April 30, 2021

PharmacologyOnLine

Archives • 2021 • vol.1 • 313-321

EFFECT OF LINEZOLID IN OXALIPLATINE ANTICANCER ACTIVITY ON HUMAN COLON CANCER CELL LINE (HCT-116)

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Abstract

Introduction: in the Western countries the colon cancer represent the second most common cancer. One of the causes of colorectal cancer treatment failure is the resistance to therapy that occurs in most patients with metastatic colon cancer. In current study, we examined the role of linezolid on oxaliplatine anticancer effect in colon cancer cells.

Materials and methods: The effect of linezolid-oxaliplatine combination in comparison to the either drugs alone as a positive control groups was examined on HCT-116 colon cancer cell line, the inhibition rate on growth was tested by crystal violet assay. The apoptotic percentages was examind by annexin-V binding assay with flowcytometry.

Results: The *in vitro* cytotoxic effect of linezolid on HCT-116 cells was evaluated by crystal violet staining, a wide range of concentrations including (31.2, 62.5, 125, 250, 500, and 1000 µg / ml) was used for linezolid- oxaliplatine combination , and for the positive control groups both linezolid and oxaliplatine. After 24 hours of incubation, result showed the concomitant use of linezolid with oxaliplatine enhanced the oxaliplatine effect as the percentages of growth inhibition was increased, and also it has been demonstrated that the apoptotic percentage was significantly increased (p value <0.05) in the linezolid- oxaliplatine combination in comparison to the positive control groups.

Conclusion: this study revealed that the concomitant use of linezolid and oxaliplatine has a synergic effect, that could become a more effective regimen for the colon cancer treatment.

Key words: *Linezolid, Oxaliplatine, Colon Cancer*

Introduction

Colorectal cancer (CRC) is one of the chief causes of death from cancer in the world [1]. Currently, surgery and chemotherapy are the main colon cancer treatments. Even though the patients survival rate has been improved with chemotherapy, resistance develops in most patients with CRC that eventually led to the failure of chemotherapy [2].

Oxaliplatine was one of the platinum chemotheraputic drugs, which had the features of dominant anticancer effects. Oxaliplatine had been extensively useful in the treatment of numerous types' of tumors [3]. At the same time low doses can lead to drug resistance ,while high doses of oxaliplatine may results in severe side effects, like nerve ending inflammation, bone marrow suppression, gastrointestinal reactions [4]. Thus, a new method to challenging CRC is required [5]. Nowadays, drug combination therapies become largely prevalent to diminish overcome chemotherapy resistance and drug side effects [6]. For example the combination of oxaliplatine with capecitabine (one of the conventional chemotherapy drugs) has improved the survival rate of CRC patients [7].

Linezolid is a member of the oxazolidinone class of antibiotics, it is used for the treatment of infections caused by gram-positive bacteria that are resistant to other antibiotics. Linezolid in addition to IV administration it can be taken orally, which is one of the advantages of linezolid as it has a very good oral bioavailability of about 100% resulting from its rapid absorption.[8]

It acts by inhibiting bacterial protein synthesis. It either exert bacteriostatic or bactericidal activity. the exact mechanism of action of linezolid appears to be in that it blocks the initiation of protein production, rather than one of the later steps. bacterial resistance to linezolid has remained low [8,9].

linezolid is a relatively safe antibiotic when given for short periods [9]. It can be used in most age groups and in patients with poor kidney function or liver disease. Side effect associated with short term use of linezolid include headache, rash, diarrhea, and nausea. While severe side effects may include bone marrow suppression, high blood lactate levels, and serotonin syndrome, which mainly occurred if usage is continued for more than two weeks. Usage for longer periods can cause irreversible nerve damage, including optic nerve damage [8,10].

In the current study, the role of linezolid in oxaliplatine anticancer effect was evaluated on colon cancer cells HCT-116.

Materials and methods

This study was accomplished in the postgraduate Cancer Research Lab, at the College of Medicine/ University of Babylon, during the period from November 2019 to March 2020.

