IJABR

International Journal of Advanced Biotechnology and Research (IJABR)
ISSN 0976-2612, Online ISSN 2278–599X,
Vol-10, Issue-2, 2019, pp512-517
http://www.bipublication.com

Research Article

Anticancer Activity in Breast Cancer CellsThrough Inhibition of DNA Cleavage, Using PHCUTR. Intermolecular Aromatic Ring Stacking of PHCUTR with DNA and Its Relation to Structure Stability

S. A. A. Sajadi

Sharif University of Technology,
P.O.Box 11155-8639, Tehran, Iran.
sajadi@sharif.ac.ir

[Received: 25/11/2018; Accepted: 19/03/2019; Published: 23/05/2019]

ABASTACT

The small complex PHCUTR (a derivative of heteroaromatic amino acid complexes), displays activity against cancer cell lines. In this study (in vitro assay), we investigated the anticancer mechanism underlying these drug activities in breast cancer cell lines. PHCUTR caused a dose-dependent decrease of both anchorage dependent and independent growth of breast cancer cells (MCF-7).In this study, we investigated the anti-proliferativepotential of PHCUTR in breast cancer cell lines and alsoclarified the mechanism of action of PHCUTR in preclinical modelsof breast cancer. PHCUTR showed a significant efficacy in killingtumor cells without causing any noticeable effect on normal breast cells. From the above results, we can conclusively state that PHCUTR has anti-breast cancer potentiality. PHCUTR induces DNAdamage and blocks the cells in S phase.Based on our previous work regarding to intramolecular interactions so called “stacking” there are significant interactions respective binding between purine moiety of nucleic base and heteroaromatic part of PHCUTR. The last plays a role of the lock between DNA strands.
Stabilization due to the intermolecular noncovalent bonding between the side chains of DNA nucleobases with 1,10-phenanthrolin- copper (II)- tryptophan complex (PHCUTR) has been evaluated from the following equilibrium:
DNA + PHCUTR $\rightleftharpoons$ DNA– PHCUTR , which lies predominantly on the right site.

KEYWORDS: PHCUTR, Anticancer Activity, Breast Cancer Cell, DNA damage

1. INTRODUCTION

Breast cancer is the major contributors to cancer-related deaths in women and despite the advances in early detection and the understanding of the molecular basis of breast cancer biology, about 30% of patients with early-stage breast cancer have recurrent disease. To offer more effective and less toxic treatments, selecting therapies including natural agents have drawn great attention of the scientific community and the general public for their ability to suppress cancers [1-3]. Some compounds such as CA (cyclophosphamide, doxorubicin) are commonly used combination regimen for advanced breast cancer [4]. Thus, topoisomerase II-inhibiting antineoplastic agents, which induce the topoisomerase II-DNA cleavable complex, are among the most effective antitumor drugs currently available for the treatment of human cancers and are key drugs in combination chemotherapy.

Noncovalent interactions are vital in the processes of biological recognition of molecules and

subsequent specific reactions [5a]. The nucleic base-base, hormone-receptor, enzyme-substrate, and antigen-antibody interactions are among the most important in biological systems, and many of them are achieved by the specific sid-chain groups of proteins involved[5b]. The receptor site has been reported to contain an imidazole group [5c] which prompted us to investigate the interaction between the tyrosine phenol and the histidine imidazole moiety in ternary copper (II) complexes with the expection that optioid peptides may be bound to the receptor through the interation with its histidine and other amino acid residues [5d].

2.MATERIAL AND METHODS

2.1.Maintenance and treatment of cell lines: the human breast cancer cell lines MCF-7 were cultured in monolayers and maintained in DMEM (Dulbecco's modified eagle medium) supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin–streptomycin and 1.5 mM L-glutamine. A control cell line (MCF-10A), which spontaneously arose from culturing MCF-10M cells, was derived from a 35-year-old parous premenopausal woman with extensive fibrocystic disease, with no family history or histological evidence of breast malignancy. MCF-10A cells are estrogen receptor-negative and have characteristics of normal cells including growth factor dependency, anchorage-dependent growth and lack of tumorigenecity in nudemice. Therefore, these cells are considered as a model fornormal breast epithelial cells [6]. MCF-10A cells were grown inDMEM/F-12, medium supplemented with 5% (v/v) horse serum,100 U/ml of penicillin, 100 mg/ml of streptomycin,0.5 mg/ml of hydrocortisone, 100 ng/ml of cholera toxin, 10mg/ml of insulin, 10 ng/ml of epidermal growth factor and1% (w/v) of L-glutamine (Sigma Co.). Allthe other cell-culture requirements were procured from HIMEDIA, India. All cells were grown in a humidifiedatmosphere of 5% CO_2 at 37°C. After 70–80% confluence,cells were treated with indicated concentrations of PHCUTR (prepared in this laboratory) and dissolved in sterile DMSO. Treatments were done for various time intervals with the addition offresh PHCUTR as shown in respective places.

