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REVIEW

An overview on preclinical and clinical experiences with photodynamic therapy for bladder cancer

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Photodynamic therapy (PDT) is one of the most interesting methods of photo treatment. In general, PDT is a modality for the treatment of non-muscle invasive tumors. PDT is very well suited in managing bladder cancer, as the bladder is accessible by endoscopy and the tumors are most often limited to the mucosa or sub-mucosa. PDT is likely more useful for patients with recurrent tumors after conventional therapies, as well as for patients with diffuse non-muscle invasive bladder carcinomas that are

refractory to standard treatments before the commitment to radical extirpative surgery, particularly in patients at surgical high risk. The treatment of tumors with PDT includes three major parameters: presence of oxygen in tumor tissue, administration of a photosensitizer, and subsequent exposure to light. The PDT mechanism relies on the in situ generation of cytotoxic agents by the activation of a light-sensitive drug, resulting in cell death. In this review, we present past and current advances in the use of PDT with urinary bladder cancer and discuss the future roles for this type of therapy in the treatment of bladder cancer.

Key Words: bladder cancer, photodynamic therapy, photodynamic diagnosis

Introduction

Bladder cancer is the ninth most common cancer worldwide. The majority of cases initially present as non-muscle invasive types. Bladder cancer has been determined to be the most expensive cancer to treat, mainly due to the frequent follow ups necessary for patients with the non-muscle invasive form,¹⁻³ which presents itself as two different types of diseases. One is a low grade, papillary, non-invasive urothelial cell carcinoma (UCC) of the bladder (stage Ta) that has a high potential to recur, but it is very unlikely to progress. The other tumor type is a high grade lesion

that often begins as a flat carcinoma *in situ* (CIS) and progresses into a solid invasive carcinoma muscle invasive form (T2-4) that is prone to metastasize.⁴

Non-muscle invasive bladder cancer is treated with transurethral resection (TUR) that is usually followed by adjuvant intravesical instillation therapy to reduce the risk of recurrence and to prevent progression to a muscle invasive disease. The majority of these patients will have a recurrence during follow up; thus, the management seems to be inadequate. Fluorescence endoscopy, i.e., photodynamic diagnosis (PDD) with intravesical application of the drug hexyl-ALA (hexaminolevulinic acid, HAL, Hexvix), was approved in Europe in 2005 for the diagnosis of bladder cancer to improve visualization techniques of CIS and tiny papillary tumors.⁵ HAL accumulates preferentially in neoplastic tissue and fluoresces in the visible region when illuminated with light of the appropriate wavelength. Combined with TUR, this technique reduces the recurrence rate.⁶

In many other fields of medicine, PDD has been developed into photodynamic therapy (PDT). The aim of this review is to present the basis and features of PDT and its application for the intravesical approach.

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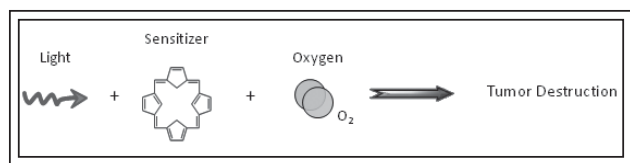


Figure 1. Three important elements in photodynamic therapy.⁷

Basis of photodynamic therapy

Photodynamic therapy (PDT) is a treatment modality consisting of three elements: a photosensitizer, light that is usually in the visible range, and molecular oxygen.⁷ A series of photochemical reactions occur, generating reactive oxygen species (ROS). They exhibit cytotoxic effects and can destroy tumor cells, see Figure 1.

