Investigation of Radiopharmaceutical Potential of an Unsymmetrical Perylene diimide (DMANPER) Radiolabeled with ^{131}I

Radyoaktif ^{131}I ile İşaretli Asimetrik Bir Perilen diimid'in (DMANPER) Radyofarmösitik Potansiyelinin İncelenmesi

Research Article

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ABSTRACT

The aim of this study was to investigate the radiopharmaceutical potential of N-(2,6-diisopropylphenyl)-N'-[4-(N,N-dimethylaminophenyl)]-perylene-3,4,9,10-tetra carboxylic diimide (DMANPER) radiolabeled with ^{131}I using scintigraphic imaging and biodistributional techniques. Radiolabeled DMANPER (^{131}I-DMANPER) was intravenously injected into an adult male Albino rabbit via its ear vein anesthetizing with a mixture of Alfazyne and Alfamine (Serva) to determine dynamic and static situations of radiolabeled compound in the rabbit metabolism. Three rats were used for biodistribution studies corresponding to different time intervals. The animals were sacrificed by heart puncture under ether anesthesia within post-injection time intervals ranging from 30 to 300 min. Results were expressed as % of the injected dose per gram tissue (% ID/g). TLRC analysis showed that DMANPER was successfully radioiodinated with 131I with a yield of practically 100 %. According to scintigraphy studies, ^{131}I-DMANPER was completely accumulated in the stomach in 30 min. In the biodistributional data for ^{131}I-DMANPER in rats, a significant amount of activity is seen in the stomach between 30-60 min. It can be concluded that ^{131}I-DMANPER may be an alternative imaging agent like N-(2,6-diisopropylphenyl)-N`-(4-pyridyl)-perylene-3,4,9,10-tetracarboxylic diimide (PYPER) to be a potential agent for diagnosis and perhaps therapy of stomach cancer.

Key words Perylene dimide, iodine-131, biodistribution, stomach imaging.

ÖZET

Bu çalışmanın amacı sintigrafik görüntüleme ve biyodağılım teknikleri kullanılarak, ^{131}I ile işaretlenmiş N-(2,6-diizopropilfenil)-N'-[4-(N,N-dimetillaminofenil)]-perilen-3,4,9,10-tetra karboksilik diimid'in (DMANPER) radyofarmösitik potansiyelini incelemektir. Radyo işaretli DMANPER (^{131}I-DMANPER), tavşan metabolizmasında radyoaktif olarak işaretlenmiş bileşiğin dinamik ve statik durumunu belirlemek için, Alfazin ve Alfamin karışımı ile anestezi verilmiş yetişkin bir erkek Albino tavşana kulak veni yoluyla enjekte edilmiştir. Biyodağılım çalışmalarında farklı zaman aralıkları için üç adet sıçan kullanılmıştır. Enjeksiyondan sonra 30 ile 300 dakika arasında değişen bir zaman aralığı içerisinde, hayvanların eter anestezisi ile kalplerinin durdurulmasıyla hayatlarına son verdirilmiştir. Sonuçlar, gram doku başına enjekte edilen dozun %'si olarak ifade edilmiştir (% ID/g). İnce tabaka radyo-kromatografi analizleri (TLRC), DMANPER'in ^{131}I ile başarılı bir biçimde, yaklaşık % 100 verimle işaretlendiğini göstermiştir. ^{131}I-DMANPER 30 dakikalık süre içerisinde tamamen midede birikmiştir. ^{131}I-DMANPER için farelerde yapılan biyodağılım sonuçlarında, 30 ile 60 dakika arasında en fazla aktivite midede görülmüştür. ^{131}I-DMANPER'in mide kanserinin teşhisi ve tedavisi için N-(2,6-diizopropilfenil-N`-(4-piridil)-perilen-3,4,9,10-tetrakarboksilik diimid (PYPER) gibi bir alternatif görüntüleme ajanı olabileceği sonucuna varılabilir.

