TUMOUR TARGETING WITH α-ACTIVE COMPOUNDS OF ^{211}At*

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The principles for use of the α-emitter ^{211}At for tumour therapy are discussed. The use of α-emitters instead of β-emitters for this purposes has the potential of providing a higher dose to the tumour with simultaneous lower dose to surrounding healthy tissue. Furthermore, β-active compounds with tumour affinity has a much higher efficiency the corresponding β-active compounds against single cell or micrometastatic cancer. Constraints and limitations of the method are discussed. Experiments with astatinated monoclonal antibodies as well as small molecules are described, as well as some possible strategies still not investigated.

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1. Introduction

Despite extensive efforts in the research and development of new modalities for cancer therapies, the prognosis for many cancer patients are poor. For some cancer forms, the situation has not really improved very much for many years. Hence, there is a strong need for new treatment modalities. Irradiation has a central role in practical oncology, very often in combination with surgery. In this context, irradiation normally means external beam irradiation, obtained by means of strong radioactive sources and linear electron accelerators, where external beams are focussed to deliver the necessary dose to the tumour in combination with minimal dose to surrounding healthy tissue. The damage to healthy tissue often limits the usefulness of external irradiation, and if the characteristics of the tumour are unfavourable, for instance if it is strongly disseminated, this approach may be completely useless. It is important to underline that the concept of tumour targeting

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with radionuclides is a niche in clinical oncology compared to the broad and wide-spread clinical application of irradiation in the treatment of cancer in general. However, the targeting methods which utilise the chemical behaviour of cancer cells may for some cancer forms offer special and alternative ways of dose delivery in situations which are beyond the reach of the external beam.

1.1. Basic principles of tumour targeting with radionuclides

Tumour targeting with radionuclides, or tumour radiotargeting, implies that the patient gets a suitable radioactive compound with some sort of chemical preference for the tumour. The chemical compound, the radionuclide and the way the compound is administered to the patient may vary, depending upon the concrete clinical situation. The dose which may be delivered to the tumour is limited by healthy tissue damage, as for external radiation. For intravenously administered sources, the dose to red bone marrow is almost always the limiting factor. The frequently used expressions "tumour-seeking compounds" and "targeting" are in principle misleading, because the "seeking" and "targeting" abilities of the molecules in question simply reflect normal chemical affinity. The classical example of a targeting "compound" is the use of $^{131}I^-$ for targeting of metastases of carcinoma of the thyroid, a method which has been applied for almost 60 years. Very frequently, this type of tumour cells have a "memory" of their origin, *i.e.* they retain the affinity of normal thyroid cells to iodide. If so, they may be targeted simply by means of this ion. Since ^{131}I is a β-emitter, the tumour dose is in many cases strong and selective enough to give full curation. If some cases, the tumour cells have lost the ability to enrich iodide, and the prognosis of metastatic thyroid carcinoma is then normally poor. A general review of the different compounds developed for tumour radiotargeting is beyond the scope of this article. Therapeutic tumour radiotargeting in the clinic is currently almost exclusively done with -emitters. The most commonly used radionuclides for this purpose are ^{131}I, ^{89}Sr, ^{90}Y, ^{153}Sm, ^{166}Ho and ^{186}, ^{188}Re. Evidently, this method requires that there exists some kind of chemical behaviour characteristic for the tumour cells which is strongly enhanced compared to normal healthy tissue. If the tumour does not possess any useful chemical properties, radiotargeting is not applicable.

