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(54) METHOD FOR DETECTION OF UROTHELIAL CANCER

VERFAHREN ZUR ERKENNUNG VON UROTHELKREBS

MÉTHODE DE DÉTECTION DU CANCER UROTHÉLIAL

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(56) References cited:
WO-A1-2009/130893 JP-A- H04 500 770
JP-A- 2006 124 372

- **TAUBER S ET AL: "Fluoreszenzzytologie der Harnblase [Fluorescencezytology of the urinary bladder]", UROLOGE AUSGABE A, SPRINGER, BERLIN, DE, vol. 40, no. 3, 1 January 2001 (2001-01-01), pages 217-221, XP009173541, ISSN: 0340-2592**

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(Cont. next page)

- PYTEL AKOS ET AL: "New aspect of photodynamic diagnosis of bladder tumors: Fluorescence cytology", UROLOGY, BELLE MEAD, NJ, US, vol. 59, no. 2, 1 February 2002 (2002-02-01), pages 216-219, XP009173544, ISSN: 0090-4295
- KIYOHIDE FUJIMOTO ET AL.: 'Fluorescence Cystoscopy-Assisted Transurethral Resection of Bladder Tumor and Photodynamic Diagnosis of Exfoliated Cells in the Urine using 5-Aminolevulinic Acid in Bladder Cancer' THE JOURNAL OF JAPAN SOCIETY FOR LASER SURGERY AND MEDICINE vol. 30, no. 4, 30 January 2010, pages 399 - 404

Description

Technical Field

[0001] The present invention relates to a method for detecting urothelial cancer, specifically to a method for detecting urothelial cancer using 5-aminolevulinic acid (ALA), a derivative thereof, or a salt of these (hereinafter may be referred to as "ALAs").

Background Art

[0002] Examination of urothelial cancer is usually performed by staining cells dropped out in the urine by Papanicolaou stain and observing them with a microscope. However, proficiency is required to diagnosis, and there are often oversights, which is a problem. The difficulty of detection leads to the delay in cancer detection, and thus to miss relapse.

[0003] On the other hand, in the endoscopic operation for a bladder cancer patient, it is known to inject ALAs into bladder, observe the fluorescence derived from cancer cells in the bladder to determine the excision site (see for example, Nonpatent Document 1). It is also known that the observation of fluorescence in the bladder is possible by administering orally or injecting intravenously ALAs (see Patent Document 1).

[0004] Further, a diagnostic agent for tumor comprising ALAs is proposed, which agent is intended to determine the presence or absence of tumor in tissues of brain, nasal tract, nasal cavity, trachea, bronchi, buccal cavity, pharynx, esophagus, stomach, breast, colorectum, lung, ovary, central nervous system, liver, bladder, urethra, urinary duct, pancreas, cervical duct, abdominal cavity, anal duct, or cervix uteri, by administering the diagnostic agent for tumor in an amount of 0.001 mg to 10 g per kg of body weight at a time, measuring protoporphyrin IX, uroporphyrin I, coproporphyrin I, etc. in a sample collected in vivo or in vitro such as blood, body fluid, tissue, urine, feces, saliva, sweat, spinal fluid, seminal fluid, or tears to diagnose tumor (see for example Patent Document 2).

[0005] Further a PDD cytology method targeting fluorescence exfoliated cells in the urine, comprising performing fluorescence cytology and flow cytometry for detecting ALA-induced fluorescence positive cells is reported, which method comprises in vitro incubation method comprising dissolving urinary sediment collected from a bladder cancer patient into a serum free culture solution with an ALA concentration adjusted to 200 μg/mL, and keeping the heat at 37°C for 2 hours in a dark room before subjecting the resultant to the test; and in vivo incubation method subjecting the urinary sediment collected from ALA solution (1.5 g ALA/50 mL buffer) kept in the bladder of a bladder cancer patient for 2 hours to the test (see for example Nonpatent Document 2).