Anahtar kelimeler Perilen diimid, iyot-131, biyodağılım, mide görüntülemesi

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INTRODUCTION

Symmetrical and unsymmetrical perylene diimides (PDIs) are one of the key ligands in photodynamic therapy, G-quadruplex DNA stabilization and inhibition of telomerase activity in cancer cells. Most cancer cells have a high level of this enzyme. Telomerase is expressed in 80 – 90 % of all tumor cells [1]. Telomerase has the ability to add DNA back to the telomeres and to accelerate the uncontrolled growth of tumor cells [2-4]. Telomeres are composed mostly of guanosines. The last few guanosines at the end of each telomere can fold into a sort of box, called a G-quadruplex. DNA G-quadruplexes comprise stacked tetrads, each of which arises from planar association of four guanosines in a cyclic Hoogsteen hydrogen bonding arrangement [5,6]. The ability of telomere DNA to adopt a G-quadruplex structure in laboratory studies is so well established that many researchers believe not only in the existence of these structures inside cells, but also in their essential role in maintaining the integrity of the telomeres. The box-like G-quadruplex structures at the end of chromosomes are thought to be the knot that prevents the chromosomes from unraveling. Many groups have designed and synthesized some molecular structures which are believed to interact with G-quadruplex DNA structures [7-11]. One of the commonly preferred and employed compounds in the G-quadruplex DNA binding studies is PDIs. A systemic therapeutic approach, called tumor targeted therapy, combines an effective chemical agent with a radionuclide [12].

The aim of the current study was to label a PDI derivative such as DMANPER with ^{131}I as a radiopharmaceutical and to investigate its radiopharmaceutical potential using biodistribution and scintigraphic studies in rats and rabbit.

MATERIALS AND METHODS

Materials

DMANPER given in Figure 1 was synthesized as described in our previous study [13] and then, it was subjected for radioiodination with Na^{131}I. Na^{131}I (74 MBq) was obtained from Ege University Department of Nuclear Medicine. All other chemicals were commercially purchased and used without further purification for medical gamma camera imaging and biodistributional studies. Thin layer radiochromatography (TLRC) studies were carried out by a Cd(Te) detector equipped with a RAD 501 single-channel analyzer.

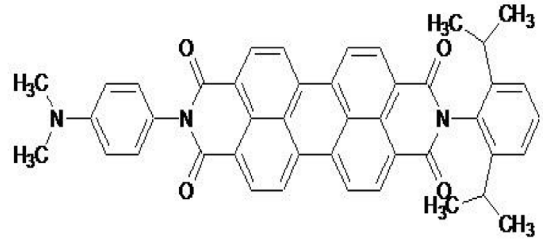

Figure 1. Chemical structure of DMANPER.

Preparing the iodogen coated tubes

1 mg amount of iodogen was dissolved in CH_2Cl_2 and transferred to closed tubes. CH_2Cl_2 was evaporated by air flow and iodogen was deposited on the walls of glass tubes as a thin film. These tubes were stored at +4°C until use.

Radioiodination procedure of DMANPER with ^{131}I

DMANPER was radioiodinated using the same procedure earlier applied for PYPER [14]. According to this procedure, 100 μL (1 mg) of DMANPER, 10 μL ethyl alcohol, and 540 μL of water were added to the iodogen coated tube and then, 350 μL of (29.6 MBq) Na^{131}I was added. This reaction mixture was kept at room temperature without stirring for 15 min. At the end of this time, the mixture was transferred to another tube by a syringe and then quality control was performed using TLRC technique as described below.

Thin layer radiochromatography (TLRC) studies

TLC Aluminum sheets (Merck, 20 x 20 cm code: 1.05552) were used for TLRC studies. Chloroform was used as the mobile phase. Each TLRC sheet was covered by adhesive band and cut as pieces of 0.5 cm width at the end of each chromatographic performance. These TLC pieces were then counted by using a Cd(Te) counting assembly. Thus, the moving of radiolabeled compound was examined.

Stability in human serum of ^{131}I-DMANPER

In vitro stability of ^{131}I-DMANPER in human serum was determined by incubating 100 μL (25 μg) of the labeled compound with 300 μL of blood serum at 37°C. The aliquots were the analyzed in time intervals of 0, 30, 60, 180 and 1440 min by TLRC technique after labeling.

