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(54) Title: ION EXCHANGE TUMOR TARGETING (IETT)

(57) Abstract

A compound for local, i.e. intratumoral or intracavitary administration, for targeting tumors comprising dextran, the charge of which has been modified through covalent binding of charged groups, such as charged amino acids. The compound further comprises a functional group for treating or imaging the tumor.

"Ion Exchange Tumor Targeting (IETT)"

Background of the invention:

Tumor specific targeting, is an intensive research field within oncology. The rationale is to reduce side effects and increase the drug dose that can be delivered to the cancer patient thus enhancing the therapeutic efficacy.

Examples of such targeting are antibody oriented targeting, i.e. immuno-targeting, and controlled drug delivery systems using various polymers as drug carriers (1, 2, 3, 4, 5, 6).

Tabata et al (11) describes intravenous administration of dextran with negative charges. This gives very low specific accumulation of the dextran to the tumor. The specific accumulation or the target to non-target ratio (ttn-ratio) is never exceeding 10 (shown in Figures 8 and 9, page 219).

Syrigos et al (12) describes intravesical administration of radiolabelled tumor-associated monoclonal antibody giving a specific accumulation or target to non-target ratio (ttn-ratio) ranging between 2 and 6.

The preceding discussion of the background art is intended to facilitate an understanding of the present invention only. It should be appreciated that the discussion is not an acknowledgement or admission that any of the material referred to was part of the common general knowledge in Australia as at the priority date of the application.

Throughout the specification, unless the context requires otherwise, the word "comprise" or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

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Summary of the invention:

This invention relates to compounds that, when administered locally, intra-tumorally or intracavitarily, show unexpectedly high accumulation to tumors and that can deliver various functional groups, including both therapeutic or imaging agents to these sites, to mediate tumor therapy or imaging.

The inventor has discovered that due to the ionic properties of tumors, polymers with an appropriate charge can be selectively targeted to tumors through electrostatic interactions in an ion exchange like reaction. The charge character of tumors could be explained by tumors having enlarged

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blood vessel surface, abnormal interstitium, and high content of sialic-acid at the cell surface. All these contain charged, mainly anionic, carbohydrates (sulphated carbohydrates) and proteins (collagen) (7, 8). The selective accumulation is unexpectedly high when administered locally, intratumorally or intracavitarily.

By using these charged groups characteristic for tumors as target, the invention avoids the general limitations of tumor specific targeting, such as low affinities between targeting compound and tumor etc. Targeting tumors with compounds according to the invention avoids the problem of tumor heterogeneity, i.e. the limitation of antibody-based cancer therapies that target only a single type of tumor cells in a tumor containing multiple cell types. Further more, tumor therapy or imaging using compounds according to present invention do not require an initial pre-targeting step, as many prior art methods do.

The compounds according to the invention show, when administered locally, intratumorally or intracavitarily, unexpectedly high affinities for tumors, i.e. they have an unexpectedly high target to non-target (ttn) ratio (see Example 2). The charge of the compounds can be adapted to the charge of the tumor to be treated, thereby increasing the target to non-target ratio of the compound to the tumor. When looking at the general properties of solid tumors, it seems probable that the principle of the invention, i.e. selective accumulation in tumor tissue through interactions between the charges of the tumor and the polymer, is functional in most human solid tumors.

In preferred embodiments, the compounds of the invention comprise a targeting compound, which is a charged polymer that binds selectively to the tumor through electrostatic interactions. The targeting compound is modified to include a functional group, which may be part of the polymer or

attached to the polymer as a tag. Where the compound is to be used therapeutically the functional group is a toxin, a drug, a radioactive molecule or a precursor thereof. Where the compound is to be used for tumor imaging rather than therapy, the functional group may also be a detectable label, such as a radioactive molecule.

Detailed description of the invention:

The compounds according to the invention are for local, i.e. intracavitary or intratumoral administration. Examples of tumors suitable for such administration are tumors growing in body-cavities such as urinary bladder tumors, ovarian tumors, certain brain tumors, and for intratumoral administration, pancreatic tumors, and head and neck tumors. When the compounds according to the invention are administered locally, intratumorally or intracavitarily the specific accumulation or ttn-ratio ranges between 700 and 2050, which far exceeds specific accumulation when administered according to prior art.

The compounds according to the invention comprise a charged polymer, which causes the targeting, and a functional group, which causes the therapy or imaging.

