Int J Pharm Bio Sci 2014 Oct; 5(4): (P) 387 - 393

Research Article Pharmacology

International Journal of Pharma and Bio Sciences ISSN 0975-6299

IN VITRO ANTICANCER ACTIVITY OF LAMOTRIGINE AND 5-FLUOROURACIL INDIVIDUALLY AND IN COMBINATION ON HUMAN K562 AND COLO320HSR CELL LINES BY TRYPAN BLUE ASSAY

KATARIKONDA SUDHAKAR*[1] AND MEDA RAMESH[2]

[1]Department of Pharmacy (pharmacology), Vagdevi College of Pharmacy and Research Centre, Brahmadevam, Nellore, A.P, India.
[2]Department of pharmacy (pharmacology), krishna teja pharmacy college, chadalawada nagar, renigunta road tirupati, chittoor dist,A.P, India.

ABSTRACT

Cancer is the leading cause of disease for high mortality all over the world. Antitumor drug resistance and side effects of antitumor drugs are the most common problems in cancer chemotherapy. It is of great importance in considering the drugs having the anticancer activity and going for the new combination of cancer therapeutics for avoiding the problems of tumor drug resistance and side effects with a new therapeutic combinations and by lowering the dose of drugs. In the present study the anticancer activity of Lamotrigine and 5-Fluorouracil individually and in combination were determined on Colo 320HSR and K562 cell lines. And our results concluded that the both drugs individually and combination have better anticancer activity at higher concentrations.

KEYWORDS: Drug resistance, Toxicity, Trypan blue, Lamotrigine, 5-Fluorouracil.

K.SUDHAKAR
Department of Pharmacy (pharmacology), Vagdevi College of Pharmacy and Research Centre,Brahmadevam, Nellore, A.P, India.

*Corresponding author

INTRODUCTION

Cancer is the leading cause of deaths worldwide. Globally there are currently 10 million new cases of cancer per annum (based on 2000 figures), but the WHO has predicted this figure to be 15 million per year by 2020[1]. In the cancer disease the host cells divides irrespectively and uncontrollably by the deregulated cell cycle[2, 3]. Significantly the cancer cell posses distinct features like self sufficiency in growth signals, insensitivity to growth signals, tissue invasion and metastasis, limitless replicative potency, angiogenesis, and evading apoptosis[4].Cancer is mainly caused due to the loss of function of tumor suppressor genes, activation of oncogenes[5], additionally various carcinogens too causes mutations in the genes and finally leads to cancer[6]. A wide range of novel therapeutic compounds have been in clinical use for the treatment of various types of cancers with a diversified mechanism of action[7]. Even then the major problem with the cancer drugs is primarily the toxicity of the drugs like neurotoxicity, haematological toxicity, gastro intestinal toxicity, oral toxicity, hair follicle toxicity, gonadal toxicity, hepatotoxicity, ototoxicity e.t.c., [8] and secondarily the drug resistance of cancer cells to the cancer therapeutic agents. It is of great deal of importance to develop new therapeutic agents to meet the best needs in treatment of cancer. And it is of much more significant of inclusion that the drugs which are used to treat a disease having the ability to cure or act against another disease one such great example which has proven and in clinical use is ASPRIN. Asprin is an NSAID used in the treatment of inflammation but it is having the ability to treat colon cancer[9]. Another example of clinically used anti epileptic drug, Lamotrigine is believed and proved to inhibit dihidrofolate reductase (weak inhibitor) [10] and can inhibit histone deacylase in cancer cells[11]. Toxicities of the drug can be managed by therapeutic monitoring and rationale usage of the drugs, avoiding or discontinuing the usage of the drugs. Drug resistance can be overcome by Combination therapy, it has been the standard of care, mostly and appreciably in cancer treatment. Since it is a rationale criteria to increase response and tolerability and to decrese resistance. Currently, there is a growing interest in combining anticancer drugs aiming at maximizing efficacy while minimizing systemic toxicity through the delivery of lower drug doses[12]. The cell cycle specific anti cancer agents like 5-Fluorouracil and Methotrexate were the best used anticancer agents, whose usage has been limited due their severe toxicities. 5-Fluorouracil is an antimetaboloite whose usage is limited due to the toxicities like, the systemic administration causes a syndrome of delayed myelin destruction in the central nervous system[13]. Methotrexate is a dihydrofolate inhibitor whose usage is minimized due to the toxicities like renal, neuro, haematological, pulmonary, cutaneous and gastro intestinal toxicities[14]. In the present study the potency and efficacy of lamotrigine and 5-Fluorouracil individually and in combination were estimated for the anticancer activity against colon cancer cell lines (COLO 320HSR) and chronic myelogenous leukemic cancer cell lines (K562) by trpan blue dye exclusion method.