Prior Art Documents

Patent Documents

[0006]

Patent Document 1: WO2009/130893
Patent Document 2: Japanese unexamined Patent Application Publication No.2006-124372

Nonpatent Documents

[0007]

Nonpatent Document 1: "Photodynamic diagnosis of bladder cancer using fluorescent cystoscope by bladder instillation of 5-aminolevulinic acid (5-ALA)"; Hirofumi Inoue, Hisashi Karashima, Masayuki Kamata, Taro Shuin, Mutsumi Kurabayashi, Yuji Otsuki; Journal of the Japanese Urological Association, Vol. 97, pp. 719-729

Nonpatent Document 2: "Photodynamic diagnosis of bladder cancer -usefulness of fluorescence cytology-"; Kiyohide Fujimoto, Makito Miyake, Kiyoshi Nakai, Yoshiaki Matsumura, Satoshi Anai, Yoshihiko Hirao; The Japanese Urological Association, Vol.101, No.2, General Assembly Special Edition, February 2010.

Tauber et al 2001 (Urologe vol 40 pages 217-221) describe 5-aminolevulinic acid induced fluorescence cystoscopy after instillation of 5-aminolevulinic acid into patients' bladders.

Pytel and Schmeller 2002 (Urology vol 59 pages 216-219) describe a diagnostic method combining the principles of photodynamic diagnosis and urinary cytology.

Summary of the Invention

Object to be Solved by the Invention

[0008] As it is stated in the above, conventional examination of urothelial cancer required proficiency, having problems to often overlook the disease, a novel and simple technique with high accuracy was awaited. For example, in the in vitro incubation, a culture apparatus to culture under dark was necessary, and the patient had to wait many hours to obtain the results after urine collection. In the in vivo incubation, there was a problem to cause burden to the patient when injecting ALA solution in the bladder, and that the detection was limited to bladder cancer.

[0009] The object of the present invention is to provide a method for detecting urothelial cancer that can detect urothelial cancer simply and with high accuracy, without need of a particular culture apparatus, and allowing determination just after urine collection.

Means to Solve the Object

[0010] The present inventors made a keen study with an idea that a technique using ALAs such as described in the above can be applied to cytology of urothelial cancer, and found out that by detecting fluorescence in free cancer cells in the urine excreted from the body of a test subject administered with ALAs, detection of urothelial cancer is possible. Further, they also found out that the dosage amount of ALAs was sufficient in an extremely small amount such as less than a half of the amount used for conventional determination of the excision site. The present invention has been thus completed.

[0011] Specifically, the present invention relates to (1) A method for detecting urothelial cancer comprising detecting fluorescence of a cell in urine collected from a test subject orally administered with 5-aminolevulinic acid (ALA), an ester derivative thereof, or a salt of these; (2) a method for detecting urothelial cancer comprising separating a cell from urine collected from a test subject orally administered with 5-aminolevulinic acid (ALA), an ester derivative thereof, or a salt of these, and detecting fluorescence in the separated cell; (3) the method for detecting urothelial cancer according to (1) or (2), wherein the orally administered 5-aminolevulinic acid (ALA), an ester derivative thereof, or a salt of these has been administered in an amount of 0.05 to 20 mg per kg of a test subject in ALA hydrochloride equivalent; (4) the method for detecting urothelial cancer according to any one of (1) to (3), wherein the test subject is a human suspected to have urothelial cancer; the method for detecting urothelial cancer according to any one of (1) to (4), wherein the ester derivative is selected from ALA methyl ester, ALA ethyl ester, ALA propyl ester, ALA butyl ester and ALA pentyl ester; (6) a 5-aminolevulinic acid (ALA), an ester derivative thereof, or a salt of these for use in the method for detecting urothelial cancer according to any one of (1) to (5); (7) a 5-aminolevulinic acid (ALA), an ester derivative thereof, or a salt of these according to (6), wherein the ester derivative is selected from ALA methyl ester, ALA ethyl ester, ALA propyl ester, ALA butyl ester and ALA pentyl ester.

Effect of the Invention

[0012] According to the present invention, no particular culture apparatus is needed, and a determination just after urine collection is possible. Further, as it can be determined that there is an abnormality when fluorescence in cells in the urine is detected, it can be immediately transferred to diagnosis through an endoscope or to transurethral resection. For example, by performing the fluorescence detection test in the cells in the urine before an endoscopic examination for determining the presence or absence of relapse, if there is no suspicion, there is no need to go through an endoscopic examination. This significantly reduces burden of patients, which will also lead to reduction of national medical expenses. As such, the method of the present invention allows rapid and simple detection with high accuracy of not only bladder cancer but all urothelial cancers, which is a remarkable technique in the medical services of this field. Further, as the detection is possible with a small dosage amount of ALAs, it is advantageous economically, and there is also an effect in security that photolesion is substantially not caused.