1.2. Radiobiology and LET

The LET (Linear Energy Transfer) of β-particles varies roughly between 0.2 to 1.0 keV μm^{-1}, with the highest value for the low energies. For α-particles with the energy typical for radioactive decay, 5–9 MeV, the LET is roughly 100 keV μm^{-1}. This difference has several important consequences

which must be considered when assessing the potential of α-emitters in tumour radiotargeting. Firstly, the high LET leads to a range for radioactive decay α's corresponding to only 4–5 cell diameters. Hence, all the energy from an α-particle emitted in tissue is deposited in only a few cells which are close to the position of the radioactive atom at the moment of decay. If one considers an idealised situation where a purely α-active source is deposited exclusively inside tumourous tissue, the surrounding healthy tissue will not become irradiated except for the closest cell layers, while the tumour receives almost the entire radiation dose. For low LET radiation, *e.g.* β-particles from the most frequently used therapeutic radionuclides, the total length of the path may be up to several millimetres in tissue. That may be suitable for solid disease but unsatisfactory for single-cell and micrometastatic situations. For these types of scenarios, ionising particles with short range leaving tracks with high ionisation density are likely to be much more efficient. Even in the most extreme situation, the single cancer cell surrounded only by healthy tissue, α-particles have a potential. For a single cell, there is a finite probability that it may be killed by a single α-particle passing through the cell nucleus and which was emitted from a radioactive atom bound to the cell itself. The corresponding probability for a β-particle is vanishingly small. For low LET radiation to be efficient, it is imperative that the tumour is sufficiently large to enable a substantial cross-fire Wheldon and O'Donoghue (1990). The short range of the α-particle also imposes strong constraints. For therapy with α-active compounds it is crucial that the radioactive compound is indeed enriched in all parts of the tumour down to the cellular level. If not, a significant amount of tumour cells may remain outside the range of the α-particles and thus avoid irradiation and survive. Due to the intense ionisation density along their path, high LET particles interact with living tissue in a way fundamentally different from low LET particles (Hall (1994)). The high LET interaction is predominantly of direct character, *i.e.* a first-order effect caused by the passage of the particle itself. The low LET interaction is predominantly indirect, *i.e.* not caused by the direct passage of the particle but mediated by radiation chemical species, *e.g.* radicals and H_2O_2. This has as a consequence that the effect of the chemical status of the cell is high for low LET radiation but not for high LET radiation.

2. Astatine

2.1. Radiochemistry of astatine

Astatine was discovered in 1940 (Corson *et al.* (1940)) and is the heaviest so far known halogen element. It has no stable isotopes, but $^{207-211}At$ have half-lives of more than an hour. Therefore, detailed experiments on astatine

chemistry using radiochemical techniques have been possible for many years. In most respects, the chemistry of astatine is easily understood from extrapolations of iodine chemistry (Hamilton and Soley (1940)). The exception to this is that At has a slightly metallic character not observed for iodine, most important being its tendency to form sulfide bonds. The astatine isotope ^{211}At was early recognised as a candidate for targeted radiotherapy. The decay pattern is shown in Fig. 1. As is seen, the α-intensity is in total 100 %. Furthermore, the decay ends in stable ^{207}Pb or very long-lived and non-radiotoxic ^{207}Bi. The EC decay gives rise to high intensity Po X-rays making ^{211}At easy to follow with γ-camera. The nuclear reaction used for the production is the same as the one used for the discovery of astatine, ^{209}Bi$(\alpha, 2n)^{211}$At, at an incident α-energy of 30–32 MeV. It is important to avoid the ^{209}Bi$(\alpha, 3n)^{210}$At reaction, because ^{210}At decays to ^{210}Po, which is strongly radiotoxic. The level of this nuclide must therefore be kept low.

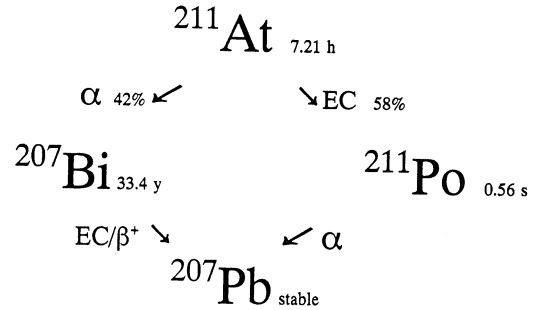

Fig. 1. The decay properties of ^{211}At.

