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RESEARCH ARTICLE

Dual Nuclear/Fluorescence Imaging Potantial of Zinc(II) Phthalocyanine in MIA PaCa-2 Cell Line

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Abstract: ***Background and Objective*:** Pancreatic cancer is very common and difficult to diagnose in early stage. Imaging systems for diagnosing cancer have many disadvantages. However, combining different imaging modalities offers synergistic advantages. Optical imaging is the most multidirectional and widely used imaging modality in both clinical practice and research.

***Methods*:** In present study, Zinc(II) phthalocyanine [Zn(II)Pc] was synthesized, labeled with iodine-131 and *in vitro* study was carried out. The intracellular uptake studies of radiolabeled Zn(II)Pc were performed in WI-38 [ATCC CCL-75™, tissue: human fibroblast lung] and MIA PaCa-2 [ATCC CRL-1420™, tissue: human epithelial pancreas carcinoma] cell lines.

***Results*:** The intracellular uptake efficiency of radiolabeled Zn(II)Pc in MIA PaCa-2 cells was determined two times higher than WI-38 cells. Also, fluorescence imaging (FI) efficiency of synthesized Zn(II)Pc was investigated in MIA PaCa-2 cells and significant uptake was observed.

***Conclusion*:** Zn(II)Pc might be used as a new agent for dual fluorescence/nuclear imaging for pancreatic cancer.

Keywords: Zinc(II) Phthalocyanine, fluorescence imaging, nuclear imaging, ^{131}I.

INTRODUCTION

Cancer, caused by abnormal cell proliferation has become a very common disease in recent years. There are many known types of cancer in medicine. Pancreatic cancer is one of the most aggressive types and difficult to diagnose in early stage. Pancreatic cancer is the fourth most common in the world and also difficult to diagnose and therapy [1-3].

There are many imaging systems such as magnetic resonance imaging (MRI), positron emission tomography (PET) and fluorescence imaging (FI). These systems have some advantages and disadvantages. MRI has high resolution but low sensitivity. Although nuclear imaging which is radionuclide-based monitoring is quite sensitive and highly quantitative. But they have poor spatial resolution. However, quantitative information is hard to get by using FI [4]. Even so FI has wide application areas because of its low cost, low energy radiation, high sensitivity and allowing non-invasive or minimally invasive applications [5, 6]. Therefore, combined imaging systems could eliminate these advantages of single imaging systems.

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An inability to penetrate depth, one of the disadvantages of fluorescence imaging is the inability to penetrate depth, can be solved using near infrared (NIR) fluorescence imaging. Deep tissues and organs could be monitored with an ideal fluorescence probe by NIR fluorescence imaging [7, 8]. Zinc phthalcyanines are very suitable candidates for fluorescence imaging due to high extinction coefficients, convenient quantum yields, high photo stabilities, tumor-localizing properties, good biocompatibility and low dark toxicity [9-11]. On the other hand radiolabeled phthalcyanines were investigated as nuclear imaging agent [12-15].

In the present study, we focused on determination of FI efficiency of a synthesized Zn(II) phthalocyanine and nuclear imaging potential of ^{131}I labeled Zn(II)Pc in WI-38 and MIA PaCa-2 cell lines as *in vitro*.

MATERIALS AND METHODS

Materials

All chemicals used for the *in vitro* studies were purchased from Biological Industries; all other chemicals were procured from Merck. Iodogen was supplied from Sigma-Aldrich. Thin-layer chromatography-cellulose gel

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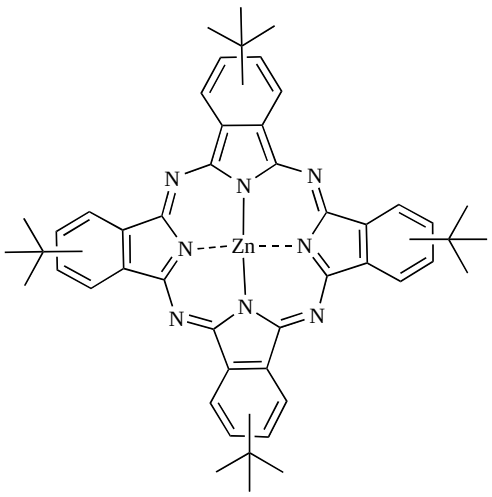

Fig. (1). Chemical structure of Zn(II)Pc.

