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ORIGINAL ARTICLE

Evaluation of cancer imaging potential and photodynamic therapy efficacy of copper (II) benzyloxypheophorbide-*a*

Kasim Ocakoglu[1,2], Özge Er[3], Fatma Yurt Lambrecht[3], Sunde Yılmaz Süslüer[4], Cağla Kayabasi[4], Cumhur Gündüz[4], and Osman Yılmaz[5]

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Abstract

The biological potential of a synthetic copper chlorophyll derivative was investigated via *in vivo* and *in vitro* experiments. The Cu-chlorophyll derivative photosensitizer (Cu-PH-A) was labeled with ^{131}I with high efficiency (92.9 ± 4.2%) using the iodogen method. Cell culture studies were performed with the MCF-7 and MDAH-2774 cell lines after radiolabeling. The photosensitizing activity of Cu-PH-A was more effective in MDAH-2774 cells than in MCF-7 cells at a concentration of 50 μM. When the biodistribution in female Albino Wistar rats was examined, uptake of the radiolabeled photosensitizer was maximal in the liver and ovaries after 60 min. It is concluded that radiolabeled Cu-chlorophyll derivative photosensitizer has high uptake in ovaries in normal rats. In addition, the intercellular uptake and PDT efficacy of the Cu-PH-A in MDAH-2774 were good compared with MCF-7 cells. This photosensitizer could be useful for both ovary tumour imaging and PDT.

Keywords

^{131}I, chlorophyll-*a*, metallo-chlorophyll, nuclear imaging, photodynamic therapy

History

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Introduction

In recent years, studies regarding photodynamic therapy have gained momentum as an alternative to surgery, chemotherapy and radiotherapy, which are commonly used to fight cancer. This method is based on exposing a photosensitive material (photosensitizer) localized in target tissues to visible light.

Radionuclide-labeled photosensitizers that have clinical effects can be used in order to understand the pharmacokinetic and pharmacodynamic characteristics of photosensitizers. Photosensitizers (PS) are used in photodynamic therapy (PDT) due to the high tumour uptake efficiencies of these compounds. The excellent tumour localization of photosensitizers allows them to be used as multi-functional agents. Because of these properties, such compounds can be used as tools for tumour imaging as well (Fluorescent, MRI and PET, SPECT) [1]. The importance of the feasibility of developing a single agent for tumour imaging (PET, fluorescence) and therapy (PDT) using such compounds is well known [2].

Photodynamic therapy is a treatment involving light and a photosensitizer, used in conjugation with molecular oxygen to obtain cell death. Moreover, PDT is a selective treatment method for the local destruction of diseased cells and tissue. The selectivity is based on the ability of the photosensitizer to preferentially accumulate in the diseased tissue. Then singlet oxygen or other highly reactive species, for example radicals, kill the target cells [3,4].

In view of the extensive variety of metal ions in biochemical systems, it is logical that metal ions are often administered as mineral supplements in order to maintain the needed daily intake for certain elements, such as calcium, iron, zinc and copper [5]. The use of inorganic compounds in therapy also has a long-standing tradition [6]. One commonly used derivative, pheophorbide-*a*, is obtained from chlorophyll-*a* in acidic medium by the removal of Mg and a phytol group by the chlorophyllase enzyme, and shows an intense absorption band at 650 nm [7].

It has been reported that the presence and nature of the central metal ion (Cu, Fe, Co, Zn, etc) bound by a number of photosensitizers strongly influences the photophysical properties of the photosensitizer [8]. Chelating of paramagnetic metals induced PS chromospheres appears to shorten triplet lifetimes, generating variations in the triplet quantum yield and triplet lifetime of the photoexcited triplet state of the metalled PS. In this context it is observed the copper metallate docta ethylbenzochlorin photosensitizer has a triplet state lifetime of less than 20 ns, and is still deemed an efficient photodynamic agent by Selman et al. [9].

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In our study, we synthesized copper (II) methyl 3-devinyl-3-{1¢-(benzyloxy)-ethyl}pheophorbide-*a* and evaluated its nuclear imaging potential *in vivo* and PDT potential *in vitro.*

Materials and methods

$Na^{131}I$ was supplied by the Department of Nuclear Medicine, Sifa University. LC-MS/MS analyses were performed using an Agilent 6460 Triple Quad LC-MS/MS (Izmir-Turkey) (80% MeOH + 20% H_2O + Formic acid, negative scan, 0.3 ml/min). Elemental analyses were performed on a LECO-CHNS-932 elemental analyser. ^1H NMR spectra were performed on a Bruker 400 MHz spectrometer using residual solvent peaks as internal standards. All chemicals were purchased from commercial sources and used as received. Spirulina maxima algae were purchased from Bienenschwarmmm (Germany). Methyl pheophorbide-*a* was synthesized according to methods reported in the literature [10–13].