The nature, location and quantity of PDT-induced reactions and the sensitivity of the target cells determine the outcome of the treatment.⁸ When the photosensitizer molecule absorbs light, the molecule will be excited from its ground state to an excited singlet state. From here, the molecule takes different pathways to return to the ground state, transferring its excess energy to nearby oxygen molecules resulting in generation of ROS, such as hydroxyl ions, hydrogen peroxide, superoxides and singlet oxygen. These react in turn with tissue and cause irreversible damages. Because the light used in PDT is of relatively low power, no tissue heating occur.⁸

The antitumor effects of PDT result from three interdependent processes: direct tumor cell kill, damage to the vasculature, and activation of a nonspecific immune response.⁸ In many cases, mitochondria are the primary targets, but plasma membranes and lysosomes may also be involved.⁹ Photosensitizers that localize

in mitochondria are more likely to induce apoptosis, while those targeting the plasma membrane primarily induce necrosis.⁸ *In vivo*, tumor destruction is likely to be the result of a combined effect.⁸ Due to a strong light attenuation, the effectiveness of PDT is decreased in deep tumor regions and tumors may relapse.⁹

The antitumor vascular effect is due to endothelial cell damage by ROS, leading to blood flow stasis, vascular collapse and vascular leakage. PDT activates the release of inflammatory cytokines, inducing leukocyte recruitment followed by tumor specific immunity, which may have a role in achieving long term control.⁹ Possible DNA damage to surrounding normal cells is a potential concern with PDT. Because of the very limited range (< 0.1 μm) and lifespan (nanoseconds) of ROS, the probability of ROS-induced DNA damage is low unless ROS is generated in close proximity of a DNA strand. Studies also indicate that ROS-induced damage to cytoplasmic proteins and mitochondria, rather than specific DNA damage, is the major cause of cell death after PDT.⁸

Photosensitizers in PDT for bladder cancer

An ideal photosensitizer must be biologically stable, photochemically efficient, selectively accumulated in or retained by the target tissue relative to surrounding normal tissues and have minimal systemic toxicity.¹⁰ Various types of photosensitizers have been studied for different types of malignancies. Table 1 lists some of the photosensitizers currently in use or under investigation for PDT for uro-oncological tumors.¹¹

Hematoporphyrin and its derivatives have been central to the development of PDT. The tumor selectivity of porphyrins has been known for many years. Photofrin was the first clinically approved

TABLE 1. Photosensitizers for uro-oncological PDT, modified¹¹ Reprinted with permission from Elsevier

Photosensitizer	Approximate main activation wavelength (nm)	Urology clinical experience
Hematoporphyrin derivatives (Photofrin)	630	Bladder and prostate
ALA	635	Bladder
ALA-ester (such as HAL)	635	Bladder
Hypericin	590	Bladder
WST09 (Tookad)- WST 11 (Padeliporfin)	755	Prostate
Benzoporphyrin (BPD)	690	Prostate
mTHPC (Foscan)	652	Prostate

photosensitizer, but it consists of over 60 compounds and is difficult to be reproducibly synthesized. In addition, its molar absorption coefficient is relatively low, and thus higher doses and fluence rates are required to produce similar effects to new generation photosensitizers. Photofrin is retained by normal cells for prolonged periods causing long lasting cutaneous photosensitivity that requires sunlight avoidance for 4-6 weeks.⁸ Intravenous Photofrin obtained its first regulatory approval for recurrent papillary tumors in the bladder in 1993. The initial response to a single treatment of the whole bladder tends to be good, but side effects such as bladder contraction and irritation are noticeable, and the incidence of relapse within a year is high.^{12,13} These factors have stimulated research leading to the development of second generation photosensitizers.⁸

The introduction of ALA in the treatment of skin malignancies was a major advancement within clinical PDT. ALA, which is the precursor of the photosensitizing compound PpIX, is a naturally occurring amino acid, which is produced in mitochondria during the normal biosynthesis of heme and accumulates temporarily in the tissue.⁷ As ALA is a hydrophilic amino acid, it experiences little cellular uptake and its distribution is somewhat heterogeneous.¹⁴

