Journal of Biology and Today's World

Journal home page: http://journals.lexispublisher.com/jbtw

Received: 15 March 2017 • Accepted: 24 May 2017

Short C

doi:10.15412/J.JBTW.01060701

Fluorescent Diagnosis of Bladder Cancer by Hexasens as a Drug

Slovokhodov Egor Konstantinovich[1], Ivanova-Radkevich Veronika Igorevna[2,3*], Brodsky Ilya Borisovich[3]

[1] City Clinical Hospital No. 40, 7 Kasatkina st., Moscow, 129301, Russian Federation

[2] State Scientific Center Scientific Research Institute Organic Intermediates and Dyes, 1/4 Bolshaya Sadovaya st., Moscow, 123995, Russian Federation

[3] Peoples Friendship University of Russia (RUDN University), 6 Miklukho-Maklaya St., Moscow, 117198, Russian Federation

*Correspondence should be addressed to Ivanova-Radkevich Veronika Igorevna, State Scientific Center Scientific Research Institute Organic Intermediates and Dyes, 1/4 Bolshaya Sadovaya st., Moscow, 123995, Russian Federation; Tel: +78001002760; Fax: +78001002760; Email: russia@prescopus.com.

ABSTRACT

This study was performed as a clinical trial conducted in Russia concerning the diagnostic efficacy of Hexasens drug. The photosensitizer based on hexyl ester of 5-aminolevulinic acid was used for the fluorescence diagnosis of bladder cancer. The study was conducted in 2015-2016 on the basis of the City Clinical Hospital No. 40 (Moscow, Russia). 53 patients were involved into study and were diagnosed as bladder cancer in T1N0M0 stage. Fluorescence diagnosis was carried out for 1 hour after the intravesical induction of 0.2% solution (50 mL or 100 mg) of Hexasens drug (FSUE "SSC NIOPIK", Russia). The results of the fluorescence diagnosis were compared with the results of the standard routine cystoscopy. The found results showed that the performance of fluorescent diagnostics (FD) led to improve diagnostic sensitivity as 13.2% compared to standard cystoscopy (from 86.8% to 100%); the diagnosis accuracy increased as 8.8% (from 91.2% to 100%) and a negative predictive value increased as 20.9% (from 79.1% to 100%). During the carrying out of fluorescence diagnosis, 8 patients (15.0%) out of 53 ones also revealed 14 fluorescing foci that not identified in white light, in which the tumorous process in them was confirmed during morphological study. The induction of Hexasens solution into a bladder and the carrying out of a fluorescent diagnosis was not accompanied by the development of any adverse reactions. Patients showed a subjective discomfort only during a prolonged exposure of the drug solution in a bladder (up to 2 hours). The developed diagnosis methods is recommended for the applying in advanced clinical diagnosis.

Key words: Fluorescent Diagnosis, Bladder Cancer, Hexasens.

Copyright © 2017 Slovokhodov Egor Konstantinovich et al. This is an open access paper distributed under the Creative Commons Attribution License.

Journal of Biology and Today's World is published by *Lexis Publisher*; Journal p-ISSN 2476-5376; Journal e-ISSN 2322-3308.

1. INTRODUCTION

Currently, fluorescent methods are considered as the most promising ones among the modern methods of cancer early diagnosis. During fluorescent diagnosis conduction, photosensitizer is induced into a patient's body, which is accumulated in a tumor selectively. Then, the tumor is irradiated by the light with the wavelength related to the maximum absorption of the photosensitizer. Under the influence of this light photosensitizer, it begins to fluoresce, and the fluorescence of the tumor tissues will be significantly harder than the fluorescence of surrounding healthy tissues. The conduction of fluorescence diagnosis helps to clarify the boundaries of tumor process prevalence and to identify additional tumor foci which are not defined by a naked eye (1-4). The FD of cancer is the most promising one for the detection of small tumors (up to 1 mm), localized in the surface layers (epidermis, mucosa epithelium), as the sensitivity of this method is significantly higher than among the other modern methods of early diagnostics (5, 6). One way of photosensitizer effective concentration creation in a tumor tissue is organism stimulation for endogenous photoactive compound production - porphyrins and, in particular, protoporphyrin IX (PPIX). One of the compounds which induces the synthesis of endogenous fluorophore PPIX effectively is 5-aminolevulinic acid (5-ALA) and its ester derivatives (methyl and hexyl esters 5-ALA). It is known that the tumor cells are capable to increase the accumulation of photoactive PPIX in the presence of exogenous 5-ALA due to an increased activity in the tumor cells of heme