All chemicals used the *in vitro* studies were purchased from Biological Industries (Kibbutz Beit-Haemak, Israel); all other chemicals were purchased from Merck (Darmstadt, Germany). Iodogen was supplied by Sigma-Aldrich (St. Louis, MO). 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 methyl3-devinyl-3-{1¢-(benzyloxy)-ethyl}pheophorbide-*a* (1)

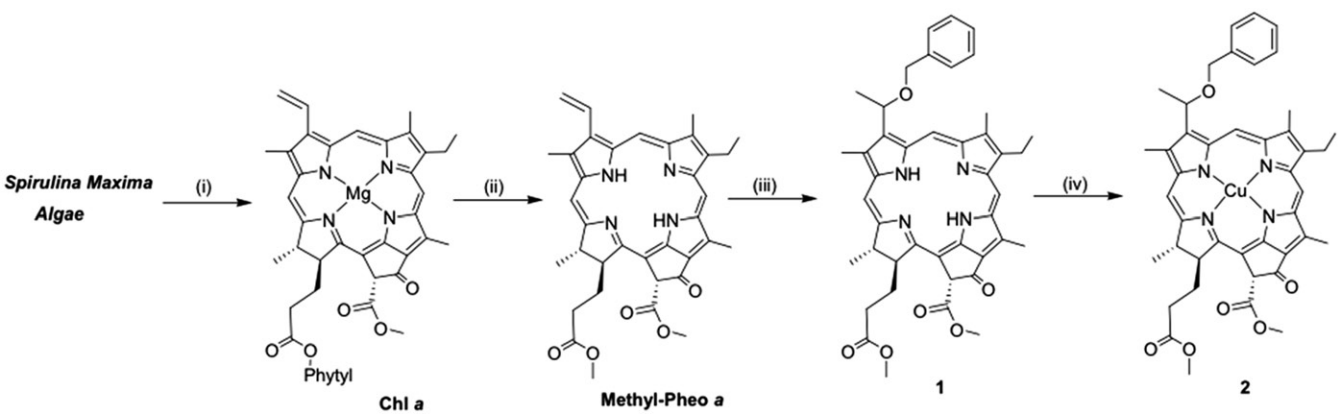
3-Devinyl-3-{1¢-(benzyloxy)-ethyl}pheophorbide-*a* (1) (Scheme 1) was obtained by following the literature procedure [2]. Methyl pheophorbide *a* (60 mg) was treated with 2 mL of 30% hydrobromic acid (in acetic acid solution), and the mixture was stirred at room temperature for 2 h. The acids were removed under high vacuum and the residue was reacted with an excess of benzyl alcohol. Dry dichloromethane (10 mL) and anhydrous potassium carbonate (40 mg) were added and the reaction mixture was stirred under a nitrogen atmosphere for 1 h. The mixture was diluted with dichloromethane and washed with aqueous sodium bicarbonate solution and then with water. The organic layer was separated and treated with diazomethane and dried over anhydrous sodium sulphate. The solvent was removed by using a rotary evaporator under vacuum. The raw product was chromatographed over a silica column using n-hexane: ethyl acetate 4:1 as eluant to remove excess benzyl alcohol, followed by n-hexane: ethyl acetate 1:1 to yield the desired compound 1 [2]. The target product **1** was characterized by NMR and mass spectrometry. Yield: 53 mg (75%). ^1H NMR (400 MHz, $CDCI_3$) δ ppm: 9.74 (s, 1H, CH-5), 9.52 (s, 1H, CH-10), 8.54 (s, 1H, CH-20), 7.39–7.30 (m, 5H, ArH), 6.00 (q, $J = 6.8$ Hz, 1H, 3^1-H), 4.79 (dd, $J = 2$ Hz, 9.6 Hz, 1H, OCH_2Ar), 4.61 (dd, $J = 4$ Hz, 8 Hz, 1H, OCH_2Ar), 4.52-4.42 (m, 1H, 18-H), 4.32–4.25 (m, 1H, 17-H), 3.87 (s, 1H, 13^2-CH), 3.72 (q, $J = 8.0$, 2H, CH_2-8^1), 3.70 (s, 3H, $COOCH_3$), 3.68 (s, 3H, $COOCH_3$), 3.60 (s, 3H, 12^1-CH_3), 3.36 (s, 3H, 2^1-CH_3), 3.16 (s, 3H, 7^1-CH_3), 2.72–2.61, 2.60–2.46, 2.38–2.19 (m, 1H + 1H + 2H, 17-CH_2CH_2), 1.82 (dd, $J = 2.8$ Hz, 4.4 Hz, CH_3-18^1), 1.70 (t, $J = 7.6$ Hz, CH_3-8^2), 0.42 (brs, 1H, NH), −1.70 (brs, 1H, NH). Anal Calcd for $C_{43}H_{46}N_4O_6$: C, 72.25; H, 6.49; N, 7.84. Found: C, 72.23; H, 6.47; N, 7.83%. MS (ESI; 80% MeOH + 20% H_2O + formic acid) *m/z*: 714.2 $[M]^+$.