To enhance lipophilicity, ester derivatives have been synthesized. For intravesical applications, hexyl ester of ALA, hexaminolevulinic acid (HAL), was introduced. This drug exhibits deeper penetration into the urothelium resulting in higher PpIX concentrations at significantly lower prodrug concentrations and shorter application times, compared to ALA.¹⁵ HAL was demonstrated to produce at least twice the fluorescence of ALA but at a concentration 45 times lower. HAL-PDD has been shown to be able to reduce the incidence of false-positive results (17%), but without significantly improving the specificity.¹⁶ To date, the major weakness of HAL-PDD is its relatively low specificity. Still HAL appears to be more promising as a PDT agent than ALA itself.¹⁶

Hypericin (HY) is a photosensitizer that has recently drawn interest for its beneficial photoactivity characteristics. Depicted in Figure 2, HY is one of the principal active constituents of *Hypericum perforatum* L. plants (St. John's wort).^{17,18} *Hypericum* plants have been of scientific interest for many years due to their widespread use in folk medicine for a range of conditions. *Hypericum* extracts are still widely used today for the treatment of depression. A daily dose of about 500 mg of extract corresponds to a total dose of 1 mg-2 mg of HY. These doses of HY, when given orally, do not provoke skin phototoxicity.

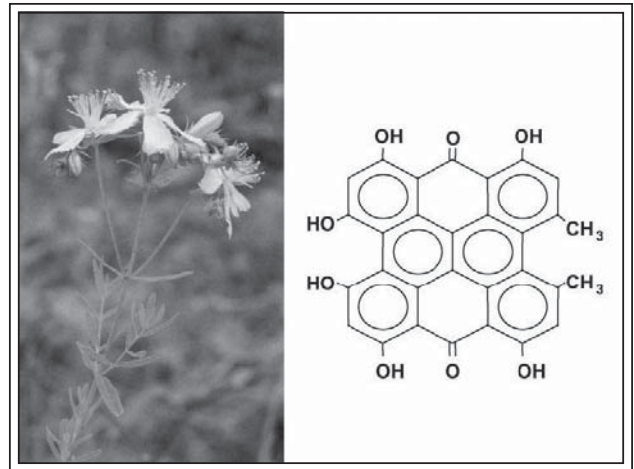


Figure 2. *Hypericum perforatum* (St. John's wort) and the structure of hypericin.¹⁸ Reprinted with permission from Elsevier.

HY is a lipophilic compound that binds to phospholipids, resulting in some degree of affinity to plasma proteins and tumors.¹⁹ Photoactivated HY mainly targets membrane structures in the cell. A number of studies using HY-PDD of bladder carcinoma *in situ* have shown high sensitivity (82%-94%) and specificity (91%-98.5%).²⁰⁻²² Notably, HY has never exhibited toxic or genotoxic effects *in vitro* or *in vivo*, and seems to have the potential to be developed for PDT of bladder carcinomas.^{18,21} HY appears to be retained in the tumor for at least 1 hour. This slow clearance is advantageous for PDT as it allows sufficient time to conduct the treatment.²³ By using the appropriate HY dose and incubation time, the compound is selectively taken up by bladder urothelial tumors. PDT with HY produces a uniform urothelial eradication, which is the only reliable proof of cure for the multifocal CIS or diffuse low grade papillary bladder tumors, while avoiding damage to the underlying muscles.²³ There are no reports of local or systemic side effects in patients with flat bladder carcinomas who received instillations for 2 to 4 hours at a maximal dose of 160 µg of HY, which corresponds to about one-tenth of the oral dose used as an antidepressant.²⁴

Studies show that HY can induce both apoptosis and necrosis in a concentration and light dose-dependent fashion.¹⁸ Accordingly, HY-induced PDT results in the activation of multiple pathways.¹⁸ The photosensitizing effects of HY are generally described as oxygen dependent, strongly supported by observations that HY in a hypoxic environment does not exhibit photocytotoxicity nor an inhibitory effect on mitochondria.^{18,25}

