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In vitro anti-cancer activities of few anti-hypertensive agents against carcinoma of cervix by MTT assay

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Abstract: Cancer disease arises from mutations and other aberrations of gene regulation and the expressed genes encode various growth factors, growth factor receptors and nuclear proteins which have a central role in the control of cellular proliferation. Though various drugs are available in the market, a therapeutic search for novel, potent anti-cancer agents is under process to combat the dreadful disease. In this study we report the anti-cancer properties of few anti-hypertensive drugs (Atenolol, Lisinopril, Nefedipine, Aldactone and Propranolol) tested against HeLa cancer cells *in vitro* by MTT assay. A gradual decrease in the viability of HeLa cells was observed in a dose-dependent manner for all the drugs used in the study. Propranolol at a tested dose of 256 µg/ml exhibited maximum percentage inhibition of 39.57%.

Key Words: cancer, HeLa, anti-hypertensive, MTT assay

Introduction

Cancer is a dreadful disease and any practical solution in combating this disease is of paramount importance to public health [1]. Though many treatment techniques are now made available, after surgical ablation of cancer, however, metastasized tumor cells continue to progress and this is one of the causes making cancer treatment difficult. At the same time, they also affect normal cells to cause serious adverse effects, such as bone marrow function inhibition, nausea, vomiting etc [2].

Cancer is primarily a genetic disease arising from mutations and other aberrations of gene regulation and expression in somatic cells. The genes involved are relatively few in number and include the cellular oncogenes which variously encode growth factors, growth factor receptors and nuclear proteins which have a central role in the control of cellular proliferation. Physiological events such as regulati-ng proliferation, apoptosis, differentiation and cell arrest modulate correct homeostasis and functionality of all tissues. A disorder in these sequential events will result in alteration of the ration between cell death, differentiation and proliferation, leading to an increase in the number of dysregulated cells [3].

It was reported in literature that various drugs have been studied to understand the role as a preventive treatment of various cancers. Ibuprofen has been studied to reduce the risk of prostate cancer [4]. In another study, a class of drugs called bisphosphonates developed to treat osteoporosis and other bone diseases were reported to have potent anti-cancer properties [5]. Moreover, growing experimental and clinical evidence indicated that NSAIDs (Non-Steroidal Anti-Inflammatory Drugs) such as Aspirin and COX-2 inhibitors also have anti-cancer activity, in particular colon [6]. The association between beta blockers and angiotensin-converting enzyme (ACE) inhibitors towards breast cancer recurrence and mortality was studied and the findings suggested that the recurrence and survival were associated with exposure to two commonly

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used classes of anti-hypertensive medications [7]. Above data has provided us the rationale to study the anti-cancer properties of few anti-hypertensive drugs and tested against the standard drug, tamoxifen.

Materials and Methods

Cancer Cells

Carcinoma of cervix (HeLa) cells were maintained in Dulbecco's modified essential medium (DMEM) supplemented with 4.5 g/L glucose, 2 mM L-glutamine and 5% fetal calf serum (FCS) (growth medium) at 37°C in 5% CO_2 incubator.

Inhibitory Compounds

Sympatholytics used to treat hypertension, anxiety and panic such as Atenolol (β-blocker), Lisinopril (angiotensin-converting enzyme inhibitor), Nefedipine (calcium channel blocker), Aldactone (renal competitive aldosterone antagonist) and Propranolol (non-selective beta blocker) and the standard drug Tamoxifen tablets were purchased.

MTT assay

The MTT assay developed by Mosmann [8] was modified and used to determine the inhibitory effects of test compounds on cell growth in vitro. This colorimetric assay is based on the capacity of mitochondrial succinate dehydrogenase enzymes in living cells to reduce the yellow water soluble substrate, 3-(4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) into an insoluble, purple colored formazan product which is measured spectrophotometrically. Since reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells.

Procedure

The monolayer cell culture was trypsinized and the cell count was adjusted to $5x10^3$ cells/well using growth medium containing 5% FCS. To each well of 96 well microtitre plates, 80 µl of growth medium, 10 µl of diluted cell suspension were added. After 24 hours, when the monolayer was formed, each well then received 10 µl of different test compounds (8, 32, 128 and 256 µg/ml) and incubated at 37º C in 5 % CO2 incubator for 48 hours. After incubation, the supernatant was discarded and 5µl of MTT dye (0.5 mg/ml in PBS) was added to each well. The plates were gently shaken and incubated for 4 hours at 37º C in 5% CO2 incubator. The supernatant was removed and 100 µl of DMSO was added, the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 570nm. All experiments were performed in triplicate.

Calculations

The percentage growth inhibition was calculated using the following formula,

$$\% \text{ cell inhibition} = 100-\{(At-Ab)/ (Ac-Ab)\}x100$$

where,

At= Absorbance value of test compound

Ab= Absorbance value of blank

Ac=Absorbance value of control.

Results and Discussion

Cancer cells were exposed to the five drugs (Atenolol, Lisinopril, Nefedipine, Aldactone and Propranolol) for 48 h and cell viability was evaluated by MTT assay. A gradual decrease in the viability of HeLa cells was observed in a dose-dependent manner for all the drugs used in the study. The percentage inhibitions were in the range 13.19 – 39.57 % (Table 1), whereas Tamoxifen reported 46.17 % at the highest tested dose (256 µg/ml) respectively.

The results from Table 1 show that the proliferation of carcinoma of cervix cells could be significantly inhibited by at least four compounds (Atenolol, Lisinopril, Nefedipine and Propranolol) in a concentration dependent manner. The morphology of the cells treated with the drugs appeared significantly different as compared to untreated control cells, which could probably due to the growth inhibitory and cell death initiating ability of the drugs.

