ISSN: 2395-0536 Impact Factor- 2.95*

INTERNATIONAL JOURNAL OF PHARMACEUTICAL RESEARCH AND NOVEL SCIENCES

IJPRNS

EVALUATION OF CYTOTOXIC ACTIVITY OF THE CUCURBITACIN DERIVATIVE IN PROSTATE CANCER CELL LINES

A.Sravya, R.Pavan Kumar, K.Hima Bindu, T.Praveen Kumar, V.V.Rama Satya, K.Vidya, Y.Ganga Sirisha, K.Nagalakshmi

Department of Pharmacology, JITS College of Pharmacy, Kalagampudi, Palakol, Andhra Pradesh,, India.

ABSTRACT

Aim is to study DNA methyl transferase inhibitory activity of cucurbitacin to treat prostate cancer by using PC cell lines. evaluation of cytotoxity of CuE in prostate cancer cell lines LNCaP (AR +ve) and PC3 (AR -ve) reveals that CuE is more selective toward AR negative cell line (PC3) than AR positive cell line (LNCaP).

Key Words: cucurbitacin, DNA methyl transferase inhibitory

Author for correspondence
A.Sravya,
Department of Pharmacology,
JITS College of Pharmacy, Kalagampudi,
Andhra Pradesh, India.
Email: sravya.allu27@gmail.com

INTRODUCTION

Prostate cancer is the most frequently diagnosed malignancy and a leading cause of cancer related deaths among men in North America. Prostate gland is a small walnut shaped gland found in men. It sits just below the bladder and surrounds the urethra that drains the bladder through the penis. Seminal fluid produced by the prostate gland which nourishes and transport sperm. Initially prostate cancer grows slowly and remains confined to the prostate gland, and other types are aggressive and spread easily. The occurrence of prostate cancer is high in developed countries and recorded lowest in central Asian countries. Risk factors associated for prostate cancer are multiple which include increasing age, race, ethnicity, family history, environmental pollution, diet, obesity, smoking, frequent sex and sexually transmitted diseases. Prostate cancer is the second most common cause of cancer deaths in men in most developed countries, and the incidence has increased significantly over recent years. In the United States the lifetime probability of developing prostate cancer is one in six. In 1997 more than 209 900 American men were diagnosed with prostate cancer and more than 41 800 died from the disease. In England and Wales death rates have trebled over the last 30 years, one in 13 men is affected, and 20 000 cases are diagnosed each year. Age is the most important risk factor. Prostate cancer is rare under the age of 40, and its incidence increases exponentially with age. There is a varied geographical incidence. The age standardised mortality rates vary from 0.1 per 100 000 in Thailand to 30 per 100 000 in some parts of the West Indies. Studies of migrant populations have suggested that environmental factors are at least as significant as race. Environmental factors implicated in prostate cancer include a high intake of saturated fat and low level of dietary selenium, vitamin E, and vitamin D. Radiation exposure may be important. In an analysis of deaths among 39 546 employees of the United Kingdom Atomic Energy Authority the only

malignancy clearly related to radiation exposure was prostate cancer. It is estimated that less than 5% of all prostate cancer is hereditary. The risk of prostate cancer is increased by a factor of 1.3 if there is an affected father in the family, and by a factor of 2.5 if there is a brother who has prostate cancer. Prostate cancer is thought to arise after a sequence of at least eight genetic mutational events. Early events appear to be the loss of tumour suppressive genes such as p53 which is mutated in up to 64% of tumours and p21 in up to 55%. The recently identified p73 tumour suppressor gene has significant homology to p53 and also appears to be mutated in prostate cancer. MMAC1/p10, however, is the most widely mutated tumour suppressor gene in prostate cancer and may contribute to the acquisition of the metastatic phenotype. The development of the hormone refractory phenotype appears to be related to the over expression of mutant p53 and bcl-2 family of proteins as well as amplification of the androgen receptor. Approximately 95% of all prostate cancers are adenocarcinomas. Roughly 4% of all prostate malignancies arise from the transitional epithelium of the urethra or ducts as transitional cell carcinoma. Primary carcinoid tumours of the prostate, sarcomas, and primary small cell carcinomas of the prostate are rare. Tumours of other organs may spread into the prostate. Histological recognition of prostate cancer depends on the overall assessment of the architecture and upon the cytology of individual cells. The prostate cancer cell cytoplasm may contain large amounts of acid phosphatase and prostate specific antigen (PSA). Using immunohistochemistry for these antigens it is possible to differentiate prostatic carcinoma cells from other tumour cells (1-3).