Cell Lines

Human colon cancer cell line (HCT-116) was obtained from the National Cell Bank of Iran (NCBI) at Pasteur Institute. The used cancer cell line was cultivated and maintained in RPMI 1640 media augmented with 100mg/ml streptomycin, 10% FBS, and 100 U/ml penicillin. It was cultured as a monolayer in a 37°C incubator and 5% CO2. [11]

Drugs stocks Preparation

Two-fold serial dilutions for both linezolid and oxaliplatine was made at concentrations of (1000, 500, 250, 125, 62.5, and 31.2) µg/ml. The combination linezolid-oxaliplatine was prepared by mixing equal volumes from each concentration of the two drugs to get half dose of each drug in the combination.

In vitro cytotoxicity assessment with crystal violet assay:

The cytotoxic effect of linezolid-oxaliplatine combination, oxaliplatine and linezolid on HCT-116 cells were evaluated *in vitro* by C.V staining. The crystal violet assay was achieved according to Feoktistova and his colleagues protocol [12].

Pre-drug exposure cellular handling: When the cells of HCT-116 line reached 70-80% confluence growth, they were trypsinized, counted, and returned in RPMI 1640 cultivation medium in sterile flat bottom 96-wells plates. Each well contain 200µl of cell suspension then cells were incubated for 24 hours at 37°C and 5% CO2 incubator.

Cellular drugs exposure: At the next day, the medium was aspirated from the plates and then they were treated with sterile

serum-free RPMI medium which was previously prepared and supplemented with a wide range of concentrations represented by (1000, 500, 250, 125, 62.5, and 31.2 µg/ml.) that is replicated in three wells of each concentration for all groups (linezolid-oxaliplatine combination, oxaliplatine group and linezolid group). While the negative control group was treated with RPMI-1640 medium. After 24 hours incubation, the absorbance was measured for each well by ELISA reader and the growth inhibition percentages were determined. and a three control wells were incubated with only culturing medium without any drug or additives. After that the plates were incubated in 37C° for 48 hour. Staining with Crystal Violet Dye

After 24hr of incubation, treated wells were washed with PBS, then 50µl of 0.5% crystal violet stain solution was poured to each well, then plates were incubated in 25C° for 20 min. After that the plates were gently washed with tap water and dried. Then, 200µl of methanol was added to each well and the plates were again incubated at room temp for 20 min with gentle rocking, the optical density of each well was read with ELIZA plate reader [13]. Finally, The percentages of cellular growth inhibition were calculated [14].

Determination of the concentration inhibits 50 % of cellular growth (IC50): To outline the dose-response curve the excel sheet was used, by plotting the different concentrations of the tested drugs and their percentages of cellular growth inhibition. The values of IC50 were calculated from it for each group [15].

Annexin V- Fluorescein isothiocyanate binding assay (FITC):

The potential anticancer effects of linezolid-oxaliplatine were investigated using annexin V-FITC in human colon cancer cell line (HCT-116) by flowcytometry.

Apoptosis detection kit with Annexin V-FITC conjugated (Invitrogen, Bioscience, Australia) was used to assess the apoptosis in colon cancer cell line induced by tested treatments. Three concentration were chosen for each group namely (1000, 250, 65.2 µg/ml) as that to assess the higher concentration, the middle concentration (which is approximately near the IC50 of each group), and the lowest concentration given inhibition in growth of the cells. The experiment was done according to the manufacturer's instructions [16].

Statistical analysis:

Statistical analysis was done by using SPSS version 23. Analysis of variance ANOVA test was used to compare mean of the different groups. P-value <0.05 was considered statistically significant. Variables were presented as mean ± SD with a 95% confidence interval. The IC50 of tested groups was calculated by Graph Pad Prism6 [17].

Results

1-The effect of linezolid-oxaliplatine combination on the growth of HCT-116 colon cancer cells:

The linezolid-oxaliplatine combination, oxaliplatine and linezolid cause highly significant ($P < 0.001$) inhibition to the growth of HCT-116 cells in compare to the negative control group in all concentrations except the concentrations of 3.1 µg/ml in which there were no significance differences from negative control groups in all treated groups (p-value > 0.05). the inhibition in growth percentages showed a dose-dependent manner in all treated groups. All concentrations of linezolid-oxaliplatine combination show highly significant ($P < 0.01$) inhibition in growth percentage in comparison to oxaliplatine and linezolid groups. The IC50 was 225 µg/ml of linezolid- oxaliplatine combination, 252 µg/ml of oxaliplatine and 237 µg/ml of linezolid, as shown in **table 1** and **figure 1.**