Brief Description of Drawings

[0013]

[Figure 1] It is a fluorescence microscopy photograph of cells separated from the urine when 1 g ALA hydrochloride is administered.

[Figure 2] It is a fluorescence microscopy photograph of cells separated from the urine when 500 mg ALA hydrochloride is administered.

Mode of Carrying Out the Invention

[0014] The method for detecting urothelial cancer of the present invention is not particularly limited as long as it is a method comprising administering 5-aminolevulinic acid, a derivative thereof, or a salt of these (ALAs) to a test subject, collecting urine from the test subject, and detecting fluorescence in the cells in the collected urine (for example detection of the presence of fluorescence or amount of fluorescence); or a method comprising separating a cell from a urine collected from a test subject administered with 5-aminolevulinic acid, a derivative thereof, or a salt of these (ALAs), and detecting fluorescence in the separated cell. The method for detecting urothelial cancer of the present invention encompasses a method for collecting data for detection. Further, the agent for detecting urothelial cancer of the present invention is not particularly limited as long as it comprises ALAs used in the above-mentioned method of the present invention. Further, the urothelial cancer which is the target of detection in the present invention is a malignant tumor developed from transitional epithelia covering the inner cavity of urinary tract (kidney, renal pelvis, urinary duct, bladder, and urethra). Furthermore, the test subject of the present invention relates to mammals including human, and specifically human being suspected of having urothelial cancer from a medical examination such as medical interview can be exemplified.

[0015] Among ALAs, an ALA derivative is exemplified by those ALAs having an ester group and an acyl group, where the preferred examples include the combinations of methyl ester group and formyl group, methyl ester group and acetyl group, methyl ester group and n-propanoyl group, methyl ester group and n-butanoyl group, ethyl ester group and formyl group, ethyl ester group and acetyl group, ethyl ester group and n-propanoyl group, and ethyl ester group and n-butanoyl group.

[0016] Among ALAs, examples of a salt of ALA or its

derivative include: an acid addition salt such as hydrochloride, hydrobromate, hydroiodide, phosphate, nitrate, hydrosulfate, acetate, propionate, toluenesulfonate, succinate, oxalate, lactate, tartrate, glycolate, methanesulfonate, butyrate, valerate, citrate, fumarate, maleate and malate; a metallic salt such as sodium salt, potassium salt and calcium salt; ammonium salt; and alkylammonium salt. When for use, these salts are used in the form of a solution and act in a similar manner to ALA and its derivatives.

[0017] Among these ALAs, 5-aminolevulinic acid; and 5-aminolevulinic acid methyl ester, 5-aminolevulinic acid ethyl ester, 5-aminolevulinic acid propyl ester, 5-aminolevulinic acid butyl ester and 5-aminolevulinic acid pentyl ester, or their hydrochloride, phosphate, hydrosulfate, etc. are preferred. ALAs mentioned above may form a hydrate or a solvate and may be used either alone or in appropriate combination of two or more kinds.

[0018] The ALAs can be produced by any known method such as production by chemical synthesis, production by microorganisms, and production using enzymes. When producing by microorganisms or using enzymes, it can be used as it is without purification, unless it contains any inconvenient inhibitor.

[0019] The dosage amount of these ALAs to a test subject is for example 0.02 to 50 mg per 1 kg of a test subject in ALA hydrochloride equivalent, preferably 0.05 to 20 mg, more preferably 0.2 to 10 mg, and furthermore preferably 5 to 10 mg.

[0020] As methods for administering ALAs in the method for detecting urothelial cancer of the present invention, oral administration including sublingual administration, intravenous administration including drip infusion, transdermal administration using a poultice and the like, suppository, drip infusion, etc. can be exemplified. From the viewpoints of reducing patients' burden or improving sensitivity of fluorescence, oral administration is preferred.