Synthesis of copper (II) methyl 3-devinyl-3-{1¢-(benzyloxy)-ethyl}pheophorbide-*a* (2)

Methyl 3-devinyl-3-{1¢-(benzyloxy)-ethyl}pyropheophorbide-*a* (2) was dissolved in dichloromethane, and saturated copper (II) acetate hydrate in methanol was added. The resulting solution was refluxed under nitrogen overnight before being diluted with dichloromethane, washed with water, dried, and evaporated. The resulting copper (II) complex (2) was crystallized from dichloromethane/n-hexane. Anal Calcd for $C_{43}H_{44}N_4O_6Cu$: C, 66.52; H, 5.71; N, 7.22. Found: C, 66.51; H, 5.68; N, 7.21%. MS (ESI; 80% MeOH + 20% H_2O + formic acid) *m/z*: 775.2 $[M]^+$.

Radiolabeling of Cu-chlorophyll derivative photosensitizer and radiochemical purity

A stock solution of Cu-chlorophyll derivative photosensitizer (3 mg) was prepared by dissolving it in 300 µl of ethanol. In each study, 10 µl of the stock solution was diluted with distilled water (100 µg/500 µl), and labeled with 250 µCi ^{131}I via the iodogen method (1 mg) after the pH was adjusted



Scheme 1. Synthesis of copper(II) methyl 3-devinyl-3-{1¢-(benzyloxy)-ethyl}pheophorbide-*a* (**2**).

to 10 with 0.2 N NH_4OH. Radiochemical purity analysis of ^{131}I-Cu-PH-A was performed by instant thin-layer chromatography on silica gel strips (ITLC-SG, Merck), using a two-solvent system – *n*-butanol:water:acetic acid (4:2:1) and chloroform:acetic acid (9:1), to determine the labeling efficiency. The TLRC sheets were dried and scanned on a TLC-scanner (BioScan AR-2000 Washington, DC).

Radio high-performance liquid chromatography

This study was performed using an HPLC system (Shimadzu, Kyoto, Japan) equipped with an RP-C-18 (250 × 4.6 mm I.D.) column, a LC-10Atvp quaternary pump, a UV detector (Shimadzu SPD-10Atvp) and a NaI (Tl) multi-channel analyser detector. The retention times (R_t) of the Cu-PH-A, ^{131}I-labeled Cu-PH-A and ^{131}I were determined using methanol:water (95:5%) as a mobile phase. The flow rate of the solvent system was 1 ml/min at a wavelength of 254 nm. The injection volume was 5 µl at 25 °C.

Electrophoresis

A paper electrophoresis procedure was carried out in a Gelman electrophoresis chamber. Cathode and anode poles were determined and then cellulose acetate strips were dampened with 100% distilled water. The samples were spotted on application points on cellulose acetate strips. Standing time and applied voltage were set at 120 min and 300 V, respectively. Next, the strips were scanned using the TLC scanner.

Lipophilicity

The n-octanol/water ratio was calculated in order to determine the lipophilicity of ^{131}I-labeled Cu-PH-A. To do this, 100 µl of photosensitizer, radiolabeled under optimum conditions (1 mg iodogen at pH 10), were added to 3 ml of *n*-octanol and 3 ml of water solution. After mixing for 1 h with a magnetic mixer, the solution was centrifuged (2500 rpm, 5 min) to separate the phases. Afterwards, a 500 µl sample from each phase was counted using a Cd(Te)-RAD-501 single channel analyser. The averaged activities from the aqueous and octanol phases were used to calculate the log *p* values. The octanol-to-water partition coefficient ($P_{o/w}$) of the radiolabeled compound was calculated by dividing the counts in the octanol phase by that in the aqueous phase.