2- Apoptotic effects of the linezolid-oxaliplatine on the colon cancer cells

The results were expressed as plots by software as the lateral scattered as in the figure2 that reflect the apoptotic percentages for each tested sample:

Linezolid-oxaliplatine combination in all 3 concentrations, oxaliplatine and linezolid treated groups in both the 1000 and 250 µg/m concentrations showed a highly sinificant ($p < 0.01$) increase in apoptotic percentages on hct-116 cells in comparison to the negative control group. The 1000 µg/ml concentration of linezolid-oxaliplatine combination, oxaliplatine and linezolid treated groups showed high significant ($p<0.01$) increase in

apoptotic percentages comparing to other two concentrations 250 and 65.2 µg/ml. Linezolid-oxaliplatine combination in all 3 concentrations showed a high significant ($p<0.01$) difference from oxaliplatine, and linezolid treated groups. regarding the concentrations 250 and 65.2 µg/ml of oxaliplatine and linezolid treated groups they cause no significance ($p > 0.05$) from other as shown in **table 2** below.

Discussion

Antibiotics used in cancer treatment have different modes of actions. Several act as potent intercalating agents while others act by damaging the DNA. Many of these chemotherapeutic drugs targets DNA and is basically observed as a non-specific target of the cytotoxic agents [18].

Up to our knowledge this is the first study aim to investigate the effect of linezolid in the chemotherapeutic activity of oxaliplatine on CRC cell line. In the present study the concomitant use of linezolid with oxaliplatine enhance the chemotherapeutic effect of the later, which indicate the synergistic effect between oxaliplatine and linezolid. This result is in agreement with that reported by Etna *et al.* (2019) study which found that cells obtained from linezolid-administered mice demonstrated a decrease in oxygen consumption rate, suggesting that linezolid may reduce tumor growth rate of breast cancer and discriminate resistant and cancer stem cells (subtype of cancer cells with stem like properties, that may be responsible for tumor recurrence) [19].

Also our result is in agreement with that found by Duewelhenke *et al.* (2007) study which revealed that linezolid had cytotoxic effect on primary human osteoblasts and explained this by the impairment of mitochondrial energetic [20]. One of the approaches that can induce mitochondrial dysfunction and deplete the supply of energy to cancer cells is through targeting mitochondria using antibiotics [21,22] Evidence showed that some bactericidal antibiotics can induce mitochondrial dysfunction, suppress cancer cells growth and, possibly, tumors [23,24]. The linezolid- induced enhancement of oxaliplatine chemotherapeutic effect may be attributed to the ability of some antibiotics including linezolid to induce mitochondrial dysfunction in eukaryotic cells and decrease cancer cell growth [19,22,23].

It was suggested that antibiotics can interfere with mitochondrial function (induce autophagy in cancer cells by increasing mitochondrial and intracellular reactive oxygen species (ROS) [19]. The enhancement to ROS presence, can amplify cellular death pathways [25]. Previous studies reported that ROS stimulation is a potential therapeutic approach for treatment of cancer as several natural products exerting antitumor effects on human cancer cells by inducing ROS generation [26]. ROS may induce genomic instability by DNA mutations, and abnormal pro-tumorigenic signaling thus they have a role in tumor growth and progression. But in contrast, ROS high levels can potentially induce cell death because antioxidant capacity cannot exceed elevated levels of ROS, that intensely suggests that high levels of ROS have a strong role in tumorigenesis block [27].

Linezolid increase the level of ROS in cancer stem cells which approves that the mitochondria is responsible for the production of ROS [19]. Also our result is in parallel to that reported by Peihai *et al.* (2019) study which demonstrated that alantolactone (a natural sesquiterpene lactone, possesses strong antitumor activities) synergized oxaliplatine antitumor effect by inducing the generation of ROS [28].

In the present study the percentages of apoptosis which was significantly increased in a dose dependent manner in the group treated with oxaliplatine – linezolid combination may be related to linezolid-induced mitochondrial dysfunction as had been found by Fuji *et al.* (2018) who suggested that apoptosis occurred in the human leukemic monocyte lymphoma U937 cell line was related to mitochondrial damage induced by linezolid [29].

On the contrary, our result disagrees with that found by Hedaya *et al.* (2016) who revealed that linezolid lack anticancer activity against breast cancer cell lines [30]. this negative result could be explained by low linezolid concentrations used by Hedaya *et al.* study as compared to the concentrations used by our study.