(ITLC-F plastic sheets 20x20) was purchased from Merck. Radiolabeling experiments were analyzed using a Bioscan AR2000 TLC Scanner. Cell culture studies were performed in a Thermo MSC Advantage 1.2 laminar air flow cabinet. An Olympus Japan inverted light microscope was used for counting cells. A Thermo Multimode microplate reader was used to determine the IC_{50} values of cell cultures.

Synthesis of Zinc(II) 2(3),9(10),16(17),23(24)-tetra*tert*-butylphthalocyaninato (2-)-N^{29}, N^{30}, N^{31}, N^{32}

Zinc (II) 2,(3),9(10),16(17),23(24)-tetra-*tert*-butyl phthalocyaninato was synthesized according to a literature procedure [16]. A mixture of 4-*tert*-butylphthalonitrile (1g, 5.42 mmol) and $ZnCl_2$ (0.36 g, 2.7 mmol) was refluxed in dimethylaminoethanol (DMAE) (5ml) under argon atmosphere for 20 h. The solvent was removed under reduced pressure and the blue solid was washed with a MeOH-H_2O (5:1) mixture. The solid residue was purified by column chromatography on silica gel (Hexane/dioxane, 3:1), to yield 310 mg, 0.38 mmol tetra-*tert*-butyl phthalocyanine as a blue solid. Yield: 28%. The characterization of the compound was carried out via ^{1}H-NMR, FTIR and MS.

Radiolabeling and Radiochemical Purity Analysis

Synthesized Zn(II)Pc shown in Figure **1** was dissolved in DMSO. Zn(II)Pc (50 µg) was diluted with distilled water to 300 µl and the solution was adjusted pH 7 for radiolabeling. Prepared Zn(II)Pc was radiolabeled with ^{131}I (9.25 MBq) using iodogen method. Iodine is oxidized by the iodogen and oxidized iodine can easily bind to benzene ring in Zn(II)Pc via an electrophilic substitution reaction. One mg iodogen was dissolved in 250 µl dichloromethane in a vial. The solvent was left to evaporate forming a thin solid layer on the wall of the reaction vial. Zn(II)Pc solution (50 µg/300 µl) was added into iodogen coated vial and then ^{131}I was added and incubated for 30 min at room temperature. The quality control of radiolabeling was determined using TLRC method [17-21]. Cellulose-coated plastic (ITLC-cellulose) sheets (1×10 cm; Merck) and two different mobile phases [mobile phase 1: chloroform-acetic acid (9:1) and mobile phase 2: n-butanol-water-acetic acid (4:2:1)] were used to determine the radiolabeling efficiency. The cellulose sheets were scanned on a TLC-scanner (BioScan AR-2000 Washington DC, USA).

Cell Culture

Cell culture studies were carried out using WI-38 and MIA PaCa-2 cell lines. The cells were cultured in MEM Non-Essential Amino Acid Solution containing 100 IU/ml penicillin G, 100 mg/ml streptomycin and 10 % heat-inactivated Fetal Bovine Serum (FBS) and were maintained at 37°C in an incubator containing 5 % CO_2.

Cytotoxicity Assay of Zn(II)Pc

The dark red-colored WST-1 reagent (2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt), was used to monitor Zn(II)Pc toxicity on MIA PaCa-2 and WI-38 cell lines. The cells were placed in a 96-well culture plate (2×10^5 cells in each well) and the IC_{50} dose of Zn(II)Pc was determined using different Zn(II)Pc concentrations (1.56 to 100.00 µM) for 24 h. The number of living cells was determined by adding 20 µl cell proliferation reagent, WST-1, to each well. The yellow-colored formazan salt was formed and the absorbance was measured in a micro plate reader (Thermo) at 450/630 nm, after 20, 40 and 60 min. Then the percentage of cytotoxicity was calculated.

Cells Imaging

Fluorescence imaging study was performed to investigate the interactions of Zn(II)Pc with MIA PaCa-2 cells. The cells were fixed on a slide 2 days before experiment. The medium was removed and Zn(II)Pc solution (25 µM) diluted with MEM (without FBS) was added on cells for 2 h at 37°C incubator after 2 days. Then the cells were washed twice with PBS and DAPI solution added for staining the cells nucleus. The cells were washed again with PBS after 15 min. Photographs were taken under green and dapi filter with Fluorescence microscope (Olympus BX53F) equipped with a CCD camera (Olympus DP72).