Lipophilicity (Partition coefficient)

The lipophilicity (logP) of the radiotracer was measured as follows: 100 μL of the radiolabeled compound, ^{131}I-DMANPER, was added to a premixed suspension of 200 μL of octanol in 200 μL pH=7 buffer. The resulting solution was mixed 15 min at room temperature and centrifuged for 30 min at 2500 rpm. Then, 0.1 mL aliquots of each phase were removed and counted by a Cd(Te) detector equipped with a RAD 501 single-channel analyzer. Experiments were conducted in triplicate.

Scintigraphic imaging studies of ^{131}I-DMANPER

^{131}I-DMANPER was intravenously injected into an adult male Albino rabbit via its ear vein anesthetizing with a mixture of Alfazyne and Alfamine (Serva) to determine dynamic and static situations of radiolabeled compound in the rabbit metabolism. For these studies, a gamma camera (Diacan Instruments) which was adjusted to detect γ-radiations of ^{131}I was used. The static scintigrams were obtained from posterior projection within different time intervals up to about 2 h following the administration of ^{131}I-DMANPER.

Biodistributional studies of ^{131}I-DMANPER on rats

All studies using the laboratory animals were approved by Ege University Institutional Animal Review Committee. ^{131}I-DMANPER was administered via tail vein injection to male Albino Wistar rats. Three rats were used for each study corresponding to different time intervals. The animals were sacrificed by heart puncture under ether anesthesia within post-injection time intervals ranging from 30 to 300 min. Following excision, the organs were weighed and their activities were counted. Results were expressed as % of the injected dose per gram tissue (% ID/g) as given in Table 1.

Table 1. R_f values for radiolabeled compound and radioactive components.

Compounds	Rf*
^{131}I-DMANPER	0.88
$^{131}I^-$	0.06
$^{131}I^+$	0.19

* developed with chloroform.

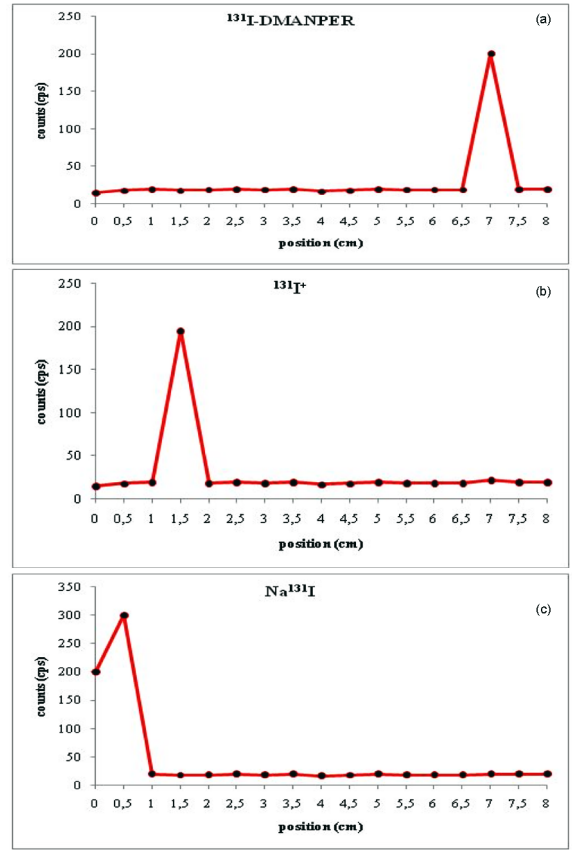

Figure 2. The radiochromatograms of ^{131}I-DMANPER (a), Na^{131}I (b), and $^{131}I^+$ (c) developed with chloroform, respectively.

Statistical analysis

Differences in the mean values of measured activities were evaluated statistically by the SPSS 13 program (Univariate Variance Analyses and Pearson Correlation). Probability values < 0.05 were considered significant. Pearson correlation was carried out between organs for ^{131}I-DMANPER.