MATERIALS AND METHODS

In-silico screening

Molecular Modelling

From the PubChem compound database the structure of cucurbitacin derivatives are downloaded. And are prepared for docking using LigPrep (Ligprep, Schrödinger). LigPrep used to convert 2D structure to 3D representation. From each input structure, LigPrep can produce a number of structures with various ionization states, tautomers, stereochemistries, and ring conformations, and eliminate molecules using various criteria including molecular weight or specified numbers and types of functional groups present. The structures were subsequently optimized by means of OPLS-2005 using a default setting in LigPrep.

Protein preparation

Proteins used in the study are downloaded from the RCSB protein data bank (PDB). By using Maestro software (Maestro, Schrödinger) the structure of protein are prepared and aligned using the protein structure alignment module in Prime (Prime, Schrödinger). Hydrogen were added to all atoms in the system. Removal of water molecules for crystallization was done from the complex except in the active site. Using the protein preparation module a brief relaxation was performed on structure in Maestro with the "Refinement only" option. This two – part procedure that consisting of optimizing hydroxyl and thiol torsion in the first stage which is followed by an all-atom constrained minimization carried out with the impact refinement module (Impref) using the OPLS-2005 force field to alleviate steric clashes that may exist in the original PDB structures. When the Root Mean Square Deviation (RMSD) reached a maximum cutoff of 0.30 Å then the minimization was terminated.

Grid generation and ligand docking

Grids were defined by centering grid on the ligand in the crystal structure using default box size setting in Glide. Hydrogen bond constraints were not applied. Then the prepared ligands were docked against the target proteins. For searching possible locations of the ligand in the active site region of the receptor Glide uses a hierarchical series of filter. In the initial filters test, examined the spatial fit of the ligand to the defined active site and the ligand-receptor interactions using a grid-based method. It involves evaluation and minimization of a grid approximation to the OPLS-AA non-bonded ligand-receptor interaction energies. Based on the energy-minimized poses the final scoring is carried out. The minimized poses are restored using Schrödinger's proprietary Glide score (G score) scoring function. G score is the modified version of ChemScore, but it includes a steric-clash

term and adds buried polar terms devised by Schrödinger to penalize electrostatic mismatches.

G Score = (a x vdW) + (b x Coul) + Lipo + Hbond + Metal + BuryP + RotB + Site

Where, vdW – van der Waal energy, Coul – Coulomb energy, Lipo – Lipophilic contact term, Hbond – Hydrogen bond, Metal – Metal binding term, BuryP – penalty for buried polar groups, RotB – penalty for freezing rotatable bonds, Site – polar interaction of the active site.

Cytotoxicity assay

Approximately 3500-4000 cells/well were added in 96 well plate from well grown culture, the viability is tested using trypan blue dye with the help of haemocytometer. It is based on the principle that live cells possess intact cell membranes that exclude trypan blue. The cytotoxic activity is measured by adding standard and test compounds (0.002 to 2µM). After 24 hours, fresh medium containing the drug replaces the old medium and incubated for another 24 hour. At the end of 48th hr 10µl of 3-[4, 5-dimethyl thiazol-2-yl] 2, 5- diphenyl tetra-zolium bromide (MTT) is added and the plates were incubated for an additional 4 hour. The formazon crystals were dissolved in 100µl of DMSO/well. The optical density was measured at 570nm. (Sanchez *et al.*, 2006). By plotting dose response curve the IC50 value will be calculated. The cell viability can be calculated using the formula (4-6),

