Ion Exchange Tumor Targeting: A New Approach

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Abstract

Connective tissues are distinguished by the types, concentrations, and organizations of material in the extracellular matrix. Many physiological functions are determined largely by the nature and organization of the extracellular components. The components are characterized by their content and distribution of charged, mostly anionic groups. The distinct roles played by the charges are sometimes modeled by analogy to the transport theory of ion exchange resins. The intent of this study was to investigate whether the properties of the tumor matrix could be used for selective, charge-dependent accumulation of charge-modified dextran. Ten patients with diagnosed superficial urinary bladder carcinoma were included in the study. They received intravesical instillations of technetium-99m-labeled charge-modified dextran derivatives (~0.1–1 mg; ~50 MBq in saline; 30-min incubation). After treatment and resection, samples were taken from normal and diseased tissue. The result clearly demonstrated a charge-dependent difference in the quotient of radioactive uptake in tumor tissue: normal tissue. Instillations of cationic dextran yielded a high quotient, up to 3000. Normal tissue had background activity. Anionic dextran yielded a low quotient, 1.8–2, with increased background (i.e., uptake in normal tissue). Neutral dextran gave a quotient of up to 90. No radioactivity could be detected in blood. The tumors in this study apparently displayed cation-exchanging properties. We will continue this investigation and determine whether this is a general property of bladder carcinomas and whether other carcinomas display ion exchange properties. If this is the case, the finding could have important implications for the local treatment of several cancers.

Introduction

Bladder carcinoma is a common cancer, the fourth most common cancer in men and the ninth most common cancer in women. The median age at diagnosis is 65 years. The annual incidence increases, whereas the mortality has decreased. At presentation, 75% of tumors are superficial, and the standard treatment approach is a complete endoscopic resection. Almost all patients develop new tumors, and 30% progress to a higher grade. Depending on a number of factors (e.g., tumor size and depth of invasion) intravesical therapy might be recommended (1). Intravesical therapies have been performed with several different agents. Examples of the most used substances are bacillus Calmette-Guérin (BCG) and mitomycin C (2). Side effects include local toxicities and whether or not the drug is systemically adsorbed. In the long term (5 years), instillation therapy does not seem to decrease tumor recurrence (3). Once the bladder cancer becomes muscle invasive, the overall prognosis is poor (4). Consequently, there is a need to improve the efficacy of intravesical therapy.

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The objective of the study was to investigate whether an eventual charge difference between normal bladder tissue and tumor tissue could be selectively targeted by intravesical instillation of charge-modified dextran.

Patients and Methods

Patients. Ten patients with diagnosed (biopsy-proven) superficial bladder cancer were included. They were all males and had a mean age of 64 years. Four of them were instilled with cationic dextran, three were instilled with anionic dextran, and three were instilled with neutral dextran (i.e., unmodified dextran). Blood samples were taken in connection with the transurethral resection.

Dextran Modification. Dextran PM40 (Pharmacia Amersham Biotech AB, Uppsala, Sweden) was used as a conjugate backbone. Sodium meta-periodate (MERCK AG, Darmstadt, Germany) was used for dextran activation. Lysine and taurine (Chemicon, Stockholm, Sweden) were used for dextran conjugation. Sodium cyanoborohydride (Chemicon, Stockholm, Sweden) was used for reductive amination. PD-10 disposable Sephadex G-25 columns were used for separation and purification (Pharmacia Amersham Biotech AB).

Activation and Coupling. As described previously (7), 20 mg of dextran PM40 were dissolved in 1 mL of 0.1 M sodium acetate buffer (pH 5.5). Thereafter, 12 mg of sodium periodate


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were added, and the solution was incubated on a magnetic stirrer for 24 h in the dark and at room temperature. After incubation, the solution was purified on a PD-10 column equilibrated with 0.2 M NaHCO₃ (pH 8.2). We added 4 mg of sodium cyanoborohydride, 28 mg of lysine (cationic), or 28 mg of taurine (anionic) to ~20 mg of activated dextran in 250 μl of 0.2 M NaHCO₃ (pH 8.2). The solution was incubated with gentle shaking for 18 h in the dark and at room temperature. After 24 h of incubation, the solution was purified on two PD-10 columns and eluted in 0.15 M NaCl.