RESULTS

Results of TLRC studies

The results of TLRC studies showed that chloroform was the most suitable developing solvent to establish their R_f values given in Table 1. TLRC analysis showed that DMANPER was successfully

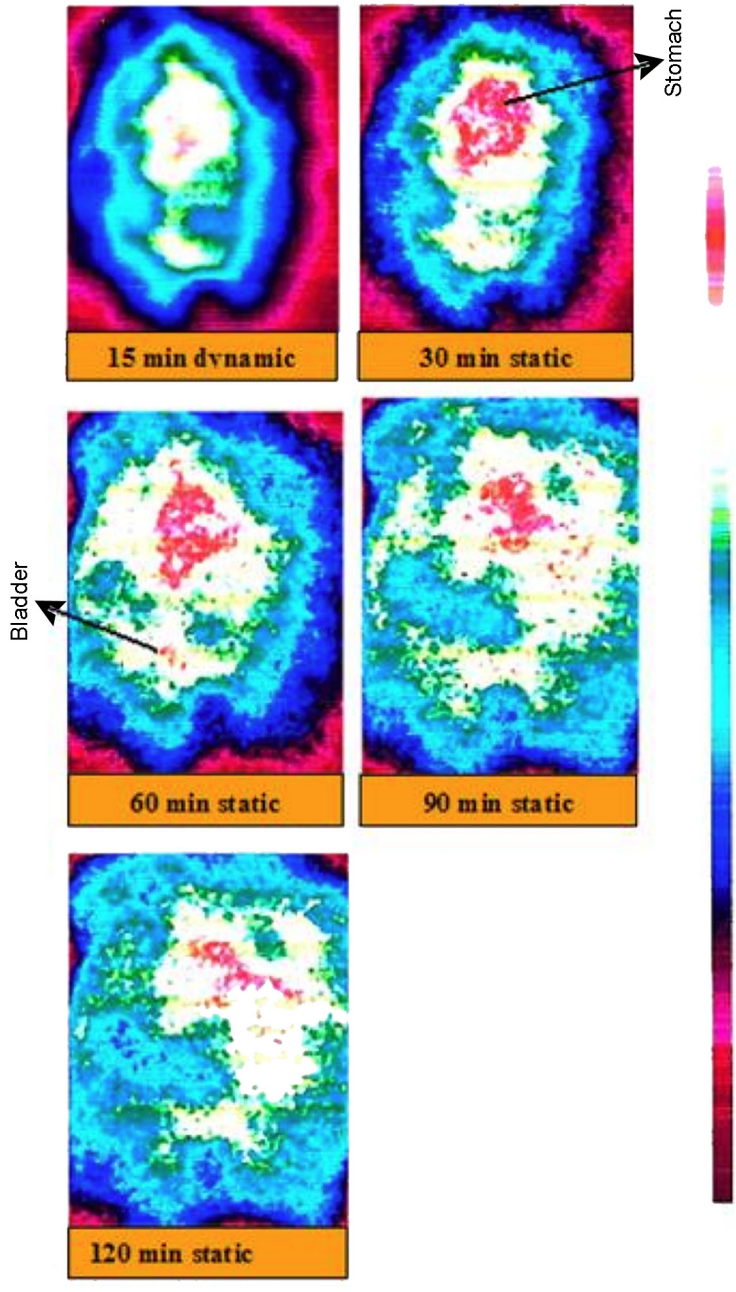

Figure 3. Static scintigrams of ^{131}I-DMANPER which was administered to a rabbit via the ear vein.

radioiodinated with ^{131}I with a yield of practically 100%. This result was in accordance with earlier results obtained in our laboratory on the radioiodination of similar compounds, which have at least one phenyl ring in their chemical structures. Radiochromatograms of ^{131}I-DMANPER and radioactive components were given in Figure 2.

Results of Lipophilicity (Partition Coefficient)

The n-octanol/water partition coefficient (lipophilicity) of ^{131}I-DMANPER was determined and the lipophilicity was found to be 7.89 ± 0.1 (n=3). It was reported that octanol/water partition coefficients of DMANPER and ^{131}I-DMANPER were 8.16 ± 0.99 and 9.44 ± 1.03 according to ACD ACD/ lopP algorithm program, respectively.