Except for the possible use of At^- for treatment of thyroid carcinomas, ^{211}At must be attached to a suitable carrier "vehicle". Therefore, the organic chemistry of At has been extensively investigated. It is closely related to the organic chemistry of iodine; most important being the instability of the C–At bonds in aliphatic hydrocarbons. The best stability at 37°C and physiological pH is obtained if At is bound to a carbon atom in an aromatic system (Berei and Vasaros (1983)). Other types of unsaturated carbon atoms have also been considered (see *e.g.* Liu *et al.*, 1985, Larsen *et al.*, (1997)). Although the potential for therapeutic use of ^{211}At was recognised early, the development of potentially useful targeting compounds containing this nuclide has taken place mainly during the last 10–15 years. With a few special exceptions, the targeting compounds investigated so far can be distinctly classified as large proteins or fragments of those, or as small molecules with affinity to a particular biological function. In the following, the most important features will be described. Results older than 10 years are included only if they are relevant with respect to the state-of-the-art situation.

2.2. Astatinated monoclonal antibodies and their fragments

Monoclonal antibodies (Köhler and Milstein (1975)) with affinity to an antigen characteristic for a tumour cell should in many ways be ideal carrier molecules for tumour radiotargeting. However, the attitude in the years immediately following the discovery of the production method was over-optimistic and unrealistic. Presently however, many of the initial problems have been overcome, and therapeutic tumour targeting with monoclonal antibodies (Mabs) has been taken into clinical use for a limited number of patient groups, so far only with -emitters. In particular, spectacular progress has been reported in the use of ^{131}I-labelled monoclonal antibodies in the treatment of lymphoma patients (Press *et al.* (1993), Kaminski *et al.* (1993)). A short and general overview of radio-immuno-targeting in the clinic is given by Bruland (1995). In this type of targeting, the radioactive compound will be bound on the cell membrane, or in some cases internalised in the cell. One of the most fundamental obstacles of radioimmunotherapy with monoclonal antibodies is the slow penetration of these large molecules in solid tissue. An important consequence of this is that solid tumours cannot be efficiently targeted with intravenously injected Mabs labelled with short-lived radionuclides. The ideal half- life of the radionuclide for that application would be approximately 2 days. For ^{211}At with 7.2 h half-life, this is an important constraint, and limits the situations for which ^{211}At-labelled Mabs are applicable. In view of this, the most promising fields of application for them seem to be clinical situations where the penetration problem is minimal, non-existent or maybe even advantageous: very strongly vascularised cancer (*e.g.* leukemia and lymphoma); treatment of surfaces in closed cavities (*e.g.* ovarian cancer with malignant ascites and cavities after removal of primary tumours); and intratumour injection in well localised and inoperable solid tumours. Several methods now exist for the labelling of Mabs with ^{211}At. Standard radiolabelling techniques used in the laboratory for labelling with radioiodine (*e.g.* IodoGen) did not work sufficiently well for astatine and were soon abandoned. Currently, astatine labelling is normally done using bi-functional esters which are first brought to react with ^{211}At. The intermediate thus formed is then purified and brought to reaction with amino groups in the monoclonal antibody molecules. This technique was developed 10–15 years ago (Zalutsky and Narula (1988), Hadley *et al.* (1991)). It has been shown that this labelling method provides sufficiently stable binding of radioiodine as well as astatine (Zalutsky *et al.* (1989), Garg *et al.* (1991)) with moderate reduction of Mab immunoreactivity. The method is illustrated in Fig. 2 for another reaction and is very general. It can be used not only for astatination of monoclonal antibodies, but also for radio- halogenation of a large variety of compounds or items containing free amino groups available for forming a very stable amide bond.