Effect of reaction pH on radiolabeling yield

In order to analyse the effect of reaction pH on radiolabeling yield, the Cu-chlorophyll derivative photosensitizer was labeled with ^{131}I at pH 3, 6 and 10. Afterwards, the radiolabeling yield was determined with the TLRC method.

In vitro stability study

To determine the shelf-life of the radiolabeled photosensitizer prepared under optimum conditions, it was measured at 30 min, 1 h, 2 h, 3 h, and 24 h by the TLRC method.

Stability in phosphate buffer/human serum

The radiolabeled photosensitizer (10 µl) was added in 90 µl phosphate buffer and human serum. Labeling efficiency of photosensitizer incubated at 37 °C was determined from samples taken at a different time points (30 min, 1 h, 2 h, 3 h, and 24 h) via the TLRC method.

Biodistribution

The animal experiment was approved by the Animal Ethics Committee of DokuzEylul University. The biodistribution of the radiolabeled photosensitizer in certain organs was investigated using female Albino Wistar rats. The photosensitizer was labeled with ^{131}I under optimum conditions (specific activity 272.05 MBq/µmol). After weighing the rats, 300 µl of adjusted pH 7 radiolabeled photosensitizer was injected intravenously into the tail vein. The animals were sacrificed at 30, 60 and 120 min post injection via anaesthesia overdose. We used three rats for each time period. After the organs were removed, they were weighed and counted using a Cd(Te)-RAD-501 single channel analyser. Then the percentage of radioactivity per gram of organ (%ID/g) was determined.

Cell culture

Cell culture studies were carried out using the MCF-7 human breast adenocarcinoma cell line and the MDAH-2774 ovarian cancer cell line. The cells were cultured in RPMI 1640 containing 100 IU/ml penicillin G, 100 mg/ml streptomycin and 10% heat-inactivated FBS and were maintained at 37 °C in an incubator containing 5% CO_2.

Cytotoxicity of the Cu-chlorophyll derivative photosensitizer

The WST-1 method was used in order to determine the IC_{50} value of Cu-PH-A. A working group consisting of MCF-7 and MDAH-2774 cells was placed in a 96-well culture plate (1×10^5 cells in each well) and, after 1 day of incubation, 1.5–100 µM of the compound prepared with RPMI 1640 (w/o FBS) were added to each well. Twenty-four hours later, the medium was removed from the cells and RPMI 1640 (with FBS) was added to each well. After 1 hour of incubation, 50 µl of the WST-1 kit was added to the wells. The absorbance of the wells was measured by a microplate reader (Varioskan Flash Multimode Reader-Thermo, Vantaa, Finland) at 450/630 nm 3 h later, and used to calculate the percentage of cytotoxicity.

Intracellular uptake of ^{131}I-Cu-chlorophyll

The *in vitro* cellular uptake of ^{131}I-labeled Cu-PH-A (25 µM/25 µCi) was examined using the MCF-7 and MDAH-2774 cell lines. The cells were placed in 24-well culture plates (1×10^5 cells in each well) at 37 °C and incubated for 2 days in the case ofMCF-7 and 1 day for the MDAH-2774 cells. The cell culture was removed and the wells were washed twice with 0.9% NaCl solution 2 days later. Afterward, the radiolabeled photosensitizer diluted with MEM (w/o FBS) was added to the cells. Ten-fold more inactive Cu-PH-A had been added as a blocking agent prior to the addition of the radiolabeled photosensitizer [14]. After 1 h incubation, the wells were counted by the Cd(Te) detector. The radioactive cell culture medium was removed and same amount of 0.9% NaCl

solution was added to each well. After counting the wells again, the data were analyzed and the percentage uptake was calculated.

In vitro photosensitizing efficacy

The MCF-7 human breast cancer cell line and MDAH-2774 human ovarian carcinoma cell line were cultured in RPMI 1640 supplemented with 10% foetal bovine serum at 95% humidity with 5% CO_2. The cells were seeded in triplicate at 1×10^5 cells/well in 96-well plates. They were incubated overnight before adding the desired Cu-PH-A compound at various concentrations (1.56–100.00 μM). After a 3-h incubation with the compound in the dark, the cells were exposed to red light (650 nm) at doses of 10–30 J/cm^2. Fresh medium was then added and the cells were incubated for 24 h after light treatment. Phototoxicity was assessed after 24 h by comparing the growth of treated cells to control cells as measured by formazan production using the WST-1 assay.

Statistical analysis

Differences in the mean values of measured activities were evaluated statistically using the SPSS 16.0 program (Chicago, IL) (Univariate Variance Analyses and Pearson Correlation). Probability values of $p < 0.05$ were considered significant.