Technetium-99m Labeling. As described previously (8, 9), we added ~40 μg of SnCl₂ in 10 μl of ethanol to 0.5 ml of conjugate in 0.15 M NaCl and 100 μg of dextran (i.e., cationic, anionic, or neutral). After mixing, ~150 MBq of sodium per-technetate was added, and the solution was incubated for 15 min. After the incubation, the radiolabeled conjugate was purified on a Sephadex G-25 column (eluted in 0.15 M NaCl). The eluate was measured in an instant gamma counter, and the labeling efficiency could be calculated. Finally the eluate was sterile-filtered and diluted in ~150 ml of saline.

Intravesical Instillation. Approximately 100 μg of the dextran derivative labeled with ~60 MBq technetium-99m, diluted in ~150 ml of saline, were instilled intravesically through a urethral catheter and then kept in the bladder for 30 min. During the incubation, the patient was encouraged to move in the bed to improve the incubation conditions. After the incubation, the bladder was washed carefully with ~200 ml of saline. The patient was then moved to the operation theater. Biopsy samples were taken from normal tissue and tumor tissue (transurethral resection). The samples were weighed and then measured in a gamma counter (including the blood samples). The radioactivity per gram of tissue and the quotient radioactive uptake in tumor tissue: uptake in normal tissue were calculated.

Results

Table 1 shows the quotient and the mean quotient of radioactive uptake in tumor tissue divided with radioactive uptake in normal bladder tissue. Instillation of cationic dextran yielded very high quotients. Normal tissue had no uptake, i.e., normal background activity (<50 cpm/g tissue). Instillation of anionic dextran yielded low quotients with increased uptake in normal bladder tissue (~6000 cpm/g tissue). Neutral dextran yielded intermediate quotients with normal background activity. No radioactivity could be detected in blood.

A careful washing procedure (i.e., the wash after the incubation), was very important to minimize the background activity (uptake in normal bladder tissue).

The bladder tumors in this investigation apparently had cation-exchanging properties, i.e. their anionic charge interacted with the cationic charge of the dextran.

Discussion

An ion exchanger consists of an insoluble matrix to which charged groups are bound. The charged groups are associated with mobile counter ions that can be exchanged with other ions of the same charge (10). In this study, the bladder tumors apparently acted as such an ion exchange matrix. The negatively charged tumor tissue-bound the positively charged dextran. Considering the anionic charge in tumor tissue, there are several sources of such charge deriving from different levels of the tumor tissue that could explain our results and that make it theoretically possible to target all of these levels. The tumor vasculature is different from normal blood vessels and is often profuse (11). The wall of the vessels contains anionic carbohydrates (the first level). The tumor interstitium (the second level), which is frequently large compared to the normal interstitium, contains negatively charged molecules such as glycosaminoglycans (12). The negative charge is mostly from sulfamino groups (i.e., strong ion exchangers). Sialic acid, another anionic molecule, is associated with tumor tissue and is often expressed at the cell membranes (the third level; Refs. 13 and 14). With these conditions in mind, which are also at least partly relevant in bladder cancer, it is reasonable that the cationic dextran was accumulated, whereas the anionic dextran was not bound. Why the neutral dextran bound to the tumor tissue is unclear, because it should not contain any positive charge.

Cationic dextran can be substituted with cytostatic drugs, e.g., anthracyclines, or with radionuclides (⁹⁰⁴Y and ¹⁸⁸⁴Re), yielding a therapeutic compound suitable for intravesical treatment. There are several questions that need to be answered. How deep does the cationic dextran penetrate? When or how early in tumor development does the ion exchange effect work? Most of the agents instilled have a modest tissue penetration, which is probably one of the factors that limits their efficacy. It might be possible to manipulate the depth of penetration by using different sizes of dextran with different molecules linked to it. If the ion exchange effect occurs early in bladder cancer development, cationic dextran could, with a suitable label, be a diagnostic tool. One consequence of successful treatment of tumors in the bladder is an increase in the frequency of extravasal recurrences (15). The upper tract tumors are difficult to manage, and cationic dextran could be of use here.

The cation exchange effect seen in this study will now be investigated in a larger number of patients to confirm that this is a general phenomenon in superficial bladder cancer. After this, therapeutic studies will start.

The findings in this investigation could have importance for the treatment of other tumors growing in body cavities, e.g., ovarian cancer and certain brain tumors, and for the treatment of inoperable tumors (intratumoral injections). It also seems reasonable to contemplate the importance of charge compatibility between target and targeting substance in systemic tumor targeting.

These issues will be explored in future studies.

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