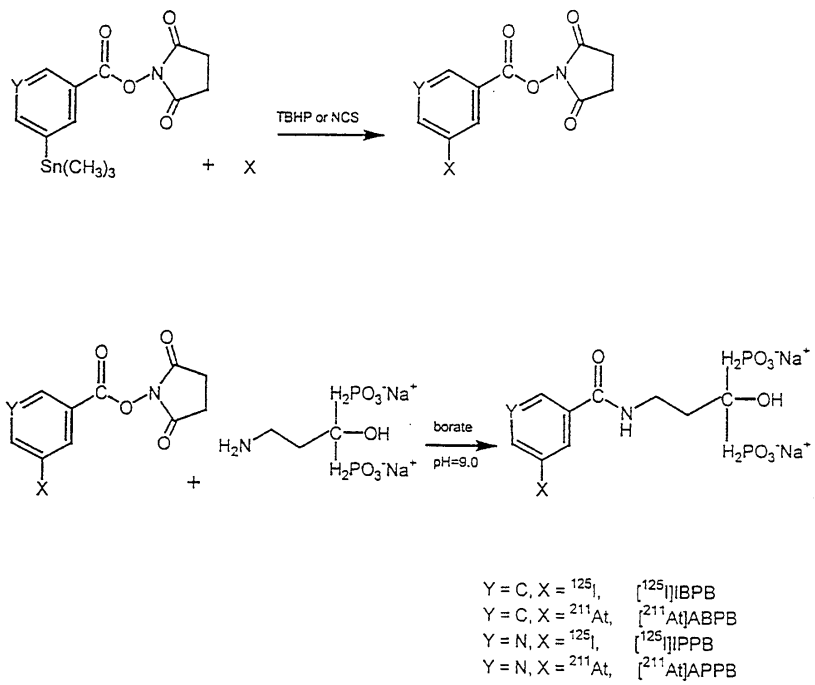

Fig. 2. The procedure for synthesis of radiolabelled ^{211}At- and ^{125}I-amidobisphosphonates by means of succinimidyl ester intermediates (Murud *et al.* (1999)). This labelling method is based on the formation of an amide bond though the reaction between the intermediate and an amino group. This labelling principle can be used for labelling of most compounds or items containing free amino groups available for attack (Zalutsky and Narula (1988)).

Experiments with astatinated monoclonal antibodies have been performed by several groups, most important being the work at Duke University Medical Centre mainly with glioma and neoplastic meningitis. Glioma are a class of brain tumours with very poor prognosis, survival for more than a year after diagnosis is rare. The primary glioma is always removed surgically, but due to the proximity of sensitive parts of the brain, surgery cannot be curative. The group at Duke has performed extensive experiments with ^{211}At-labelled Mabs in animal models for these malignancies, a review can be found in Zalutsky and Bigner (1996). In the clinic, ^{131}I-labelled monoclonal antibodies has been used in the treatment of glioma patients (Brown *et al.* (1996)). It is expected that a higher tumour dose can be administered by means of ^{211}At, since almost the entire dose then is related to short-range α-particles not influencing healthy parts of the brain.

Based on their extensive experiments in animal models, a clinical trial with ^{211}At-labelled monoclonal antibodies on human glioma patients has recently started. A large variety of quite different clinical situations have also been investigated with ^{211}At-labelled Mabs in different animal models. Some of the situations simulated are intraperitoneally restricted ovarian cancer with ascites (Vergote *et al.* (1992a,b); Larsen *et al.* (1995)), osteosarcoma (Larsen *et al.* (1994a,b)), intratumour injections (Larsen and Bruland (1998)) and lymphoma (Aurlien *et al.* (1999)). Evidently, care must be exercised in the extrapolation of results obtained with rodent models into the clinical treatment of human patients. However, numerous model experiments have shown different types of significant positive effects at acceptable doses, *e.g.* tumour radiotoxicity, strong tumour cell/normal cell selectivity, hampering of ascites production or decreased tumour progression. Some of these experiments also clarified the foreseen constraints of the method imposed by the 7.2 h half-life of ^{211}At. The slow transport process in solid tissue must always be taken into account. Additionally, the well-known problem of poor vascularisation may cause insufficient uptake of the radioactive Mabs in parts of the tumour. A possible method to circumvent the slow penetration of Mabs in solid tissue might be multi-layer targeting, which will be discussed in the next chapter. In addition to the already discussed penetration problem for monoclonal antibodies, the main fundamental obstacle seems to be the diversity of antigen expression. The antigen expression of a tumour is seldom homogenous. Very frequently, there is a sub-population of tumour cells which is virtually antigen negative and therefore does not bind the radiolabelled monoclonal antibody. If these cells do not receive their dose from cross-fire, they will survive the treatment like normal cells and can form a basis for a new tumour. This problem is fundamental for the whole strategy of targeting with monoclonal antibodies. One way of solving it might be to target several different antigens on the same tumour with a mixture of different monoclonal antibodies.