Intracellular Uptake of ^{131}I Labeled Zn(II)Pc

The *in vitro* intracellular uptake of ^{131}I labeled Zn(II)Pc [^{131}I-Zn(II)Pc)] was performed using WI-38 and MIA PaCa-2 cell lines. The cells were placed in 24-well culture plates (1×10^5 cells in each well) in 37°C incubator for 2 days. The medium on cells was removed, the wells were washed 2 times with 0.9 % NaCl solution and radiolabeled Zn(II)Pc (25 µM, activity: 0.9 Bq) diluted with MEM (without FBS) was added on cells after 2 days. At the same conditions we assayed to control intracellular uptake of $Na^{131}I$. The wells were counted by Cd (Te) RAD 501 signal channel analyzer after determined time period incubation (1, 2, 4, 6 and 24 h). Then, the radioactive medium on cells was pulled and the wells were washed one time in each time period. After that, 500 ml of 0.9 % NaCl solution was added in each well and the wells were counted again. The data were analyzed and the percentage of uptake was calculated.

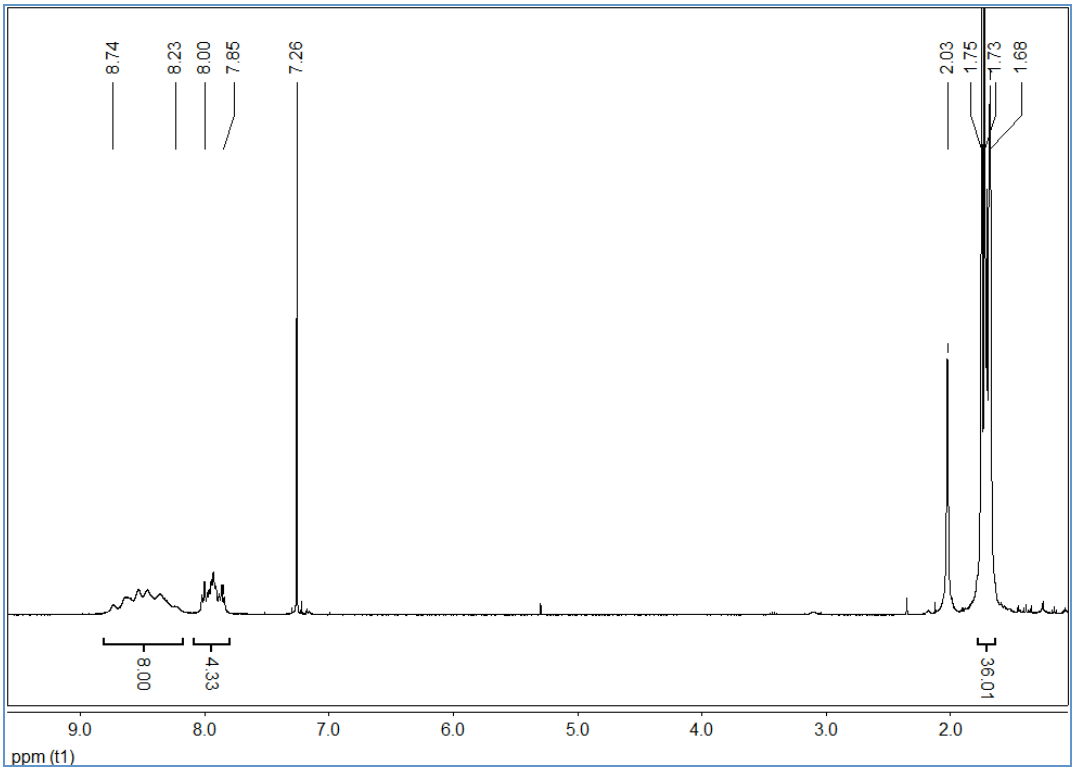

Fig. (2). ^{1}H-NMR ($CDCl_3$) spectrum of Zn(II)Pc.