[0021] The agent for detecting urothelial cancer of the present invention comprising the ALAs can contain, as necessary, other ingredients such as other medicinal ingredients, nutrients, carriers, etc. As a carrier that can be blended with the detecting agent of the present invention, an organic or inorganic, solid or liquid, pharmacologically acceptable carrier material, which is suitable for intake and is generally inactive, can be used. Specific examples of such a carrier include crystalline cellulose, gelatin, lactose, starch, magnesium stearate, talc, vegetable or animal fat, fat and oil, gum and polyalkylene glycol. Examples of dosage forms of orally administered agents include powders, granules, tablet, capsule, syrup and suspension. These preparations can be produced using a solvent, a disperser, a thickener, an excipient, etc., as appropriate, according to an ordinary method. When preparing the detecting agent of the present invention as an aqueous solution, it is preferred to prepare so that it does not become alkaline in order to prevent decomposition of ALAs. If it is alkaline, decomposition can be prevented by removing oxygen.

[0022] The method for detecting urothelial cancer of the present invention allows to detect urothelial cancer by collecting urine after administering ALAs (the detecting agent of the present invention), and detecting the presence or absence of fluorescence or the amount of fluorescence in the cells in the collected urine with a fluorescence microscope, etc. Further, by further adding ALAs to the collected urine, leaving it for a predetermined time according to need, and detecting the presence or absence of fluorescence or the amount of fluorescence, the sensitivity can be further improved. According to the method for detecting urothelial cancer of the present invention, detection (determination) of urothelial cancer can be mechanically performed based on the presence of fluorescence, one without any particular knowledge such as a medical doctor, can easily make the detection. Further, detection can be made using an apparatus.

[0023] For the collection of urine, it is preferred that the urine is the first discharged after administering ALAs (the detecting agent of the present invention). It is more preferred to collect urine after 1 hour and within 12 hours after the administration, and more preferably after 2 hours and within 12 hours. The method of taking urine is not limited, and can be from spontaneous urination or collection by catheter.

[0024] When detecting fluorescence in the cells, cells are separated from urine by centrifugation or filtration, similarly as for normal cytology. When detecting fluorescence, excitation light containing ultraviolet rays comparable to Soret band to visible rays of violet to blue is irradiated, and generated fluorescence is detected with fluorescence microscope, etc.

Examples

[0025] The present invention will be more specifically described in the following examples. However, these examples are not intended to limit the technical scope of the present invention.

Example 1

[0026] 1 g of ALA hydrochloride was dissolved in 50 ml of orange juice, and was given to a patient suspected of having bladder cancer (body weight about 60 kg). Urine was collected after about 4 hours, which was immediately centrifuged (3000 rpm/min., 15 min.) in a light shielded state, and was confirmed by microscopic visualization usingsediments. The results are shown in Fig. 1. The microscope used was OLYMPUS BX50CCD mounted with camera OLYMPUS DP70. The mirror set was Exciter: XF1076 400AF30 Dichroic:XF2007 475DCLP, Emitter: XF3090 585ALP (OMEGA OPTICAL), all of which being a standard fluorescence microscope system.

[0027] As it is clear from Fig. 1, a clear PPIX fluorescence derived from cancer cell was observed. From a

normal pathological diagnosis of the cells, it was shown to be cancer cells.

[0028] From the above, it has been revealed that according to the method of the present invention, urothelial cancer can be detected by detecting fluorescence of the cells in the urine, without observing in the bladder as in the conventional way. When orally administering ALAs, as ALAs are supplied to cancer affected areas from blood, conventionally, it was not assumed that PPIX derived from ALAs accumulates not only in cancer affected areas but also in cancer cells exfoliated from cancer affected areas. However, actually, PPIX derived from ALAs were accumulated in cancer cells in the urine exfoliated from cancer affected areas, and an unexpected result was obtained that by detecting fluorescence in the cells in the urine, urothelial cancer can be detected.

Example 2

[0029] The detection method was performed in the same way as Example 1, except that the dosage amount was changed to 500 mg, which is the half amount of Example 1. As a result, the picture of Fig. 2 was obtained. From a normal pathological diagnosis of the cells, it was shown to be cancer cells.

Industrial Applicability

[0030] The method for detecting urothelial cancer of the present invention allows a very rapid, simple and accurate detection with fewer burdens to a patient, and as the dosage amount of ALAs suffices with half of the conventional amount, there is an economic merit. Further, as the risk of vomiting, liver impairment, and photosensitivity which are sometimes observed can be substantially eliminated, it can be said to be a remarkable method that contributes to an early detection of urothelial cancer.

Claims

1. A method for detecting urothelial cancer comprising detecting fluorescence of a cell in urine collected from a test subject orally administered with 5-aminolevulinic acid (ALA), an ester derivative thereof, or a salt of these.