References

1- Stein A, Atanackovic D, Bokemeyer C. Current standards and new trends in the primary treatment of colorectal cancer. Eur. J. Cancer; 2011, 47 Suppl 3:S312-4.

2- Dallas N, Xia L, Fan F, Gray M, Gaur P, van Buren G, *et al,* Chemoresistant colorectal cancer cells, the cancer stem cell phenotype, and increased sensitivity to insulin-like growth factor-I receptor inhibition. Cancer Res. 2009;69(5):1951-7.

3- Noh SH, Park SR, Yang HK, Hyun Cheol Chung , Ik-Joo Chung , Sang-Woon Kim. *et al,*. Adjuvant capecitabine plus oxaliplatine for gastric cancer after D2 gastrectomy (CLASSIC): 5-year follow-up of an open-label, randomised phase 3 trial. Lancet Oncol; 2014, 15(12):1389-96.

4- Ramanathan RK, Clark JW, Kemeny NE, Lenz HJ, Gococo KO, Haller DG, *et al,* Safety and toxicity analysis of oxaliplatine combined with fluorouracil or as a single agent in patients with previously treated advanced colorectal cancer. J. Clin. Oncol. 2003;21(15):2904-11.

5- Thomas H, Coley H. Overcoming multidrug resistance in cancer: an update on the clinical strategy of inhibiting p-glycoprotein. Cancer Control, 2003;10(2):159-65.

6- Demols A, Peeters M, Polus M, Marechal R, Gay F, Monsaert E, *et al,* Gemcitabine and oxaliplatine (GEMOX) in gemcitabine refractory advanced pancreatic adenocarcinoma: a phase II study. Br. J. Cancer 2006;94(4):481-5.

7- Petrioli R, Francini E, Cherri S, Torre P, Fiaschi A, Miano S, *et al,*. Capecitabine Plus Oxaliplatine and Bevacizumab, Followed by Maintenance Treatment With Capecitabine and Bevacizumab for Patients Aged > 75 Years With Metastatic Colorectal Cancer. Clin Colorectal Cancer 2018.

8- Roger C, Roberts JA, Muller L). "Clinical Pharmacokinetics and Pharmacodynamics of Oxazolidinones". *Clinical Pharmacokinetics*. May 2018**; 57** (5): 559–575.

9- Mendes RE, Deshpande LM, Jones RN. "Linezolid update: stable in vitro activity following more than a decade of clinical use and summary of associated resistance mechanisms". *Drug Resistance Updates*. April 2014; **17** (1–2): 1-12.

10- Marino PL, Sutin KM "Antimicrobial therapy". *The ICU book*. Hagerstown, MD: Lippincott Williams & Wilkins. 2007; p. 817. ISBN 978-0-7817-4802-5.

11- Adams T, Anwar R, Mfarej M, Rundatz T, Coyle M, Mclaughlin JS. Nutritional Stress of Cultured Vero Cells Causes Altered Growth and Morphology as Seen in Neoplastic Transformation. Am J Undergrad Res. 2015;12(3):63–75.

12- Feoktistova M, Geserick P, Leverkus M. Crystal violet assay for determining viability of cultured cells. Cold Spring Harb Protoc.;2016(4):343–6.

13- Karpakavalli M, Kumar P, Sanglimuthu A, Kumar EP. Docking, synthesis, characterization and anticancer activity of 4-(4'-hydroxy, 3'-methoxy) phenyl, but-2-one-3-ene, a curcumin analogue precursor. Int J Curr Pharm Rev Res. 2016;8(4):1–5.

14- Kuppusamy, P. Ichwan SJ, Al-Zikri PN, Suriyah WH, Soundharrajan I, *et al,*. 'In Vitro Anticancer Activity of Au, Ag Nanoparticles Synthesized Using Commelina nudiflora L. Aqueous Extract Against HCT-116 Colon Cancer Cells', Biological Trace Element Research, 2016;173(2), pp. 297–305.

15-Tong, G.-X., Jing Chai, Jing Cheng, Yi Xia, Rui Feng, *et al,*. 'Diagnostic Value of Rectal Bleeding in Predicting Colorectal Cancer: a Systematic Review', Asian Pacific Journal of Cancer Prevention, 2014; 15(2), pp. 1015–1021.

