CERN SPSC 2005-037
SPSC-M-743
October 31, 2005

Status Report and Future Plans for Experiment AD-4 Biological Effectiveness of Antiproton Annihilation

Michael H. Holzscheiter
for the AD-4 Collaboration.

Summary

A first round of experiments conducted in 2003 and 2004 at CERN and at TRIUMF have shown a significant enhancement of the biological effective dose ratio (BEDR) for antiprotons compared to protons. These results have been submitted for publication (see appendix B). Preliminary analyses of data available for carbon ions have indicated that the BEDR of antiprotons is also significantly higher than for carbon ions, but the uncertainty of the carbon analysis is too high to allow a definite statement. To overcome this shortfall we have proposed a direct comparison experiment using carbon ions at the GSI facility. This experiment was approved for beam time for 2006 and 2007 and will be conducted in collaboration with the biophysics group of Prof.G. Kraft at GSI.

Parallel to the biological measurements we have spent a significant amount of our beam time on studies of different approaches to the dosimetry of the antiproton beam. Two problems need to be overcome. Not only is the response to high LET radiation for most dosimeters not well understood, but in addition, the pulsed time structure of the beam makes the instantaneous dose rate too high for most standard dosimeters to handle. We have collected a number of data sets using different methods (TLD's, Alanin, GAFchromic films) which we can compare to theoretical predictions using Monte Carlo calculations.

Following the experimental work at CERN and TRIUMF we concluded that further studies are clearly warranted. We also realized that we could make best use of the relatively scarce beam time available, by developing proper tools for model calculations of antiprotons annihilating in biological targets which could then be certified using existing and future data sets. We have assembled an informal working group bringing together experts in three major Monte Carlo codes, GEANT4, MCNPX, and SHIELD-HIT. We have started to compare these codes using as simple as possible beam/target combinations and have identified first problems in the application of some of these codes to our specific problem. We are continuing our work to resolve these conflicts and to identify the best approach to the complex problem of antiproton annihilation in biological targets. This should allow us to generate the necessary data set which, in combination with an adaptation of the Local Effect Model developed at GSI, can then be used to assemble a complete dose planning tool. Our goal is to use a few well defined experiments to validate the Monte Carlo package and then use computer modeling to compare the efficacy of antiprotons to carbon ions and protons in typical clinical treatment scenarios.

While the development of real time imaging technology was not explicitly described in the original proposal, this advantage of antiprotons over all other particle beam methods was mentioned and at the very end of our run time in October 2004 we conducted first demonstration experiments using two different detector types. Further experiments are necessary to identify the advantages and disadvantages of the two methods and to generate a working design for a real time imaging system.

I. **Biological Measurements:**

To study the biological effectiveness of antiprotons we defined the term Biological Effective Dose Ratio (BEDR) as the ratio of the biological effects in the entrance channel ('plateau') and the annihilation region ('peak') of an antiproton beam entering a biological target. As this measurement is self contained for a specific type of particle beam (antiprotons, protons, carbon ions, etc) this definition alleviates the need for absolute dosimetry vs. penetration depth. The cross comparison between particle types is simply performed by comparing the BEDR for antiprotons to the BEDR for protons or carbon ions obtained using the identical experimental set-up and the identical definition of biological endpoint studied. In our case the experiment consisted of injecting a beam of (nominally) 50 MeV protons or antiprotons into a target of V-79 Chinese Hamster cells, immobilized in gelatin and kept at a temperature below 4 ^{o}C from the time of initial sample preparation until the point of plating the cells from a specific depth in the target in a culture medium. The analysis was based on a clonogenic assay, extracting the particle fluence which resulted in a 20% survival in both the plateau and the peak region. These results are described in detail in the attached paper submitted to Science Magazine for publication. The summary of the results is shown in figure 1 below, exhibiting for the specific experiment under study a BEDR for antiprotons of 9.8, compared to 2.5 for protons.

