Preclinical Studies And Clinical Trial Of Targeted Alpha Therapy For Cancer

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1. INTRODUCTION

The control of cancer continues to be an elusive objective. While current therapies can be effective, in general they fail to change the prognosis of the more common cancers. Melanoma has special significance in Australia, and while surgery for early stage melanoma can be curative, there is no systemic therapy available to control metastatic melanoma Melanoma, colorectal, prostate and breast cancer are all curative on early stage presentation. Thus an early systemic therapy could be effective in preventing the development of metastatic cancer if targeting of micrometastases, ie isolated cells, cell clusters and preangiogenic lesions, could be achieved. While current therapies have considerable value and play an important palliative role, the majority of patients with metastatic cancer will die of their disease.

1.1 Targeting Cancer Cells

Two targeting approaches are investigated in this paper:
a) monoclonal antibodies (mab) against antigens expressed by melanoma, colorectal cancer and prostate cancer.
We use the mab 9.2.27 that is directed against an antigen expressed on most melanoma (9.2.27) cell surfaces [1]; the c30.6, (chimeric version of the mouse antibody 30.6) and 35A7 are expressed against antigens on colorectal cancer cell surfaces; the mab J591 targets an external domain of the prostate specific membrane antigen (PSMA) [2]. PSMA expression is seen in all prostate cancers and in most metastatic lesions.
b) plasminogen activation inhibitor (PAI-2) which targets the urokinase plasminogen activator (uPA) for breast and prostate cancers. uPA has high affinity and specificity for the cell-surface localized receptor uPAR, where it can be inhibited by PAI-2 [3,4]. PAI-2 is more likely involved in pericellular proteolysis in which uPA-mediated proteolysis plays an important role, such as tumour cell invasion and metastasis. Exogenously administered PAI-2 targets uPA expressing tumour cells, particularly those that have metastasised or are likely to. Targeting uPA-over-expressing cells by its natural inhibitor remains an unexploited mode of attack for prostate cancer malignancy.

1.2 Targeted Alpha Therapy

Alpha emitting radionuclides [5] emit alpha particles with energies up to an order of magnitude greater than most beta rays, yet their ranges are two orders of magnitude less as alpha particles have a linear energy transfer (LET) which is about ~100 times greater. This is manifested by a high relative biological effectiveness (RBE). As a result, a much greater fraction of the total energy is deposited in cells with alphas and very few nuclear hits are required to kill a cell. Consequently, 100 fold enhancement in radiation dose would be delivered to the nucleus of a cancer cell if a "smart" carrier is employed to take the alpha-radionuclide to that cancer cell. The availability of the alpha nuclides has been the major problem in the past for their large scale scientific and clinical application. While studies have been carried out on ^{149}Tb, ^{211}At and ^{212}Bi with encouraging results, Bi-213 can be obtained from the Ac-225 generator and the Memorial Sloan Kettering Cancer Centre has pioneered the use of this isotope in the first phase 1 clinical trial for advanced leukaemia [6]. McDevitt et al [7] and Allen [8] have recently reviewed these and other data that point to the potential efficacy of alpha-immunotherapy for subclinical and clinical disease.

1.3 Requirements for control of metastases

A therapeutic modality must be able to control the growth of metastatic cancer by killing isolated cells and contiguous non-targeted cancer cells, while sparing dose limiting stem cells [9]. Only alphas can kill isolated cells at tolerable dose limits, whereas the low LET of betas makes this a very difficult task within human dose tolerance limits. Alphas have ranges of several cell diameters and can traverse the nucleus from any position in the cell, causing double strand breaks in DNA that are difficult to repair. Further, the high cytotoxicity of alphas means that targeted cells can be killed even for quite low levels of epitopic expression; some hundreds or thousand times lower than for betas for the same endpoint,

reducing the effect of variable epitope expression. Alphas can reach through several cell diameters, killing any cell in their path with only a few traverses of the nucleus, eg contiguous non-targeted cancer cells, but can also spare distant stem cells because of their limited range of effect.

