Inssaf IH. Dr. Al - Shemmary /J. Pharm. Sci. & Res. Vol. 10(2), 2018, 333-336

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

A combination effect of Nilotinib and Daunorubicin on the Tumor Cells from Patients with the Chronic Myelogenous Leukemia *in vitro*

Inssaf IH. Dr. Al - Shemmary[*,1]
College of Medicine, University of Anbar, Al-Ramadi, Iraq. [*]

Abstract

In vitro, the cytotoxicity testing for anticancer drugs combination (nilotinib and daunorubicin) was carried out on the leukemic myeloid stem cells. This cells were isolated from bone marrow by using Ficoll -paque from 20 patients (10males and10 females) with CML (new diagnosis) with the ages ranged between 40 -7o years who attended to the National Center of Hematology/ Al – Mustansiyria University in the period from August 2016 to October 2017. Myeloid stem cells were cultured in the RPMI- 1640 media, the viable cells count was 5× 10^6 cells. Nilotinib and daunorubicin were prepared in different concentrations (300,150, 75, 37.5, 18.75µg/ml) and five combinations from each concentration of two drugs, incubation at 37°C for 72 hours. The viable cells were measured by MTT(Methyl thiazolyl tetrazolium) assay.This study showed that the cytotoxic effect or inhibition growth rate of nilotinib was higher than daunorubicin on the leukemic myeloid stem cells at 72hr and the inhibition growth rate in both drugs depended on the dose or concentration. There was significant differences (P<0.01) between concentrations in the same drug. In the high concentration (300 µg/ml), the inhibition rates were 94.03%, 79.26% for nilotinib and daunorubicin respectively. In combinations, the inhibition rate was higher in comparison to each drug alone and also its depend on the dose, thus the inhibition rate in high concentration (300 µg/ml) was 98.67% and in low concentration (18.75 µg/ml) was 63.49%.

Key words: daunorubicin, nilotinib, myeloid culture, MTT assay.

INTRODUCTION

Nilotinib (Tasigna)[R] is a second generation of tyrosine kinase inhibitor (TKI) anticancer drug [1,2], is a phenylamino-pyrimidine molecule. It acts through inhibits BCR-ABL, c-kit, and PDGFR- ß tyrosine kinases. Nilotinib is orally drug with greater potency and selectivity for BCR-ABL than imatinib. It was approved by FDA on 2007 [3,4]. Nilotinib is used for the treatment of adult patients with chronic phase and accelerated phase of chronic myelogenous leukemia (CML) with positive Philadelphia chromosome (Ph+) resistant to or intolerant of prior treatment with imatinib [5,6].

Daunorubicin is an anthracycline antibiotic antitumor agent. The anthracycline antibiotics isolated from *Streptomyces peucetius* [7,8]. The cytotoxic effect of anthracyclines through four major mechanisms: (1) inhibition of topoisomerase II; (2) high-affinity binding to DNA through intercalation, with consequent blockade of the synthesis of DNA and RNA, and DNA strand scission lead to stop the process of replication result in the cell cannot split into two new cancer cells; (3) generation of semiquinone free radicals and oxygen free radicals through an iron-dependent, enzyme-mediated reductive process; and (4) binding to cellular membranes to alter fluidity and ion transport. This interactions lead to stop the process of replication result in the cell cannot split into two new cancer cells[9,10,11]. Daunorubicin is used in the treatment of acute myeloid leukemia, acute lymphocytic leukemia and Kaposi's sarcoma [12].

Chronic myelogenous or myeloid leukemia (CML) is a malignant clonal, myeloproliferative disorder of the pluripotent hematopoietic stem cells [13.14].It is characterized by the chromosomal translocation 9;22, known as the Philadelphia chromosome (Ph) [15,16,17].The important clinical hallmarks of the disease are ganulocytosis, shift to the left in the differential WBCs count, increase in basophils , eosinphils and splenomegaly [18].

Hematopoietic stem cells (HSCs) are the stem cells from which all the blood cells are derived. HSCs gives both of myeloid and lymphoid lineages by haematopoiesis process. Myeloid stem cells give neutrophils, eosinophils, basophils, monocytes, macrophages, erythrocytes, dendritic cells and megakaryocytes . Lymphoid stem cells give T cells, B cells, and natural killer cells[19].