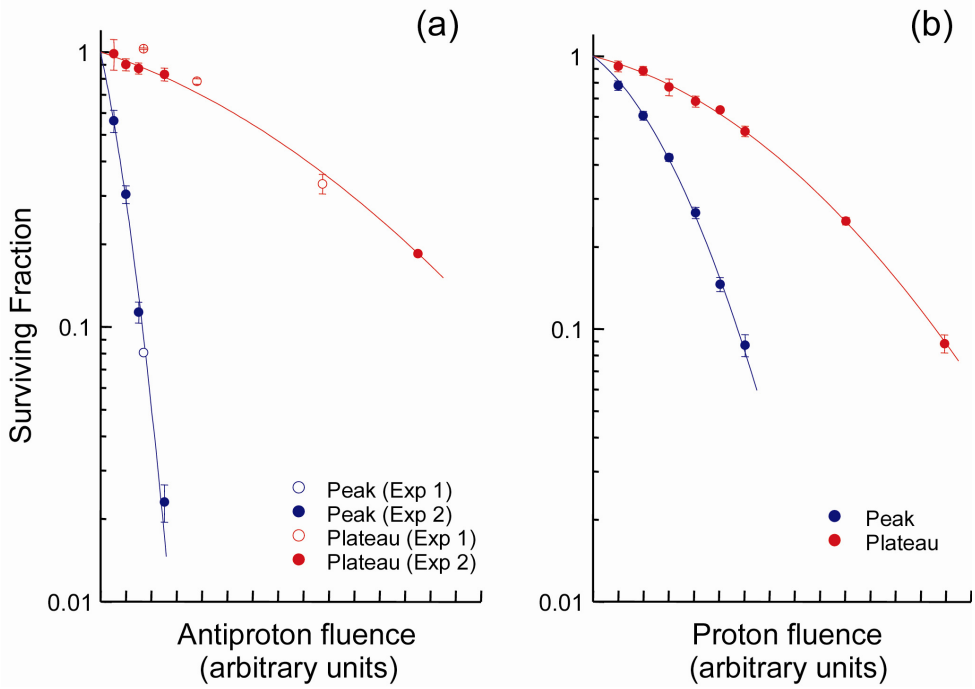

Figure 1: Survival for Peak and Plateau regions of V79 Chinese Hamster cells irradiated with an antiproton beam (a) and a proton beam (b) of 50 MeV kinetic energy. The BEDR for 20% survival is 9.8 and 2.5 respectively for the two cases.

For continuing studies we have defined two main objectives:

1. Using beam geometries more closely resembling treatment relevant situations. We propose to perform a set of experiments using an antiproton beam of higher starting energy offering a penetration depth of around 10 – 15 cm. In this case the natural straggling will spread out the Bragg peak enough so we can use our standard slicing protocol without adding a multi step degrader, therefore eliminating the problem of dose modulation in the Bragg peak. In addition, in a dedicated experiment, the Bragg peak will be spread out (SOBP) in such a way as to provide a flat dose (within 5%) over a depth of ~5cm.

Clonogenic survival measurements will be made as a function of depth in both the entrance region (plateau) of the beam as well as within the Bragg peak. These clonogenic survival measurements will allow determination of BEDR and (assuming that accurate dosimetry becomes possible from either Monte Carlo calculations or from the dosimetry development mentioned below) also RBE.

Even though the measurements of BEDR/RBE in beams in which the Bragg peak has been spread out are considered the most clinically relevant, the interpretation is complicated by the complexity of the antiproton annihilation event. Although the physical dose within the Bragg peak can be made constant, the relative contribution to dose from antiprotons of various energies and their annihilation products changes throughout the peak. It is thus expected that the BEDR/RBE will also change throughout the SOBP. Assuming that the BEDR/RBE depth relationship is known for a beam of specific energy, it is theoretically possible to predict this value. Therefore the initial measurement of the BEDR/RBE for a non-spread (un-modulated) beam is an important input for subsequent work.