Results and discussion

Radiolabeling

The labeling efficiency of ^{131}I-Cu-PH-A was determined using the TLRC and HPLC methods. R_f values of Na^{131}I and ^{131}I-labeled Cu-PH-A were determined as 0.45 and 0.92 by TLRC, respectively, when mobile phase 1[n-butanol/water/acetic acid (4:2:1)] was used. When mobile phase 2 [chloroform/acetic acid 9:1] was used, the R_f values of Na^{131}I and ^{131}I-labeled Cu-PH-A were 0.05 and 0.95, respectively. According to the RHPLC results, the R_t of ^{131}I and ^{131}I-Cu-chlorophyll were determined as 3.8 and 4.3 min, respectively. The radiolabeling yield was determined as 92.9 ± 4.2% ($n = 6$), as acquired via RHPLC and also TLRC. In previous studies, a Cu-chlorophyll derivative photosensitizer was labeled with ^{124}I and showed quite high labeling yields. Moreover, the charge of the radiolabeled compound was found to be neutral according to the electrophoresis results [2,15].

On the other hand, PH-A and some photosensitizers similar to PH-A (T3, 4BCPC, PH-A-BSANPs, photosan-3, hematoporphyrin-linked albumin nanoparticles) have been labeled with ^{99m}Tc [12,13,16,17]. In addition, Smith et al. created different metal complexes of PH-A (Ni, Cu, and Zn) (10).

Effect of reaction pH

pH experiments performed in a 1 mg iodination tube showed that the radiolabeled yield of ^{131}I-Cu-PH-A was low at pH 3 (59.4 ± 2.1%) and pH 6 (68.5 ± 4.4%). The best radiolabeling efficiency was found to be 92.9 ± 4.2% at pH 10 (Figure 1). Labeling with radioiodine is usually accomplished by promotion the electrophilic species I+ into an aromatic ring in the molecule of interest. Oxidized iodine can easily bind with a hydrogen atom in benzene ring in an electrophilic substitution reaction in order to generation iodine complex [18–22]. It is observed in some literature [19,20] that pH of radioiodination changes depending on the molecular structure.

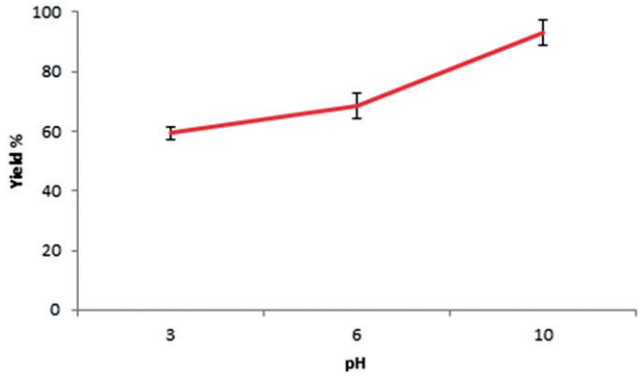


Figure 1. Labeling yield of ^{131}I-Cu-PH-A at different pH values.

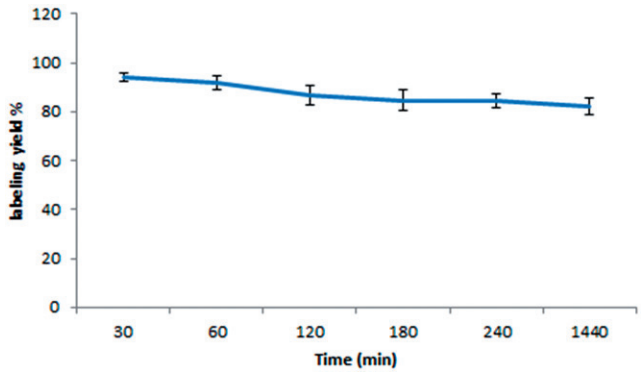


Figure 2. *In vitro* stability of ^{131}I labeled Cu-PH-A at different times after labeling.

Stability studies

The complex was found to be stable in *in-vitro* conditions. While the radiolabeling efficiency in the first 30 min was found to be 94.2 ± 1.8%, the radiolabeled yield at the end of 1440 min decreased to 82.4 ± 3.4% (Figure 2). Obtained results indicated that radiolabeled compound has enough shelf life for imaging study.

Stability in phosphate buffer showed that the radiolabeling efficiency at the end of the 1440 minutes was 78.5 ± 0.7 (Figure 3). The results demonstrated that the radiolabeled compound is stable in neutral pH.