MATERIALS AND METHODS

MATERIALS

Drugs used

Lamotrigine was obtained from RACHEM Pvt. Ltd Hyderabad, A.P. India. Fluorouracil injection 500 mg/10 ml was collected from Vishwa Bharati multi specialty hospitals Kurnool, A.P. India.

Chemicals used

RPMI 1640 (GLIBCO®), Fetal bovine serum (GLIBCO®). Trypsin, 70% (v/v) isopropanol in sterile water, Dimethyl sulphoxide (Sigma), Penicillin - Streptomycin (Gibco), Trypan blue (Sigma).

Cell lines used

In the present study K562 cells (derived from human Chronic Myelogenous Leukemia cells) and COLO 320HSR cells (derived from human Colon cancer cell lines) were used. These cells are obtained obtained from Sugen life sciences Pvt. Ltd. Tirupati A.P. Inida.

Cellculture

K562 cells and Colo 320HSR cells were cultured by using RPMI 1640 (GLIBCO®) containing 10% of fetal bovine serum (GLIBCO®). The cells were seeded in a 48 well plates at the concentration of $1x10^4$ cells/ml and incubated in 5% CO_2 incubator at 37^0 C for 24 hours[15].

Drug treatment

Cells were maintained in 48 well plate and triplicates were maintained for each concentration, and treated with different concentrations (10, 25, 50, 100µM) of Lamotrigine and 5-Fluorouracil respectively. The combination of Lamotrigine and 5-Fluorouracil were taken as half of each concentration of both the drugs and both were taken combinely to make the final concentration and DMSO is used as negative control. The treated wells were incubated for 48 hours in 5% CO_2 incubator at 37^0 C after 24 hrs of incubation.

Determination of anticancer activity by Trypan blue dye exclusion method [(16)]:

Principle

This method is based on the principle that viable (live) cells actively pumps out the dye by efflux mechanism and appears in white colour, where as the dead cells do not efflux the dye and appears in violet colour.

Procedure

i. Prepare a suspension of approximately 1×10^6 cells/ml. Ensure that the sample is thoroughly mixed.

ii. Make a 1:1 mixture of the cell suspension and the 0.4% trypan blue solution. The sample can be as small as 10 ml to several ml in volume. Gently mix and let stand for 5 min at room temperature.

iii. Prior to use, wash the hemocytometer with 70% (v/v) ethanol and allow drying.

iv. Wash a cover slip with 70% (v/v) ethanol, allow to dry, and place on top of the hemocytometer counting chamber.

v. Apply 15 µl of cell suspension to the edge of the chamber between the cover slip and the V-shaped groove in the chamber. Allow the cell suspension to be drawn into the chamber by capillary action.

vi. Let sit the cells for 1–2 min and then count.

vii. Count the stained and unstained cells in all the four corner squares of hemocytometer of dimensions 1 mm × 1 mm and is divided into 16 small squares in a four-by four array. The volume of each of these four cornersis thus 0.1 mm3 or 1 × 10−4 mL.

viii. The percentage of unstained cells represents the percentage of viable cells in the suspension.

ix. The percentage of viability is calculated by

$$\%\text{Viablecells} = \frac{\text{Number of viable cells}}{\text{Total number of cells}} \times 100$$

Statistical analysis

Results were calculated as mean ± S.E and S.E. where appropriate, one-way ANOVA followed by Post hoc Dunnet's t-test was employed. Values $P<0.05$ were considered significant. And graphs were designed by using Microsoft Excel soft ware (2007 edition).