All these experimenyts will be complemented by determinations of BEDR/RBE for protons (Aarhus) and carbon ions (GSI) using beam parameters designed to have similar depth dose characteristics as those for antiprotons.

2. Determination of the OER/RBE relationship for antiprotons and the influence of DNA repair. Over the past several decades it has become clear that several factors influence the response of mammalian cells to conventional low LET irradiation. Two of the most important factors are the level of oxygenation and the repair capacity of the cell. For conventional irradiation, oxygen provides an approximately 3-fold reduction in dose required to kill cells. Consequently, hypoxic cells in tumors are thought to limit the efficacy of current radiotherapy. For high LET radiation it has been observed that the OER is much reduced, likely due to the fact that a larger fraction of DNA damage is caused directly by the radiation itself as opposed to byproducts of water irradiation. Thus, with high LET radiation, hypoxia has a smaller consequence to the treatment outcome. When evaluating the potential merit of new radiation treatments, such as antiprotons, it is thus important to characterize the dose modifying effect of oxygen. We propose to extend our experimental setup to allow irradiation under conditions of hypoxia. Cell samples will be made hypoxic prior to loading. Irradiation tubes will be filled within a hypoxia workstation and subsequently sealed within a second tube containing a deoxygenated glycerine solution. This second tube will seal the inner tube from the environment allowing transport and irradiation under normal conditions at CERN. Irradiation of these tubes will be conducted using a higher range of doses in order to produce a survival response over the same range as that for aerobic conditions. We expect that the OER will be similar in the plateau region of the curve, but drop significantly in the peak.

For low LET radiation, the vast majority of DNA damage is effectively repaired. This is easily observable in cells deficient for DNA double-strand break (DSB) repair, which are several fold more sensitive to low-LET irradiation. However, for high LET radiation the influence of DNA repair is smaller. This is due to the fact that the type of damage induced by high LET is more complex and more difficult to repair even in repair proficient cells. The difference in response between repair proficient and repair deficient cells gives an indication of the relative fraction of repairable lesions. This is also an important parameter for evaluation of new radiation treatments. A number of elegant studies with carbon ions have demonstrated this fact. We propose to use both repair proficient and deficient cells in these experiments, in order to directly compare the contribution of DNA repair on clonogenic survival as a function of depth/energy in the antiproton beam. All our measurements performed and proposed are using V79 Chinese Hamster cells. This cell line has a radiation survival response typical of many other tumor cells, including human tumor cell lines. Importantly, this cell line has been used frequently to assess the biological properties of other particle beams including protons and carbon ions and as such acts as a 'reference' for comparison of studies. For the studies involving the influence of DNA repair, we propose to use genetically matched cell lines that have been derived from the V79 cells. These cells differ only in the expression of important DNA repair genes. This will eliminate other potential contributions to radio-sensitivity that may arise due to additional genetic differences.

II. Dosimetry Studies

One of the important elements in the understanding and subsequent use of radiation therapy is the determination of the physical dose delivered to the tissue, in the entrance channel of the radiation, in the tumour itself, and in the peripheral region (outside the direct beam path). Knowledge about the physical dose is necessary in combination with the biological effect of the radiation for adequate dose planning.

The main challenge is that, due to the annihilation of the antiprotons, the particle field is highly mixed, consisting of both low- and high- energy particles like photons, neutrons, pions, muons, protons, and nuclear fragments. Most, if not all, dosimeters are dependent on the particle type and the LET (linear energy transfer), and hence different dosimeters are needed to obtain the real physical dose. An additional complication arises in the present experiment, since the beam presently available at the CERN AD is pulsed, which excludes some types of standard dosimeters. Dosimeters applicable in a pulsed beam include films, Thermo Luminescent Detectors (TLD's), Alanine Detectors and so-called "bubble detectors" (for neutron measurements). If a quasi-dc beam could be developed, more standard dosimeters like ionization chambers could be used. We have performed initial studies with a variety of detectors but need to increase our data set in order to make conclusive statements about the relative sensitivity of different detectors for antiproton annihilation and to be able to benchmark Monte Carlo calculations.