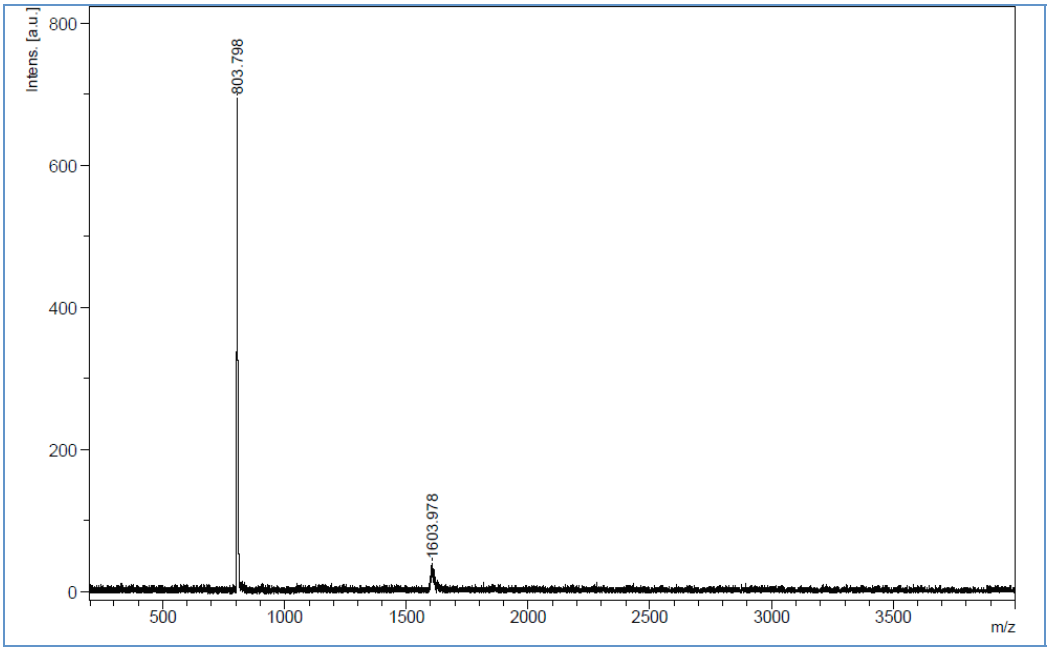

Fig. (3). MALDI-TOFspectrum of Zn(II)Pc.

RESULTS AND DISCUSSION

The Charecterization Results of Zn(II)Pc

^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) = 8.7-8.2 (m, 8H), 8.0-7.8 (m, 4H), 1.7-1.6 (m, 36H, $C(CH_3)_3$) (Fig. **2**). FTIR (ATR): ν (cm^{-1}) 2954, 2895, 1613, 1487, 1391, 1255, 1087, 921, 744 cm-1. UV/Vis (THF): λmax (log ε) = 672 (5.79), 607 (5.09), 346 (5.03). MS (MALDI-TOF): m/z: 803.798 $[M+H]^+$ (Fig. **3**).

Radiolabeling Efficiency of Zn(II)Pc

The radiolabeling quality control was performed by TLRC method and R_f values of $Na^{131}I$, ^{131}I-Zn(II)Pc were determined as 0.05 and 0.95, respectively, when mobile phase 1was used. When mobile phase 2 was used, the R_f values of $Na^{131}I$, ^{131}I-Zn(II)Pc were found to be 0.4 and 0.93, respectively. The data showed that the radiolabeling efficiency of ^{131}I-Zn(II)Pc was obtained as 93.4±1.6 % at pH 7, room temperature and 1 mg iodogen.

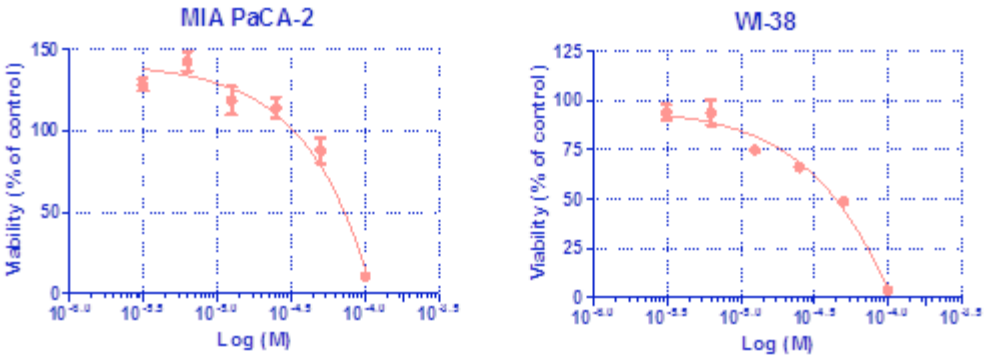

Fig. (4). Cytotoxicity of 3.13-100.00 µM concentration of Zn(II)Pc in MIA PaCa-2 and WI-38 cell lines.