2.2.Clonogenic assay

Clonogenic assay was performed to screen the survival andproliferation of cells after treatment with PHCUTR . For this, ~500cells of MCF-7 and MCF-10A (control)were seeded in 12-well plates and grown for 24 hr and thentreated with different concentrations of PHCUTR (0.5–10 μM) fornext 24 hr. Thereafter, medium was replaced with fresh medium, and plate was returned to the incubator for five to sixdoublings. After colony formation, medium was removed andplate was air dried and stained with 0.2% crystal violet. Then,the wells were washed twice with distilled water, and colonieswere counted using gel documentation system (UVP,Germany).

2.3.MTT assay

To check the anchorage-dependent cell growth, a colorimetricassay [3-(4,5-dimethylthiazol-2yl-)-2,5-diphenyl tetrazoliumbromide] (MTT) was carried out. MTT assay is based on theprinciple that the viable cell number is directly proportionalto the purple formazan color of the reduced MTT dye, whichcan be quantitatively measured by spectrophotometer. In 96-well flat-bottomed tissue-culture plates, ~8,000–10,000 cellsof MCF-7 and MCF-10A (control) wereseeded in triplicates and incubated for 24 hr and then treatedwith PHCUTR at a wide concentration range from 2 to 200 μM.

After 24 hr of treatment, cells were washed with PBS, andMTT (Sigma) solution was added at a concentration of0.05 μg/μl diluted in PBS. Cells were incubated at 37°C for4 hr to allow the formation of purple formazan crystals dueto mitochondrial dehydrogenase activity. About 100 μl of detergentsolution (10% NP-40 with 4 mM HCl in isopropanol)was added to each well and incubated for 30 min at 37°C.The color intensity was then measured spectrophotometricallyat 570 nm using a microplate reader (Multimode ELISAreader). All theexperiments were done in

triplicates in at least three culturesfrom each cell line, and the data were represented.

3. RESULTS

PHCUTR reduces the cell migration of MCF-7 cells.Figure 1 demonstrates the migration and proliferation ratesof MCF-7 cells (wound-healing assay) after exposure withPHCUTR. After making a wound, cells were treated with varied concentrations of PHCUTR , and then the wound was inspected microscopically over time as the cells migrated to fill the damaged area. For the untreated cells, migration occurred, and the wound was healed in 24 hr. However, cell migration was inhibited in treated cells in a dose-dependent manner. Ata concentration of 5 μM PHCUTR, wound healing was slowed, while at 10μM, PHCUTR migration was inhibited. Higher doses of PHCUTR caused widespread apparent cell death, which suggested that apoptosis was occurring (Fig. 1).

4.DISCUSSION

Breast cancer is the most common invasive cancer in females worldwide. It accounts for 16% of all female cancers and 22.9% of invasive cancers in women. Breast cancer rates are much higher in developed nations compared to developing ones. There are several reasons for this, with possibly life-expectancy being one of the key factors - breast cancer is more common in elderly women; women in the richest countries live much longer than those in the poorest nations [7].To offer more effective and lesstoxic treatments, selecting therapies including natural agentshave drawn great attention of the scientific community andthe general public for their ability to suppress cancers. Similarly, the known bioactivecompoundswere recently shown to possess anticancer activityin pancreatic cancer and colorectal cancer [8,9].In this study, we investigated the anti-proliferativepotential of PHCUTR in breast cancer cell lines and alsoclarified the mechanism of action of PHCUTR in preclinical modelsof breast cancer. PHCUTR [10] showed a significant efficacy in killingtumor cells without causing any noticeable effect onnormal breast cells (MCF-10A) (Fig. 1). PHCUTR also causedbreast cancer cell death in an anchorage-independent mannerwhen plated on soft agar (Fig. 2). Taken together,we can conclude that PHCUTR has anticancer activity inbreast cancer cell lines. Further detailed biochemical investigations of PHCUTR against breast cancer are required that will help to improve its chemotherapeutic potential against breast cancer. The optimal dozes for PHCUTRwas found1-10 μM.