Specificity of the dose to particular particles, for example neutrons, will be obtained with dosimeters of varying sensitivity to particular particle types. Additionally irradiation tracks in GAFChromic films will be investigated to provide information about the LET. The hereby acquired information about the particle energy spectrum is useful for benchmarking Monte-Carlo simulations, which are needed for optimizing dose planning systems for both physical dose and biological effect. In the peripheral region the neutron field in particular will be investigated in order to assess

the risk of stochastic radiation effects. The prime example here is the use of "bubble detectors" sensitive to neutrons with only a low sensitivity to low LET radiation. Similarly a comparison between ^{6}Li and ^{7}Li TLD's can be used to determine the neutron doses and background.

III.Peripheral Damage:

Initial studies have shown no or low biological effect on cells located outside the direct beam. As this is one of the most critical issues in radiation therapy, these studies need to be intensified and expanded. Based on our previous experience we propose three different experimental techniques to assess the degree of peripheral damage that may be caused outside of the primary antiproton beam. Two of these techniques are based on identifying the presence of DNA damage in mammalian cells placed at various distances outside of the antiproton beam. The third technique is the clonogenic assay as used for the biological measurements.

Comet Assay: Prior to performing the comet assay on an irradiated sample cell viability will need to be tested and analyzed with a light microscope. If the viability in cell sample is to be lower than 70% comet assay can not be performed due too high percentage of apoptotic and necrotic cells that would interfere with the results. After this preparatory step the alkaline comet assay will be carried out on the irradiated samples and the control samples of cells simultaneously using the exact same method. The quantification of DNA damage in individual cells of samples embedded on slides under alkaline conditions is to be done according to Singh et al. (1988). The analysis is performed with a fully automated image analysis system that acquires images of individual cells, computes the integrated intensity profiles for each cell, and then evaluates the range of derived parameters. Tail length and tail moment are used to describe the amount of damage inflicted to a cell. Migration length is related directly to fragment size and is proportional to the extent of DNA damage. Tail intensity represents a percentage of DNA that migrated from the nucleus into the tail and is proportional to the number of alkali label sites. The analysis is performed on approximately 100 cells per slide and statistical averages are derived.

The study of peripheral damage using the comet assay will be conducted at the Institute for Medical Research and Occupational Health in Zagreb, Croatia. This institute has many years of experience using this assay for the study of industrial workers exposed to low level pollutants. Our initial studies were performed there, as and the results obtained encouraged us to continue using this method as one of the ways of attacking this important problem.

Gamma-H2AX: In the past several years a new technique has emerged allowing the measurement of DNA double strand breaks (DSBs) with exceptional sensitivity. DSBs are considered the most important DNA lesion that occurs after radiation treatment. Previous efforts to measure DSBs required using very large doses (~200Gy) in order to create enough damage to be measurable. The new technique is based on identifying a particular phosphorylation event on the histone protein H2AX. This protein becomes rapidly phosphorylated at the sites of DSBs (within minutes) and can be detected with an antibody. Individual DSBs in cells can thus be counted by an observable foci. Moreover, the removal of the gamma-H2AX foci correlates with repair of the DSB. This technique has been used recently to measure DNA damage after very low doses of radiation, such as those given in a common CT radiological exam. We propose to develop this technique to look for evidence of DSB in the peripheral area of the antiproton beam.