Radiolabeling yield in human serum was determined as 81.1 ± 0.3% in the first 30 min and end of the 1440 min the radiolabeling yield was decreased to 71.0 ± 0.5% (Figure 4). Although radiolabeling yield values of human serum and phosphate buffer are quite close each other, radiolabeling efficiency in human serum reduces faster than phosphate buffer. Both stabilities results show that the radiolabeled compound is stable for using *in vivo* study.

Biodistribution

An *in vivo* study of ^{131}I-labeled Cu-PH-A was carried out using female Albino Wistar rats. The results can be seen

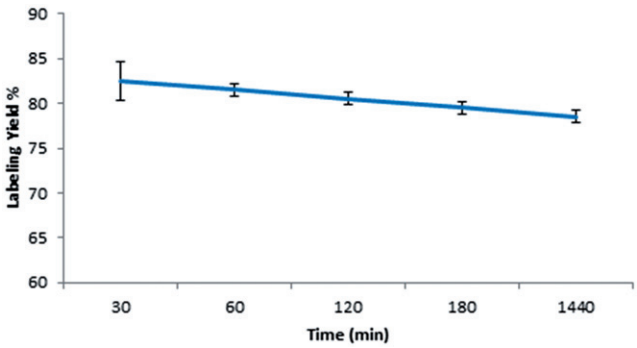


Figure 3. The stability of ^{131}I labeled Cu-PH-A in phosphate buffer at different times after labeling.

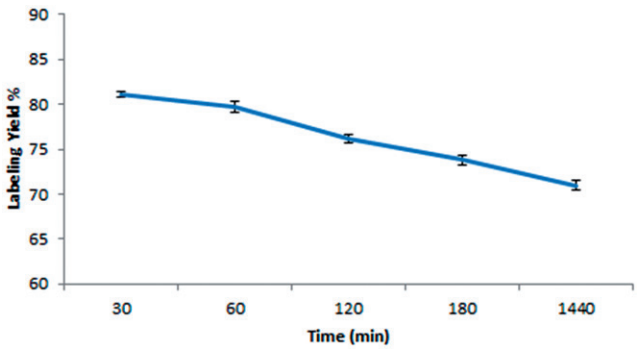


Figure 4. The stability of ^{131}I labeled Cu-PH-A in human serum at different times after labeling.

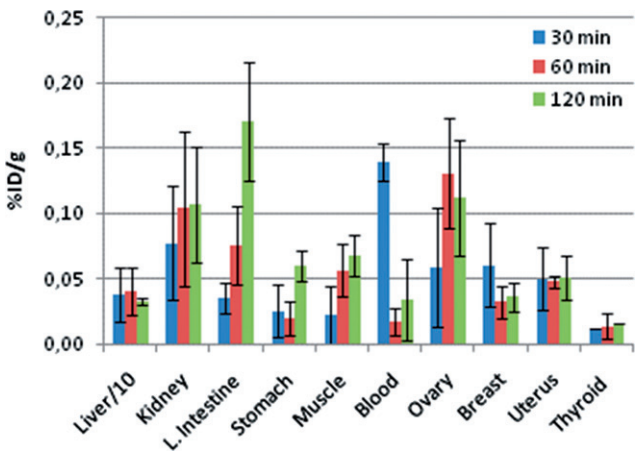


Figure 5. Biodistribution results of ^{131}I-labeled Cu-PH-A.

in Figure 5. The uptake of ^{131}I-Cu-PH-A reached a maximum in the kidney (%ID/g: 0.11) and large intestine (%ID/g: 0.17, $p < 0.05$) at 120 min. While the uptake in breast (%ID/g: 0.06) and uterus (%ID/g: 0.05) could be seen to be high at 30 min, the highest uptake was shown in ovary (%ID/g: 0.13, $p < 0.05$) and liver (%ID/g: 0.40) at 60 min. It was reported by Ozgur et al. that the maximum uptake of ^{99m}Tc-PH-A-BSANPs was in breast and uterus at 120 min [13]. However, the uptake of ^{99m}Tc-PH-A was seen to be the highest in ovary and uterus at 120 min. and in breast at 60 min. Our results demonstrate that ^{131}I-labeled Cu-PH-A has a higher uptake in ovary at 60 min, owing to ^{99m}Tc-PH-A-BSANPs and ^{99m}Tc-PH-A having a high affinity for the organ. On the other hand, the thyroid gland was controlled, and the activity in the thyroid gland was low. It also showed that the ^{131}I-compound is stable against biological decomposition *in vivo*.