In view of the importance of the hydrophobic interaction involving a heteroaromatic moiety, quanitative evaluation of the extent of such interactions would offer a key to the mechanism of the activity and similar biological phenomena.An efficient approach to this goal is the thermodynamic stabilization and spectral behavior due to noncovalent bonds within ternary metal complexes, where the central metal ion may serve as a positive center, and a template mimicking the three dimensional environment at the receptor site.

Ligand-ligand interactions in ternary complexes as biological models have been studied for amino acids and nucleotides by varous methods. It is revealed the evidence of electrostatic interactions between the charged side chains of a protonated basic amino acid and an acidic amino acid both coordinated to Cu(II). We suggest a mechanism such as intercalation for inhibition of DNA replication of cancer cells presented in Fig. 1. In figures 3 and 4 are shown the schematic structure of DNA- PHCUTR complex. The complex of PHCUTR act as an intercalator.

In biochemistry, intercalation is the insertion of molecules between the planar bases of DNA. This process is used as a method for analyzing DNA and it is also the basis of certain kinds of poisoning.There are several ways molecules (in this case, also known as ligands) can interact with DNA. Ligands may interact with DNA by covalently binding, electrostatically binding, or intercalating [11]. Intercalation occurs when

ligands of an appropriate size and chemical nature fit themselves in between base pairs of DNA. These ligands are mostly polycyclic, aromatic, and planar, and therefore often make good nucleic acid stains.These structural modifications can lead to functional changes, often to the inhibition of transcription and replication and DNA repair processes, which makes intercalators potent mutagens. Intercalation as a mechanism of interaction between cationic, planar, polycyclic aromatic systems of the correct size (on the order of a base pair) was first proposed by Leonard Lerman in 1961 [11]. One proposed mechanism of intercalation is as follows: In aqueous isotonic solution, the cationic intercalator is attracted electrostatically to the surface of the polyanionic DNA.

From this position, the ligand diffuses along the surface of the DNA and may slide into the hydrophobic environment found between two base pairs that may transiently "open" to form an intercalation site, allowing the ethidium to move away from the hydrophilic (aqueous) environment surrounding the DNA and into the intercalation site. The base pairs transiently form such openings due to energy absorbed during collisions with solvent molecules.

As shown in Figures 3 and 4 the complex of PHCUTR lies between the nucleobases. The points between them present the hydrophobic interaction between the heteroaromatic rings, which increases the stability of DNA-PHCUTR complex.This leads to the agglomeration of cancer cells (see Fig.1).

ACKNOWLEDGMENT

I thank Iran national Science foundation for financial support.

5.REFERENCES

1. Sigel, R. K.O.; Song, B.; Sigel, H.; Stabilities and Structures of Metal Ion Complexes of Adenosine 5‘-*O*-Thiomonophosphate ($AMPS^{2-}$) in Comparison with Those of Its Parent Nucleotide (AMP^{2-}) in Aqueous Solution, *J. Am. Chem. Soc*., 119, pp. 744-755, (1997).
2. Tabassum, S.; Zaki, M.; Afzal, M.; Arjmand,F.;New modulated design and synthesis of quercetin–$Cu^{II}/Zn^{II}–Sn_2^{IV}$ scaffold as anticancer agents: *in vitro* DNA binding profile, DNA cleavage pathway and Topo-I activity, *Dalton Trans.*, 42**,**pp. 10029-10041, (2013).
3. Tyagi, N.; Chakraborty, A.; Singh, U.P.; Roy, P.; Ghosh, K.;Mononuclear iron(III) complexes of tridentate ligands with efficient nuclease activity and studies of their cytotoxicity, *Org. Biomol. Chem.*, 13**,**pp. 11445-11458, (2015).
4. Preet, R.;Mohapatra, P.;Mohanty, S.;Sahu, S.K.;Choudhuri T, Wyatt MD, Kundu CN,Quinacrine has anticancer activity in breast cancer cells through inhibition of topoisomerase activity, *Int. J. Cancer.*,130(7): pp.1660-70, (2012).
5. Tranum, B. L.; McDonald, B.; Thigpen, T.; Vaughn, C.; Wilson, H.; Maloney, T.; Costanzi, J.; Bickers, J.; el Mawli, N. G.; Palmer, R.; Hoogstraten, B.; Heilburn, L.; Rasmusen, S.; Adriamycin combinations in advanced breast cancer. A Southwest Oncology Group Study.Cancer (Phila.), 49, pp. 835 –839, (1982).
6. a) Frieden, E; J. Chem. Edu. 1975;**52**:754-761. b) Barker, R., Organic Chemistry of Biological Compounds, Prentice Hall, 1971. c) Roy, B., Biochem. Biophys. Res. Commun., 1982; **109**: 518-526. d) Yamauchi, O., *Inorg. Chim. Acta*, 100, pp. 165-172, (1985).
7. Soule, H.D.; Maloney, T.M.; Wolman, S.R.;Peterson, W.D.; Jr, Brenz, R.; McGrath, C.M.;Russo J, Pauley RJ, Jones RF, Brooks SC.Isolation and characterization of aspontaneously immortalized human breastepithelial cell line, MCF-10. *Cancer Res*,50, pp. 6075–86, (1990).
8. Gonzalez-Angulo, A.M.; Morales-Vasquez, F.;Hortobagyi, G.N.; Overview of resistance