3. Astatinated small molecules

For many purposes, small molecules with special affinity to certain biochemical functions target tumours better than tumour specific monoclonal antibodies. Cancers are often characterised by particular types of pathological chemistry. Frequently, this abnormal chemical behaviour is simply an enhancement of a process also taking place in the normal cells in the organ of origin. If the pathological chemistry is unique or is more pronounced than the same process in the normal cell population, it may be used for targeting. Two examples of such processes are the synthesis of a primitive bone-like substance (osteoid) in osteoblastic osteosarcoma and the

enhanced production of melanin in strongly pigmented (melanotic) malignant melanoma. Whereas the molecular weight of monoclonal antibodies or antibody fragments typically are of the order of 50 000 to 200 000, the targeting small molecules are normally < 1000, even including the astatine. This difference causes a much more rapid transportation process and is the main advantage of small molecules for tumour targeting. Several low molecular targeting compounds have been labelled with ^{211}At, some of them are quite promising, whereas others are unstable and not sufficiently specific. Sometimes, the chemical interaction mechanism is clear, in other cases, the functional affinity may be strong but have a dubious explanation.

3.1. Bisphosphonates

Some of the most well-known chemical mechanisms occur in the case of compounds with affinity to bone. It has been known for many years that a number of bis- and polyphosphonates replace phosphate in the bone metabolism and are enriched in regions with enhanced build-up of new bone (see *e.g.* Fleisch (1995), Lewington (1996), Farhanghi *et al.* (1992)). On the basis of these results, astatinated bisphoshonates have been developed (Murud *et al.* (1999), Larsen *et al.* (1999)). The labelling method is the same as the one used for labelling of monoclonal antibodies (see above), and is shown in Fig. 2. The biodistributions of the compounds are shown in Fig. 3 demonstrating the outspoken bone affinity. In skeleton metastases and osteosarcoma, this affinity may be enhanced by factors of the order of 4–8. These compounds might have a potential for targeting of bone metastases of soft-tissue carcinomas and of disseminated osteosarcoma, two very different clinical situations but with a similar affinity for "bone-seeking" compounds. The micro-distribution has not yet been measured and will be decisive for their further clinical use.

3.2. Melanin-affinity compounds

Malignant melanoma has for several decades been one of the most rapidly increasing cancers in the world. It metastasises rapidly, and early detection is therefore imperative. If it is surgically removed before it has metastasised, it is normally completely cured. If not, the prognosis is very poor. Hence, there is a strong need for new treatment modalities for disseminated melanoma. Malignant melanoma may occur at very different stages of pigmentation depending on the content of melanin, the most important component of dark skin pigment. Enhanced melanin production is a type of pathological chemical process which might be useful for targeting. Several attempts have been made. Tyrosine is one of the compounds used in the biosynthesis of melanin, and this compound and slight modifications of it have been