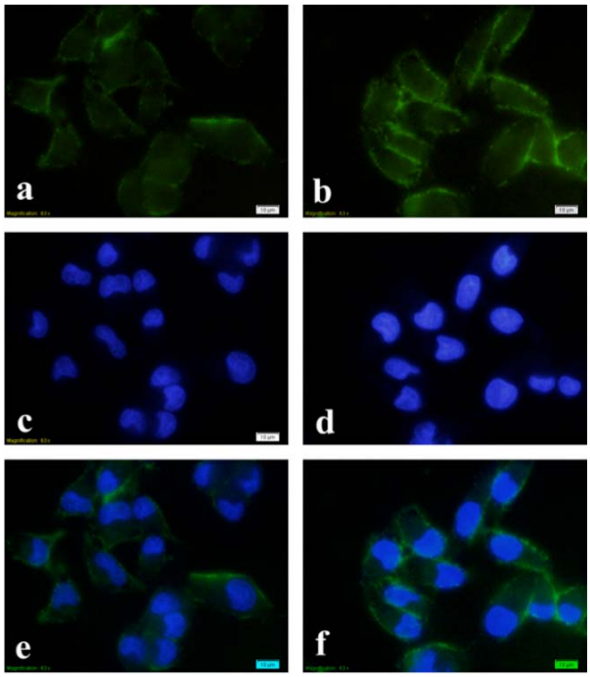

Fig. (5). Fluorescence microscope images of Zn(II)Pc on MIA PaCa-2 cells with 10 µm scale bars **(a)** and **(b)**; Zn(II)Pc on MIA PaCa-2 cells with green filter, **(c)** and **(d)**; Zn(II)Pc on MIA PaCa-2 cells with DAPI filter,**(e)**; superimposed image of **(a)** and **(c)**, **(f)**; superimposed image of **(b)** and **(d)**.

Cytotoxicity of Zn(II)Pc

IC_{50} doses of Zn(II)Pc were 55.47 µM in MIA PaCa-2 pancreatic cancer cell line and 120.50 µM in WI-38 human fetal lung fibroblast cell line (Fig. **4**). In the another study IC_{50} value of Zn(II)Phthalocyanine derivatives had been determined to be nM level in human colon adenocarcinoma cells [22]. Hairong *et al.* evaluated the dark-toxicity of Zn(II)Phthalocyanines in HEp2 [ATCC CCL-23™, tissue: human HeLa contaminant carcinoma] cells. Although IC_{50} value of Zn(II)Pc 11 was found as to be around 85 µM, Si(IV)-Pcs were stated to be nontoxic [23].

Cells Imaging

The fluorescence imaging experiment was carried out to determine the relations between MIA PaCa-2 cells and Zn(II)Pc in Figure **5**. For this reason Zn(II)Pc compound was added on the cells and fluorescence microscope images were taken under green and DAPI filter. The images show that Zn(II)Pc has significant uptake by MIA PaCa-2 cells.

Hydrophobic ZnPc structures are transported by lipoproteins. Therefore, they are directly taken up with tumor cells and localized in subcellular membrane structures [24, 25]. It's clearly seen in our cell imaging study, Zn(II)Pc was not uptaken by cell nucleus, however Zn(II)Pc was localized in the cell cytoplasm. In the fluorescence imaging studies, ZnPcs are localized in Golgi apparatus at shorter incubation periods. However, there is still the presence of ZnPc in Golgi apparatus and also mitochondrial localization could be observed in the longer incubation periods [25].

In literature Lv *et al*. synthesized lactose and galactose substituted Zn(II)Pc. And *in vivo* fluorescence imaging ability of the compounds was determined for liver cancer, lung cancer and melonama cancer with tumor bearing mice. Zn(II)Pc showed potential as a targeting optical probe in the diagnosis of liver cancer [26, 27]. As seen in several studies, Zn(II)Pc and derivatives could be used for fluorescence imaging. For these reason we observed as *in vitro* that Zn(II)Pc has FI ability in pancreatic cancer. Hence FI ability of Zn(II)Pc will be investigated *in vivo* in future.

The Intracellular Uptake

The intracellular uptake study results in WI-38 and MIA PaCa-2 cell lines are shown in Figure **6**. It is observed that the intracellular uptake of ^{131}I-Zn(II)Pc increased with time and reached maximum at 4 hours (1.61±0.12 %) in MIA PaCa-2 cell lines. However, ^{131}I-Zn(II)Pc depicted maximum uptake in WI-38 cell line at 1 h (0.92±0.21 %) and began to decrease with time. As a result, it was observed that the intracellular uptake of ^{131}I-Zn(II)Pc was higher in MIA PaCa-2 cells than WI-38 cells. At the same time, the uptake of ^{131}I was examined as control group in both cell lines. The results showed that the intracellular uptake of ^{131}I was much lower than uptake of ^{131}I-Zn(II)Pc in both cell lines. The ratio of intracellular uptake of the cancer cell lines (MIA PaCa-2) to normal cell line (WI-38) is enough high for nuclear imaging. Tumor-to nontumor (T/NT) ratio of 2 is adequate for imaging of tumor [28]. Thus the radiolabeled Pc may be useful in pancreatic cancer nuclear imaging. In another study, new synthesized water-soluble sulfonated zinc phthalocyanine radiolabeled with ^{68}Ga and ^{64}Cu was