tosystemic therapy in patients with breastcancer. *Adv Exp Med Biol*, 608, pp. 1–22, (2007).
9. Goodell, J.R.; Ougolkov, A.V.; Hiasa, H.; Kaur,H.; Remmel, R.; Billadeau, D.D.; Ferguson,D.M.; Acridine-based agents with topoisomerase II activity inhibitpancreatic cancer cell proliferation andinduce apoptosis. *J Med Chem*, 51, pp. 179–82, (2008).
10. Jani, T.S.; DeVecchio, J.; Mazumdar, T.;Agyeman, A.; Houghton, J.A.; Inhibition ofNF-jB signaling by quinacrine is cytotoxicto human colon carcinoma cell lines (cc)and is synergistic in combination withTRAIL or oxaliplatin. *J Biol Chem*,285, pp. 162–72, (2010).
11. Sajadi, S.A.A.;Equilibria and Stability in Glycine, Tartrate and Tryptophan Complexes, Investigation on Interactions in Cu(II) Binary and Ternary Systems in Aqueous Solution, *Open Journal of Inorganic Non-Metallic Materials*, 4, pp. 1-6, (2014).
12. a) Richards, A.D.; Rodgers, A.; "Synthetic metallomolecules as agents for the control of DNA structure". *Chemical Society Reviews*. 36 (3),pp. 471–83. *doi*:*10.1039/b609495c*, (2007). *PMID* *17325786*.
13. b) Lerman, L.S.;Structural considerations in the interaction of DNA and acridines. *Journal of Molecular Biology*.3(1): pp. 18–30, (1961). *doi*:10.1016/S0022-2836(61)80004-1. PMID 13761054.
14. c) Luzzati, V.; Masson, F.; Lerman, L. S.; "Interaction of DNA and proflavine: A small-angle x-ray scattering study". *Journal of Molecular Biology*. 3(5),pp. 634–9, (1961). *doi*:10.1016/S0022-2836(61)80026-0. PMID 14467543.
15. *d)* Lerman, L.S.;The structure of the DNA-acridine complex. Proceedings of the National Academy of Sciences of the United States of America. 49(1), pp. 94–102, (1963). *doi*:10.1073/pnas.49.1.94. *PMC* *300634*. PMID 13929834.

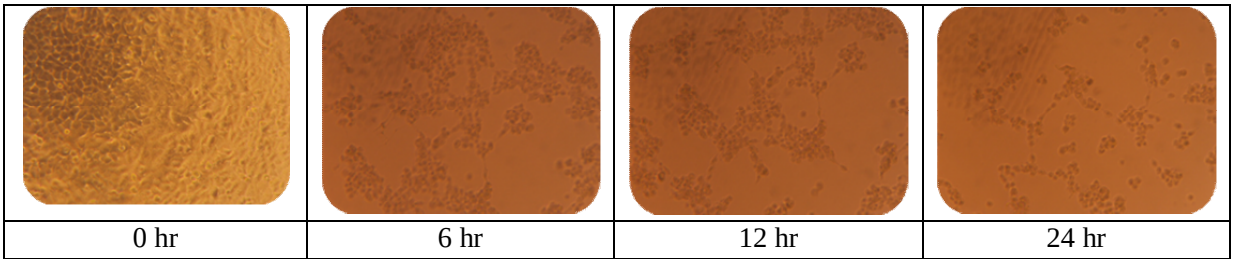

Figure 1. Effect of PHCUTR on cell proliferation of breast cancer cell line (MCF-7). Cells were seeded in six well plates for 24 h. After the cells were reached to 90 % confluence, a wound was made by scratching with 100 µl micro tip at an angle of 30°, and then cells were treated with different concentrations of PHCUTR. Photographs were taken at intervals of 0, 6, 12 and 24 hr after treatment to check the healing of wound at 40× magnification (scale bar is 50 µm). Photographs presented here are representative of four independent experiments.