PATIENTS AND METHODS

Twenty patients (10 males and 10 females) with CML (newly diagnosis) with the ages ranged between 40 -70 years who attended to the National Center of Hematology/Al– Mustansiyria University in the period from August 2016 to October 2017.

Human bone marrow was obtained from the posterior iliac crest by an aspiration needle under local anesthesia (10 ml xylocaine).[20]. Ficoll- opaque was added for isolation of myeloid cells [21]. Cells were placed into 25 cm falcon after adding 10 ml of RPMI – 1640 (20 % FCS), the medium was prepared by dissolving 16.35g powder of RPMI – 1640 with Hepes buffer and L– glutamine. 2 g of sodium bicarbonate, 1ml of ampicillin, 0.5 ml of streptomycin and 200 ml of fetal calf serum were added to one liter of medium. Incubated at 37° C [22,23]. The viable cells count was 5× 10^6 Cells.

Nilotinib (Novartis, Switzerland. Lot S0060) and daunorubicin (Pfizer. Actavis, Italy S.P.D,) were prepared in different concentrations (300,150, 75, 37.5, 18.75µg/ml) and combination of two drugs from each concentration. 200µl of each drug and combination were added to the cells culture (200 µl of cell suspension in the each well of micro titration plate of 96 wells flat bottom). Four replicates were done. Incubated at 37° C for 72hr [24,25].

MTT(Methyl thiazolyltetrazolium) solution 28 µl (2 mg /ml) was added to calculate cells viability. Read at 550 nm by ELISA reader [26]. The percentage of inhibition rate for cells growth was calculated as: (A – B) / A X 100. A : is the mean of optical density for untreated wells (control). B: is the mean of optical density for treated wells [27].

Statistical analysis

The descriptive data of the results was demonstrated as ranges, percentages, means, standard errors and LSD (P≤0.05) for comparison [28].

RESULTS

This study showed that the cytotoxic effect or inhibition growth rate of nilotinib and daunorubicin on the leukemic myeloid cells at 72h depended on the dose of drugs as in the table1, 2 and figure1, 2. There was significant differences (P<0.01) between concentrations for each drug. Also this study recorded that the inhibition rate of nilotinib was higher than the inhibition rate of daunorubicin. In the high concentration (300 µg/ml), the inhibition rates were 94.03%, 79.26% for nilotinib and daunorubicin respectively while in the low concentration of drugs

(18.75 µg/ml),the inhibition rate were 41.51%, 25.74% for nilotinib and daunorubicin respectively.

In combinations (nilotinib and daunorubicin), the present study showed that the inhibition rate of combinations also depend on the dose, thus the inhibition rate in high concentration (300 µg/ml) was 98.67% and in low concentration (18.75 µg/ml) was 63.49% as in table 3 and figure 3. Also there was significant differences (P<0.01) in low and high concentrations as in table 3. The inhibition rate of combinations was higher in comparison to each drug alone as in table 4 and figure 4.

Table 1:Inhibition rate of daunorubicin on the leukemic myeloid cells at 72 hr.

Concentration (µg/ml)	Mean ± SEM of IR %
18.75	25.74 ± 1.07 e
37.5	32.31 ± 1.36 d
75	43.51 ± 2.53 c
150	57.12 ± 2.93 b
300	79.26 ± 3.06 a
LSD value	5.924 **
P-value	0.0001
** (P<0.01).	

IR=inhibition rate, SEM=standard error of mean, different letter=differ significantly, **=significant differences.

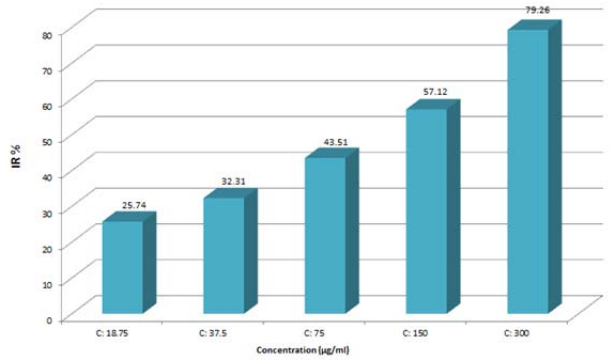

Figure1: Inhibition rate of daunorubicin on the leukemic myeloid stem cells at 72 hr.