RESULTS AND DISCUSSION

The cytotoxic effect of Lmotrigine, 5-Fluorouracil and combination of both the drugs on K562 and colo 320 HSR cell lines was assayed by trypan blue dye exclusion method. Trypan blue assay is a preliminary step to assess the anticancer property of wide range of chemical compounds. The dead cells will be stained by trypan blue and viable cells remain unstained. The maximum amount of inhibition of K562 cells was exhibited by 5-Fluorouracil followed by the combination of Lamotrigine and 5-Fluorouracil followed by Lamotrigine at the highest concentrations (100 µM). The results were shown in table-1 and the relative comparision of viability is shown in graph-1 and figure-1. The maximum amount of inhibition of Colo 320HSR cells was exhibited by 5-Fluorouracil followed by the combination of Lamotrigine and 5-Fluorouracil followed by Lamotrigine at the highest concentrations (100 µM). The results were shown in table-2 and the relative comparision of viability is shown in graph-2 and figure-2.

TABLE-1
percentage of viability values of lamotrigine and 5-flurouracil on K562 cells

S.No	Lamotrigine (L) in µM	Combination in µM (L + 5FU)		5 Fluoro uracil (5FU) in µM	% of viability		
		L	5FU		L	L+5FU	5FU
1	10	5	5	10	74.04±3.66* 74.04±2.11**	67.28±3.19* 67.28±1.84**	30.64±1.86* 30.64±1.07**
2	25	12.5	12.5	25	66.71±1.36* 66.71±0.78**	55.41±1.92* 55.41±1.11**	20.13±2.023* 20.13±1.16**
3	50	25	25	50	63.61±2.245* 63.61±1.29**	48.46±1.78* 48.46±1.02**	1.3±0.31* 1.3±0.18**
4	100	50	50	100	58.97±2.07* 58.97±1.19**	37.21±1.80* 37.21±1.04**	0.1833±0.31* 0.1833±0.18**

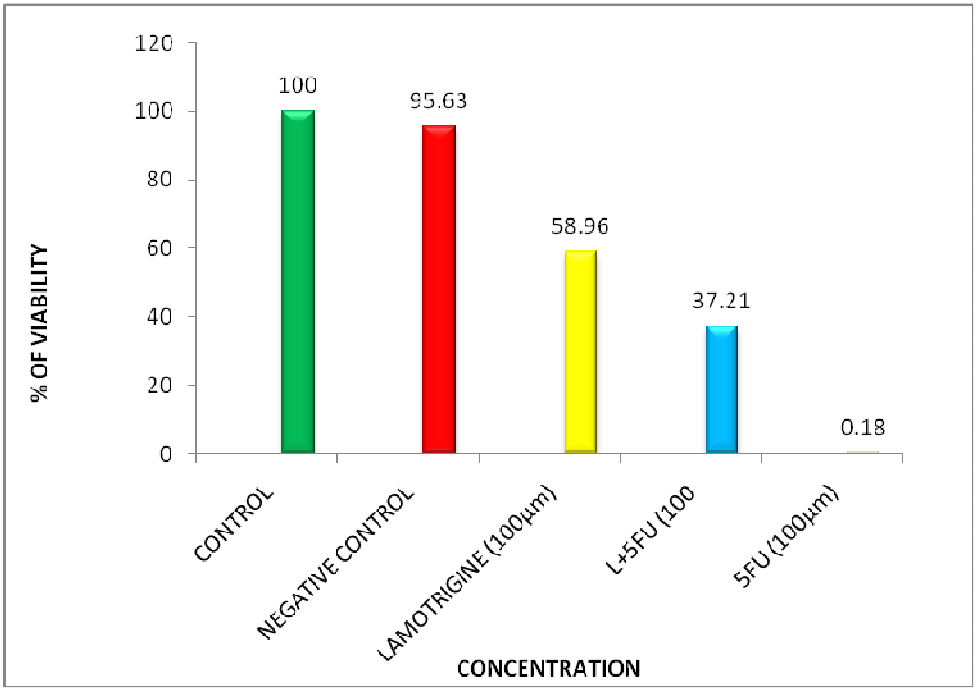
Inference: % of viability values of Lamotrigine and 5Flurouracil on K562 cells. MEAN±S.D*, MEAN±S.E**. Value P<0.05 were considered significant.