Pure HY, now synthetically produced, is characterized by a number of drawbacks, such as low solubility, costly production, and a lack of stability in solution,¹⁷ thus prompting the development of new drugs based on HY being less expensive and more soluble. A polar methanolic fraction (PMF) of HY is an alternative. PMF-HY has interesting properties for PDT. Several studies have established its powerful *in vivo* and *in vitro* antineoplastic activity following PDT. It has been tested against bladder cancer *in vitro* using PDT.^{17,18,26} These results were compared with the results obtained in the same cell lines using Photofrin. Significantly better photocytotoxicity and selective localization were found with this new drug.²⁶

A poly-N-vinylamide of various degrees of polymerization (PVP) is another alternative formulation. HY forms liquid molecular chromophore complexes in water and binds to PVP, providing improved biodistribution properties. PVP-HY forms intermolecular cross-links interesting for diagnostic and therapeutic applications, but so far clinical studies are lacking. In PVP-HY 100 g or less of PVP binds more than 1000 mg of HY and is soluble in 1 liter of water. Aqueous dispersions of PVP-HY display a characteristic absorption spectrum and fluorescence emission band around 600 nm. Its photochemical properties are conducive for diagnostic investigations both *in vitro* and *in vivo*. Furthermore, PVP-HY exhibits high photostability in the presence of oxygen and broad band light, which ensures reproducible photodynamic therapy and diagnosis.²⁷

Oxygenation

The availability of molecular oxygen during irradiation has a profound effect on the treatment outcome. Without oxygen, PDT will have no antitumor effect; accordingly, hypoxic tumor cells of solid tumors are generally resistant to PDT. The generation of ROS and PDT efficacy depends on intra-tumor oxygen tension (pO_2 in cellular-targeting PDT), hemoglobin oxygen saturation (SO_2 in vascular-targeting PDT) and the microenvironment of solid tumors. It can therefore be valuable to monitoring tumor tissue oxygenation during PDT.²⁸

At high fluence rates of light, the supply rate of oxygen cannot compete with its use in the PDT reaction.²⁹ Thus, the oxygen tension decreases during treatment. At low fluence rates of light, long exposure times are, on the other hand, required. Vessel damage may occur during the light exposure, and this will again lead to oxygen depletion.

Several techniques have been proposed to deal with tissue oxygen depletion during PDT, including fractionating of light irradiation into controlled light/dark periods, and by reducing the fluence rate.^{28,30} This affects oxygen depletion by providing sufficient time for reoxygenation during the treatment.³¹ Additionally, relocalization of the particular photosensitizing agent and induction of apoptosis by reperfusion injury have been reported to occur and support the success of photodynamic therapy.³¹

Light in PDT for bladder cancer

The proper light delivery depends on the organ site. In the bladder, treatment light is delivered endoscopically via optical fibers. To treat the target area uniformly, the light is diffused by specialized diffusing fiber tips or by balloon catheters filled with a scattering liquid.¹¹

Bladder tissue is relatively translucent compared to many other human tissues with no difference in light penetration between malignant and normal bladder tissue at any of the wavelengths of interest for PDT. The light wavelength is very important for light penetration. Tissue absorption generally decreases with wavelength in the visible and near-infrared regions. Therefore, the best light penetration is in the near-infrared region. Light in the red region of the spectrum is sufficient to achieve effective treatment depths of approximately 5 mm to 8 mm by surface illumination. In PDT for clinical use, the activating light is usually between 600 nm and 900 nm. The strong absorption of hemoglobin below 600 nm and insufficiency to generate ROS for longer wavelengths defines the limits of the effective PDT range.⁸