Table 2:Inhibition rate of nilotinib on the leukemic myeloid stem cells at 72 hr.

Concentration (µg/ml)	Mean ± SEM of IR %
18.75	41.52 ± 1.96 e
37.5	52.19 ± 2.37 d
75	59.90 ± 2.84 c
150	81.44 ± 4.61 b
300	94.03 ± 4.73 a
LSD value	7.031 **
P-value	0.0001
** (P<0.01)..	

IR=inhibition rate, SEM=standard error of mean, different letter=differ significantly, **=significant differences.

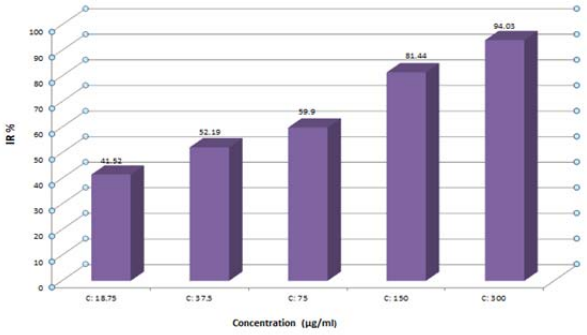

Figure 2: Inhibition rate of nilotinib on the leukemic myeloid stem cells at 72hr

Table 3: Inhibition rate of combinations (daunorubicin & nilotinib) on the leukemic myeloid stem cells at 72hr.

Concentration of combinations (µg/ml)	Mean ± SEM of IR %
18.75	63.50 ± 3.52 c
37.5	76.08 ± 3.87 b
75	81.44 ± 4.07 b
150	84.28 ± 4.61 b
300	98.67 ± 5.93 a
LSD value	8.266 **
P-value	0.0001
** (P<0.01).	

IR=inhibition rate, SEM=standard error of mean, different letter=differ significantly, **=significant differences.

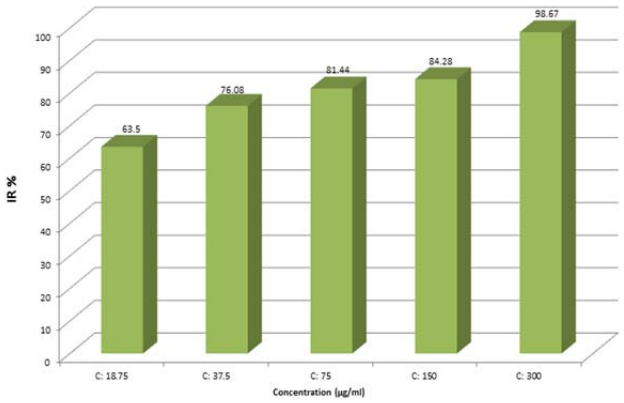

Figure 3. Inhibition rate of combinations (daunorubicin & nilotinib) on the leukemic myeloid stem cells at 72 hr.

Table 4: Comparison of inhibition rate between combination and each drug alone on the leukemic myeloid stem cells at 72hr.

Concentration (µg/ml)	Mean ± SEM of IR %			LSD value
	D	N	Combination (D &N)	
18.75	25.74 ± 1.07	41.52 ± 1.96	63.50 ± 3.52	8.953 **
37.5	32.31 ± 1.36	52.19 ± 2.37	76.08 ± 3.87	7.461 **
75	43.51 ± 2.53	59.90 ± 2.84	81.44 ± 4.07	7.952 **
150	57.12 ± 2.93	81.44 ± 4.61	84.28 ± 4.61	7.912 **
300	79.26 ± 3.06	94.03 ± 4.73	98.67 ± 5.93	8.577 **
** (P<0.01).				

IR=inhibition rate, SEM=standard error of mean, **=significant differences, D= daunorubicin, N = nilotinib. D&N= combination of daunorubicin and nilotinib.

DISCUSSION

The present study showed that the cytotoxic effect or inhibition growth rate of nilotinib and daunorubicin depend on the dose, therefore the inhibition growth rate was increased with the increase concentrations in both drugs. Nilotinib was higher inhibition rate than daunorubicin on the leukemic myeloid stem cells at 72hr that isolated from patients with CML, but the combination of two anticancer drugs showed higher inhibition rate than each drug alone. The study was investigated combination from available anticancer drugs such as nilotinib and daunorubicin to obtain a good effect against leukemic cells from patients with CML. This may be the foundation for further study on the potential of the applied combinations in a clinical setting.