TABLE-2
percentage of viability values of lamotrigine and 5-flurouracil on COLO 320 HSRcells

S.No	Lamotrigine (L) in µM	Combination in µM (L + 5FU)		5 Fluoro uracil (5FU) in µM	% of viability		
		L	5FU		L	L+5FU	5FU
1	10	5	5	10	82.96±1.28* 82.96±0.74**	73.52±4.45* 73.52±2.57**	52.54±1.64* 52.54±0.95**
2	25	12.5	12.5	25	74.82±1.12* 74.82±0.65**	59.26±1.28* 59.26±0.74**	23.87±0.99* 23.87±0.57**
3	50	25	25	50	67.4±2.563* 67.4±1.48**	50.37±1.74* 50.37±1.01**	4.717±3.12* 4.717±1.80**
4	100	50	50	100	64.95±0.44* 64.95±0.25**	48.14±1.28* 48.14±0.74**	0 ±0.00* 0 ±0.00**

Inference: % of viability values of Lamotrigine and 5Flurouracil on Colo 320HSR cells. MEAN±S.D*, MEAN±S.E**. Values P<0.05 were considered significant.

GRAPH-1
% of viability values of lamotrigine and 5-flurouracil on K562 cells



GRAPH-2

% of viability values of lamotrigine and 5-flurouracil on Colo 320 HSR cells

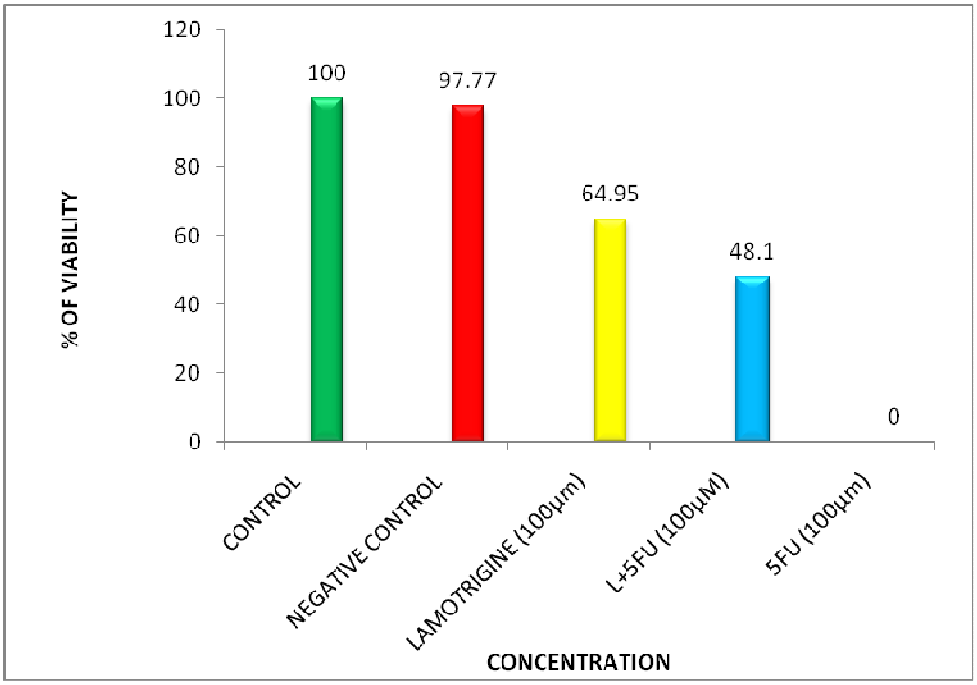


FIGURE-1

Anticancer activity of Lamotrigine and 5-Fluorouracil on K562 cells

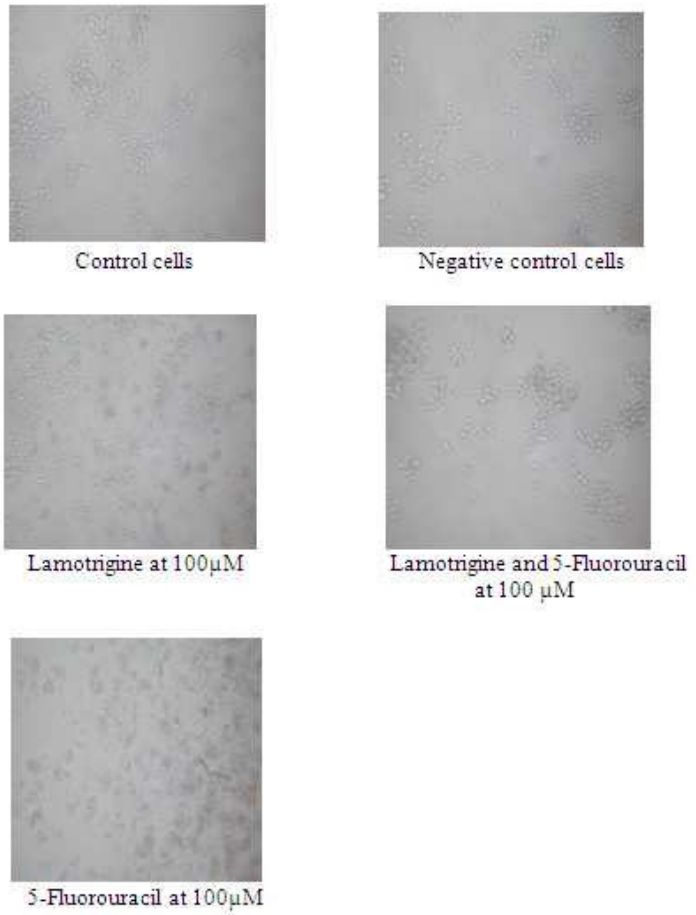