The experimental protocol will be identical to that used for assessment of clonogenic survival within the primary antiproton beam. Following solidification at 4C, the tubes containing cells will be placed in a radial direction away from the primary annihilation point. Gel slices will then be made at various distances away from the annihilation point. Cells from these slices will be harvested using methods identical to that used for the clonogenic assay. These cells will then be attached to a microscope slide using a Cytospin, and then fixed and stained with antibodies against gamma-H2AX. 1000 cells will be examined by microscopy and the average number of gamma-H2AX foci determined. Results will be compared with non-irradiated controls. For these experiments 3 different antiproton doses will be used to validate a dose response relationship. In a subset of these experiments, the rate of gamma-H2AX loss (DNA repair) will also be evaluated by allowing various periods of repair time prior to fixation of the cells and examination of foci.

Clonogenic survival: In addition to measurement of gamma-H2AX foci, cells isolated as described above will be examined for clonogenic survival. For these experiments we will use 5 different antiproton doses. Sufficiently large fluences of antiprotons will be used to ensure that the level of peripheral damage is sufficiently high to become measurable by clonogenic assay. This is a very important experiment to define the level of peripheral damage in a biologically relevant context.

With the exception of the high does irradiations for the clonogenic assay studies all experiments on peripheral damage can be performed parasitically to the main clonogenic studies described in section one.

III. Real Time Imaging.

The annihilation of antiprotons in a target offers the possibility of true real-time imaging of the energy deposition at very low dose. We have been studying two different methods, one using the high energy gamma's from the conversion of neutral pions and the other one relying on the detection of the charged pions from the annihilation event.

The system proposed to be used for the detection of the gamma's is based on a system developed for medical imaging applications by BioScan, S.A. in Geneva. It consists of: (a) a converter/scintillator which emits photons when traversed by high energy gammas; (b) a large area flat panel a-Si:H detector matrix which detects photons of visible light with high efficiency; (c) a fast real-time electronic system for readout and digitization of images, protected from radiation damage by its peripheral layout and additional shielding; and (d) appropriate computer tools for control, on-line and off-line analysis, reproduction of images and network transfer.

In order to reconstruct a 3D image of the energy deposition profile using this system a mask needs to be placed between the annihilation vertex and the detector. This mask will allow only those gamma's to reach the detector which travel parallel to the mask's channels, therefore producing a true 2D shadow of the source of the particles. Using several 2D images from different observation angles one can, in principle, reconstruct the full 3D image.

Early tests of the system showed that the converter/detector combination, originally developed for 30 MeV gamma's is capable of adequately detecting the high energy gamma's in this application. Placing a simple slit between the target source and the detector allowed identification of background to noise levels. Recent Monte Carlo studies revealed issues concerning the production of secondary showers in the

wall of a mask with structures sufficiently small to reach high resolution shadow images. Further work, both theoretically and experimentally, will be necessary to validate or reject this method for our application.

Alternatively one can use standard silicon pixel detectors to detect the track of a charged particle traversing several layers of detector planes and then reconstruct the particle track back to the annihilation vertex. Doing this from several angles (preferably approaching 4π) one can achieve a full 3D tomography of the annihilation volume.

At the very end of our run period we were able to perform some preliminary tests using two planes of a prototype chip of the ALICE silicon detector. Continuing work will be necessary to quantify the results from these tests and to further develop the necessary strategies to reconstruct the 3D annihilation volume. In our current application this is further complicated significantly by the high instantaneous dose rate.

IV. Monte Carlo Development.

Considering the scarcity of antiproton beam time available, the development of a computer model package capable of predicting with high accuracy the physical dose deposited by an antiproton beam of specific energy, energy spread, and spatial profile in a human tissue equivalent target is an extremely important task. One of the main challenges faced in this task is the complex character of the annihilation event. A variety of different secondary particles with a broad spectrum of energies and biological effectiveness is generated in the annihilation and standard dose planning instruments are not capable of generating a reliable treatment plan. If the code developed is also capable of giving a detailed compilation of all secondary particles generated with their individual energies it will be possible to interface the above code with a modified version of the Local Effect Model (LEM), thereby producing complete treatment plans for antiproton, carbon, and proton treatments. This would allow a direct comparison of the different methods without using extensive experimental testing. Essentially, the experimental work with antiprotons could be reduced to a set of very specific experiments which can be used to benchmark the computer code developed.