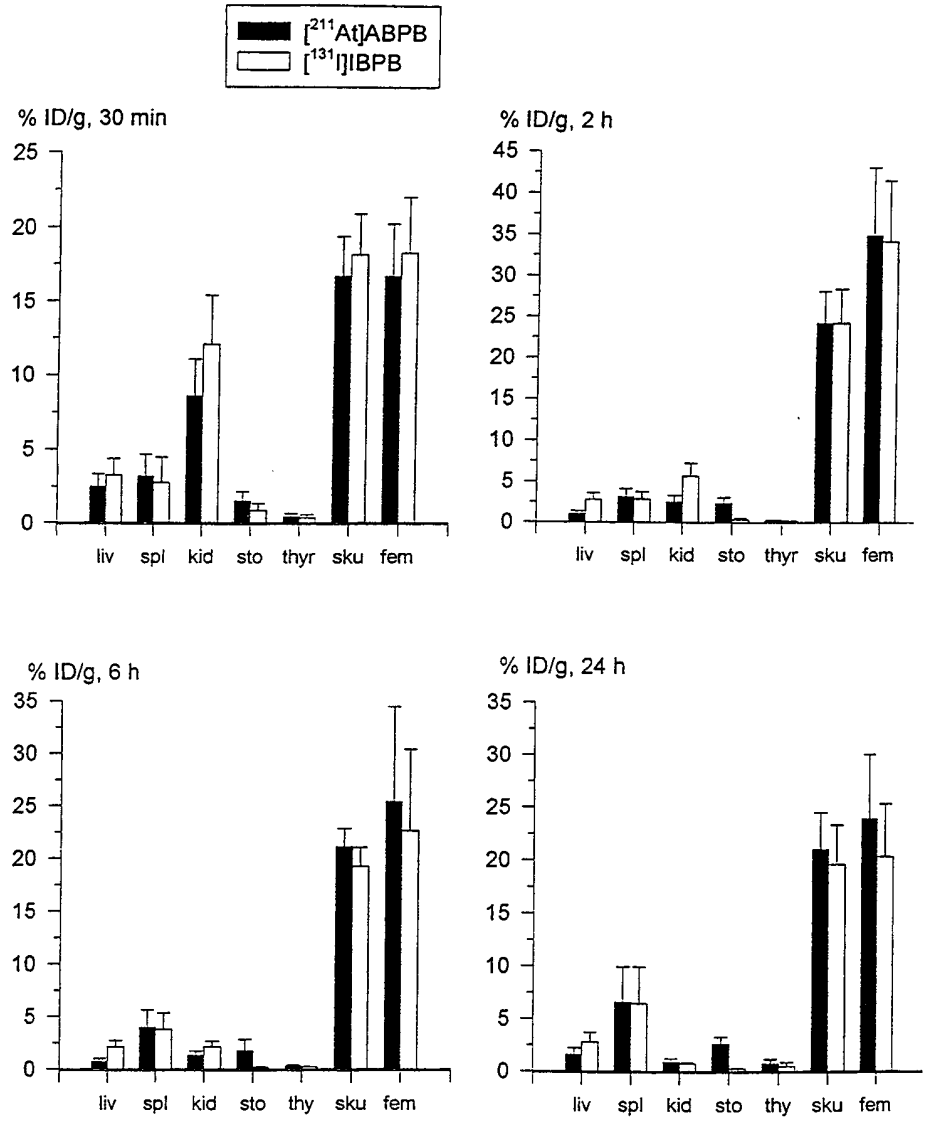

Fig. 3. The biodistributions of carrier free [^{211}At]ABPB and [^{131}I]IBPB in immunocompetent Balb/c mice, from data in (Larsen *et al.* (1999)). liv — liver; spl — spleen; kid — kidney; sto — stomach; thy — throat region with thyroid, sku — skull; fem — femur. High bone uptake is observed. The low value in the throat region is a strong indication for chemical stability. The values are given in terms of % ID (Injected Dose) per gram organ weight.

radioiodinated as well as astatinated (McLaughlin *et al.* (1988)). Others have used the affinity of methylene blue for targeting (Link *et al.* (1989), (1992), (1996)) and reported therapeutic effects on experimental melanomas in mice. Several other identified melanin-affinity compounds *e.g.* certain benzamides (Michelot *et al.* (1991), (1993) , John *et al.* (1993), Mohammed *et al.* (1997), Nicholl *et al.* (1997)), as well as cystaminylphenol and related compounds (Jimbow *et al.* (1989), (1993), Miura *et al.* (1990), Alena *et al.* (1990)) still remain to be astatinated and tested in that form. The "problem organ" for these compounds seems to be the eye (Labarre *et al.* (1999)) and the tendency to enhanced uptake in melanin-containing parts of the eye need to be clarified and if necessary overcome before therapeutic applications of astatinated melanin seekers can be realised.