2. A method for detecting urothelial cancer comprising separating a cell from urine collected from a test subject orally administered with 5-aminolevulinic acid (ALA), an ester derivative thereof, or a salt of these, and detecting fluorescence in the separated cell.

3. The method for detecting urothelial cancer according to claim 1 or 2, wherein the orally administered 5-aminolevulinic acid (ALA), an ester derivative thereof, or a salt of these has been administered in an amount of 0.05 to 20 mg per kg of a test subject in ALA hydrochloride equivalent.

4. The method for detecting urothelial cancer according to any one of claims 1 to 3, wherein the test subject is a human suspected to have urothelial cancer.

5. The method for detecting urothelial cancer according to any one of claims 1 to 4, wherein the ester derivative is selected from ALA methyl ester, ALA ethyl ester, ALA propyl ester, ALA butyl ester and ALA pentyl ester.

6. Use of a 5-aminolevulinic acid (ALA), an ester derivative thereof, or a salt of these in the method in vitro for detecting urothelial cancer according to any one of claims 1 to 5.

7. Use of a 5-aminolevulinic acid (ALA), an ester derivative thereof, or a salt of these according to claim 6, wherein the ester derivative is selected from ALA methyl ester, ALA ethyl ester, ALA propyl ester, ALA butyl ester and ALA pentyl ester.

Patentansprüche

1. Verfahren zum Erkennen von Urothelkrebs, umfassend das Erkennen von Fluoreszenz einer Zelle in Urin, der von einem Testsubjekt gesammelt worden ist, welchem oral 5-Aminolevulinsäure (ALA), ein Esterderivat davon oder ein Salz von diesen verabreicht worden ist.

2. Verfahren zum Erkennen von Urothelkrebs, umfassend das Trennen einer Zelle aus Urin, der von einem Testsubjekt gesammelt worden ist, welchem oral 5-Aminolevulinsäure (ALA), ein Esterderivat davon oder ein Salz von diesen verabreicht worden ist, und Erkennen von Fluoreszenz in der ausgetrennten Zelle.

3. Verfahren zum Erkennen von Urothelkrebs nach Anspruch 1 oder 2, wobei die oral verabreichte 5-Aminolevulinsäure (ALA), ein Esterderivat davon oder ein Salz von diesen in einer Menge von 0,05 bis 20 mg pro kg eines Testsubjekts in ALA-Hydrochlorid-Äquivalenz verabreicht worden ist.

4. Verfahren zum Erkennen von Urothelkrebs nach einem der Ansprüche 1 bis 3, wobei das Testsubjekt ein Mensch ist, von dem vermutet wird, dass er an Urothelkrebs leidet.

5. Verfahren zum Erkennen von Urothelkrebs nach einem der Ansprüche 1 bis 4, wobei das Esterderivat ausgewählt ist aus ALA-Methylester, ALA-Ethylester, ALA-Propylester, ALA-Butylester und ALA-Pentylester.

6. Gebrauch einer 5-Aminolevulinsäure (ALA), eines Esterderivats davon oder eines Salzes von diesen in dem Verfahren in vitro zum Erkennen von Urothelkrebs nach einem der Ansprüche 1 bis 5.

7. Gebrauch einer 5-Aminolevulinsäure (ALA), eines Esterderivats davon oder eines Salzes von diesen nach Anspruch 6, wobei das Esterderivat ausgewählt ist aus ALA-Methylester, ALA-Ethylester, ALA-Propylester, ALA-Butylester und ALA-Pentylester.

Revendications

1. Procédé de détection du cancer urothélial comprenant la détection de la fluorescence d'une cellule de l'urine recueillie auprès d'un sujet d'essai administrée par voie orale avec de l'acide 5-aminolévulinique (ALA), un dérivé d'ester de celui-ci ou un sel de ces derniers.

2. Procédé de détection du cancer urothélial comprenant la séparation d'une cellule de l'urine recueillie auprès d'un sujet d'essai administrée par voie orale avec de l'acide 5-aminolévulinique (ALA), un dérivé d'ester de celui-ci ou un sel de ces derniers, et la détection de la fluorescence dans la cellule séparée.