synthesis initial stage, and ferrochelatase deficient in them, the enzyme converting PPIX into a heme (7). PPIX accumulation in tumor cells occurs within a few hours, and its high level is held up to 6 hours, while PPIX is utilized more quickly by the way of its conversion into a photopassive heme. The result of it is a high fluorescence contrast between a tumor and a surrounding tissue, which manifests itself during this period of time, and increasing 10-15 fold for different tumors, which is an important factor for the visualization of tumor boundaries during FD conduction (7, 8). 5-ALA is of great interest for clinical use as PPIX may be an effective agent for FD. So far, a number of drugs on the basis of 5-ALA were registered and approved for the medical using on the basis of 5-ALA - Levulan (Norway), Dusa (Canada) and Alasens (Russia). These drugs showed a very high efficiency for the diagnosis of malignant tumors in a number of locations; and FD method with their application is used in the leading clinics of the world as one of the most sensitive one to clarify the prevalence of tumor process (7-9). Hexasens drug was developed in Russia at FSUE "SSC NIOPIK" together with MNIOI named after P.A. Herzen based on hexyl ether 5-ALA synthesized according to the an original technology (10). The preclinical studies of a developed drug were performed at the department of modifiers and anticancer therapy protectors MNIOI named after P.A. Herzen on different animal species (10, 11). Currently, the first phase of Hexasens drug clinical studies in Russia organized by FSUE "SSC NIOPIK" was completed successfully under the Protocol No. 01-(FD-GE)-201 "controlled open study of tolerability and diagnostic efficacy of Hexasens drug - the photosensitizer for the fluorescence diagnosis of bladder cancer". The studies were conducted on the basis of MNIOI named after P.A. Herzen. FD methods were developed during the research and it was recommended for further studies: the induction of Hexasens drug solution into an urinary bladder within 100 mg (50 ml of 0.2% solution) with the exposure of 1.5-2 hours, followed by FD conduction. Based on these results the conclusion was made about the feasibility of Hexasens drug clinical research continuation (12). The aim of this study was to establish the safety and the efficacy of Hexasens drug for the fluorescence diagnosis of bladder cancer compared to standard cystoscopy.

2. MATERIALS AND METHODS

The study was conducted as the part of the 3rd phase of clinical trials in respect of Hexasens drug under the Protocol of clinical studies number 02-(FD-GE)-2014 "Controlled open study of Hexasens drug diagnostic efficiency - the photosensitiser for the fluorescence diagnosis of bladder cancer". The permission from the RF Ministry of Health No: 304 issued on 06.09.2015 was received for the study performance. Study conduction terms - 2015-2016. The place of study performance - City clinical hospital No: 40 (Moscow, Russia). All patients participating in the study met the following inclusion criteria:

- age - from 18 to 70 (inclusive);
- verified diagnosis (instrumentally and morphologically confirmed) of bladder cancer;
- tumors without the signs of decay or necrosis;
- objectively measured and measurable tumor lesions;
- the prevalence of tumor process according to TNM classification: Ta-isNoMo - T1N0M0
- Life expectancy - at least 6 months;
- blood pressure no more than 140/90 mmHg, creatinine level in blood plasma no more than 97 mol/l, ALT, AST not above 40 u/l, Bilirubin - no more than 20 mmol/l;
- hematological parameters: white blood cell count > 4000/mm^3, platelets > 150,000/mm^3, hemoglobin 12 g/dl;
- the satisfactory condition of the patients (0-2 according to world Health Organization (WHO) scale);
- the absence of therapeutic control in respect of comorbidities;
- a patient's ability to perform the procedure of investigation and provide a written informed consent in accordance with good clinical practice (GCP) and local legislation.