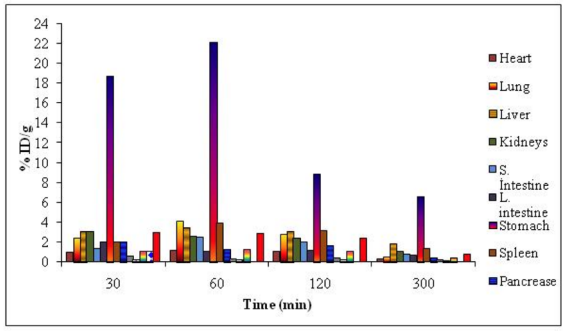

Figure 4. *Ganoderma lucidum* mycelium structure. Black arrow=initial of mycelium; White arrows= septa

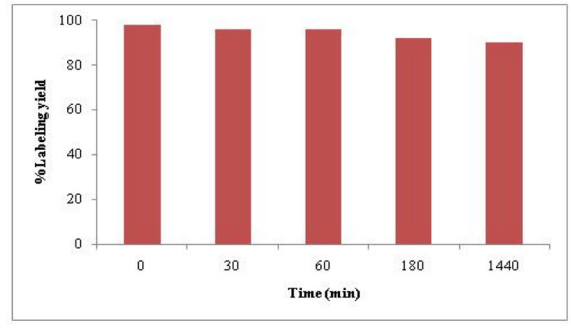

Figure 5. Biodistributional data graphic of ^{131}I-DMANPER in the metabolism of rats.

Results of Stability Studies

Stability in human serum was investigated at time 0, 30, 60, 180, and 1440 min after radiolabeling. The results of the serum stability experiments demonstrated that approximately 90-98% of ^{131}I-DMANPER existed as an intact complex in human serum within 1440 min as seen in Figure 5. The stability of the radioiodinated compound was good enough to allow scintigraphic studies. For this reason, the radioiodinated compound was directly injected to a rabbit without needing for any separation or purification procedure.

Results of scintigraphic studies

The serial static image scans of the rabbit administered with ^{131}I-DMANPER were carried out at different time intervals (30 min, 1 h, 1.5 h, and 2 h). The whole-body images of rabbits after the intravenous administration of ^{131}I-DMANPER are shown in Figure 3. It is seen that ^{131}I-DMANPER was completely accumulated in the stomach in 30 min. After 2 h, the activity was not completely cleared from the stomach. As seen from static scintigrams, no thyroid uptake was observed at the end of 2 h and this has also indicated a high radioiodination yield of DMANPER with ^{131}I. A low activity was seen in the bladder after 1 h following the administration of the compound. On the other hand, it was also observed that the background radioactivity of ^{131}I remained for sufficiently long time in the metabolism (longer than 2 h) and was not cleared rapidly.

Results of biodistributional studies

Biodistributional data for ^{131}I-DMANPER in rats following its intravenous administration were

Table 2. Biodistributional data obtained from ^{131}I-DMANPER in rats (n=3).

	%ID/g of tissue (mean ± SD)			
Organ	30 min	60 min	120 min	300 min
Heart	0.96 ± 0.66	1.10 ± 0.20	1.02 ± 0.08	0.32 ± 0.07
Lung	2.48 ± 0.34	4.16 ± 0.66	2.78 ± 0.29	0.55 ± 0.21
Liver	3.11 ± 0.11	3.44 ± 0.58	3.12 ± 0.11	1.88 ± 0.33
Kidney	3.00 ± 0.14	2.53 ± 0.43	2.38 ± 0.14	1.08 ± 0.64
S. Intestine	1.32 ± 0.77	2.45 ± 0.18	2.00 ± 0.22	0.80 ± 0.15
L. Intestine	1.99 ± 0.56	1.01 ± 0.50	1.10 ± 0.28	0.66 ± 0.09
Stomach	18.76 ± 0.78	22.14 ± 1.11	8.88 ± 0.65	6.57 ± 0.98
Spleen	2.03 ± 0.43	3.90 ± 0.21	3.14 ± 0.78	1.29 ± 0.10
Pancreas	2.11 ± 0.22	1.31 ± 0.32	1.73 ± 0.49	0.47± 0.22
Muscle	0.61 ± 0.18	0.29 ± 0.04	0.39 ± 0.11	0.15 ± 0.03
Brain	0.19 ± 0.02	0.19 ± 0.04	0.66 ± 0.34	0.05 ± 0.01
Fat	1.08 ± 0.06	1.35 ± 0.48	1.11 ± 0.45	0.48 ± 0.02
Blood	2.97 ± 0.27	2.86 ± 0.33	2.39 ± 0.25	0.80 ± 0.21

given in Table 2. Figure 4 shows these data as a graphical representation. A significant amount of activity was seen in the stomach between 30– 60 min. As seen from the Table 2, the stomach activity is as high as about 22.14% of the injected dose per gram tissue at the end of 60 min. This activity was not cleared even after 5 h. These biodistributional results are agreed well with that of scintigraphic results obtained.