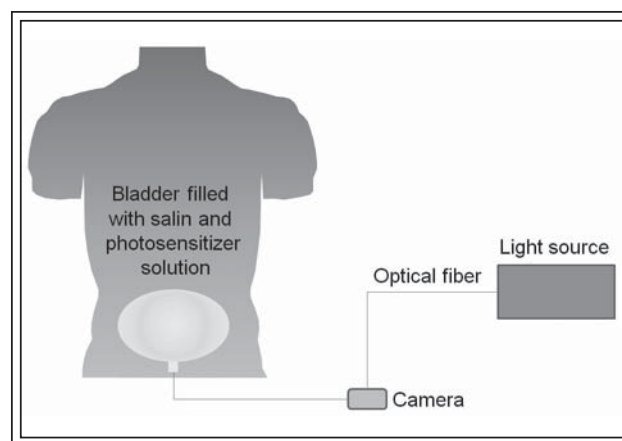


Figure 3. Schematic representation of PDT after the administration of a photosensitizer and local anesthesia.

The light source can be a laser, a filtered, high brightness lamp³² or an array of light-emitting diodes. The most commonly used PDT light sources are lasers. The laser light can be coupled into and passed down an optical fiber for convenient light delivery. Because bladder cancer tends to occur multifocally and is often not cytoscopically visible, the entire bladder wall should be illuminated as uniformly as possible.³³ The most commonly used procedure is to expand the bladder cavity by filling it with 100 mL-200 mL saline to smooth the mucosal folds. A spherical light-diffuser is then positioned in the "optical center" of the bladder cavity, Figure 3.³⁴

In cases of superficial tumors, a fluence rate of light at the tissue surface is often sufficient.³⁵ However, the effect of multiple scattered light, which causes the fluence rate in the outermost cell layers to be higher than the applied light intensity, must also be considered.⁷ Because the bladder is a confined hollow organ, any light reflected from the bladder surface will reach another part of the bladder wall, so comparing to PDT for cutaneous malignancies much less light is required in PDT for bladder cancer.³⁴

Preclinical and clinical results

A major development in the history of bladder cancer-PDT happened in 1976, when human bladder tumor cells transplanted into mice were destroyed for the first time using Photofrin-PDT. When normal or smooth muscle cells were implanted, minimal, if any, damage occurred. These results initiated the first human study of Photofrin-PDT, in which five patients with bladder

cancer were treated. PDT induced tumor necrosis with negligible effects to the untreated areas.³⁶ Several rather small trials have been reported with a complete response (CR) rate of 44%-84% at 3 months. The largest study, including 58 patients who had failed prior intravesical therapy, showed CR rates of 84% and 75% for patients with papillary and CIS, respectively.³⁷ At longer follow ups, most had recurrent disease and bladder contracture was quite common, which limited the appeal of this technique. PDT with intravesically applied ALA was later tested and a CR rate with different follow up times, Table 2, were reported. The therapy was well tolerated, and side effects occurred in all patients in the form of irritating urinary symptoms, but usually were resolved within 2 weeks.³⁸ Side effects, such as reduced bladder capacity, which occurred frequently with first-generation photosensitizers, have not been observed in any patients following ALA-PDT. These results can be explained by the absence of PpIX in the endothelium of the subepithelial vessels and bladder muscle.³⁹

PDT after oral administration of ALA has been tested in a trial. Twenty-four patients were treated and 21% and 60% CR was observed for Ta-T1 and CIS, respectively, with a median follow up of 36 months.³⁸ No phototoxic skin reaction or decreased bladder capacity was observed in this study. A small study used electromotive diffusion (EMD) to increase the uptake of ALA. Five of six patients with CIS biopsy-proven recurrent carcinoma *in situ* of the bladder were tumor free after a follow up of 10-16 months.⁴⁰ In a phase I study, sequential mitomycin C and ALA were applied in 24 patients, most with a prophylactic indication. Mitomycin C instillation was followed by

TABLE 2. Clinical studies of PDT in urinary bladder cancer

References	No. patients	Photosensitizer	Tumor type	Previous treatment	Complete response
Manyak and Dean ⁴³	34	Photofrin	29 with TCC carcinoma <i>in situ</i> (CIS) and 5 with multiple small papillary stage T(a) or T(1) lesions	Chemotherapy and/or immunotherapy	44%
Berger et al ³⁸	31	ALA	Recurrent superficial bladder carcinoma Ta, T1, CIS	BCG*	52%
Waidelich et al ⁴²	12	ALA	Ta grade I and III, CIS	Multiple transurethral resections, chemotherapy and/or immunotherapy	47%
Shackley ³³	19	ALA	Ta grade I and III, CIS	Mitomycin-C, BCG*	58%

*BCG = Bacillus Calmette Guerin.