The accumulation of activity in the liver at 60 min post injection was the highest, but had fallen at 2 h. The decline in liver uptake was accounted for by increased activity in the intestines at 2 h (Figure 5). The major route of excretion is through the renal and hepatobiliary pathways. It was also shown in previous studies that many photosensitizers similar to ^{99m}Tc-PH-A-BSANPs, ^{99m}Tc-PH-A, ^{99m}Tc-HP, PH-A and T3, 4BCPC, such as Photosan-3, were removed from the body via the urinary system [12,13,16,17,23,24].

Even as ^{131}I-labeled Cu-PH-A was eliminated by being carried out of the body, there was no change in the uptake of ^{131}I-labeled Cu-PH-A in ovary at 120 min. On the one hand, while the uptake in blood decreased, the uptake was almost stable in ovary in this time frame. These results demonstrated that ^{131}I-labeled Cu-PH-A shows specific localization in ovary. It has been reported by Murugesan et al. that ^{99m}Tc-T3, 4BCPC showed high accumulation in liver and kidney in normal rats through 4 h post injection. Hence, the accumulation they observed was higher in these organs than ours [23]. The lipophilicity of our compound is 2.07 and we obtained high uptake in liver because of its lipophilicity. The compound has the highest affinities in liver as similar compound because of their lipophilicity [2]. Actually, the uptake in ovary was higher than other organs in 60 min, and this can be an advantage for imaging. First we carried out biodistribution, and then the *in vitro* studies in MCF-7 and MDAH-2774 cells were designed according to the obtained results.

Cell culture studies on the MCF-7 and MDAH-2774 cell line

According to the WST-1 cytotoxicity test results, 50% cell viability was observed at a concentration of 100 μM Cu-PH-A for the MCF-7 cell line and 135 μM for the MDAH-2774 ovarian cancer cell line (Figure 6). ^{131}I-labeled Cu-PH-A showed specific binding to MCF-7 cells. When the uptake by MCF-7 was significantly blocked by a 10 fold molar excess of unlabeled Cu-PH-A, cell-associated radioactivity decreased to 30 % (Figure 7) [19].

Cell culture studies performed with MDAH-2774 cells indicated that ^{131}I-labeled Cu-PH-A showed more specific binding to this line than to MCF-7 cells. When comparing blocked uptake with unblocked for MDAH-2774 cells, cell-associated radioactivity declined to 68 % (Figure 8).

The MCF-7 breast cancer cell model was investigated earlier for *in vitro* evaluation of ^{99m}Tc-PH-A-BSANPs and ^{99m}Tc-PH-A [12,13]. These studies showed that the intracellular uptake of both by MCF-7 is specific. In another study by Yang et al., ^{99m}Tc-HP-ANP showed increased penetration of murine lung tumours induced by CT-26 colon cancer cells compared to normal lungs, and ^{99m}Tc-HP-ANP demonstrated good radioimaging properties according to a scintigraphic application in rabbits [17]. In a previous study PHSA was also

determined to have greater phototoxicity than Pheo in Jurkat cells [15].

In vitro photosensitizing efficacy

The photosensitizing ability of Cu-PH-A was analysed in MCF-7 and MDAH-2774 cells at two concentrations (25 and 50 μM) and different light doses (10, 20, 30 J/cm^2) with a 24 h incubation period. The light dose-dependent response was determined by the WST-1 assay. The results are summarized in Figure 8. Cu-PH-A was more effective in MDAH-2774 than in MCF-7 cells at the same 50 μM concentration (Figure 9).

In a study made by Selman et al., copper octaethylbenzochlorin demonstrated an unexpected result. It appeared to be more photoactive towards leukaemia cells in vitro and in a rat bladder tumour model despite an undetectable triplet state [9,25]. Goma et al. reported that a light-activated chlorophyll derivative decreased MCF-7 cell viability with increasing light dose. They explained this may be due to the increased production rate of highly reactive singlet oxygen species that cause tumour cell death [26]. In contrast, our results indicated Cu-PH-A decreased the cell viability of MDAH-2774 cells with increasing light dose, but not that of MCF-7 cells.

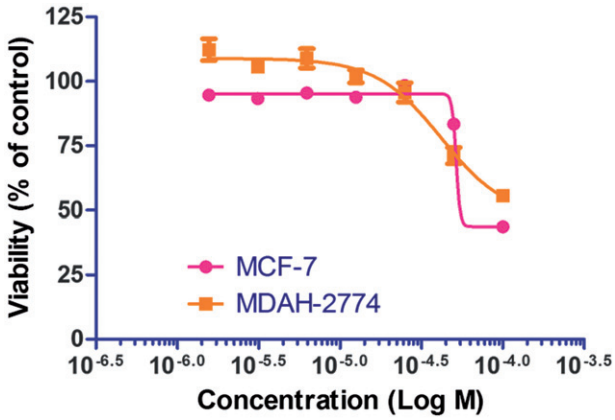


Figure 6. Cytotoxicity of Cu-PH-A in MCF-7 and MDAH-2774 cells, $IC_{50} = 100$ μM in MCF-7, $IC_{50} = 135$ μM in MDAH-2774.