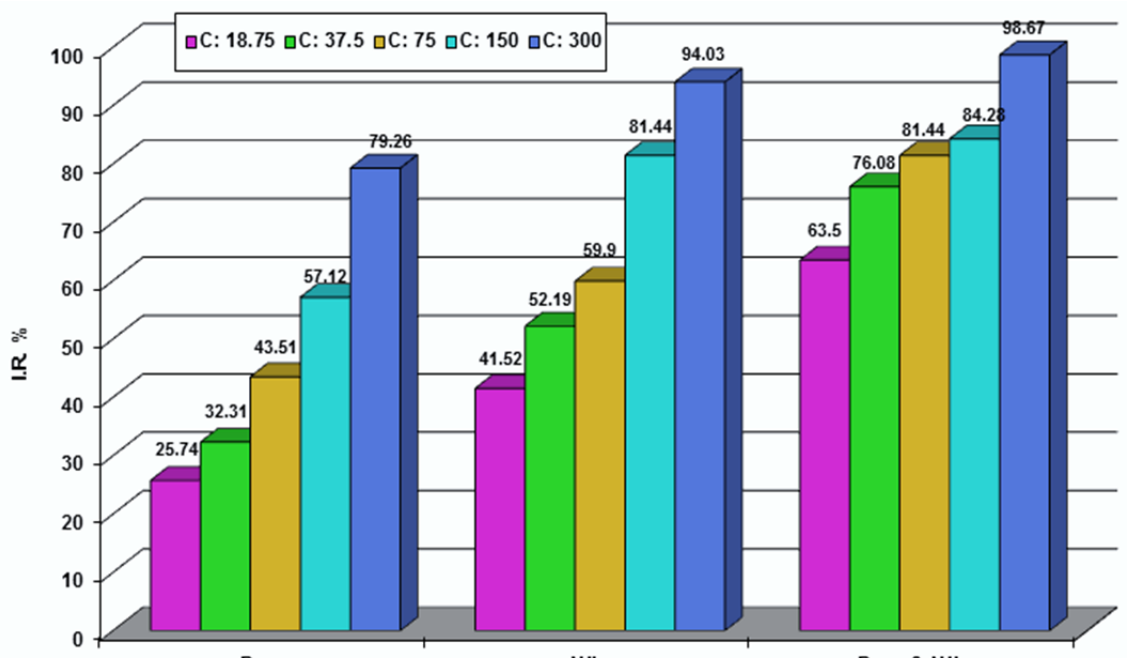

Figure 4: Comparison of inhibition rate between combinations and each drug alone on the leukemic myeloid stem cells at 72 hr.

Because of the incidence of leukemias in the human is about 10 / 100000 of population and its more common in males than in females such as the ratio 1.4 : 1 in the CML[29,30]. Moreover, resistance was developed to target drugs against cancer cells especially resistance to target drugs in CML therapy, this is lead to prevent or decrease the chance of cure. Therefore many efforts can impact on overall therapeutic outcomes in the treatments of patients, especially in late staged or resistance to cancer therapy. The anticancer drug combinations is one of these effort that lead to obtain a good outcomes in patients with CML and reduce resistance [31, 32].

In the previous studies were investigated various combination treatment regimens employing nilotinib with other anticancer drugs in order to establish the high effective anticancer treatment [33,34] as well as the Combination therapy or polytherapy is the therapy that uses more than one medication versus monotherapy which is any therapy consist of one medication, these therapies used to treat a single disease. Combination therapy is used in oncology in recent years, with various studies demonstrating higher response of combinations in compared to monotherapies and the FDA recently approving therapeutic combination regimens that demonstrated superior safety and efficacy to monotherapies [35,36,37].

CONCLUSIONS

The present study Showed that the cytotoxic effect of nilotinib was higher than daunorubicin and combinations of two drugs were highly inhibition rate than each drug alone on the leukemic myeloid stem cells at 72 hr.