16- Longley DB, Johnston PG. Molecular mechanisms of drug resistance. J Pathol.2005;205:275–92.

17- Tacar, O., Sriamornsak, P. and Dass, C.R. Doxorubicin: An Update on Anticancer Molecular Action, Toxicity and Novel Drug Delivery Systems. *Journal of Pharmacy and Pharmacology*, 2013; **65**, 157-170.

18- Nelson H, Petrelli N, Carlin A, Couture J, Fleshman J, Guillem J, et al. Guidelines for colon and rectal cancer surgery. J Natl Cancer Inst. 2001;93(8):583–596.

19-Etna Abad, Yoelsis García-Mayea, Cristina Mir, David Sebastian, Antonio Zorzano, David Potesil, *et*

al,. Common Metabolic Pathways Implicated in Resistance to Chemotherapy Point to a Key Mitochondrial Role in Breast Cancer. Molecular and Cellular Proteomics. 2019; 18: 231–244.

20-Duewelhenke N., Krut O., and Eysel P. Influence on Mitochondria and Cytotoxicity of Different Antibiotics Administered in High Concentrations on Primary Human Osteoblasts and Cell Lines. Antimicrobial Agents and Chemotherapy. 2007; 51 (1): 54–63.

21-Esner, M., Graifer, D., Lleonart, M. E., and Lyakhovich, A. Targeting cancer cells through antibiotics-induced mitochondrial dysfunction requires autophagy inhibition. *Cancer Lett.* 2017; **384,** 60–69.

22-Lleonart, M. E., Grodzicki, R., Graifer, D. M., and Lyakhovich, A. Mitochondrial dysfunction and potential anticancer therapy. *Med. Res. Rev.* 2017; **37,** 1275–1298.

23-Kalghatgi, S., Spina, C. S., Costello, J. C., Liesa, M., Morones-Ramirez, J. R., *et al,*. Bactericidal antibiotics induce mitochondrial dysfunction and oxidative damage in Mammalian cells. *Sci. Transl. Med.* 2013; **5,** 192ra85.

24-Moullan, N., Mouchiroud, L., Wang, X., Ryu, D., Williams, E. G., *et al,*. Tetracyclines disturb mitochondrial function across eukaryotic models: A call for caution in biomedical research. *Cell Rep*. 2015; **10,** 1681–1691.

25-Lyakhovich, A., and Graifer, D. Mitochondria-mediated oxidative stress: Old target for new drugs. *Curr. Med. Chem.* 2015; **22,** 3040–3053

26- Peng Zou, Yiqun Xia, Jiansong Ji, Weiqian Chen, Jinsan Zhang, Xi Chen. *et al.* Piperlongumine as a direct TrxR1 inhibitor with suppressive activity against gastric cancer. Cancer Lett. 2016;375(1):114-126;

27-Andrea Glasauer and Navdeep S.Chandel. Targeting antioxidants for cancer therapy. Biochemical Pharmacology. 2014; 92 (1): 90-101.

28-Fuji Satoshi, Muraoka Sanae, Miyamoto Atsushi and Sakurai Koichi. Linezolid-induced Apoptosis through Mitochondrial Damage and Role of Superoxide Dismutase-1 in Human Monocytic Cell Line U937. Yakugaku zasshi journal of the Pharmaceutical Society of Japan. 2018; 138(1):73-81.

29-Peihai Cao, Yiqun Xia, Wei He1, Tingting Zhang, Lin Hong, Peisen Zheng, *et al,*. Enhancement of oxaliplatine-induced colon cancer cell apoptosis by alantolactone, a natural product inducer of ROS. International Journal of Biological Sciences. 2019; 15(8): 1676-1684.