To tackle this issue we have joint forces with some of the lead institutions in this field and have initiated a two step approach to this problem:

1. Determination of the complete physics of the annihilation event. Using MCNPX, GEANT4, and the code SHIELD-HIT, specifically developed for radiation therapy with heavy ions, we will calculate the integral dose distribution and the complete spectrum of secondary particles and their energy spectra. Special attention will be given to particles heavier than mass 4 and carefully benchmarking of the codes against each other and against experimental data for protons and heavy ions available in the literature. Agreement of the three codes for a number of benchmark models would lead to a high level of confidence of the final code package. Disagreements between codes can be used to identify specific physics issues leading to such disagreement, and can help us in improving the code package.

2. Development of a dose planning tool for antiproton treatments. Using the output data from section 1 we can use the local effect model (LEM) developed at GSI to generate a biological effective dose distribution. The results of these calculations can be benchmarked against antiproton irradiation experiments with cell samples.

Once tested against these benchmarks, this model can be used to compare different treatment methods for specific indications.

In addition we can use the different codes to characterize the response of different dosimetry methods to antiproton beams. Again, the ultimate goal here consists of combining physical dose calculations and the local effect model to produce an overall response for a specific dosimeter.

V. Carbon Ion Comparison.

While the initial focus of our experiments was on a direct comparison between protons and antiprotons, the collaboration realizes that the most appropriate benchmark for antiproton therapy is in the comparison to the most advanced particle beam therapy method currently available, i.e. the therapy using high LET carbon ions as developed and used at GSI in Darmstadt, Germany and in several centers in Japan.

We have joined forces with the biophysics group of Prof. G. Kraft at GSI and have submitted a proposal for beam time for 2006 and 2007 to perform a set of experiments identical to the experiments performed and proposed with antiprotons here at CERN. We will use identical methods and materials to allow direct comparisons and quantitative statements about the differences in efficacy. Together with continued studies using protons and X-rays, we will generate a complete cross comparison of different treatment modalities.

Appendix A:

Current list of Collaborators in AD-4

Aarhus University Hospital, Norrebrogade 44, DK-8000 Aarhaus, Denmark
Jorgen PETERSEN

CERN, Division EP, CH-1211Geneva, Switzerland
Michael DOSER, Rolf LANDUA

David Geffen School of Medicine at UCLA, Los Angeles, CA 90095, USA
John J. DeMARCO,Keisuke S. IWAMOTO, William H. McBRIDE, Rodney WITHERS

Geneva University Hospital, 1 rue Michel Servet, CH-1211 Geneva, Switzerland
Gerd BEYER, Oliver HARTLEY

Institute for Medical Research and Occupational Health, Ksaverska Cesta 2, HR 10001 Zagreb
Vera GARAJ-VRHOVAC

PBarLabs, LLC, 410 Old Santa Fe Trail, Suite D, Santa Fe, NM 87501, U.S.A.
Michael H. HOLZSCHEITER, Carl MAGGIORE

University of Aarhus, Dept. of Phys. & Astronomy, DK-8000 Aarhus C, Denmark
Niels BASSLER, Helge KNUDSEN, Soren Pape MØLLER, Ulrik I. UGGERHØJ

University of Maastricht, Res. Institute Growth and Development, The Netherlands
Bradly G. WOUTERS

University of Montenegro, Nikca od Rovina 53, Podgorica, Montenegro
Dragan HAJDUKOVIC, Sandra Kovacevic, Danijela SCEPANOVIC

Vinca Institute of Nuclear Science, Belgrade, Serbia & Montenegro
Sanja VRANJES