Before the start of the study, the participants were informed in detail about the studied properties of Hexasens drug, indications, contraindications, administration route, safety precautions to prevent adverse reactions, as well as about their participation in the study of fluorescence diagnosis efficacy and tolerability. The consent to participate in the study was given by the patients in writing. The study included 53 volunteer patients diagnosed with bladder cancer in T1N0M0 stage, including 41 males and 12 females. The mean age was 59 ± 11 (from 22 to 69) years. 26 patients were diagnosed with a primary tumor of the bladder prior to the inclusion in the study, 27 had relapse after previous treatment. The degree of tumor differentiation was defined as G1 among 18 patients, as G2 among 16 patients and as G3 among 19 patients. 43 patients had a variety of related clinically significant diseases (coronary heart disease, hypertension, etc.). Hexasens drug powder (Federal State Unitary Enterprise "SSC NIOPIK", Russia) in the dose of 100 mg dissolved immediately before the use of 50 ml of isotonic sodium chloride solution to the concentration of 0.2% and was injected through the catheter into the urinary bladder of the patient. The exposure of the drug solution in a bladder was 1-2 hours. Fluorescence diagnostics was carried out for 1 hour after the removal of the drug solution from the bladder. In order to perform the fluorescence cystoscopy, they used the tools and equipment of "Karl Storz, D-Light» company (Germany) with the wavelength of 420 nm. The inspection of the bladder mucosa was performed in two modes: white light mode and fluorescence mode using a rigid cystoscope and fluorescence optics. During the study, the attention was paid to the mucous membrane color, as well as to the presence of pathological formations on a bladder mucosa. All found fluorescence foci were

measured and recorded. After FD, the patients underwent a transurethral resection of a bladder, during which the mucous membrane parts were removed which were suspicious of tumor lesions in white light as well as the fluorescent portions of a mucous membrane during FD performance. Each tested person provided the test "blind" biopsy from a visually unchanged and a non-fluorescent mucosa.

All removed material was sent to a remote morphological study.

Thus, the following lesions were examined in each patient:

- The lesions determined as tumors in white light (both fluorescent and non-fluorescent ones);
- Additional fluorescence foci (if any);
- Control lesions (non-fluorescent and determined in white light as an unmodified mucosa for the taking of control "blind" biopsy - 1 focus in each patient).

Biopsy was taken from all studied foci. Morphological study was the final method of intravesical pathology diagnosis, which allowed determining the nature of morphostructural changes in a bladder mucosa.

The evaluation of FD and cystoscopy effectiveness in white light was performed according to the following parameters: sensitivity, specificity, accuracy, the diagnostics positive and negative predictive value. According to the results of the morphological study, in order to evaluate the diagnostic efficacy of a standard white light cystoscopy, each investigated focus had the following marking:

- RP (really positive result) - visually suspicious tumor tissues for the presence of tumor process;
- RN (really negative result) - visually non-suspicious tissues for the presence of tumor process;
- FP (false positive result) - visually suspicious healthy tissues for the presence of tumor process;
- FN (false negative result) - visually non-suspicious tumor tissues for the presence of tumor process.

According to the results of morphological studies in order to evaluate the diagnostic efficacy of fluorescent diagnostics each studied focus had the following marking:

- RP (really positive result) - fluorescent tumor tissues;
- RN (really negative result) - non fluorescing healthy tissues;
- FP (false positive result) - fluorescent healthy tissues;
- FN (false negative result) - non-fluorescent tumor tissues.

Then the sensitivity and specificity values were calculated for the both types of diagnosis (cystoscopy and FD) according to the formulae:

$$\text{Specificity} = \frac{RN}{FP + RN} * 100\%$$

$$\text{Sensitivity} = \frac{RP}{RP + FN} * 100\%$$

The accuracy of each diagnostic mode, a positive predictive value and a negative predictive value was calculated additionally according to the following formulas:

$$\text{Accuracy} = \frac{RP + RN}{RP + FN + RN + FP} * 100\%$$

$$\text{Positive predictive value} = \frac{RP}{RP + FP} * 100\%$$

$$\text{Negative predictive value} = \frac{RN}{RN + FN} * 100\%$$

The safety assessment was carried out according to the frequency and the severity of adverse events. The emergence and the development of adverse events were controlled according to regular clinical examination data and the results of urine and blood tests.

During the study period the patients were examined according to the Protocol scheme No. 02-(FD-GE)-2014, presented in the Table 1.