In preferred embodiments the targeting polymer of the compound according to the invention is a poly-alcohol, such as dextran. When using dextran, a preferred molecular weight is between 10^3-10^6 Dalton. In a preferred embodiment the charged side groups of the polymer are amino acids having charged side chains, or derivatives thereof, such as ornithine, lysine, arginine, histidine, glutamic acid, aspartic acid etc. The amino acids are coupled to dextran by activating the hydroxyl groups of dextran through partial oxidation to aldehydes which react with the free amino groups of the amino acids. This procedure is described in Foster, RL. 1975 (10). An example of a

resulting compound (lysine-dextran) according to the invention is shown in Figure 1. In a preferred embodiment an amino acid is coupled to between 15-30% of the glucose residues of dextran.

The resulting charge of the compound can be positive, negative or neutral depending of the requirements, i.e. the electrostatic properties of the type of tumor to be targeted.

Examples of the functional group of the compound according to the invention include drugs (e.g. antibiotics, anti-virals, anti-fungals), toxins (e.g. ricin), radio-nuclides (e.g. Cu-64, Cu-67, Sr-89, Y-90, Tc-99m, I-131, Sm-153, Ho-166, Re-186, Re-188, Bi-212), hormone antagonists (e.g. tamoxifen), heavy metal complexes (e.g. cisplatin), oligo-nucleotides (e.g.antisense oligo-nucleotides), chemoterapeutic nucleotides, peptides, non-specific (non-antibody) proteins, boron containing compounds (e.g. carborane), photodynamic agents (e.g. rhodamine), enediynes (e.g. calichesmicins), and transcriptions based pharmaceuticals.

Coupling of the functional group could be done either directly to the polymer or through a bifunctional chelate, such as an aminated radio-metal chelate. Direct coupling of the functional group to the poly-alcohol could be achieved in the same manner as the charged side-groups are coupled to the poly-alcohol. The binding of the functional group must not allow release of the functional group in vivo.

The compounds according to the invention can be administered alone, or in conjunction with a pharmaceutically acceptable carrier.

EXAMPLES:

This invention will now be described in greater detail by reference to the following non-limiting examples, in which:

FIG. 1 shows an example of a compound according to the invention wherein the amino acid lysine is coupled to dextran, according to the invention.

Example 1.

Synthesis of Tc-99m-lysine-dextran according to the invention.

Activation of the hydroxyl groups of dextran.

20 mg of dextran (mean mw 40 000 Dalton, Pharmacia-Amersham Biotech, Sweden) is mixed with 12 mg sodium periodate in a total volume of 1mL 0.15 M sodium acetate, pH 5.5 and incubated during stirring at room-temperature for 5h. The polymer is then purified on a disposable Sephadex®-G25 (Pharmacia-Amersham Biotech, Sweden) gel filtration column.

Coupling of lysine to activated dextran.

20 mg of the activated dextran is mixed with a large molar excess of lysine (28 mg lysine), in 2.1 mL of 0.1M $NaHCO_3$ and 4mg of sodium cyanoborohydride is added. The mixture is incubated during stirring at room temperature for ~15h. After incubation the compound is purified on a PD10 column and the buffer is exchanged to 0.1M sodium acetate.

This results in the coupling of a lysine to approximately 15-30% of the glucose residues of dextran.

Labeling of lysine-dextran with technetium (Tc-99m).

Labeling of lysine-dextran with Tc-99m is performed according to the method of Henze et al. (9), i.e.,

50 µg $SnCl_2$ (10µL of konc. 5g/L, in 99% ethanol) is added to 50 µg lysine-dextran in 1 mL 0.1M sodium acetate. Then ~100MBq sodium pertechnetate

(~100µL) is added and the reaction mixture is incubated for 15 minutes at room temperature. The solution is purified on a Sephadex®-G25 PD10 column (Pharmacia-Amersham Biotech, Sweden), sterile filtrated, and dilute in 150-200ml saline. After labeling the compound should be used directly.
All solutions above are degassed.
The resulting Tc-99m lysine dextran contained 1MBq per µg.

Example 2.
This example serves to demonstrate that Tc-99m-lysine dextran according to the invention is selectively accumulated in tumor tissue.

Nine patients having epidermal growth factor positive superficial transitional cell bladder carcinoma were studied. Tc-99m-dextran with different charges and containing the same total amount of radio-activity were instilled in the bladder of these patients. The instilled polymers were Tc-99m-epidermal growth factor (EGF)-dextran, neutral dextran, i.e. Tc-99m-dextran, anionic dextran, i.e. Tc-99m-taurin dextran, and cationic dextran, i.e. Tc-99m-lysine dextran (according to Example 1).

200 mL of the polymers, of the concentration 0.25 µg/mL in saline, were instilled through an urethral catether and kept in the bladder for 30 minutes and were then carefully washed out with approximately 400 mL saline. The patients were then operated on and samples were taken from normal bladder tissue and bladder tumor tissue, respectively. The samples were weighed and counted in gamma counter and the radioactive uptake was calculated as cpm/g tissue. The target to non-target (ttn) ratio was calculated. Results (mean values) are shown in Table 1.