3. Procédé de détection du cancer urothélial selon la revendication 1 ou la revendication 2, dans lequel l'acide 5-aminolévulinique (ALA) administré par voie orale, un dérivé d'ester de celui-ci ou un sel de ces derniers a été administré en quantité de 0,05 à 20 mg par kg d'un sujet d'essai en équivalent de chlorhydrate d'ALA.

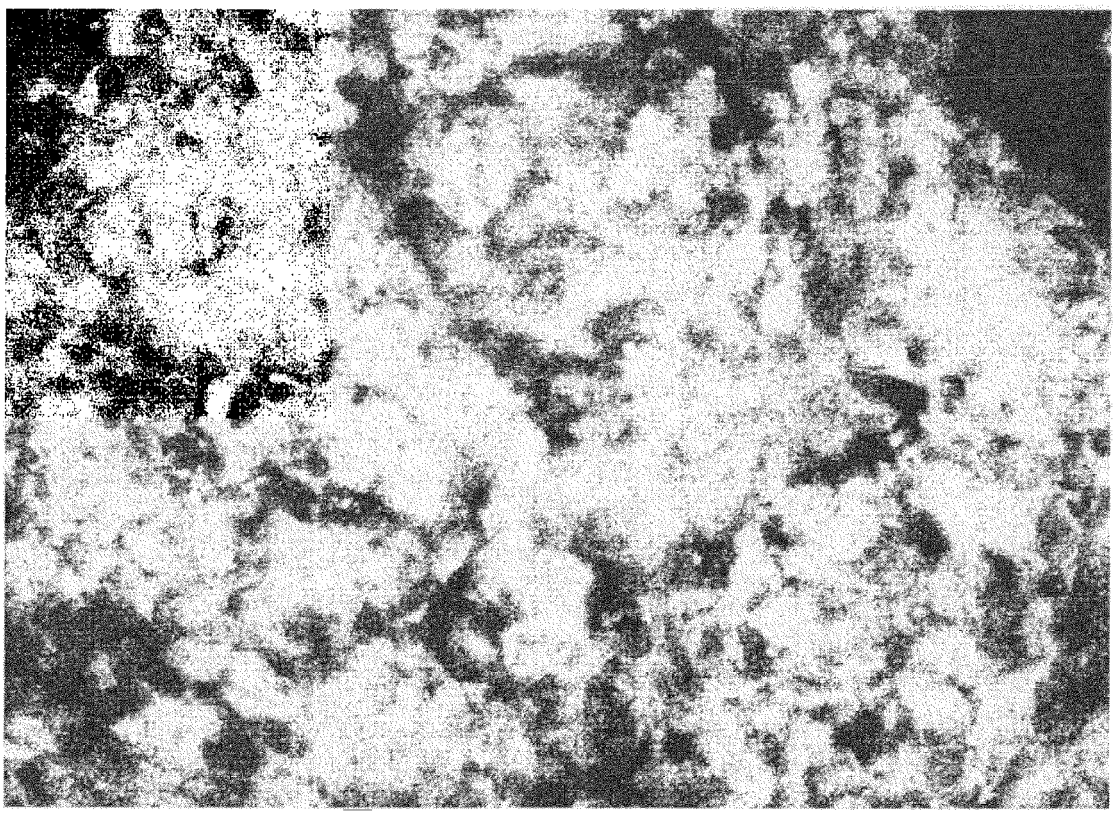
4. Procédé de détection du cancer urothélial selon l'une quelconque des revendications 1 à 3, dans lequel le sujet d'essai est un humain suspecté d'avoir un cancer urothélial.

5. Procédé de détection du cancer urothélial selon l'une quelconque des revendications 1 à 4, dans lequel le dérivé d'ester est choisi parmi un ester méthylique d'ALA, un ester éthylique d'ALA, un ester propylique d'ALA, un ester butylique d'ALA et un ester pentylique d'ALA.