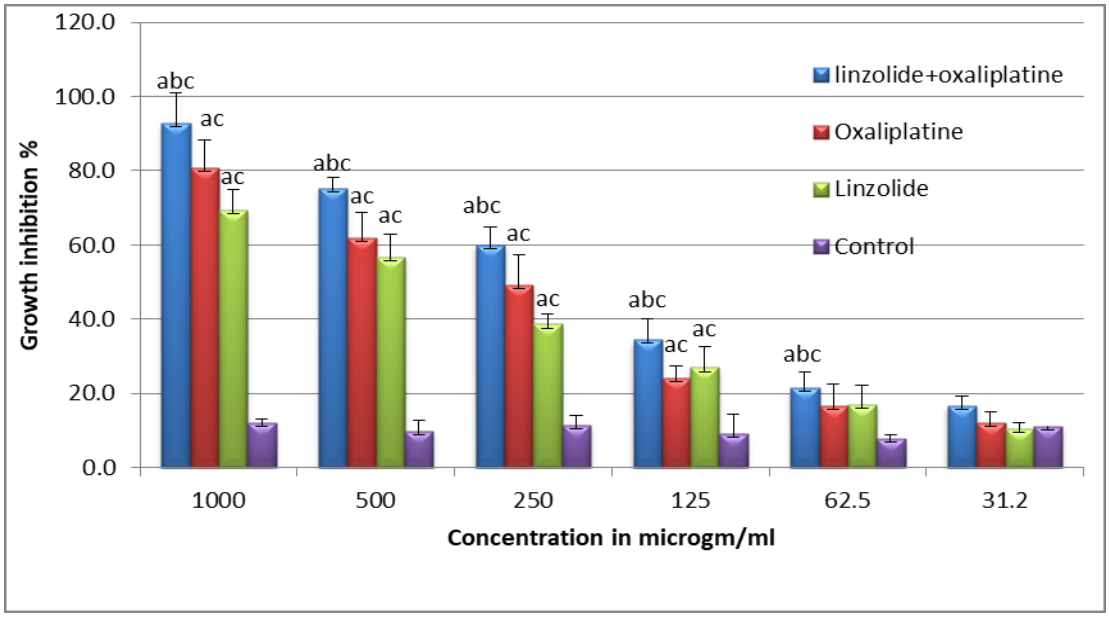
30-Hedaya M. Omar, Mathew M. Princy, Mohamed H. Fatima, Phillips A. Oludotun and Luqmani A. Yunus. Antiproliferative activity of a series of 5-(1H-1,2,3-triazolyl) methyl- and 5-acetamidomethyl-oxazolidinone derivatives. Molecular Medicine Reports. 2016; 13: 3311-3318.

Table (1): the Percentage of inhibition of growth of colon cancer cells and the IC50 produced by different concentrations of different tested groups

Conc. in µg / ml	Inhibition of cell growth percentages (Mean ± SD) for each concentration			
	Linezolid-oxaliplatine combination group	Oxaliplatine group	Linezolid group	Negative control group
1000	92.8±8.3 [abc]	80.75±7.6 [ac]	69.33±5.5 [ac]	12±1
500	75.14±3 [abc]	61.94±6.7 [ac]	56.72±6 [ac]	9.93±1.3
250	60.04±4.6 [abc]	49.12±8 [ac]	38.62±2.6 [ac]	11.43±1.5
125	34.49±5.5 [abc]	24.16±3 [ac]	26.89±5.7 [ac]	9.03±3.5
62.5	21.47±4.2 [abc]	16.77±5.8	16.82±5.2	7.72±1
31.2	16.77±2.4	12.08±3	10.51±1.5	11.05±0.6
IC50	225 µg / ml	252 µg / ml	237 µg / ml	

p-values <0.01.

a= significant difference from negative control group. b= significant difference from other treated groups. c= significant difference from other concentrations within same treated group.



Figure(1): Percentage of inhibition of growth of colon cancer cells produced by different concentrations of different tested groups. a= significant difference from negative control group. b= significant difference from other treated groups. c= significant difference from other concentrations within same treated group. p-values <0.01.