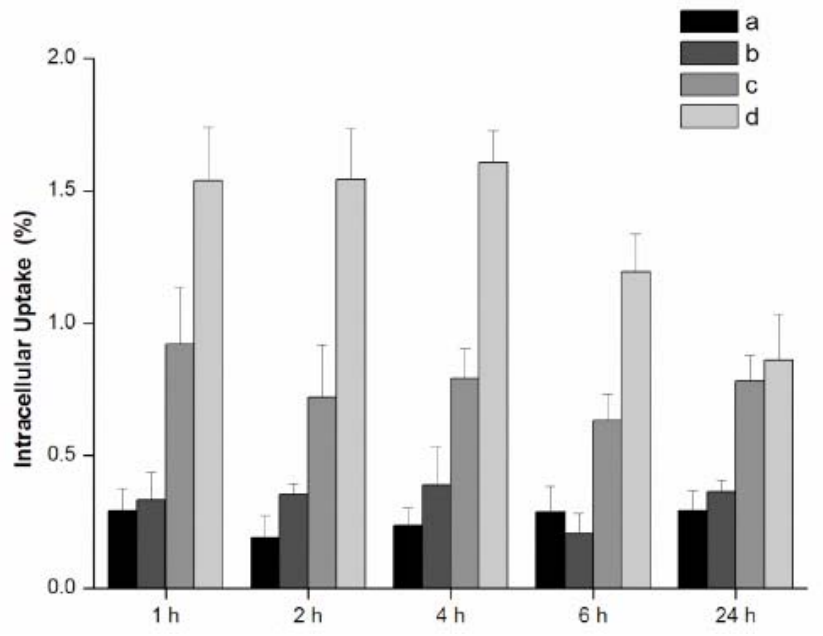

Fig. (6). (a): Intracellular Uptake (%) of ^{131}I in WI-38 cells; (b): Intracellular Uptake (%) of ^{131}I in MIA PaCa-2 cells; (c): Intracellular Uptake (%) of ^{131}I-Zn(II)Pc in WI-38 cells; (d): Intracellular Uptake (%) of ^{131}I-Zn(II)Pc in M IA PaCa-2 cells.

investigated and the compound showed promising results for dual fluorescence/PET imaging [14].

Thereby, in the literature, Pcs show significant uptake in human nasopharynx KB carcinoma cells, human colon adenocarcinoma cells and human HeLa contaminant carcinoma cells [22, 23, 29, 30].

The lipophilicty of ^{131}I-Zn(II)Pc had been found to be highly lipophilic (13.3±3.0) by our group [20, 21]. For this reason, the intracellular uptake of ^{131}I-Zn(II)Pc in MIA PaCa-2 cells is higher than WI-38 cells. In literature, it is observed that lipophilic Zn(II)Pcs have high uptake in HSC-2 human oral squamous carcinoma cell line at 2 hours and uptake of more hydrophilic compounds was poor [31].

It is known that tumor tissues are more acidic than normal tissues due to excessive lactic acid production in cells [32]. However, cellular localization of hematoporphyrin derivatives is more effective in lower pH [33]. Therefore, it is possible that Zn(II)Pc had showed higher uptake in tumor cells.

As shown in our study, Zn(II)Pc is convenient to use as multifunctional agent for combining imaging studies owing to its tumor-localizing efficiency and low toxicity and sufficient to radiolabeling features.

CONCLUSION

As a result, we synthesized a Zn(II)Phthalocyanine and investigated its fluorescence and nuclear imaging ability for pancreatic cancer. Zn(II)Pc showed high radiolabeled efficiency with ^{131}I and significant intracellular uptake in MIA PaCa-2 cell lines. At the same time, *in vitro* fluorescence imaging study with MIA PaCa-2 cells showed good results. The results form the basis further in vivo study. Thus, the synthesized Zn(II)Phthalocyanine migh be a promising candidate for dual fluorescence/nuclear imaging pancreatic cancer.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare with respect to this research.

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REFERENCES

[1] Kleeff, J.; Michalski, C.; Friess, H.; Buechler, M.W. Pancreatic cancer - From bench to 5-year survival. *Pancreas,* **2006,** *33* (2), 111-118.

