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Comparison of two routes of photosensitizer administration for photodynamic therapy of bladder cancer

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Abstract Photodynamic therapy (PDT) consists in administration of a photosensitizer and subsequent irradiation of the tumor with visible light. Routinely the photosensitizer is given intravenously (i.v.). The goal of our study was to examine whether intravesical (i.b.) instillation of the photosensitizer for PDT of bladder cancer might be feasible. Therefore, the uptake of chlor-aluminum-sulfonated phthalocyanine (CASPc) in bladder, bladder tumor, skin, and muscle in a rat bladder cancer model after i.v. injection and i.b. instillation was compared. The efficacy of PDT after either method of administration was also evaluated. The CASPc concentration in bladder tumor after i.v. injection was approximately 1.5-fold that after i.b. instillation. The ratio of CASPc concentration between bladder tumor and normal bladder was approximately 2:1 after administration by either route. There was no systemic absorption of CASPc after i.b. instillation; hence no systemic side effects are expected. PDT showed similar effects on bladder tumor after either method of administration, but less side effects on normal bladder wall after i.b. instillation. Our results demonstrate that i.b. instillation of CASP for PDT of superficial bladder cancer seems to have advantages over i.v. injection.

Key words Bladder cancer · Photodynamic therapy · Intravenous injection · Intravesical instillation

Photodynamic therapy (PDT) consists in administration of a photosensitizer and subsequent irradiation of the tumor with visible light. Usually red light is used because it has better tissue penetration depth than other wavelength of the visible spectrum. For endoscopic irradiation such as is applied in PDT of urinary bladder cancer, laser light that can be coupled into laser fibers with high energy is necessary. Light absorption by the photosensitizer leads to a photochemical reaction that destroys the tumor by forming singlet oxygen and/or free radicals [2, 6]. Thus far, a variety of tumors encountered in different specialties have been treated. In urology, PDT is used clinically for superficial bladder carcinoma, especially carcinoma in situ [1, 5, 8–10, 13]. Up to now a hematoporphyrin derivative (HPD) has been administered i.v. Since HPD has some drawbacks, such as chemical impurity, weak absorption in the therapeutically used red light and skin phototoxicity, new photosensitizers are being sought. Promising second-generation photosensitizers are phthalocyanines [16]. One derivative of this group, chlor-aluminum-sulfonated phthalocyanine (CASPc), was used in this study. CASPc showed good results in vitro and in vivo and should now be tested in a bladder cancer model. A new method for PDT of bladder cancer might be intravesical (i.b.) instillation of the photosensitizer to enhance the photosensitizer concentration in bladder carcinoma and to lower side effects. The goal of our study was to investigate the biodistribution and photodynamic efficacy of CASPc after i.v. injection and i.b. instillation in a rat bladder cancer model.

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Materials and methods

Tumor cell line

A bladder carcinoma cell line (AY 27) derived from rats after administration of a carcinogen was grown as monolayer culture (donated by Dr. S. Selman, Toledo, Ohio and Dr. Chi Whi Lin, Boston, Mass.).

Animal model

Female Fischer rats were anesthetized and a laparotomy was performed. After exposure of the urinary bladder a suspension of AY-27 cells was injected into the bladder wall as described elsewhere

Table 1 Concentrations of chlor-aluminum-sulfonated phthalocyanine (μg/g tissue; mean SE) in bladder tumor, normal bladder wall, skin and muscle 48 h after intravenous injection or 4 h after intravesical i.b. instillation

	i.v. (48 h)	i.b. (4 h)
Bladder tumor	5.89, 2.59	4.48, 2.39
Bladder	3.61, 0.65	2.09, 0.79
Skin	1.21, 0.25	0.21, 0.13
Muscle	0.25, 0.02	0.02, 0.01

[3, 4, 7]. One week after tumor implantation, when tumors reached 0.5 cm in diameter, experiments were carried out.

Distribution of the photosensitizer

CASPc, (Ciba-Geigy, Basel, Switzerland) a largely tetrasulfonated phthalocyanine with traces of mono-, di-, and trisulfonated derivatives, was used. It was injected i.v. into a tail vein at a concentration of 5 mg/kg body weight, or 0.5 ml Dulbecco's phosphate-buffered saline containing 1 mg CASPc per ml was instilled into the urinary bladder for 2 or 4 h. Bladder, bladder tumor, skin and muscle were taken out 24 and 48 h after i.v. injection or immediately after the blader instillation. CASPc was extracted after homogenization of the tissue. CASPc concentration was determined by fluorescence measurements and compared with a standard curve at known concentration of CASPc. The distribution of CASPc in normal bladder wall and bladder tumor was also evaluated by fluorescence microscopy on frozen sections. These slides were compared with slides stained with hematoxylin and eosin. Animals treated without CASPc served as controls.

Photodynamic therapy

For PDT CASPc was administered as described before. An argon-pumped dye-laser emitting a wavelength of 675 nm was used as light source. A laser fiber was inserted into the center of the bladder through a catheter. At the end of the fiber was a light-diffusing bulb for homogenous light distribution over the bladder wall. Before irradiation the bladder was filled with 0.6 ml saline. The fluence used was between 100 and 300 J/cm^2 at an irradiance between 160 and 200 mW/cm^2, where no thermal damage occurred. The light dose chosen was dependent on the results. Since only little treatment effect was observed after irradiation with low fluences a relatively high light dose was applied compared with other reported studies. At 24 h after treatment animals were sacrificed and examined macroscopically. The whole urinary bladder was taken out for histological examination. Untreated rats or rats treated with light or CASPc alone served as controls.