ACKNOWLEDGEMENTS

I grateful to the patients who gave me bone marrow and to staff of the National Center of Hematology / Al – Mustansiyria University for helping me in the carrying out this study.

REFERENCES

1. Deremer DL., Ustun C., Natarajan K. Nilotinib: a second-generation tyrosine kinase inhibitor for the treatment of chronic myelogenous leukemia. Clin Ther. 2008; 30(11):1956-75.
2. Tiwari A., Sodani K., Wang S. Nilotinib (AMN107, Tasigna®) reverses multidrug resistance by inhibiting the activity of the ABCB1/Pgp and ABCG2/BCRP/MXR transporters. Biochemical Pharmacology. 2009; 78(2): 153–161.
3. Hochhaus A., Saglio G., Hughes TP. Long-term benefits and risks of frontline nilotinib vs imatinib for chronic myeloid leukemia in chronic phase: 5-year update of the randomized ENESTnd trial. Leukemia. 2016; 30:1044–54.
4. Kantarjian HM., Hochhaus A., Saglio G. et al. Nilotinib versus imatinib for the treatment of patients with newly diagnosed chronic phase, Philadelphia chromosome-positive, chronic myeloid leukaemia: 24-month minimum follow-up of the phase3 randomised ENESTnd trial. Lancet Oncol. 2011; 12: 841–51.
5. Saglio G., Kim DW., Issaragrisil S., et al. Nilotinib versus Imatinib for Newly Diagnosed Chronic Myeloid Leukemia. N Engl J Med. 2010; 362: 2251-2259.
6. British national formulary : BNF 69. British Medical Association. 69 ed. 2015; pp. 581–583.
7. Bennett TE. (11th ed), Antineoplastic agents. In : Goodman & Gilman. The pharmacological Basic of therapeutic. McGraw – Hill, New York, 2008.
8. Liu F., Jin H., Wang Y., et al. Anti-CD123 antibody-modified niosomes for targeted delivery of daunorubicin against acute myeloid leukemia. Journal of Drug Delivery. 2017; 24(1): 883 -890.
9. katzung BG.,Master SB., Trevor AJ. (12th ed.), Basic and clinical pharmacology. Lange, McGraw- Hill, 2012.
10. Harvey RA., Champe PC., Finkel R., *et al*. Lippincott Illustrated Reviews Pharmacology. 5th ed. Wolters Kluwer, Lippincott Williams & Wilkins. 2012; PP.494 -495.
11. Pang B., de Jong J., Qiao X., Wessels LF, Neefjes J. Chemical profiling of the genome with anti-cancer drugs defines target specificities. Nature Chemical Biology. 2015; 11(7): 472–480.
12. Olsson-Strömberg U., Aleskog A., Björnberg A. et al. Imatinib activity in vitro in tumor cells from patients with chronic myeloid leukemia in chronic phase and blast crisis. 2006; 17(6): 631-9.
13. Hochhaus A., Masszi T., Giles FJ. Chronic myelogenous leukemia Treatment-free remission following frontline nilotinib in patients with chronic myeloid leukemia in chronic phase: results from the ENESTfreedom study. Leukemia. 2017; 31: 1525–1531.
14. Lompardía SL., Díaz M., Papademetrio DL., et al. 4-methylumbelliferone and imatinib combination enhances senescence induction in chronic myeloid leukemia cell lines. Investigational New Drugs. 2017; 35(1): 1-10.