Conclusions

In the current study, the biodistribution of ^{131}I-labeled Cu-PH-A in healthy female rats indicated high uptake in the ovary. ^{131}I-labeled Cu-PH-A has potential for scintigraphic imaging of ovarian tumours.^{131}I-labeled Cu-PH-A showed specific uptake in MDAH-2774 cell line. Nevertheless, the PDT efficacy of the Cu-PH-A in MDAH-2774 was good compared with MCF-7 cells. In conclusion, Cu-PH-A may be used as a site-specific tumour imaging and PDT agent in ovarian tumours.

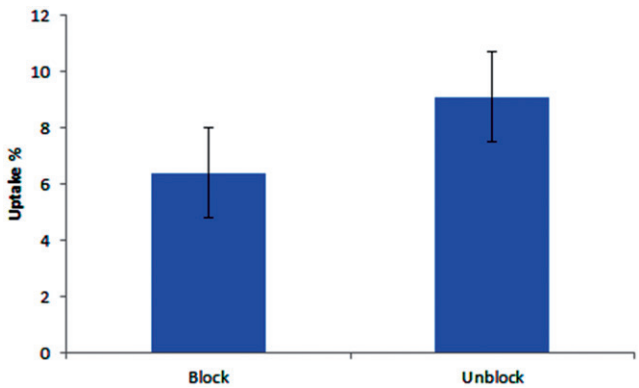


Figure 7. Intracellular uptake of ^{131}I-labeled Cu-PH-A in MCF-7 cells.

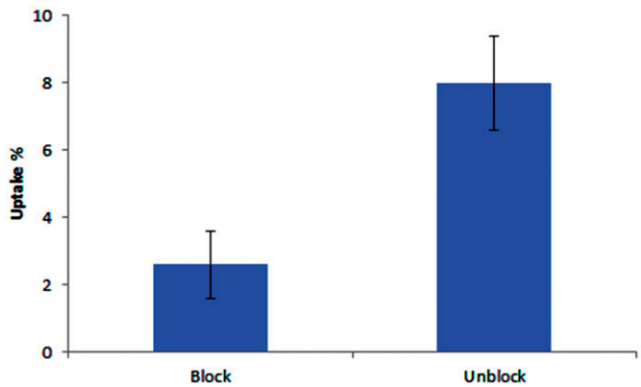


Figure 8. Intracellular uptake of ^{131}I-labeled Cu-PH-A in MDAH-2774 cells.

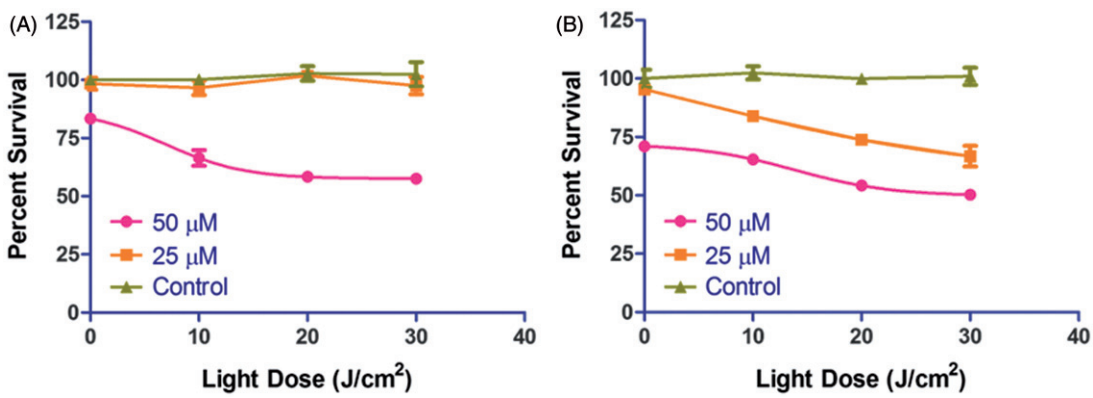


Figure 9. The *in vitro* photosensitizing activity of Cu-PH-A in (A) MCF-7, (B) MDAH-27 tumor cells (25 and 50 μM) at 24 h incubation. Control: Cell exposed to light without photosensitizer.

Declaration of interest

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