Briefly, this study has expressed that DMANPER radioiodinated with ^{131}I or which will be radioiodinated with ^{125}I and some other similar radioiodines has potential applications in cancer research of stomach.

DISCUSSION

Perylene diimides are one of the most widely used organic photosensitive pigments which have shown great photo and thermal stability in the visible region [18]. In the presence of molecular oxygen, they produce the super oxide anion radical (O_2^-) which is known as a highly efficient reactive reagent for oxidation of many organic compounds, especially those containing double-bonds [19-21]. Due to their excellent photosensitive properties, they are expected to be developed as new phototherapeutic medicines [22-24]. Many investigations have demonstrated that perylene diimide derivatives have a strong photodynamic effect on tumors [25,26] and impressive antiviral activity against human immunodeficiency virus type 1 (HIV-1) [27,28]. The data of the biodistribution experiments were seen in Figure 4.

The %ID/g values of the radiolabeled DMANPER in the stomach, the lung, the spleen, and the liver were 22.14 ± 1.11, 4.16 ± 0.66, 3.90 ± 0.21, 3.44 ± 058 at 60 min, respectively. The uptake in these organs decreased through time. In this study, significantly positive correlation was shown in lung-kidney ($r=0.905$, $p < 0.01$), lung-liver ($r=0.970$, $p < 0.07$), kidney-spleen ($r=0.888$, $p < 0.01$), throid-blood ($r=0.958$, $p < 0.02$), stomach-liver ($r=0.942$,$p < 0.04$), blood-kidney ($r=0.950$, $p < 0.02$), and lung-stomach ($r=0.791$, $p < 0.02$) in rat's organs.

As seen in Figure 3, ^{131}I-DMANPER was significantly localized in first 30 minutes in three different centers in the abdominal zone; these were probably the stomach, the kidney and the bladder; but, in 3 hours the activity was mostly concentrated in the thyroid. Scintigraphic results agreed with that of biodistribution results. It is now well established that guanine-rich sequences of DNA can adopt secondary structures quite distinct from typical Watson-Crick duplex DNA [17] . These secondary structures include "frayed wires" and quadruplex DNA. Quadruplexes are of particular interest in cancer biology as they are thought to play a role in the stabilization of telomeric DNA and its binding to the enzyme telomerase, which is active in a number of tumor types. Consequently, agents that target telomeric quadruplexes are thought to be potential antitumor agents [15,16]. More recently, quadruplexes have also been identified in promoters of specific genes, notably c-MYC, and agents that bind quadruplex DNA can affect transcription of genes regulated by these promoters. Although a number of different small molecules are known to interact with quadruplex DNA, the most often molecules which were studied are the derivatives of porphyrin and perylene. The perylene diimide has been shown to promote formation of quadruplex DNA from single strands [11]. Starting from this considerations, Dinçalp et al have carried out a study on spectral properties and G-quadruplex DNA binding selectivity of a series of unsymmetrical perylene diimides such as PYPER, N-(2,6-diisopropylphenyl)-N'-[4-(aminophenyl)]-perylene-3,4,9,10-tetracarboxylic diimide (ANPER), and DMANPER [13].

Both absorption binding and fluorescence quenching experiments of unsymmetrical PDI ligands (PYPER, ANPER, and DMANPER) with different nucleotides have demonstrated that these ligands bind to G-quadruplex DNA. These results were shown that unsymmetrical PDI ligands offer promising molecular architectures for the design of new G-quadruplex DNA-interactive ligands based on PDI structure with therapeutic application potentials in nuclear medicine.