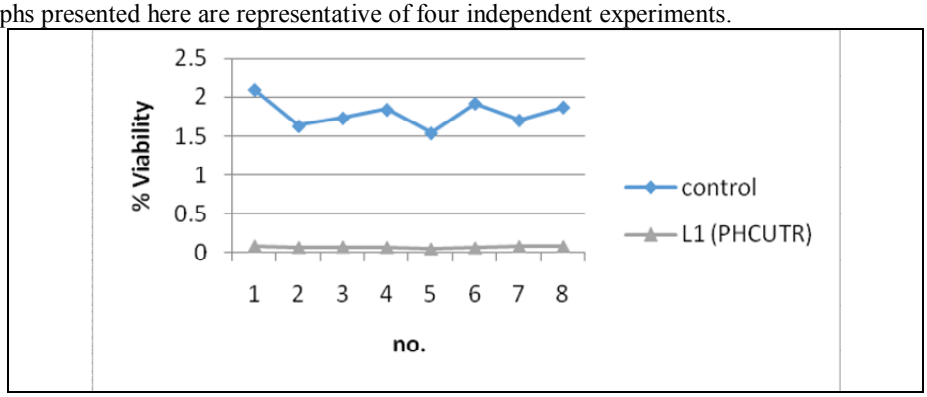

Figure 2. Anchorage-dependent cell viability of breast cancer (MCF-7) cell lines and MCF-10A (control) after treatment with PHCUTR. Cells were plated in each well (~5,000–6,000 cells/well) of the 96-well tissue-culture plates,

after 70% confluency were treated with PHCUTR for 24 hr. The plates were harvested by adding MTT reagent and the developed purple color crystals were solubilized using detergent and finally color intensity was measured by spectrophotometer at 570 nm in microplate reader. Data were plotted as percent viability (% control).

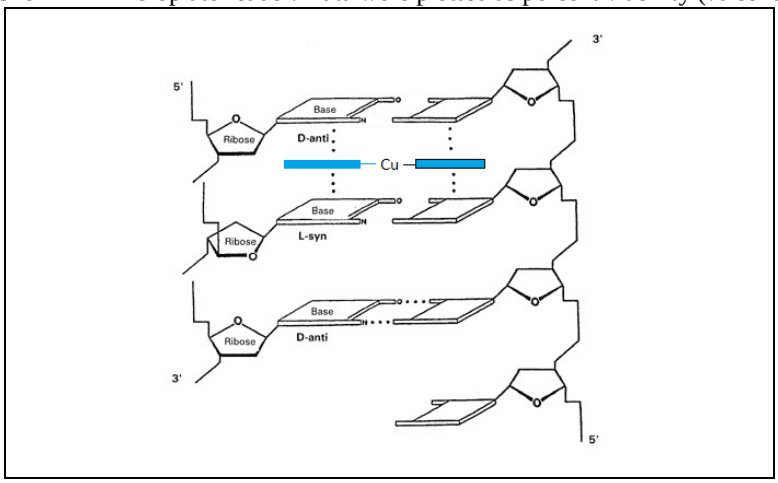

Figure 3: shematic structure of DNA-PHCUTR complex

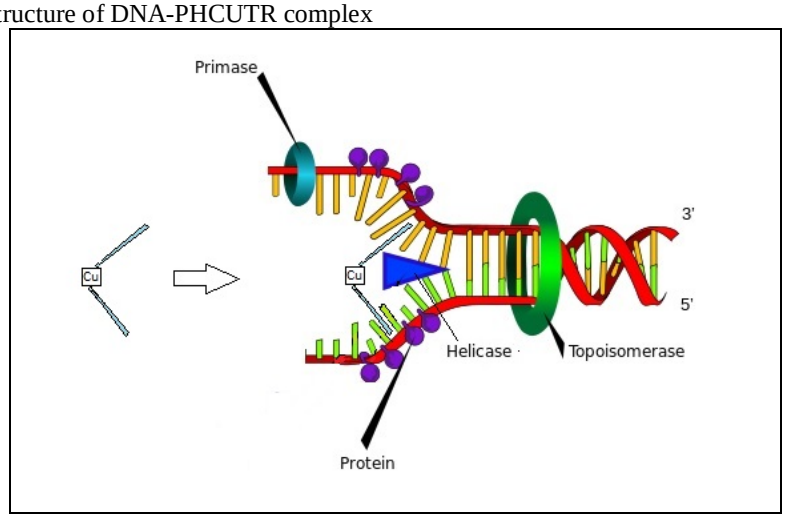

Figure 4: shematic structure of DNA-PHCUTR complex