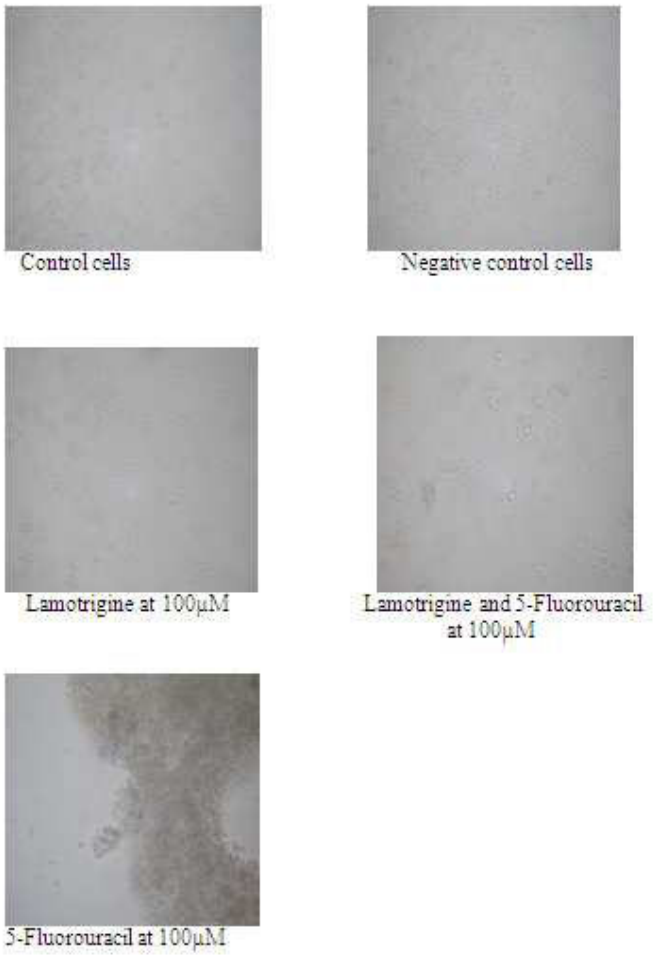


FIGURE-2
Anticancer activity of Lamotrigine and 5-Fluorouracil on Colo 320 HSR cells



CONCLUSION

Based upon the results we got in the present study, we conclude that Lamotrigine individually and in combination with 5-Fluorouracil can inhibit the growth of chronic myelogenous leukemic cells and colon cancer cells effectively at higher concentration (100M). But the relative inhibition of cancer cell growth by Lamotrigine alone is significantly better on chronic myelogenous leukemic cell lines than colon cancer cell lines at higher concentrations (100µM). Thus, considering the results we got from the present study, we conclude that Lamotrigine can be tested for the both Chronic myelogenous leukemia and colon cancer individually and in combination with 5-Fluorouracil for clinical trails only after getting a good preclinical dossier tested in various animal species for the toxicology, efficacy and determination of maximum tolerable dose of this combination of drugs.