% Inhibition = ((O.D of control cells - O.D of test cell) / O.D of control cell) X 100

RESULTS AND DISCUSSION

Cucurbitacin derivatives were evaluated for their binding affinity with DNMT3A-DNMT3L (PDB ID: 2QRV), DNMT1 (PDB ID: 3PT6, 3PTA, 3SWR) and DNMT3B (PDB ID: 3QKJ). Cucurbitacins had binding affinity with DNMT1, DNMT3A and DNMT3B. Cucurbitacin S and U had no binding affinity with DNMT1 (PDB ID: 3SWR). Among all these proteins which are studied, cucurbitacin exhibited better binding affinity with DNMT1 (PDB ID: 3PTA). In DNMT1 protein receptor, cucurbitacins had similar and more binding affinity as of decitabine (G score -6.546) (Table-1). Cucurbitacin derivatives were further evaluated for their ADME and toxicity properties using QikProp 4.0, Schrodinger software. Cucurbitacin derivatives G, A, B, H, K, J and P showed deviation from the prescribed range of ADME properties (Table-5 &6). Among the cucurbitacins, cucurbitacin E is within the range of the required properties. Based upon binding affinity and predicted ADME properties, we have selected cucurbitacin E as a lead compound and used for the further studies. The standard drug decitabine forms H-bond interactions with amino acids ASN 1578, ASH 700 and ASN 1267. Similarly CuE had H-bond interaction with amino acid MET 696, GLU 698, GLN 1227.

Table-1 Docking of cucurbitacin derivatives with various isoforms of DNMT

COMPOUND	DOCKING SCORE	COMPOUND	DOCKING SCORE	COMPOUND	DOCKING SCORE
DNMT3A-DNMT3L (2QRV)	DNMT1(3PT6)	DNMT3A-DNMT3L (2QRV)	DNMT1(3PT6)	DNMT3A-DNMT3L (2QRV)	DNMT1(3PT6)
CU-A	-3.793	CU-A	-3.793	CU-A	-3.793
CU-B	-0.099	CU-B	-0.099	CU-B	-0.099
CU-C	-3.49	CU-C	-3.49	CU-C	-3.49
CU-D	-3.776	CU-D	-3.776	CU-D	-3.776
CU-E	-4.025	CU-E	-4.025	CU-E	-4.025
CU-F	-3.92	CU-F	-3.92	CU-F	-3.92
CU-G	-5.477	-7.074	-9.476	-5.577	-9.074
CU-H	-3.71	-5.534	-7.769	-4.884	-6.362
CU-I	-3.834	-5.103	-7.462	-4.848	-5.306

CU-J	-3.681	-5.803	-7.265	-5.221	-6.522
CU-K	-3.92	-6.075	-7.265	-5.963	-7.516
CU-L	-3.302	-5.732	-6.976	-4.654	-2.046
CU-O	-3.806	-4.783	-7.602	-5.04	-5.617
CU-P	-3.143	-5.099	-6.797	-4.564	-6.123
CU-Q	-4.097	-5.063	-7.519	-5.352	-6.627
CU-R	-3.314	-5.642	-6.492	-4.773	-6.854
CU-S	-3.329	-4.653	-6.853	-4.716	----
CU-U	-3.554	-6.258	-6.407	-4.607	----
ZEBULARINE	-7.306	-6.194	-7.277	-5.048	----
DECITABINE	-6.492	-5.802	-6.546	-5.509	----
5-AZACYTIDINE	-6.008	-6.875	-5.964	-6.229	----