Table 1. Patient examination scheme before, during and after the diagnosis

Examination methods and procedures	Examination terms			
	During screening	On the day of FD performance	7 days after FD performance	21 days after FD performance
Clinical picture description	+	+	+	+
Pregnancy test	+	+	+	+
Body temperature	+	+	+	+
AP	+	+	+	+
ECG	+	-	-	-
Urinalysis (specific density, glucose, protein, blood elements, color, precipitate)	+	-	+	+
Total blood count (erythrocytes, leukocytes, leukocytic formula, hemoglobin, platelets)	+	-	+	+
Biochemical blood analysis (ALT, AST, bilirubin, serum creatinine, total protein, albumin, glucose)	+	-	+	+
Pelvic ultrasound	+	-	-	-
Cystoscopy	+	+	-	-
Morphological examination (biopsy)	+	+	-	-
Transurethral resection	-	+	-	-
The analysis of side effects	All symptoms are described in detail with the exact time of appearance and disappearance			

3. RESULTS AND DISCUSSION

The total number of studied foci among all patients was 159. The average number of studied lesions among one patient was 3.0. When a standard cystoscopy was performed 92 foci were detected which were suspicious for the presence of bladder cancer. During FD performance, all these lesions demonstrated fluorescence. The presence of the tumor process was confirmed morphologically and these lesions were evaluated as really positive for FD and for standard cystoscopy. During FD performance, 8 patients demonstrated 14 foci of fluorescence in bladder mucosa areas additionally, unchanged in white light. Morphological study confirmed the presence of tumor process in them, and these lesions were evaluated as really positive for FD, and as really negative for a standard cystoscopy. Additionally, each patient provided one control biopsy from visually unchanged and non-fluorescent bladder mucosa. These foci did not demonstrate tumor cells and they were evaluated as really negative for both types of diagnostics. The results are shown in Table 2.

Table 2. FD and standard cystoscopy results

Parameter	FD	Cystoscopy
Number of RP results	106	92
Number of RN results	53	53
Number of FP results	0	0
Number of FN results	0	14
Specificity	100%	100%
Sensitivity	100%	86,8%
Accuracy	100%	91,2%
Positive predictive value	100%	100%
Negative predictive value	100%	79,1%

As can be seen from the presented data in Table 2, the conducting of FD allowed increasing the diagnostic sensitivity as 13.2% (from 86.8% to 100%), the diagnosis accuracy as 8.8% (from 91.2% to 100%) and negative predictive value as 20.9% (from 79.1% to 100%). According to the designed protocol, the difference in similar indicators for different diagnostic modes of 10% and more was considered to be clinically significant one. Therefore, in terms of sensitivity and negative predictive value the effectiveness of FD method fairly exceeds the

efficiency of standard cystoscopy in white light. The difference in accuracy indicators was less than 10% (8.8%), and therefore it cannot be considered as clinically significant one. Sensitivity index (and its high values for FD method as compared to standard cystoscopy) is a very important one for the preoperative diagnosis, as it characterizes the ability of tumor lesion detection not identified during a standard cystoscopy, and the possibility of a more detailed surgical removal of a tumor. In this study, during FD performance 8 patients (15.0%) out of 53 ones revealed 14 fluorescing foci additionally not determined in white light, in which the neoplastic process was confirmed during morphological study.

The second task after the study of the developed FD mode efficiency was the evaluation of its safety. Immediately during the process of the drug Hexasens solution introduction along the catheter into the urinary bladder of the patient, as well as after the drug administration no complaints and adverse reactions were noted. Both groups of patients throughout the whole exposure time of the drug solution in the bladder (up to 2 hours) did not reveal any discomfort or inconvenience, except of subjective difficulties with the need for a long-term retention of the drug solution in the bladder. No adverse reactions were reported during and after FD session performance and during follow-up visits.

During the patient observation period provided by the Protocol and included in the study, there were no significant changes in their general condition, blood pressure, heart rate and body temperature. There was no development of allergies, local and general toxic reactions, the exacerbation of co-morbidities. The clinical and biochemical analysis of patient urine and blood did not show any significant deviations (beyond age norm). Subjectively the status of all patients was satisfactory during the treatment cycle. There were no complaints. The obtained results allow to recommend the method of fluorescent diagnostics with Hexasens drug at the dose of 100 mg in the form of 0.2% solution (50 ml) for the practical application among the patients with primary bladder cancer diagnosis. In this study we found that, the conducting of fluorescence diagnosis using Hexasens drug among 53 patients with early bladder cancer diagnosis allowed to increase the diagnostics sensitivity by 13.2% as compared to standard cystoscopy (from 86.8% to 100%), diagnostic accuracy by 8.8% (from 91.2% to 100%) and negative predictive value by 20.9% (from 79.1% to 100%). During the performance of fluorescence diagnosis, 8 patients (15.0%) out of 53 ones revealed 14 fluorescing foci additionally not identified in white light, in which the neoplastic process was confirmed during morphological study. During the carrying out of fluorescence diagnosis, none of 53 patients who took part in the study received false-positive or false-negative results. The introduction of Hexasens drug solution into a bladder and the carrying out of a fluorescent diagnosis was not associated with the development of any adverse reactions. The patients had a subjective discomfort only at the prolonged exposure of the drug solution in the bladder (up to 2 hours).