Propranolol was found to be the most active drug with 39.57 % inhibitory activity against Hela cells tested at a dose of 256 µg/ml. Nefedipine reported 27.15 % inhibition, which is higher than the standard drug tamoxifen (15.34 %) at 8 mg/ml concentration, however, at 32 and 128 µg/ml tested doses, the percentage inhibition was around 30 %. The sensitivity of HeLa cells when treated with the drug Propranolol from 32 to 128 µg/ml concentration was indeed around 30% but exposing cells to the final concentration resulted in 39.57 % inhibition. From this study, it can be understood that the Propranolol has the ability to reduce the proliferation of HeLa cells *in vitro*.

Table 1: Optical Density (OD) and inhibitory data of Tamoxifen and 5 drugs at various concentrations

Conc.	% Inhibition					
(µg/ml)	Tamoxifen	Atenolol	Lisinopril	Nefedipine	Aldactone	Propranolol
8	15.34	13.19	31.13	27.15	13.22	26.38
32	29.91	21.32	33.74	30.06	13.80	29.91
128	30.98	25.46	36.66	30.52	15.18	30.52
256	46.17	31.90	37.27	32.06	24.23	39.57

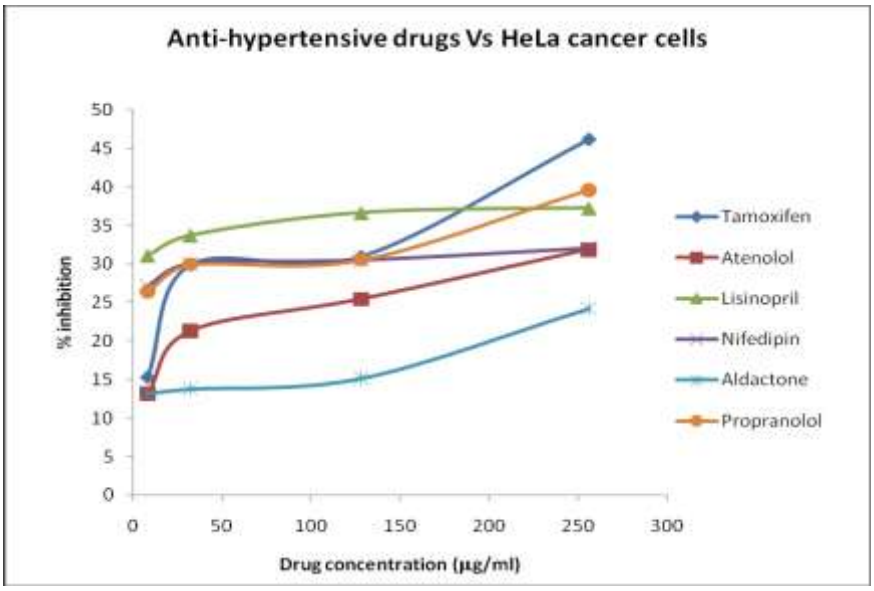

Figure 1: Percentage inhibitory values of five anti-hypertensive drugs against carcinoma of cervix.

Conclusion

The current investigation has been carried out to test the ability of five anti-hypertensive drugs (Atenolol, Lisinopril, Nefedipine, Aldactone and Propranolol) as anti-proliferative agents on growth of HeLa cells using MTT assay. In the present work, about four compounds showed growth inhibitory effects with percentage inhibitions nearer to the standard drug Tamoxifen. Moreover, further *in vitro* investigations shall reveal valuable insights into the binding of these compounds against specific protein targets expressed in carcinoma of cervix. Based on the result, it can be suggested that Propranolol might represent potential agent for the treatment of cervical cancer.

References

1. Patel PR, Akhil AN, Rikin CP, Dhara KR and Vishal RP. *Invitro* anticancer activity of *rubia cordifolia* against hela and hep2 cell lines. *Int J Pharm Pharm Sci*, Vol 3, Suppl 2, 2011, 7071
2. Ueda J, Yasuhiro T, Banskota AH, Tran QL, Tran QK, Harimaya Y, Saiki I and Kadota S. Antiproliferative Activity of Vietnamese Medicinal Plants. *Biol. Pharm. Bull.* **25**(6) 753-760 (2002)
3. Chadha R, Kapoor VK, Deepika T, Rupinder K, Poonam A and Jain DVS. Drug carrier systems for anticancer agents: A review. *Journal of Scientific & Industrial Reseach* 67, 2008, 185-197
4. Timothy JW, Pauline JW, Andrew ST, Thomas JH, Georgina RS, Ping J, Michael DT and Matthew DL. Chiral inversion of 2-arylpropionyl-CoA esters by human -methylacyl-CoA racemase 1A (P504S)-a potential mechanism for the anti-cancer effects of ibuprofen. *Chem. Commun.* 2011, 47, 7332-7334
5. http://www.sciencedaily.com/releases/2009/03/09032513 2458.htm
6. Xu XC. COX-2 inhibitors in cancer treatment and prevention, a recent development. *Anticancer Drugs* 2002 13(2):127-137
7. Ganz PA, Habel LA, Weltzien EK, Caan BJ, Cole SW. Examining the influence of beta blockers and ACE inhibitors on the risk for breast cancer recurrence: results from the LACE cohort. *Breast Cancer Res Treat.* 2011 DOI: 10.1007/s10549-011-1505-3
8. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods* 65, 1983, 55.

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