The test and standard compounds were evaluated in PC3 (AR-) and LNCaP (AR +) cell lines. The test and standard compounds were initially evaluated for their cytotoxicity from 0.002µM to 2µM. CuE exhibits 42% cytotoxic effect at the tested dose (2µM) on PC3 cell lines. CuE inhibited the proliferation of PC3 cells in a dose dependent manner. The IC50 value of CuE in PC3 cell line was found to be 0.028µM. Zebularine exhibits 45.9% cytotoxic effect at 200µM concentration. CuE exhibits 27.73% cytotoxic effect at the tested dose (2µM) on LNCaP cell line. CuE inhibited the proliferation of LNCaP cells in a dose dependent manner. The IC50 value of CuE in LNCaP cell line was found to be 0.0123µM. Zebularine exhibits 10% cytotoxic effect at 2µM concentration. The results indicate that the CuE was more cytotoxic towards both LNCaP & PC3 cell lines than the standard drug zebularine (Table-2 and 3). CuE was found to be 2.26 times selective towards the AR negative cell line than the AR positive cell line.

Table-2 % Inhibition of Cucurbitacin E in PC3 cells

Conc (µM)	**% Inhibition**	**% Cell Viability**
Control	--------	100
Cu-E 0.002	20.40	79.60
Cu-E 0.02	28.37	71.63
Cu-E 0.2	41.93	58.07
Cu-E 2	42.88	57.12
Zebularine 200	45.9	54.1

Table-3 % Inhibition of cucurbitacin E in LNCaP cells

Conc (µM)	**% Inhibition**	**% Cell Viability**
Control	--------	100
Cu-E 0.002	NA	NA
Cu-E 0.02	19.33	80.67
Cu-E 0.2	21.01	78.99
Cu-E 2	27.73	72.27
Zebularine 2	10.08	89.92

CONCLUSION

The present study identified that the CuE had a better binding affinity towards DNMTs and had H-bond interaction with DNMT1. CuE satisfies the ADME and toxicity parameter studies. Further evaluation of cytotoxity of CuE in prostate cancer cell lines LNCaP (AR +ve) and PC3 (AR -ve) reveals that CuE is more selective toward AR negative cell line (PC3) than AR positive cell line (LNCaP). In addition CuE inhibited DNMT1 and DNMT3B enzyme. Moreover CuE is more cytotoxic towards PC3 and LNCaP cells than standard zebularine.

REFERENCES

1. Anne-Marie Arel-Dubeau, Fanny Longpré, Julie Bournival, Cindy Tremblay, Julie Demers-Lamarche, Pavlina Haskova, Everaldo Attard, Marc Germain, and Maria-Grazia Martinoli. Cucurbitacin E Has Neuroprotective Properties and Autophagic Modulating Activities on Dopaminergic Neurons. *Oxid Med Cell Longev*. 2014 Dec 9: 425496.
2. Balkema-Boomstra AG, Zijlstra S, Verstappen FW, Inggamer H, Mercke PE, Jongsma MA, et al. Role of Cucurbitacin c in resistance to spider mite (Tetranychus urticae) in Cucumber (Cucumis sativus L.) *J Chem Ecol*. 2003; 29: 225–35.
3. Bilian Jin, Yajun Li, and Keith D. Robertson. DNA Methylation. *Genes Cancer*. 2011 Jun; 2(6): 607–617.
4. Escandell JM, Recio, Manez S, Giner, Cerda-Nicolas M, Gil-Benso R. Dihydrocucurbitacin B inhibits delayed type hypersensitivity reactions by suppressing lymphocyte proliferation. *J Pharmacol Exp Ther*. 2007; 322: 1261– 8.
5. Eunsohl Lee, Jingcheng Wang, Kenji Yumoto, Younghun Jung, Frank C. Cackowski, Ann M. Decker, Yan Li, Renny T. Franceschi, Kenneth J. Pienta, and Russell S. Taichman. DNMT1 Regulates Epithelial-Mesenchymal Transition and Cancer Stem Cells, Which Promotes Prostate Cancer Metastasis. *Neoplasia*. 2016 Sep; 18(9): 553–566.
6. Heinlein CA, Chang C. Androgen receptor in prostate cancer. *Endocr Rev*. 2004 Apr 25(2): 276-308.