TABLE 3. Comparison of ALA-, HAL- and HY-PDD, modified.²⁰ Reprinted with permission from Begell House

Characteristics	ALA	HAL	HY
Form	Prodrug	Prodrug	Active form
Sensitivity	77.8%-100%	96%	82%-94%
Specificity	33%-80% (many false positives)	43%	91%-98.5%
Stability	Easily photobleached during process	Easily photobleached during process	Greater stability
Permeability	Charged molecule; difficulty in penetration	Hydrophobic	Hydrophobic

ALA concentrations of 6%, 8% or 10%. A total fluence of 25 J/cm² represented the upper light dose for the tolerability of this procedure by patients, and the prophylactic effect was promising.⁴¹

Twelve patients with papillary tumors and CIS were treated in a study using white light ALA-PDT. The CR rate was 50% and 43%, respectively, at 18 months of follow up.⁴² No decreased bladder capacity or systemic side effects were observed. In that study, irradiation of 100 J/cm² was applied with an irradiation time of 60 to 150 minutes (mean 102 min.).

ALA-PDT is painful and requires some form of anesthesia. Two different doses of ALA (3% and 6%) were administered in a study with and without local anesthesia with lignocaine. The discomfort was immediate and was a function of the ALA concentration rather than the total light dose. The procedure was well-tolerated using local anesthetic at the lower dose, but more effective anesthesia seems to be required at higher doses.³³

Esterification of ALA resulted in more rapid build up of PpIX fluorescence at lower concentrations and for a longer period *in vitro*.⁴⁴ In humans, intravesical instillation of 8 mM of HAL for 2 hours induced the same fluorescence intensity as 6 hours of 180 mM of ALA.⁴⁵⁻⁴⁶ Histology has shown that a fluence of 20 J/cm² (20 mW/cm²) combined with 8 mM of HAL (1 h) is an appropriate dose for HAL-based PDT treatment of rat TCC.⁴⁷

In PDD of bladder cancer using HY, very high sensitivity and specificity were reported, see Table 3.²⁰ HY-PDD has been shown to increase the amount of detected tumor by 30%, resulting in a reduction of tumor recurrence by 20%.²²

Both preclinical and clinical studies have introduced HY as a potent and safe photosensitizer for PDT. The biodistribution of HY in an orthotopic bladder cancer model was investigated. The tumor-to-normal-bladder ratio was 12:1 after 4 hr of hypericin (30 µM) instillation. The drug was retained in the tumor for at least 1 hr and

penetration was restricted to the urothelial tumor and normal urothelium.²³

As mentioned, PMF-HY and PVP-HY have recently been developed and tested as novel, natural photosensitizers for use in PDT and PDD to overcome the problem of low water solubility of HY. PMF-HY has been tested on HL-60 leukemic cells and cord blood hemopoietic progenitors.²⁶ The type of cell death induced by PMF-HY photoactivation has been studied using flow cytometry and DNA laddering. The reported significant photocytotoxicity, selective localization, natural abundance, and easy and inexpensive preparation underscore that the PMF-HY extract may be a novel, effective PDT photosensitizer.²⁶ Studies on the effect of PMF-HY-PDT against RT4 and T24 human bladder cells showed that 60 µg/mL of the extract with 4-8 J/cm² appeared to be an effective dose, with significant 86% cell killing of RT4s. The same drug and light dose was sufficient for killing of 80% of T24 cells. Cell death by PMF photodynamic action in these two bladder cell lines is caused predominantly by apoptosis. Although the absorption maximum of PMF is located at 590 nm, photoexcitation is achieved with a laser light at 630 nm.