2. MATERIALS AND METHODS

2.1 Radioisotopes, chelators, cell lines

Actinium-225 was purchased from United States Department of Energy. Bismuth-213 was eluted from the Actinium-225 column using 0.15 M distilled and stabilised Hydriodic Acid. Two chelators were used to couple the radioisotopes to the carrier, viz cyclic anhydride of DTPA (cDTPA) and DTPA-CHX-A''[10,11]. Both are known to form stable complexes with monoclonal antibodies. The chelation and labeling methods have been described by Rizvi et al [11]. Instant Thin Layer Chromatography (ITLC) was used to separate the radiolabeled and/or cold conjugate from the free radioisotope.

MM138: A non-pigmented melanoma cell line supplied by Dr A Henniker, Westmead Hospital; positive to the anti-melanoma mab 9.2.27.

HT-29: Colorectal cancer cell line, positive to anti-colorectal cancer (CRC) mab c30.6 supplied through Dr R Ward, Garvan Institute, Sydney.

SV174T: Colorectal cancer cell line, positive to anti-CRC mab 35A7, supplied by University Hospital Geneva.

MDA-MB-231: Breast cancer cell line, positive to uPA .

PC3, LNCap-LN3: Prostate cancer cell lines supplied by the Prof P Russell, Prince of Wales Hospital, NSW Australia, and DR C Pettiway, MD Anderson Hospital, Houston TX, USA. PC3 is positive to uPA, LN3 positive to the mab J591 against the PSMA.

2.2 Cell survival assay

The CellTiter 96 Aqueous non-radioactive cell proliferation assay (Promega, WI, USA) was used for cell survival assays.

2.3 In Vivo Model

Balb/c and ARC (S) *nu/nu* mice were used in all studies. Mice received sc inoculations of 1-2 million cancer cells (in 100 –150 μL sterile PBS) in the flank (melanoma) or mammary fat pads (breast cancer). Mice received local or systemic (ip) injections at 2 d post-inoculation. This model has been shown by us to simulate micrometastatic cancer. The University of New South Wales Animal Care Ethics Committee approved all mouse studies.

2.4 Immunohistochemistry

The expression of target antigens in cultured cells, frozen sections, paraffin sections and lymph node tissues obtained from sacrificed mice were investigated using an indirect conjugated peroxidase or alkaline phosphatase anti-alkaline phosphatase (APAAP) immunostaining methods. A mouse mab # 394 (American Diagnostic Inc) was used for uPA expression.

2.5 TUNEL assay for apoptotic cells *in vitro* and *in vivo*.

Apoptotic cells in cancer cell lines and tumour sections were detected using standard TUNEL methodology with the TdT-fragELTM *in situ* apoptotic detection kit according to the manufacturer's instruction (Oncogene Research Products Bosston, MA).

3. RESULTS

3.1 Radioisotope production and labeling

Ac:Bi generator technology has been developed to prepare and test several TAT approaches for melanoma [11, 12]; leukaemia [13]; colorectal cancer [14]; breast cancer [15] and prostate cancer [16,17]. Labeling efficiencies of up to 85-95% were obtained for both cDTPAa and CHX-A" chelators, which produced stable AIC and API with ~20% leaching after 2 half lives in serum.

3.2 In vitro studies

37% survival values (D_0 μCi) were measured for the alpha conjugates. Melanoma cell line MM138 treated by ^{213}Bi-9.2.27 (2.1 μCi); breast cancer cell line MDA-MB-231 treated by ^{213}Bi-PAI2 (2.3 μCi); prostate cancer cell line LNCaP-LN3 treated by ^{213}Bi-J591 (4.1 μCi); prostate cancer cell line PC3 treated by ^{213}Bi-PAI2 (3.4 μCi); colorectal cancer cell line HT-29 treated by ^{213}Bi-C30.6 (2.8 μCi); leukaemia HL-60 treated by ^{213}Bi-WM53 (2.7 μCi)

3.3 In Vivo studies

3.3.1 Melanoma

In vivo results have shown that local TAT can completely inhibit the growth of sc melanoma in nude mice with only 25 μCi injection at 2 days post-inoculation [13]. Further, large melanomas can be completely regressed by intra-lesional TAT of ~100 μCi. There is no evidence of recurrence at 4 months.