In an earlier study, a perylene derivative (PYPER) was synthesized then radioiodinated with ^{131}I and its radiopharmaceutical potential was examined on a male Albino rabbit and male Albino Wistar rats by Avcıbaşı et al and the results obtained showed that ^{131}I-PYPER might be used as a scintigraphic agent for imaging stomach [14]. The results obtained in

this study also showed that DMANPER is entirely similar to that of PYPER. Thus, it can be concluded that ^{131}I-DMANPER may be an alternative imaging agent like PYPER to be a potential agent for diagnosis and perhaps therapy of stomach cancer. The photodynamic-, chemo-, and radio-therapy would be able to concentrate into the tumor cell to increase damage caused by PDI's.

CONCLUSION

The conclusions of this study were: ^{131}I-DMANPER which has diagnostic and therapeutic application potentials in nuclear medicine was first radioiodinated with this study using the iodogen method. Radiolabeling of DMANPER with ^{131}I means that it can also be radioiodinated with other radioiodine isotopes such as ^{123}I, ^{124}I, ^{125}I under similar conditions. ^{131}I-DMANPER has shown specifity in the stomach, the lung, the spleen, and the liver, so it may be proposed as an imaging agent for the tumors of these organs. At the next step of this study, the selective incorporation of ^{125}I-DMANPER into the stomach, the lung, the spleen, and the liver cancer cells should be examined using tumor bearing laboratory animals or cultured human cancer cell lines.

REFERENCES

1. N.W. Kim, M.A. Piatyszek, K.R. Prowse, C.B. Harley, M.D. West, P.L.C. Ho, Specific association of human telomerase activity with immortal cells and cancer Science, 266 (1994) 2011.
2. J. Lingner, T.R. Hughes, A. Shevchenko, M. Mann, V. Lundblad, Cech TR, Reverse transcriptase motifs in the catalytic subunit of telomerase, Science, 276 (1997) 561.
3. T.M. Nakamura, G.B. Morin, K.B. Chapman, S.L. Weinrich, W.H. Andrews, J. Lingner, Telomerase catalytic subunit homologs from fission yeast and human, Science, 277 (1997) 955.
4. D. Sun, B. Thompson, B.E. Cathers, M. Salazar, S.M. Kerwin, J.O. Trent, Inhibition of human telomerase by a G–quadruplex–interactive compound, J. Med. Chem, 40 (1997) 2113.
5. G. Laughlan, A.I.H. Murchie, D.G. Norman, M.H. Moore, P.C.E. Moody, D.M.J. Lilley, The high–resolution crystal structure of a parallel–stranded guanine tetraplex, Science, 265 (1994) 520.
6. D.E. Gilbert, J. Feigon, Multi–stranded DNA structures, Curr. Opin. Struct. Biol., 9 (1999) 305.
7. H. Arthanari, S. Basu, T.L. Kawano, P.H. Bolton, Fluorescent dyes specific for quadruplex DNA, Nucleic. Acids Res, 26 (1998) 3724.
8. I. Haq, J.O. Trent, B.Z. Chowdhry, T.C. Jenkins, intercalative G–tetraplex stabilization of telomeric DNA by a cationic porphyrin, J. Am. Chem. Soc, 121 (1999) 1768.
9. M.A. Read, S. Neidle, Structural characterization of a guanine–quadruplex ligand complex, Biochemistry, 39 (2000) 13422.
10. S.M. Kerwin, D. Sun, J.T. Kern, A. Rangan, P.W. Thomas, G–quadruplex DNA binding by a series of carbocyanine dyes, Bioorg. Med. Chem. Lett, 11 (2001) 2411.
11. P. Alberti, P. Schmitt, C.H. Nguyen, C. Rivalle, M. Hoarau, D.S. Grierson, Benzoindoloquinolines interact with DNA tetraplexes and inhibit telomerase, Bioorg. Med. Chem. Lett, 12 (2002) 1071.
12. T. Ünak, Potential use of radiolabeled glucuronide prodrugs with auger and/or alpha emitters in combined chemo– and radio–therapy of cancer, Curr. Pharm. Design, 6 (2000) 1127.
13. H. Dinçalp, N. Avcıbaşı, S. Içli, Spectral properties and G–quadruplex DNA binding selectivities of a series of unsymmetrical perylene diimides, J. Photoch. Photobio. ***A***, 185 (2007) 1.
14. U. Avcıbaşı, H. Dinçalp, T. Ünak, Y. Yıldırım, N. Avcıbaşı, Y. Duman, Preliminary tests of the radiopharmaceutical potential of N–(2,6–diisopropylphenyl)–N'–(4–pyridyl)–perylene–3,4,9,10–tetracarboxylic diimide radiolabeled with iodine–131, J. Radioanal. Nucl. Ch, 273(3) (2007) 669.
15. E. Protozanova, R.B. Macgregor, Kinetic footprinting of DNA triplex formation, Anal. Biochem, 243 (1996) 92.
16. W. Guschlbauer, J.F. Chantot, D. Thiele, Four–stranded nucleic acid structures 25 years later: From guanosine gels to telomer DNA, J. Biomol. Struct. Dyn, 8 (1990) 491.
17. R. Samudrala, X. Zhang, R.M. Wadkins, D.L. Mattern, Synthesis of a non–cationic, water–soluble perylenetetracarboxylic diimide and its interactions with G–quadruplex–forming DNA, Anal. Biochem, 15 (2007) 186.
18. L. Ma, H. Tai, C. Li, Y. Zhang, Z. Wang, W.Z. Ji, Photodynamic inhibitory effects of three perylenequinones on human colorectal carcinoma cell line and primate embryonic stem cell line, World J. Gastroenterol., 9(3) (2003) 485.

