung infection were treated with ^{213}Bi -18B7 mAb and then after six months their

lungs were evaluated histologically for signs of long-term radiation damage. Lungs of treated mice had no evidence of radiation fibrosis.

In addition, when using a radioactive therapy in patients there is always a concern of long-term effects such as neoplasms arising from radiation-induced mutations. However, this risk would likely be outweighed by the benefits of treating or preventing life-threatening infections.

CONCLUSIONS

RIT with alpha-emitter ^{213}Bi involves the application of established technology developed for the treatment of malignancies to infectious diseases. The development of RIT for infectious diseases is potentially easier than its application to tumor therapy given antigenic and tissue perfusion differences between sites of microbial infection and tumor infiltration. Nevertheless, considerable pre-clinical and clinical development work is likely to be required to learn how to use RIT for infection optimally.

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Fig. 1.

Radioimmunotherapy of experimental fungal, bacterial and viral infections with ^{213}Bi -labeled mAbs: a) Kaplan-Meier survival curves for A/JCr mice infected IV with 10^5 *C. neoformans* cells 24 hr prior to treatment with 50–200 μCi ^{213}Bi -labeled mAbs. Animals injected with PBS (phosphate buffered saline) or 50 μg "cold" 18B7 served as controls; b) RIT of *S. pneumoniae* infection with ^{213}Bi -labeled mAbs in C57BL/6 mice. 8–10 mice per group were used. Mice were infected IP with 1,000 organisms 1 hr before treatment with mAbs; c) RIT of SCID mice infected intrasplenically with JR-CSF-infected human PBMCs with ^{188}Re - and ^{213}Bi -labeled human anti-gp41 mAb 246-D: mice received either 20 μg "cold" anti-gp41 mAb 246-D, 100 μCi (20 μg) ^{213}Bi -1418 or 80 μCi (20 μg) ^{188}Re -1418 as isotype-matching controls, 80 μCi (20 μg) ^{188}Re -246-D, or 100 μCi (20 μg) ^{213}Bi -246-D IP 1 hour after infection with PBMCs. In some experiments mice were given 80 μCi (20 μg) ^{188}Re -246-D IP 1 h prior to infection with PBMCs.

Fig. 2. Biodistribution of ^{111}In -18B7 IgG1 and its F(ab)2 and Fab fragments in AJ/Cr mice systemically infected with 10^5 *C. neoformans* cells 24 hr prior to administration of radiolabeled mAbs: a) 24 hr post administration of mAbs; b) 48 hr.

Fig. 3. Comparison of the percentage of non-viable *C. neoformans* cells by colony forming units (CFUs), PI permeability, apoptosis levels by FLICA and metabolic activity by XTT assay. The cells were treated with ^{213}Bi -18B7 mAb or with its "cross-fire" component only: a) CFUs and PI permeability; b) apoptosis levels by FLICA; c) XTT results for ^{213}Bi -18B7; d) CFUs for ^{213}Bi -18B7.

Fig. 4.

The levels of cytokines in the lungs and the brains of AJ/Cr mice infected IV with *C. neoformans* and treated with ^{213}Bi -18B7 mAb. Three groups of 10 AJ/Cr mice were infected IV with 10^5 *C. neoformans* cells and 24 h after infection: treated IP with 150 μCi ^{213}Bi -18B7 (30 μg per animal), or with 30 μg unlabeled 18B7, or left untreated.

Table 1Susceptibility of *C. neoformans* B3501 biofilms to ^{213}Bi -labeled mAbs^a

Antibody (radioactivity level, μCi)	% Metabolic activity	
	B3501 biofilm	B3501 planktonic cells
^{213}Bi -18B7 (15)	96 (5)	75 (0.3)
^{213}Bi -18B7 (30)	47 (5)	31 (1.5)
^{213}Bi -MOPC21 (15)	99 (0.8)	94 (0.3)
^{213}Bi -MOPC21 (30)	100 (2)	94 (0.6)
^{213}Bi -13F1 (15)	100 (1)	86 (2)
^{213}Bi -13F1 (30)	98 (1)	34 (1)

^aThe metabolic activity of age-matched biofilms and planktonic cells was measured using a 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino) carbonyl]-2H-tetrazolium hydroxide (XTT) reduction assay. The values represent the averages of three XTT measurements, and standard deviations are given in parentheses. 18B7 and MOPC21 mAbs - IgG1; 13F1 mAb- IgM.