In a study in which the biodistribution of PVP-HY and HAL in normal and orthotopic tumor-bearing rat urinary bladder was investigated and compared, 30 µM of HY-PVP accumulated about 3.5-fold more in malignant urothelial tissue compared to normal urothelium, whereas PpIX accumulated to the same extent in malignant and normal urothelium after intrabladder infusion of 8 or 16 mM HAL.⁴⁸ In that study, PVP-HY and PpIX selectively accumulated in the urothelium with a tumor-to-muscle ratio of 30.6 for PVP-HY and 3.7-8.3 for 16 and 8 mM HAL, respectively. One can thus conclude that several studies have suggested the great potential of PVP-HY as a photodynamic agent against non-muscle invasive bladder cancers after intravesical administration, with limited risk of affecting the deeper layers of the urothelium.^{48,49}

Discussion and conclusion

This review article presents a collection of studies performed on the use of PDT for urothelium cancer. The advantages of PDT over other conventional cancer treatments are its low systemic toxicity and its ability to selectively destroy tumors that are accessible to light.⁵⁰ Therefore, PDT is being used for the treatment of endoscopically accessible tumors such as bladder cancer tumors. Considering that about 70% of patients with superficial bladder tumor relapse within only 2 years after primary conventional surgical therapy, PDT may be recommended as a second-line or immediate therapy for patients in which multiple transurethral resections, chemotherapy and/or intravesical BCG immunotherapy alone had failed.^{38,39,51-53}

The selective retention of the photosensitizer in neoplastic tissues and the *in situ* activation of the drug by irradiation gives PDT an obvious advantage over conventional chemotherapeutic or radiation cancer treatments, as it combines a minimal systemic toxicity with a highly selective photodynamic destruction of tumor cells.¹⁸ The risk for mutagenic alterations due to PDT is very small, as most photosensitizers are not localized in the cell nucleus. However, further studies are necessary.

The selection of the proper photosensitizer for bladder PDT is still debated. So far, HAL is the only drug that has been approved in Europe for clinical use. However, this drug has a relatively low tumor specific uptake and suffers from photo degradation upon irradiation, which might hamper the PDT efficacy. Therefore, HY has been suggested to be a good alternative. HY has a minimal photobleaching effect with highly specific uptake.⁵⁴

The oral administration of HY extract is used clinically as an antidepressive agent with no side effects.^{24,55} Its high specificity and selective mucosal uptake seem to be promising for the future use of HY in clinical PDT.^{55,56} However, despite the fact that HY has been found to be superior to ALA and HAL in some aspects, it is a very lipophilic compound and only sparingly soluble in water. This apparent water insolubility makes the formulation of HY for clinical applications difficult.²¹ Nevertheless, PMF and PVP formulations of HY are being introduced to disperse HY in water. Investigations on PMF-HY and PVP-HY suggest that these photosensitizers have great potential as photodynamic agents both for PDD and PDT of bladder cancer.^{48,49}

Current photosensitizers accumulate preferentially in tumor tissue, though the mechanisms are still not fully understood.¹⁶ By improving the accumulation of

the photosensitizing agent in malignant tissue, the PDT response could be enhanced.⁴⁵ The intravesical instillation of photosensitizer has been shown to cause less damage to normal tissue than intravenous administration and still yields the same PDT efficacy.⁴⁷

Because the side effects in PDT are dose dependent, fractionating drug and light might subside cancerous cells and reduce local toxicity.⁴³ As there is no difference in light penetration between UCC and normal bladder walls, a tumor-specific PDT response with diffuse intravesical light will depend on drug localization within the tumor.⁵⁷

Because of the complex mechanisms in PDT and dependence on a large number of parameters, there is still room for improvements. However, strong evidence suggests that PDT has many clinical advantages and promising potential for managing bladder cancer. □

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