15. Smith DL, Burthem J., Whetton AD. Molecular pathogenesis of chronic myeloid leukaemia. Expert Rev Mol Med. 2003; 5:1–27.
16. Menon NM., Katsanis E., Khalpey Z., Whitlow P. Pediatric secondary chronic myeloid leukemia following cardiac transplantation for anthracycline-induced cardiomyopathy. Pediatric Blood & Cancer. 2015; 62 (1): 166–168.
17. Peterson LF.,Chia Lo M., Liu Y. *et al*. Induction of p53 suppresses chronic myeloid leukemia. Journal of Leukemia & Lymphoma. 2017; 58 (9): 2165-2175.
18. Yalin G., Michael L., Monika E. CD34- Hematopoietic Stem Cells: Current Concepts and Controversies. Stem Cells. 2003; 21 (1): 15–20.
19. Hoffbrand AV., Catovsky D., Edward GD.,Green AR. (6th ed.), Postgraduate Haematology. Blackwell Publishing Ltd, 2011.
20. Lewis SM., Bain BJ.,Bates I. (10th ed.), Dacie and Lewis Practical Haematology. Churchill Livingstone Elsevie , 2006; PP.25-75,311-333.
21. Petersen FB., Weinberg P., Hansen JA., Thomas ED. Collection and transportation of human bone marrow cells from unrelated donors. Transfus Sci. 1991; 12(3):155-9.
22. Freshney R.(4rded.),Culture of Animal Cells: A Manual of Basic Technique. Wiley- Liss. New York, USA. 2000; PP. 329 – 344.
23. Gao S.,Yu B., Li Y., et al. Antiproliferative effect of octreotide on gastric cancer cells mediated by inhibition of AKt/PKB and telomerase. W.J. G. 2003; 9 (10): 2362 – 2365.
24. Betancur- Galvis LA., Morales GE., Forero JE., Roldon J. Cytotoxic and antiviral activity of Colombian medicinal plant extract of the Euphorbia Genus. Mem Inst. Oswaldo Cruz, Rio de Janeiro. 2002 ; 97 (4): 541 – 546.
25. Marley SB., Davidson RJ., Goldman JM., Gordon MY. Effects of combinations of therapeutic agents on the proliferation of progenitor cells in chronic myeloid leukaemia. Br J Haematol. 2002;116:162–165.
26. Pieters R., Huismans DR., Leyva A., Veerman AJ. Adaptation of the rapid automated tetrazolium dye based (MTT) assay for chemosensitivity testing in childhood leukemia. Cancer Lett. 1988; 41:323–332.
27. Chiang W., Chang MY., Lin CC. In vitro cytotoxic antiviral and immunomodulatory effects of Plantago major and Plantago asiatica. American Journal of Chinese Medicine. 2003; 31(2): 225-234.
28. SAS. Statistical Analysis System, User's Guide. Statistical. Version 9. 1st ed. SAS. Inst. Inc. Cary. N.C. USA. 2012.
29. Sanford SA., Harold SR., William RR. Handbook of hematologic pathology. New York, N.Y: Marcel Dekker. 2000; pp. 193–194.
30. Provan D., Gribben JG. (3rd ed.),Chronic myelogenous leukemia.In: Molecular Hematology. Singapore, Wiley-Blackwell. 2010; PP. 76.
31. Lu DY., Chen EH., Lu TR., Wu HY., Ding J. Anticancer Drug Combinations, Studies for All Possibilities. Advances in Pharmacoepidemiology and Drug Safety. Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 2016; 5:138.
32. Aritro Nath, Jacqueline Wang,R. Stephanie Huang. Pharmacogenetics and Pharmacogenomics of Targeted Therapeutics in Chronic Myeloid Leukemia. Molecular Diagnosis & Therapy. 2017 ; 21 (6): 621–631.
33. Radujkovic A., Fruehauf S., Zeller WJ., Anthony D., Topaly J. Synergistic activity of nilotinib and established chemotherapeutic drugs in imatinib-sensitive and -resistant *BCR-ABL*-positive cells. Cancer Chemotherapy and Pharmacology. 2010 ; 66 (2): 255–264.
34. Emmanuelle NV., Matthias JL., Damien W. Combined Chemotherapy (daunorubicin + cytarabine) and Dasatinib as Salvage Therapy of Chronic Myeloid Leukemia (CML) in Myeloid Blast Crisis, a Pilot Study. Blood. 2009; 114 (22):2195.
35. Ali I., Rahis-ud-din, Saleem K., Aboul-Enein HY., Rather A. Social aspects of cancer genesis. Cancer Therapy, 2011; 8: 6-14.
36. Janku F., Hong DS., Fu S., Piha-Paul SA., *et al*. Assessing PIK3CA and PTEN in early-phase trials with PI3K/AKT/mTOR inhibitors. Cell Reports. 2014; 6 (2): 377–387.
37. Musgrove EA., Caldon C E., Barraclough J., Stone A., Sutherland RL. Cyclin D as a therapeutic target in cancer. Nature Reviews. Cancer. 2011; 11 (8): 558–572.