Results

Both after i.v. injection and after i.b. instillation, the highest CASPc concentration was found in bladder tumor, followed by normal bladder wall. The CASPc concentration in bladder tumor after i.v. injection was approximately 1.5-fold that after i.b. instillation. After i.b. instillation no fluorescence in skin and muscle was detected. The ratio between bladder tumor and normal bladder was approximately 2 after either mode of administration. The detailed extraction data are listed in Table 1. Fluorescence microscopy showed different patterns of CASPc fluorescence. After i.v. injection CASPc was found within the tumor around the vasculature and in the musculature of the normal bladder wall. In contrast, after i.b. instillation CASPc fluorescence was seen only on the tumor surface and in the urothelium.

After i.v. injection and whole-bladder irradiation at 200 J/cm^2 animals showed 25–100% necrosis of their tumors. In normal bladder wall partial ulceration, an inflammatory reaction and necrosis of up to 25% of the wall was seen. After i.b. instillation and irradiation at 300 J/cm^2, 75–100% tumor necrosis was found. The urothelium alone exhibited partial ulceration, but no inflammation, or in particular, necrosis of normal bladder wall was detected.

Discussion

In bladder cancer it might be better to give the photosensitizer i.b. instead of systemically (i.v.) because of the following hypothetical advantages: (1) enhanced photosensitizer concentration in bladder tumor after i.b. instillation compared with i.v. injection and therefore (2) higher phototoxic efficiency; (3) fewer side effects to normal bladder wall; (4) fewer systemic side effects. It has been reported that after PDT of bladder cancer with HPD as photosensitizer irritative symptoms, such as frequency and dysuria, have occurred [5, 8, 13]. Even vesico-ureteral reflux and bladder shrinkage caused by fibrosis of the bladder muscle have been described [5, 8]. After i.v. injection of HPD, the most frequently used photosensitizer in clinical PDT, another severe side effect is skin phototoxicity, which means that patients have to avoid direct sun exposure for several weeks. Our study was carried out to test a different photosensitzer and to compare systemic and intravesical administration. Phthalocyanines are a group of promising photosensitizers with different central atoms, such as aluminum, zinc, or gallium [16]. They are easy to synthesize and chemically stable [16]. Another interesting subject for study would be a comparison of phthalocyanines with different central ions, since they exhibit different biological activities. In our study CASPc was chosen because of the good results obtained with it in vitro and in vivo [15, 16]. It is also reported to have lower cutaneous skin phototoxicity than HPD [14]. When it is sulfonated, in order to achieve water solubility, a mixture of mono-, di-, tri-, and tetrasulfonated phthalocyanine usually occurs. Therefore, CASPc is chemically impure, but differs from photofrin in that the mixture components are well-characterized and dominated by the tetrasulfonated derivative. The composition of the HPD mixture varies in different preparations and with duration of storage [16]. The results show the highest CASPc concentration in bladder tumor followed by normal bladder after either administration. The total CASPc concentration in bladder tumor after i.v. injection was slightly higher than after i.b. instillation. However,

fluorescence microscopy demonstrated a different distribution of CASPc within the bladder. After i.b. instillation CASPc fluorescence was detected only on the tumor surface and normal urothelium. Therefore, a higher CASPc concentration was seen in the superficial layers of the bladder tumor. As superficial bladder cancer, especially carcinoma in situ, is an indication for PDT, this distribution of the photosensitizer should be advantageous. Another advantage of i.b. instillation is the lack of CASPc in normal bladder wall except for the urothelium. Hence, less irritative bladder symptoms are expected. Furthermore, after i.b. instillation no absorption of CASPc was observed. Therefore, no systemic side effects, such as skin phototoxicity can occur.

Photodynamic treatments showed similar effects on tumor necrosis after either mode of administration, but fewer side effects to normal bladder wall after i.b. instillation. In particular, no necrosis of normal bladder wall was seen. Pope reports on a study in which CASPc was injected i.v. to rats or instilled into normal rat bladders [11, 12]. At 1 h after i.v. injection CASPc fluorescence was seen mainly in the endothelium of vasculature and in well-vascularized layers of the submucosa and serosa [12]. At 24 h after injection a more marked gradient of 3.5–4 was found between superficial and muscle layers [12]. After intravesical instillation a patchy, unreliable distribution of the photosensitizer was described [12]. However, compared with our study, the CASPc bladder instillation time was only 0.5 and 1 h [12]. The distribution of CASPc seems to be more homogeneous after longer instillation times of 2 h and 4 h. Furthermore, for PDT in bladder carcinoma the photosensitizer uptake in tumor and the ratio between tumor and normal bladder wall are crucial. Taari studied morphological effects of PDT on normal rabbit cancer after i.v. and i.b. administration of Photofrin II and Photosan [17]. After i.b. instillation of the photosensitizers and whole-bladder irradiation at 12 and 24 J/cm^2 minimal inflammation of or epithelial injury to the bladder wall was found when it was studied by light microscopy [17]. In contrast, after i.v. injection of the photosensitizers and irradiation, such changes as inflammation and epithelial injury of the bladder wall could be seen even macroscopically [17]. In conclusion, our data demonstrate that i.b. instillation of CASPc, as against i.v. injection, seems to be possible for PDT of carcinoma in situ and for prophylaxis of recurrent superficial bladder carcinoma. Further studies are required to examine the efficacy of CASPc in papillary tumors after topical administration. Similar CASPc concentrations in bladder tumor and normal bladder wall were found after either mode of administration. Furthermore, similar tumoricidal effects but fewer side effects to normal bladder wall were observed in our animal model after i.b. instillation.

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