3.3. 5-astato deoxiuridine

A different targeting strategy is based on the enhanced DNA synthesis in cancer cells. Targeting by means of compounds structurally similar to DNA building-blocks may have a general cancer affinity, and 5-astato deoxiuridine is such a candidate (Larsen *et al.* (1997)). Although promising results have been reported, the stability of the compound seems to be somewhat too low, calling for a chemical modification of the compound not affecting its biological function.

3.4. meta-astatobenzylguanidine

Radioactive *meta*-iodobenzylguanidine has been used for several years with ^{123}I or ^{131}I for the diagnosis and therapy of neuroendocrine tumours, preferentially neuroblastoma and malignant pheochromocytoma (see *e.g.* Troncone *et al.* (1990)). Since this was an already approved radiopharmaceutical containing a halogen, methods were developed for its astatination (Vaidyanathan *et al.* (1996a)). *Meta*-iodobenzylguanidine has an affinity to neuroendochrine tumours due to the similarity to substances used in the metabolism of these tumours. Tumour uptake was observed for the astatinated compound, but there was a problematic heart uptake as well (Vaidyanathan *et al.* (1996b)), which was found to be higher than the heart uptake of the radioiodinated analog used for therapy. If the heart uptake can be inhibited, it seems likely that the astatinated bezylguanidine might have a therapeutic potential.

3.5. Avidin-biotin

As discussed above, the heavy monoclonal antibodies penetrate tissue too slowly to be useful in combination with ^{211}At for treatment of solid dis-

ease. One possible method to circumvent this problem is the avidin-biotin system, which is based on the strong binding between a protein (avidin or strept-avidin) and a small molecule, biotin (also known as Vitamin H). Each avidin molecule has four binding-sites for biotin. In the simple version of this method, the monoclonal antibodies are first linked to a number of biotin molecules, which are then injected into the patient. 24–36 hours later, avidin and *strept*-avidin are administered. After another 24 hours, the radioactive biotin compound is injected. This molecule will then bind to one of the non-occupied binding-sites on the avidin molecules, which are already located at the tumour cells. With this method, the actual tumour targeting is done by a very selective monoclonal antibody, whereas the radioactivity is transported by a separate, low molecular compound which is rapidly distributed. Several biotin derivatives have been developed for radio-iodination (Wilbur *et al.* (1998)) but not yet taken into clinical use. Astatinated biotin compounds have also recently been made (Foulon *et al.* (1997ab), (1998)) . A version of the three-step avidin/biotin method is already applied in patients for targeting with metallic β-emitters (see *e.g.* Paganelli *et al.* (1999), Cremonesi *et al.* (1999)).

Even the low molecular compounds have fundamental difficulties. Very often, the tumour enrichment is not homogenous. Parts of the tumour may for some reason have lower uptake. Therefore, a combination of different targeting principles may in many cases be needed.

4. Conclusion and outlook

The concomitant development of improved methods for labelling and of tumour targeting methods during the last 10–15 years may enable the use of ^{211}At-labelled compounds for cancer therapy in the not so distant future. Due to the limited cyclotron capacity available for production of the radionuclide, it seems likely that the first clinical applications will be treatment of well- defined closed cavities with ^{211}At-labelled Mabs after surgical removal of cancers with a bad prognosis. Examples of such cancers are glioma and chemotherapy resistant ovarian cancer. It also seems likely that ^{211}At-labelled Mabs may come rapidly into use in the treatment of lymphomas and leukemias resistant to all other known therapy modalities. Several small molecules have shown a high targeting potential, but more documentation about their microdistribution in tumours and healthy tissue as well as their normal-organ toxicity is needed before application to humans can be realised.

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