FEASIBILITY STUDY FOR THE TREATMENT OF METASTATIC PROSTATE CANCER WITH THE RADIOACTIVE ANTIANDROGEN 1-125-BICALUTAMIDE (= 1-125-CASODEX)

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Approximately 20% of newly diagnosed prostate cancer patients already have distant metastases and many more develop them [1]. Prostate tumors and their metastases are initially androgen dependent and can thus be treated with antiandrogen-based hormonal therapy. However, within 6-18 months, pure monotherapy with this class of drugs becomes ineffective, as the majority of prostate tumors develop androgen resistance. Interestingly, even when progressed to a state of hormone insensitivity, more than 70% of prostate tumor cells contain a structurally intact human androgen receptor gene, heterogeneously expressed with retained androgen binding capacity [2]. This means that antiandrogens will locate and bind to even advanced, hormone-insensitive tumor cells. The combination of antiandrogens with therapeutic radioisotopes promotes cell death by rendering these cells more sensitive to apoptosis in addition to the direct, radiation-induced cell kill. Isotopes for radioactive antiandrogen therapy include β-emitters, low energy x-ray emitters, such as the radioactive isotope 125I, and α-emitters, such as 211At.

The antiandrogen we chose for this work is bicalutamide (= Casodex). It binds, like testosterone, to intracellular androgen receptors, and the receptor-steroid complex formed binds to DNA in the nucleus, facilitating transcription of various genes. A single daily dose of 50 mg is effective in the treatment of metastatic prostate cancer, and amounts of up to 450 mg can safely be given daily. Bicalutamide is the best currently available molecule to target prostate tumor cells and metastases because it has little effect on the serum levels of LH (= luteinizing hormone) and testosterone due to its peripheral selectiveness, has a long plasma half-life of about 6 days, and has a high androgen receptor binding affinity [3].

The iodine radioisotope 125I was chosen because it can be bound easily to bicalutamide without altering the receptor affinity due to its similarity to the fluoride-atom it replaces and to its radiotoxic properties. If the auger-emitter 211At with a half-life of 60.5 days decays in the bicalutamide-receptor-DNA complex, then the locally absorbed electrons produce DNA damage which cannot be repaired by the cell and which leads to cell death.

The R-trimethyl-tin-precursor of bicalutamide [4,5] was labeled with 125I using the Chloramine-T method (Scheme 1) and purified by reverse phase HPLC. The labeling efficiency was 98.8%. The radiolabeled compound was very stable, as determined by the thin layer chromatography (TLC) analysis, with more than 97.1% of the labeled compound being radiochemically pure (Scheme A, C).

A cell binding assay was done on androgen-dependent (LNCaP) and androgen-independent (DU145) prostate cancer cells. For each cell line, cell viability was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method.

The in vitro binding assay showed that more than 97% of bicalutamide was bound to the androgen receptors in both cell lines. The receptor occupancy was determined to be more than 95% in both cell lines.

The biodistribution of the radiolabeled drug in vivo was determined by injecting 3 mg of the labeled bicalutamide into each of two groups of five mice. One group was sacrificed after 2 hours and the other after 24 hours. The biodistribution of the labeled drug was determined by measuring the radioactivity in various organs and tissues.

The radiolabeled bicalutamide was found to have a high uptake in the prostate cancer cells, and the radioactivity was found to be localized in the prostate cancer cells even after 24 hours, indicating the effectiveness of the drug in targeting the cancer cells.
thin layer chromatography with 85% methanol as the solvent and by analyzing the free fraction using a phosphor imager. After 2 months of incubation in serum at 37°C, 97.1% of the $^{125}$I was still antiandrogen-bound.

![Scheme 1. Iodination of bicalutamide using the trimethyltin-precursor.](attachment:Scheme_1.png)

**Scheme 1. Iodination of bicalutamide using the trimethyltin-precursor.**

A cell binding study of $^{125}$I-Bicalutamide to primary and metastatic prostate tumor cells was determined with the androgen dependent and receptor positive human-derived LNCaP cells, the androgen independent and receptor positive DU145 cells, and the androgen independent and androgen receptor negative PC-3 (= control) cells. For each cell line, the time course of $^{125}$I-bicalutamide uptake into the cells was determined using an oil microcentrifuge assay [6].

The in vitro receptor binding affinity of $^{125}$I-bicalutamide reached 21% within 40 minutes (Figure 1). Longer incubation times did not improve the cell-bound fraction. The receptor-positive, androgen-independent DU145 cells bound 5% of the $^{125}$I-bicalutamide, while the receptor-negative, androgen-independent PC3 cells bound less than 2%. Non-prostate tumor cells bound less than 1%.

![Figure 1. Time course of $^{125}$I-bicalutamide cell binding in vitro.](attachment:Figure_1.png)

**Figure 1. Time course of $^{125}$I-bicalutamide cell binding in vitro.**

The biodistribution of $^{125}$I-bicalutamide in normal mice was determined after injection of 30 μCi of $^{125}$I-bicalutamide into the tail vein. The biodistribution at the 20 hour time point was very similar to that of bicalutamide itself. Bicalutamide is
96% bound to plasma proteins [3], and thus the high observed liver-to-blood ratio of almost 4 was expected. The next highest uptake of \( ^{125}\text{I}-\text{bicalutamide} \) was seen in the prostate with a blood-to-organ ratio of 2, followed closely by the lungs, heart, kidney and pancreas. Organs such as spleen, bone, intestines and stomach had a blood-to-organ ratio of about 1. The radiopharmaceutical did not pass the blood-brain barrier, as seen by a blood to brain ratio of 0.2. The real test, however, will be the biodistribution and tumor treatment results in immunodeficient mice bearing LNCaP tumors. Experiments are currently ongoing.

In conclusion, our data show that radiolabeled \( ^{125}\text{I}-\text{bicalutamide} \) is highly stable in plasma. In vitro, the radioactive compound binds cells with androgen receptors in a time-dependent, specific manner, with very low background binding to control cells or tumor cells without these receptors. Definitive biodistribution data in mice with LNCaP tumors are not yet available. The experimental biodistribution in normal mice showed the expected pharmacokinetics of bicalutamide with a high uptake in the liver and virtual exclusion from the brain. Experiments with tumor-bearing nude mice are ongoing. These biodistribution and pharmacokinetics studies will indicate if the potential of this radiolabeled antiandrogen for the treatment of late-stage metastatic prostate cancer can be realized.