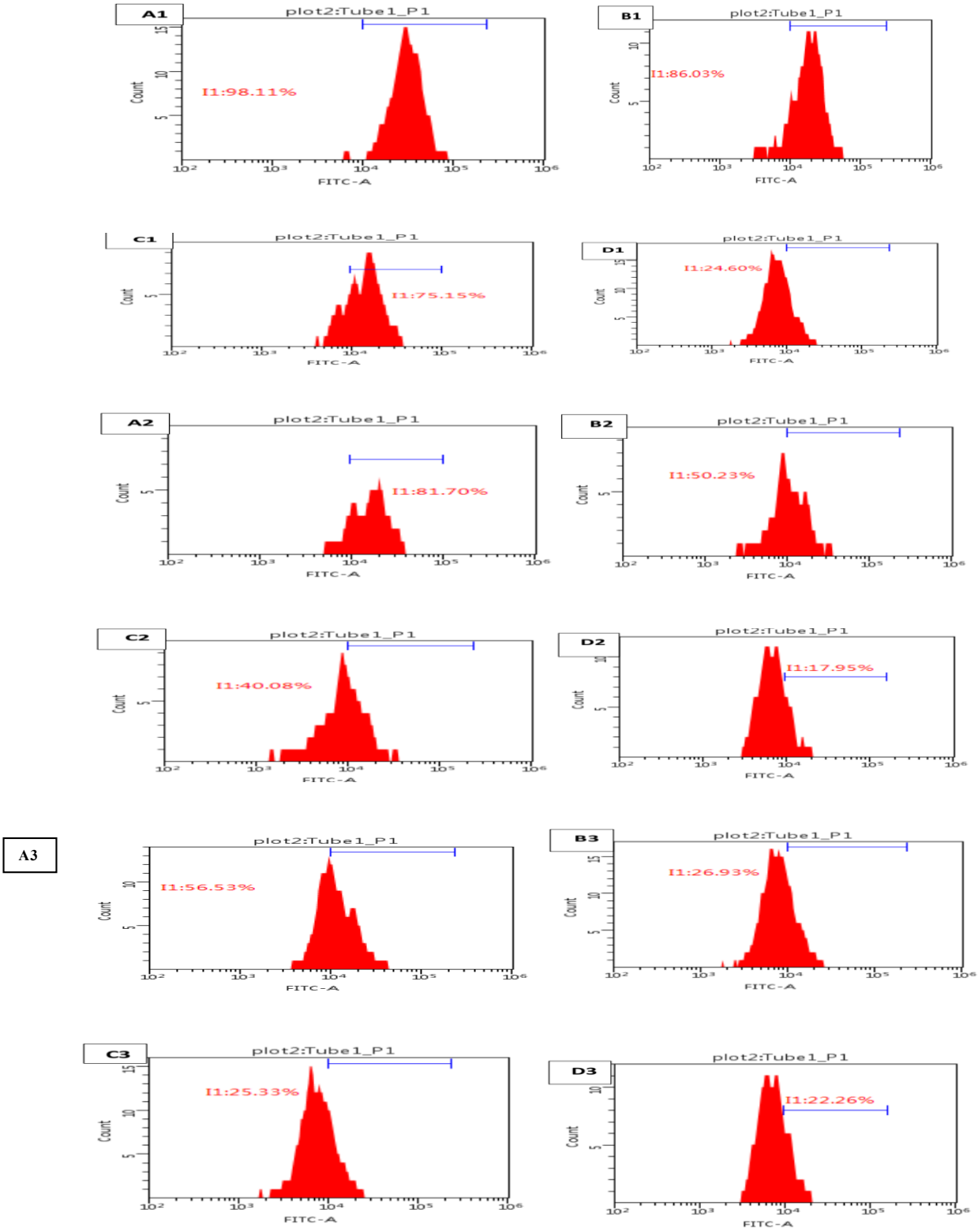


Figure (2) Flowcytograms of apoptotic percentages for different tested groups on HCT-116 colon cancer cells

A: linezolid oxaliplatine combination group B: oxaliplatine group C: linezolid group D: control group.

While 1,2,3 refer to the three concentrations tested: 1= 1000 µg/ml, 2= 250 µg/ml, 3= 65.2 µg/ml.

Table 2: Apoptotic percentages of different tested groups on human colon cancer cell line

	Percentages of apoptosis percentages in (Mean ± SD) for each concentration		
drugs	**1000 (µg/ml)**	**250 (µg/ml)**	**62.5 (µg/ml)**
linzolide+oxaliplatine	97.6+2.1 [abc]	80.3+1.4 [abc]	53.4+2.8 [abc]
oxaliplatine	84.5+1.5 [abc]	55.8+5.4 [ab]	26.5+2.5
Linezolid	73.7+2.3 [ac]	38.3+1.7 [a]	24+2.8
-ve control	22.4+2.2	19+2.1	23.3+3.1

p-values <0.01.

a: significant difference from negative control group. b: significant difference from other treated groups. c: significant difference from other concentrations within same treated group .