[2] Costello, E.; Neoptolemos, J.P. Pancreatic cancer in 2010: new insights for early intervention and detection. *Nat. Rev. Gastroenterol. Hepatol.,* **2011,** *8* (2), 71-73.

[3] Vincent, A.; Herman, J.; Schulick, R.; Hruban, R.H.; Goggins, M. Pancreatic cancer. *Lancet,* **2011,** *378* (9791), 607-620.

[4] Liu, Y.; Yu, G.; Tian, M.; Zhang, H. Optical probes and the applications in multimodality imaging. *Contrast Media Mol. Imaging,* **2011,** *6* (4), 169-177.

[5] Luker, G.D.; Luker, K.E. Optical imaging: Current applications and future directions. *J. Nucl. Med.,* **2008,** *49* (1), 1-4.

[6] Weissleder, R.; Pittet, M.J. Imaging in the era of molecular oncology. *Nature,* **2008,** *452* (7187), 580-589.

[7] Hilderbrand, S.A.; Weissleder, R. Near-infrared fluorescence: application to in vivo molecular imaging. *Curr. Opinion Chem. Biol.,* **2010,** *14* (1), 71-79.

[8] Kobayashi, H.; Ogawa, M.; Alford, R.; Choyke, P.L.; Urano, Y. New Strategies for Fluorescent Probe Design in Medical Diagnostic Imaging. *Chem. Rev.,* **2010,** *110* (5), 2620-2640.

[9] Witjes, M.J.H.; Speelman, O.C.; Nikkels, P.G.J.; Nooren, C.; Nauta, J.M.; vanderHolt, B.; vanLeengoed, H.; Star, W.M.; Roodenburg, J.L.N. In vivo fluorescence kinetics and localisation of aluminium phthalocyanine disulphonate in an autologous tumour model. *Br. J. Cancer,* **1996,** *73* (5), 573-580.

[10] Moan, J.; Iani, V.; Ma, L.W. In vivo fluorescence of phthalocyanines during light exposure. *J. Photochem. Photobiol. B-Biol.,* **1998,** *42* (2), 100-103.

[11] Nesterova, I.V.; Erdem, S.S.; Pakhomov, S.; Hammer, R.P.; Soper, S.A. Phthalocyanine Dimerization-Based Molecular Beacons Using Near-IR Fluorescence. *J. Am. Chem. Soc.*, **2009,** *131* (7), 2432-2433.

[12] Ma, B.; Li, G.L.; Kanter, P.; Lamonica, D.; Grossman, Z.; Pandey, R.K. Bifunctional HPPH-N2S2-Tc-99m conjugates as tumor imaging agents: synthesis and biodistribution studies. *J. Porphyrins and Phthalocyanines*, **2003,** *7* (7), 500-507.

[13] Ranyuk, E.; Ali, H.; Guerin, B.; van Lier, J.E. A new approach for the synthesis of F-18-radiolabelled phthalocyanines and porphyrins as potential bimodal/theranostic agents. *J. Porphyrins and Phthalocyanines*, **2013,** *17* (8-9), 850-856.

[14] Ranyuk, E.; Lebel, R.; Berube-Lauziere, Y.; Klarskov, K.; Lecomte, R.; van Lier, J.E.; Guerin, B. Ga-68/DOTA- and Cu-64/NOTA-Phthalocyanine Conjugates as Fluorescent/PET Bimodal Imaging Probes. *Bioconjugate Chem.*, **2013,** *24* (9), 1624-1633.

[15] Scasnar, V.; Vanlier, J.E. Biological-Activities of Phthalocyanines-XV. Radiolabeling of The Differently Sulfonated Ga-67 Phthalocyanines for Photodynamic Therapy and Tumor Imaging. *Nucl. Med. Biol.*, **1993,** *20* (3), 257-262.

[16] Maya, E.M.; Vazquez, P.; Torres, T. Synthesis of alkynyl-linked phthalocyanine dyads: Push-pull homo- and heterodimetallic bisphthalocyaninato complexes. *Chem. Eur. J.*, **1999,** *5* (7), 2004-2013.

[17] Lambrecht, F.Y.; Durkan, K.; Yildrim, Y.; Acar, C. Labeling of acetaminophen with I-131 and biodistribution in rats. *Chem. Pharm. Bull.*, **2006,** *54* (2), 245-247.

[18] Seyitoglu, B.; Lambrecht, F.Y.; Durkan, K. Labeling of apigenin with I-131 and bioactivity of I-131-apigenin in male and female rats. *J. Radioanal. Nucl. Chem.*, **2009,** *279* (3), 867-873.