19. J.S. Ma, F. Yan, C.Q. Wang, J.Y. An, Hypocrellin–A sensitized photooxidation of bilirubin, Photochem. Photobiol., 50 (1989) 827.
20. G.G. Miller, K. Brown, A.M. Ballangrud, O. Barajas, Z. Xiao, J. Tulip, J.W. Lown, J.M. Leithoff, M.J. Allalunis–Turner, R.D. Mehta and R.B. Moore, Preclinical assessment of hypocrellin B and hypocrellin B derivatives as sensitizers for photodynamic therapy of cancer: progress update, Photochem Photobiol, 65 (1997) 714.
21. Y.Y. He, J.Y. An, L.J. Jiang, Glycoconjugated hypocrellin: synthesis of [(b–D–glucosyl)ethylthiyl] hypocrellins and photosensitized generation of singlet oxygen, Biochim. Biophys. Acta, 1472 (1999) 232.
22. Z.Diwu, Novel therapeutic and diagnostic application of hypocrellins and hypericins, Photochem. Photobiol., 61 (1995) 529.
23. C. Schleger, N. Krebsfaenger, A. Kalkuhl, R. Bader, T. Singer, Innovative cell culture methods in drug development, ALTEX, 18 (2001) 8.
24. J. Rohwedel, K. Guan, C. Hegert, A.M. Wobus, Embryonic stem cells as an in vitro model for mutagenicity, cytotoxicity and embryotoxicity studies: present state and future prospects, Toxicol. in vitro, 15 (2001) 741.
25. Z.J. Diwu, R.P. Haugland, J.X. Liu, J.W. Lown, G.G. Miller, R.B. Moore, K. Brown, J. Tulip and M.S. McPhee, Photosensitization by anticancer agents 21: New perylene– and aminonaphthoquinones, Free Radic Biol Med, 20 (1996) 589.
26. Z.J. Wang, Y.Y. He, C.G. Huang, J.S. Huang, Y.C. Huang, J.Y. An, Y. Gu, L.J. Jiang, Pharmacokinetics, tissue distribution and photodynamic therapy efficacy of liposomal–delivered hypocrellin A, a potential photosensitizer for tumor therapy, Photochem. Photobiol., 70 (1999) 773.
27. J. Hirayama, K. Ikebuchi, H. Abe, K.W. Kwon, Y. Ohnishi, M. Horiuchi, M. Shinagawa, K. Ikuta, N. Kamo and S. Sekiguchi, Photoinactivation of virus infectivity by hypocrellin A, Photochem. Photobiol., 66 (1997) 697.
28. J.B. Hudson, J. Zhou, J. Chen, L. Harris, L. Yip, G.H. Towers, Hypocrellin, from Hypocrella bambuase, is phototoxic to human immunodeficiency virus, Photochem. Photobiol., 60 (1994) 253.