Abstract. Background: Estramustine phosphate (EMP) and estramustine binding protein antibody (EMBP-AB) accumulate in the mouse prostate. The distribution of radioiodinated EMBP-AB in tumor mice was investigated to assess its therapeutic potential against prostate cancer. Materials and Methods: Mice with DU-145 prostate cancer xenografts received 243 µCi of I-125-labeled EMBP-AB (RI-EMBP-AB). The concentration of activity in different organs was measured 4 h after the injection. Results: The blood contained 0.45% of the injected dose per gram, the prostate 2.4%, the testes 0.95% and the tumor 0.65%. Radioactivity in these organs decreased more rapidly than in other organs. The doses of radiation absorbed by the prostate and the tumor, assuming a 1 mCi injected dose, were 1.81 and 0.92 cGy, respectively. Conclusion: Most other organs would receive relatively high doses of radioactivity, were I-125 to be used in therapeutic doses. Therefore, RI-EMBP-AB is not beneficial in radionuclide treatment as compared to possible EMP applications.

The treatment of hormone-refractory prostate cancer is a challenge to the clinical oncologist and urologist. The treatment methods available include secondary hormonal therapy, radiotherapy and chemotherapy with both cytotoxic and other antineoplastic drugs. The most important drugs for this indication are docetaxel and estramustine. Despite recent findings of prolonged survival with use of docetaxel, the response of hormone refractory prostate cancer remains suboptimal and calls for the study of new treatment methods to improve the 11-month median survival in metastatic, hormone-refractory prostate cancer treated by traditional methods (1, 2).

Newer treatment options presently under clinical evaluation include various other chemotherapeutic agents, radiation enhancement with pre-adjvant chemotherapy and receptor-targeted radiotherapy using radioactive isotopes incorporated into a carrier molecule that accumulates specifically in the cancer cells being treated. The aim of this study was to assess the feasibility of radioiodinated estramustine binding protein antibody for the treatment of hormone-refractory prostate cancer.

Estramustine binding protein. Estramustine phosphate (EMP) is an antimitotic drug used for the treatment of advanced prostate cancer. EMP is dephosphorized in vivo and estramustine (or metabolite estromustine) accumulates in tissues that contain estramustine binding protein (EMBP). EMBP is found in high concentrations in acinar lumina and cytoplasmic vesicles of epithelial cells of the prostate. The biological function of EMBP is not fully understood, but it is a secretory protein of the prostate and may have an immunosuppressive function. It is also found in the pancreas and other glands and, in the rat, in parts of the normal brain (3-6).

Some tumors, among them prostatic and pancreatic cancer, melanomas, brain tumors and meningomas, breast cancer and renal cell cancer contain high concentrations of EMBP. In some cases, the concentration of systemically injected EMP in such tumors has reached six to twenty times the plasma concentration (7-10). The concentration of EMBP in cases of prostate cancer is typically high in well differentiated tumors and in some tumors that have lost androgenic control (11-12). A lot of experimental work has been done on cultured cells and rodents, revealing the presence of EMBP in DU-145 human prostate cancer cells (13), Dunning R3327 rat prostate cancer (14) and in the BT4C rat glioma model (15).

Antibodies against EMBP have been developed to detect EMBP in different tissues, the most commonly used are rabbit or mouse anti-rat-EMBP antibodies (5-9, 12, 16, 17).
cell cultures and animal models. Radioiodine associated with tumor-targeted antibodies has shown promising results in experiments with animals and even in clinical trials (18-22). The Auger-electron radiation of I-123 and I-125 has a short range. They are most effective when incorporated into DNA; this increases the biological effect of radiation by a factor of about ten as compared with extracellular radiation. The medium-energy beta-emitter I-131 has a longer range and a greater capacity to penetrate tissue, and the effect is little enhanced by proximity to DNA (19, 21-24).

Our aim was to combine a radioactive isotope with a carrier molecule that will selectively take the radioisotope into cancer cells. We have demonstrated that I-125-labelled EMP not only accumulates in the prostate, but also in the liver, gallbladder and lungs of a mouse, while radioactive iodine-labelled estramustine binding protein antibody (RIEMBP-AB) mainly accumulates in the prostate (25). In this study, we determined the uptake of a therapeutic dose of RI-EMBP-AB in human prostate cancer tumors implanted in nude mice to determine whether the accumulation of radioactivity in prostate cancer is sufficient to achieve therapeutic effects. We used a cultured prostate cancer cell line, DU 145, which is known to contain EMBP (13).

Materials and Methods

Mice. Twelve-week-old male Balb/c nude mice were used in the experiment. The mice had free access to food and water; their average weight was 18 g. Three to five mice were used for each observation point. The study was approved by the ethical committee of the hospital and the committee for animal experiments.

DU-145 xenografts. Human prostate cancer cell line DU-145 cells were grown in modified Eagle’s medium supplemented with 10% fetal bovine serum in a 5% CO\textsubscript{2} atmosphere. The presence of EMBP in the cell line was verified by immunofluorescence. Approximately, 3.4 million DU-145 cells in an exponential growth phase were injected subcutaneously into the flank of each mouse. After 30 days, subcutaneous tumors had developed at the injection site, ranging from 5 to 15 mm in greatest diameter. The tumor xenografts had no other visible effect on the animals.

Estramustine binding protein antibody. Clone A8-G11-C10-F9-B2 mouse antibody against rat EMBP was provided by Pharmacia & Upjohn, Lund, Sweden. The antibody has previously been described in detail. The antibody adheres to its targets intracellularly. It has been shown to cross-react with human EMBP and produces a similar staining of purified rat EMBP and EMBP in DU 145 human prostate cancer cells (16-17). The antibody used in the experiment was tested by the supplier by Western blotting and showed high affinity to the relevant epitopes.