ACKNOWLEDGEMENT

I, K. Sudhakar thankfully acknowledge SUGEN LIFE SCIENCES Pvt Ltd., Tirupati for providing all necessary facilities and also grateful to C. Krishna kumari for her valuable guidance and assistance in pursuing the research. I am thankful to Mr. M. Ramesh for his kind cooperation. It is great privilege to me to mention my professor Dr. D. Sivaraman from Vagdevi College of Pharmacy and Research Centre, Nellore and I would like to thank all the individuals who have contributed for the successful completion of the work for their encouragement and support.

REFERENCES

1. Vinodh Jaganti , Sukirti das and T. Sai sampath, A review on cancer vaccines. International Journal of Pharma and Bio Sciences, 2 (3): 86-97, (2011).
2. Humphrey P. Rang, Maureen M. Dale, James M. Ritter and R. J Flower. A Text book of RANG and DALE'S Pharmacology. 7th. Edn. Elsevier publisher; 673-688, (2007).
3. Charles J. Sherr, Cancer Cell Cycles. SCIENCE, 274: 1672-1677, (1996).
4. Douglas Hanahan and Robert A. Weinberg, The Hallmarks of Cancer. Cell, 100: 57–70, (2000).
5. Niklaus Fankhauser, Igor Cima, Peter Wild and Wilhelm Krek, Identification of a Gene Expression Signature Common to Distinct Cancer Pathways. Cancer Informatics, 11: 139–146, (2012).
6. Geoffrey M. Cooper ans Robert E. Hausman. THE CELL a molecular approach, 4th Edn, ASM Press (Washington) publisher, 719-762, (2007).
7. Laurence L. Brunton, Bruce A. Chabner, Bjorn Christian Knollmann. Goodman and Gilman's The Pharmacological Basis of Therapeutics, 12th Edn, McGraw-Hill Professional publisher: 1665-1755, (2010).
8. Ambili Remesh, Toxicities of anticancer drugs and its management. International Journal of Basic & Clinical Pharmacology, 11 (1): 1-12, (2012).
9. Melania Dovizio, Stefania Tacconelli, Carlos Sostres, Emanuela Ricciotti and Paola Patrignani, Mechanistic and Pharmacological Issues of Aspirin as an Anticancer Agent. Pharmaceuticals, 5: 1346-1371, (2012).
10. Richard H. Weisler, Joseph R. Calabrese, Charles L. Bowden, John A. Ascher, Joseph DeVeaugh-Geiss and Gary Evoniuk, Discovery and development of lamotrigine for bipolar disorder: A story of serendipity, clinical observations, risk taking, and persistence. Journal of Affective Disorders, 108: 1-8, (2008).
11. Fathia Zaky El Sharkawi, Hany Abdelaziz El Shemy and Hussein Moustafa Khaled, Possible Anticancer Activity of Rosuvastatine, Doxazosin, Repaglinide and Oxcarbazepin. Asian Pacific Journal of Cancer Prevention, 15: 199-203, (2014).
12. Ana Catarina Pinto, Joao Nuno Moreira and Sergio Simoes, Combination Chemotherapy in Cancer: Principles, Evaluation and Drug Delivery Strategies. In: Oner Ozdemir(ed), Current Cancer Treatment – Novel Beyond Conventional Approaches, In Tech, Croatia, 2011, pp. 693-714.
13. Ruolan Han, Yin M Yang, Joerg Dietrich, Anne Luebke, Margot Mayer-Proschel and Mark Noble, Systemic 5-fluorouracil treatment causes a syndrome of delayed myelin destruction in the central nervous system. Journal of Biology, 7 (12): 1-22, (2008).
14. Emna Gaies, Nadia Jebabli, Sameh Trabelsi, Issam Salouage, Rim Charfi, Mohamed Lakhal and Anis Klouz, Methotrexate Side Effects: Review Article. Drug Metabolism & Toxicology, 3 (4): 1-5, (2012).
15. Syed Kamil Mulla and Paramjyothi Swamy, Anticancer activity of ethanol and polyphenol extracts of Portulaca Quadrifida Linn. On human colon cancer cell lines. International Journal of Pharma and Bio Sciences, 3 (3): 488-498, (2012).
16. Kristine S. Louis and Andre C. Siegel. Cell Viability Analysis Using Trypan Blue: Manual and Automated Methods. In: Martin J. Stoddart (ed), Mammalian Cell Viability Methods and Protocols, New York, 2011, pp.7-12.