The patients participating in the study did not report any side effects (general toxicity, hematologic, hepato- and nephrotoxicity) and complications during FD performance. There were no statistically significant changes in the indicators of clinical and biochemical blood and urine tests during control observations performance as compared with screening results. The developed set of methods is recommended for the use in clinical practice.

4. CONCLUSION

According to performed research, the clinical using of Hexasens drug is recommended for the early fluorescence diagnostics of the bladder cancer. Hexasens drug at the dosage of 100 mg was dissolved in 50 ml of isotonic sodium chloride solution immediately before using. The concentration of 0.2% is induced into the cavity of a bladder along a catheter. The drug solution that used for exposure period in a bladder was between 1-2 hours. Fluorescent diagnosis was carried out as soon as possible, but no later than 1 hour after a bladder emptying. The inspection of a bladder mucosa was performed in fluorescence mode using a standard cystoscopy and the device of "Karl Storz, D-Light" company (Germany) with the wavelength of 420 nm.

ACKNOWLEDGMENT

Not mentioned any acknowledgment by authors.

FUNDING/SUPPORT

Not mentioned any Funding/Support by authors.

AUTHORS CONTRIBUTION

This work was carried out in collaboration among all authors.

CONFLICT OF INTEREST

The authors declared no potential conflicts of interests with respect to the authorship and/or publication of this paper.

REFERENCES

1. Allison RR, Downie GH, Cuenca R, Hu X-H, Childs CJ, Sibata CH. Photosensitizers in clinical PDT. Photodiagnosis and photodynamic therapy. 2004;1(1):27-42.
2. Calin M, Parasca S. Photodynamic therapy in oncology. Journal of optoelectronics and advanced materials. 2006;8(3):1173.
3. Castano AP, Demidova TN, Hamblin MR. Mechanisms in photodynamic therapy: part one—photosensitizers, photochemistry and cellular localization. Photodiagnosis and photodynamic therapy. 2004;1(4):279-93.
4. Detty MR, Gibson SL, Wagner SJ. Current clinical and preclinical photosensitizers for use in photodynamic therapy. Journal of medicinal chemistry. 2004;47(16):3897-915.
5. Huang Z. A review of progress in clinical photodynamic therapy. Technology in cancer research & treatment. 2005;4(3):283-93.
6. de Leeuw J, van der Beek N, Neugebauer WD, Bjerring P, Neumann H.

Fluorescence detection and diagnosis of non-melanoma skin cancer at an early stage. Lasers in surgery and medicine. 2009;41(2):96-103.
7. Fotinos N, Campo MA, Popowycz F, Gurny R, Lange N. 5-Aminolevulinic Acid Derivatives in Photomedicine: Characteristics, Application and Perspectives. Photochemistry and photobiology. 2006;82(4):994-1015.
8. Goh AC, Lerner SP. Application of new technology in bladder cancer diagnosis and treatment. World journal of urology. 2009;27(3):301-7.
9. Peng Q, Berg K, Moan J, Kongshaug M, Nesland JM. 5-Aminolevulinic Acid-Based Photodynamic Therapy: Principles and Experimental Research. Photochemistry and photobiology. 1997;65(2):235-51.
10. Yakubovskaya R, Morozova N, Pankratov A, Kazachkina N, Plyutinskaya A, Karmakova T, et al. Experimental photodynamic therapy: 15 years of development. Russian Journal of General Chemistry. 2015;85(1).
11. BONE PO. of the World Association for Laser Therapy October 19–22, 2008. Photomedicine and Laser Surgery. 2009;27(1).
12. Ivanova-Radkevich V, Filonenko E, Slovokhodov E, Smirnova I, Kuznetsova O, Lobaeva T, et al. Organization of Clinical Trials of Photosensitizer based on 5-Aminolevulinic Acid Hexyl Ester. Indian Journal of Science and Technology. 2016;9(18).