3.3.2 Breast Cancer

Human breast cancer tumours xenografts were successfully induced in nude mice and xenografts appeared at 2-3 weeks post-inoculation. After 4-5 weeks, cancer cells were identified histologically in sections taken from primary tumour tissue and the axillary and cervical lymph nodes. Invasion of the regional lymph nodes by the breast cancer cells was clearly seen, thus establishing a genuine metastatic cancer model. Similar sections were stained with mab against uPA. Positive uPA antigenic expression in both the primary tumour and in metastatic cancer cells in the lymph nodes were identified, indicating that metastatic cancer cells could be targeted by API (^{213}Bi-PAI2). Indeed, sub-cutaneous local injection of API completely inhibited the growth of sc breast cancer in nude mice with only a 25 μCi injection at 2 days post-inoculation [18]. However, unlike melanoma, intralesional API injections were not able to regress established tumours, but in all cases a partial response was observed.

3.3.3 Prostate

For the LNCaP-LN3 animal model, a single local injection at 2 days post-inoculation showed complete inhibition of tumourogenesis with 50 μCi AIC (^{213}Bi-J591), whereas non-specific TAT (^{213}Bi-9.2.27) at the same dose had no inhibitory effect. Intra-lesional injection of tumours with volume ~40-60 mm^3 were completely regressed for 50μCi of AIC, whereas 100 μCi was required to regress 70-80 mm^3 tumours. For the PC3 animal model, a single local or i.p. injection of API was able to completely regress the growth of tumours and lymph node metastases at 2 days post s.c. inoculation, and obvious tumour regression was achieved in the therapy groups compared with control groups with API when the tumours measured 30-40 mm^3 and 85-100 mm^3.

3.4 Toxicology

Our toxicology studies show that nude mice can tolerate up to 6 mCi/kg of alpha chelate (API) by systemic administration, causing a 5-10% short term weight loss. Up to 10 mCi/kg of AIC via intra-lesional injection causes <20% short term weight loss [18].

3.5 Apoptosis

A high percentage of TUNEL-positive LNCaP-LN3 cells were observed after AIC, and for PC3 and MDA-MB-231 cells after API incubation.

3.6 Clinical trial

A phase 1&2 trial has commenced at St George Hospital for intra-lesional TAT of secondary melanoma. Three patients received the starting dose of 50 μCi, which was increased by 100 μCi for the next 3 patients, up to a maximum value of 1 mCi. 15 subjects have been treated up to 450 μCi , and TAT has been found to be safe and efficacious.

4. CONCLUSION

In vitro results in this study show highly selective cytotoxicity to targeted cancer cells. The *in vivo* data demonstrate that complete inhibition of tumourogenesis by single local or systemic (i.p.) TAT at 2 days post-inoculation is possible for all cancers examined. Intralesional injection of larger melanomas can achieve complete regression without recurrence, but this is not the case for breast cancer tumours. API can reduce lymph node metastases in the PC3 prostate model.

The successful results for melanoma point to the potential application of local TAT in the management of secondary melanoma when surgery is undesirable. Anti-tumourogenesis has implications for the control of subclinical micrometastatic cancer after excision of a high risk primary tumours or isolated limb infusion, and ultimate control of early stage micrometastases. Apoptosis may be the lethal pathway of prostate and breast cancers.

This study shows that TAT may well be an efficacious and safe therapeutic modality and that AICs and API may be able to address the problems of isolated and small cell cluster toxicity, preangiogenic lesions and variable antigenic expression.

ACKNOWLEDGEMENTS

We are grateful to PI2 Pty Ltd for providing PAI-2; the Scripps Research Institute for providing the 9.2.27 mab; G. Smith, Garvan Institute, NSW, Australia for providing the c30.6 mab; BZL Biologics, Inc for providing the J591 mab. The API work was funded by the US Department of Defence (#DAMD17-99-1-9383). The prostate AIC work was funded by the Masonic Lodge Carlton-Kogarah No. 305.

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