Table 1.

polymer	radioactive uptake, ttn	number of patients studied
Tc-99m-EGF-dextran	700:1	4
Tc-99m-dextran	90:1	1
Tc-99m-taurin dextran	2:1	1
Tc-99m-lysine dextran	2050:1	2

The Tc-99m-lysine-dextran according to the invention resulted in the highest tumor uptake of radioactivity, i.e. the highest target to non-target ratio. Tc-99m-EGF-dextran serves as a comparison with prior art techniques, i.e. targeting to tumors by antibodies directed to proteins that normally are over-expressed in tumors. However, the conditions in this study were unfavorable for the interaction between the Tc-99m-EGF-dextran and EGF-receptors expressed by tumors, hence the large ttn ratio was interpreted as charge dependent through charged side groups of Tc-99m-EGF-dextran.

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NEW CLAIMS (submitted on December 1, 2000)

1. Use of a compound comprising dextran, the charge of which has been modified through covalent binding of charged groups for the manufacture of a medicament for selectively targeting charged tumours by an ion exchange reaction, for local, intratumoral or intracavitary administration.

2. Use of a compound according to claim 1, comprising dextran, the charge of which has been modified through covalent binding of cationic groups for the manufacture of a medicament for targeting anionic tumours, for local intratumoural or intracavitary administration.

3. Use according to claim 1 or 2, wherein said charged groups are selected from amino acids with charged side chains, or derivatives thereof.

4. Use according to claim 1, wherein said amino acids are selected from ornithine, lysine, taurin, arginine, histidine, glutamic acid, and aspartic acid.

5. Use according to claim 1 or 2, wherein said charged groups are bound to the activated hydroxyl groups of said dextran.

6. Use according to claim 1 or 2, wherein a charged group is bound to 15-30% of the glucose residues of said dextran.

7. Use according to claim 1 or 2, wherein said dextran contains functional groups.

8. Use according to claim 7, wherein said functional groups are bound to the activated hydroxyl groups of said dextran.

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9. Use according to claim 8, wherein said functional groups consist of a radio-nuclide.

10. Use according to claim 1 or 2, wherein said dextran has a molecular weight of 10^3-10^6 daltons.

11. Use according to claim 1 or 2, wherein said medicament becomes localized to the tumor at a target to non-target ratio of at least 600:1.

12. Use according to claim 1 or 2, wherein the charge of said dextran is adapted to the charge of the tumor to be treated, thereby increasing the target to non-target ratio of the compound to the tumor.

13. Use according to claim 1 or 2, wherein the tumor to be treated is a bladder carcinoma tumor.

14. Use of a compound as hereinbefore described, with reference to Examples 2.

Dated this Tenth day of January 2003.

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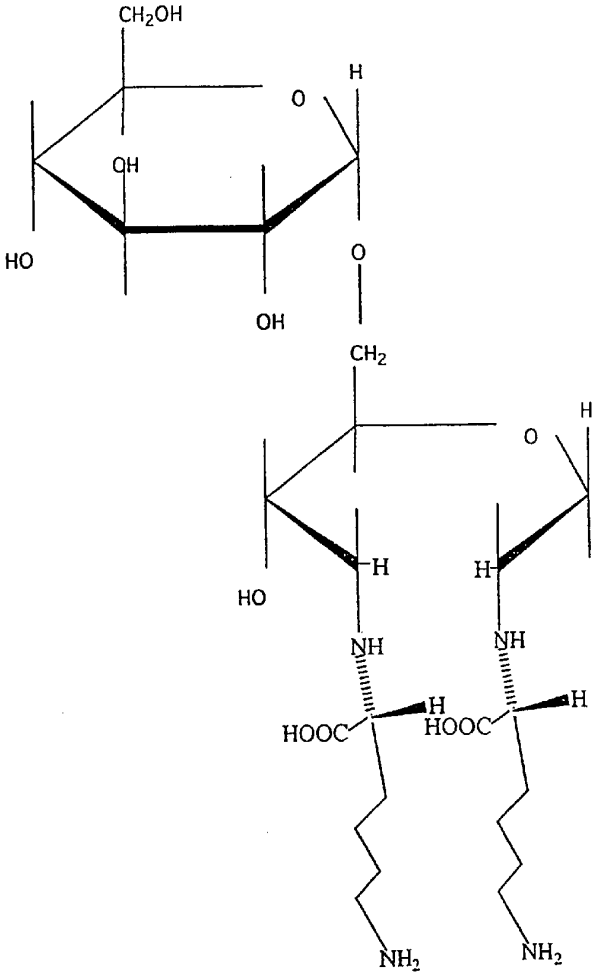


Fig. 1