[19] Bayrak, E.; Lambrecht, F.Y.; Durkan, K.; Yilmaz, O. In vitro evaluation, biodistribution in rats of radiolabeled raloxifene. *Appl. Radiat. Isot.*, **2010,** *68* (1), 33-36.

[20] Ocakoglu, K.; Yurt Lambrecht, F.; Gunduz, C. Tümör Görüntüleme ve Fotodinamik Terapi için Simetrik/Asimetrik Makrosiklik Yapıların Sentezi ve Biyolojik Potansiyellerinin Karşılaştırılması. TUBITAK, 2012-2014; Vol. 112T565.

[21] Ocakoglu, K.; Er, O.; Ersoz, O.A.; Lambrecht, F.Y.; Ince, M.; Kayabasi, C.; Gunduz, C. Evaluation of nuclear imaging potential and photodynamic therapy efficacy of symmetrical and asymmetrical zinc phthalocyanines. *J. Drug Del. Sci. Tech.*, **2016,** *33*, 164-169.

[22] Lo, P-C.; Leung, S.C.H.; Chan, E.Y.M.; Fong, W-P.; Ko, W-H.; Ng, D.K.P. Photodynamic effects of a novel series of silicon(IV) phthalocyanines against human colon adenocarcinoma cells. *Photodiagnosis Photodynamic Ther.*, **2007,** *4* (2), 117-123.

[23] Li, H.; Jensen, T.J.; Fronczek, F.R.; Vicente, M.G.H. Syntheses and properties of a series of cationic water-soluble phthalocyanines. *J. Med. Chem.*, **2008,** *51* (3), 502-511.

[24] Berard, V.; Rousseau, J.A.; Cadorette, J.; Hubert, L.; Bentourkia, M.; van Lier, J.E.; Lecomte, R. Dynamic imaging of transient metabolic processes by small-animal PET for the evaluation of photo sensitizers in photodynamic therapy of cancer. *J. Nucl. Med.*, **2006,** *47* (7), 1119-1126.

[25] Fabris, C.; Valduga, G.; Miotto, G.; Borsetto, L.; Jori, G.; Garbisa, S.; Reddi, E. Photosensitization with zinc (II) phthalocyanine as a switch in the decision between apoptosis and necrosis. *Cancer Res.*, **2001,** *61* (20), 7495-7500.

[26] Lv, F.; Li, Y.Z.; Cao, B.; Liu, T.J. Galactose substituted zinc phthalocyanines as near infrared fluorescence probes for liver cancer imaging. *J. Mater. Sci. Mater. Med.*, **2013,** *24* (3), 811-819.

[27] Lv, F.; He, X.; Wu, L.; Liu, T. Lactose substituted zinc phthalocyanine: A near infrared fluorescence imaging probe for liver cancer targeting. *Bioorg. Med. Chem. Lett.*, **2013,** *23* (6), 1878-1882.

[28] Deland, F.H.; Shih, W.J. The Status of Spect in Tumor-Diagnosis. *J. Nucl. Med.*, **1984,** *25* (12), 1375-1379.

[29] Gauna, G.A.; Marino, J.; Garcia Vior, M.C.; Roguin, L.P.; Awruch, J. Synthesis and comparative photodynamic properties of two isosteric alkyl substituted zinc(II) phthalocyanines. *Eur. J. Med. Chem.*, **2011,** *46* (11), 5532-5539.

[30] Ongarora, B.G.; Hu, X.; Verberne-Sutton, S.D.; Garno, J.C.; Vicente, M.G.H. Syntheses and Photodynamic Activity of Pegylated Cationic Zn(II)-Phthalocyanines in HEp2 Cells. *Theranostics*, **2012,** *2* (9), 850-870.

[31] Chin, Y.; Lim, S.H.; Zorlu, Y.; Ahsen, V.; Kiew, L.V.; Chung, L.Y.; Dumoulin, F.; Lee, H.B. Improved Photodynamic Efficacy of Zn(II) Phthalocyanines via Glycerol Substitution. *Plos One*, **2014,** *9* (5).

[32] Vaupel, P.W.; Frinak, S.; Bicher, H.I. Heterogeneous Oxygen Partial-Pressure and pH Distribution in C3h Mouse Mammary Adenocarcinoma. *Cancer Res.*, **1981,** *41* (5), 2008-2013.

[33] Bohmer, R.M.; Morstyn, G. Uptake of Hematoporphyrin Derivative by Normal and Malignant-Cells - Effect of Serum, pH, Temperature, and Cell-Size. *Cancer Res.*, **1985,** *45* (11), 5328-5334.

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