Radioiodination. EMBP-AB was iodinated using lactoperoxidase sorbent (LPS) as follows: 10 µl of LPS suspension, 25 µl of stock EMBP-AB solution and 40 µl of acetate buffer, 0.1 mol/l pH 6.0, were mixed. Two mCi of Na I-125 were added to the mixture and iodination was started with 20 µl of hydrogen peroxide solution (1:1000 of perhydrol). After iodination the precipitate was centrifuged and the supernatant diluted with isotonic sodium chloride and filtered through a silver disc (Millipore, Biclerica, MA, USA). Tested by thin layer chromatography (ITLC Gelman Sciences, Ann Arbor, MI, USA), the labeling efficiency was 60-70%, with purity over 90% after the filtering.

The experiment was repeated using I-131-labeled EMBP-AB. This time, labeling was performed as in our earlier experiment, with solid lactoperoxidase (25).

Biodistribution. Each mouse received a 0.2 ml intraperitoneal injection of I-125-EMBP-AB in saline solution. The average injected dose of radioactivity was 243 µCi (9.00 MBq). In the group treated with I-131-EMBP-AB, the average injected dose was 19 µCi (0.70 MBq). The mice were decapitated and dissected 4, 24 or 48 h from the injection. Specimens of organs were weighed and their radioactivities were measured with a gamma counter (LKB 1282 Compugamma, Wallac Oy, Turku, Finland).

Dose of radiation. The effective half-lives of radioactivity were calculated by fitting the biodistribution data to an exponential curve. To calculate the absorbed dose of radiation in the tumor and in the prostate, the effective half-lives were used with the S-factors calculated earlier for the mouse testes, assuming the activity to be evenly distributed within a 100 mg sphere (25). The dose of I-125 was set at about 250 µCi per mouse, which is high enough to cause therapeutic effects in organs with affinity to the radioactive agent (26).

Results

The injections had no visible side-effects on the mice. The distribution of RI-EMBP-AB in organs of interest at 4, 24 and 48 h is shown in Figure 1. Four hours after the injection, the prostate contained on average 2.4% and the
testes 0.95% of the injected dose (ID) per gram of tissue. The amount of RI-EMBP-AB in the tumor graft (0.65% ID/g) was slightly higher than in blood (0.45% ID/g) and most other organs. The prostate contained 5.2-fold and the tumor 1.4-fold the amount in the blood at 4 h (Figure 2).

The organs which contained most RI-EMBP-AB at 4 h, the prostate, testes and the tumor, showed very short half-lives of radioactivity: 8.6, 10.4 and 15.8 h, respectively. Half-lives in organs not accumulating much RI-EMBP were as follows: 37.6 h in the lungs, 44.9 h in the blood, 50.6 h in the liver and 94.7 h in the kidney. The doses of radiation absorbed by the prostate and the tumor, assuming the injected dose to be 1 mCi, were 1.81 and 0.92 cGy, respectively.

The experiment was repeated with another group of Balb/c nude mice with similar DU-145 tumors, this time using I-131-labeled EMBP-AB. The prostate-blood ratio of activity was 4.5, but the tumor contained no more activity than the blood at 4 h (Figure 2).

Discussion

The distribution of RI-EMBP-AB in the measured organs is fairly consistent with our earlier findings (25). In our previous study, the prostates of mice contained 2.9% ID/g and the blood 0.9% ID/g 7 h after the injection.

A known target for the antibody, the tumor, was expected to accumulate activity at least as much as the prostate. Contrary to expectations, the concentration of activity at its highest was not much higher in the tumor than in the blood. The dose of radiation in the prostate was 2-fold greater than that than in the tumor. This is an interesting finding, as it means that the antibody could function as a carrier of radioiodine into the prostate but not into the tumor, whilst both contain the relevant antigen. The reason for this could be a difference in the affinity of EMBP-AB to the antigen between the mouse prostate and the human tumor. Produced in mice against rat prostatic EMBP, the antibody has nevertheless been shown to have high affinity to human EMBP (16, 17). The relative affinities between species are not known and should be studied. Another explanation for our finding might be that a differential effect of the labeling of EMBP-AB affects its ability to be incorporated into cells in healthy tissue and tumor cells.

As can be seen in Figure 1, the male reproductive glands and the tumor, containing high amounts of EMBP, initially accumulate RI-EMBP-AB but then lose it rapidly, while the descent of activity in other organs takes place slower. The half-lives of activity in the blood and liver were about three-fold, and in the kidney about six-fold, as compared with the tumor graft, while the concentrations of RI-EMBP-AB in these organs at 4 h were only slightly lower than in the tumor. Thus most organs received doses of radioactivity comparable with that of the tumor, which would cause intolerable side-effects when using I-125-EMBP-AB at therapeutic doses (26). We suggest that other agents should be studied to find better carrier substances for the treatment of hormone-refractory prostate cancer. Promising examples of those already studied include strontium-89 against bone metastases (27), (177)Lu-DOTA-8-AOC-BBN (7-14)NH(2) with an affinity to gastrin-releasing protein also found in prostate cancer (28), and antibodies against prostate-specific antigen (PSA) (29).

Conclusion

At therapeutic doses, iodine-125-labeled estramustine binding protein antibody accumulated in the prostate and the testes, but only marginally in a DU-145 human prostate cancer xenograft in nude mice. The initial concentration in these organs was higher than in blood, but decreased to levels lower than in most other organs within 24 h. We conclude that I-125-EMBP-AB is not suitable for the treatment of prostate cancer, because the concentration and dose of activity in the tumor, containing the target protein for the antibody, are not high enough compared with other organs. Instead, other carrier substances should be considered for this kind of treatment.

References