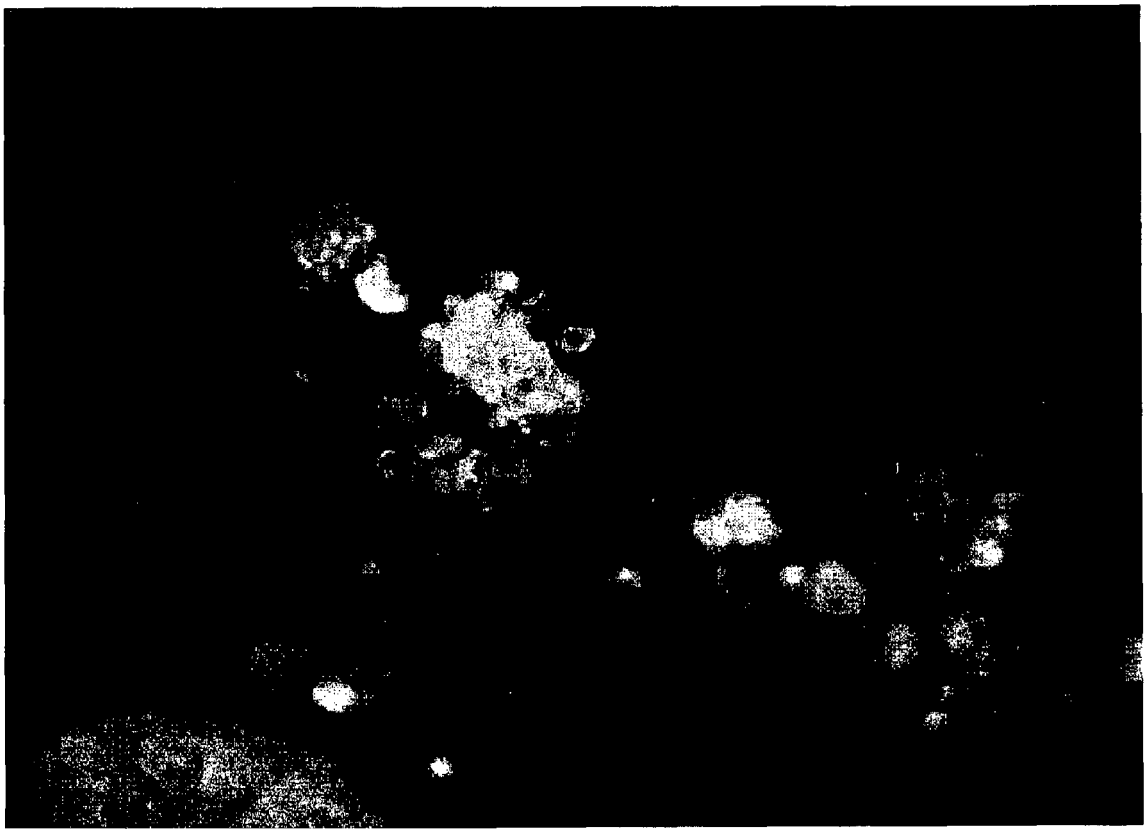
6. Utilisation d'un ester 5-aminolévulinique (ALA), d'un dérivé d'ester de celui-ci ou d'un sel de ces derniers dans le procédé *in vitro* de détection du cancer urothélial selon l'une quelconque des revendications 1 à 5.

7. Utilisation d'un ester 5-aminolévulinique (ALA), d'un dérivé d'ester de celui-ci ou d'un sel de ces derniers selon la revendication 6, dans laquelle le dérivé d'ester est choisi parmi un ester méthylique d'ALA, un ester éthylique d'ALA, un ester propylique d'ALA, un ester butylique d'ALA et un ester pentylique d'ALA.

[Fig. 1]



[Fig. 2]



REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

- WO 2009130893 A **[0006]**
- JP 2006124372 A **[0006]**

Non-patent literature cited in the description

- **HIROFUMI INOUE ; HISASHI KARASHIMA ; MASAYUKI KAMATA ; TARO SHUIN ; MUTSUMI KURABAYASHI ; YUJI OTSUKI;.** Photodynamic diagnosis of bladder cancer using fluorescent cystoscope by bladder instillation of 5-aminolevulinic acid (5-ALA). *Journal of the Japanese Urological Association,* vol. 97, 719-729 **[0007]**
- Photodynamic diagnosis of bladder cancer -usefulness of fluorescence cytology. **KIYOHIDE FUJIMOTO ; MAKITO MIYAKE ; KIYOSHI NAKAI ; YOSHIAKI MATSUMURA ; SATOSHI ANAI ; YOSHIHIKO HIRAO.** The Japanese Urological Association. February 2010, vol. 101 **[0007]**
- **TAUBER et al.** *Urologe,* 2001, vol. 40, 217-221 **[0007]**
- **PYTEL ; SCHMELLER.** *Urology,* 2002, vol. 59, 216-219 **[0007]**