



Potential of Cisplatin Activity in Colorectal Cancer Cells by Lovastatin

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Author's contributions

The sole author designed, analysed, interpreted and prepared the manuscript.

Article Information

DOI: 10.9734/JPRI/2019/v28i130191

Editor(s):

(1) Dr. Wenbin Zeng, Professor, Xiangya School of Pharmaceutical Sciences, Central South University, Changsha, China.

Reviewers:

(1) Michael Bordonaro, Geisinger Commonwealth School of Medicine, USA.

(2) Irfan Ahmad Khan, Aligarh Muslim University, India.

Complete Peer review History: <http://www.sdiarticle3.com/review-history/49489>

Original Research Article

Received 24 March 2019

Accepted 05 June 2019

Published 12 June 2019

ABSTRACT

Cisplatin (CIS) is an anticancer drug used in the treatment of several solid tumors with nephrotoxicity as its main toxic effect. The current study was directed to assess the role of hypolipidemic drug (lovastatin) on sensitization of human colorectal cancer cells (HCT -116) to the action of CIS.

This study assessed the action of lovastatin on sensitization of colorectal cancer cells to cisplatin by examining cisplatin cytotoxicity, cisplatin cellular uptake and P-glycoprotein (P-gp) activity in presence and absence of Lovastatin 10 and 30 µg/ml. Lovastatin treatment at dose level of 10 and 30 µg/ml potentiated the cytotoxic effect of cisplatin against the growth of HCT-116 cells with IC₅₀ 7.3 and 5.4 µg/ml, respectively compare to 18 µg/ml after treatment with cisplatin alone.

Moreover, lovastatin increased the uptake of cisplatin into colorectal cancer cells with marked inhibition of P-glycoprotein pump.

On conclusion Lovastatin treatment increases the antiproliferative activity of cisplatin against the growth of colorectal cancer cells due to inhibition the activity P-glycoprotein pump with marked increase in its cellular uptake.

Keywords: *Cisplatin; lovastatin; colorectal cancer cells; interaction.*

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1. INTRODUCTION

Low incidences of CRC have been evident in the Kingdom of Saudi Arabia. Nevertheless, incidence of the CRC and the relative rates of mortality have been seen to rise constantly in this nation for more than twenty years ago. Saudi Arabia now leads in the ranking of cancer in men and falls in the third position in women [1,2]. The major regimen for cancer treatment is surgery followed by chemotherapy. CIS is among the active cytotoxic elements in the present use. There is evidence of its efficacy in a cancer disease when use alone or in combination with other cytotoxic drugs. The detrimental effects such as nephrotoxicity has been associated with its use which may have hindered its clinical importance [3,4]. Additionally, the development of chemoresistance by the tumors presents a major obstacle in cancer treatment. Among the strategies that can help to overcome the challenge of chemoresistance is chemosensitization. This strategy involves the use of a single drug to improve the action of another drug via modulation of one or more resistance mechanisms. Different approaches have been adopted to minimize the side effects of CIS while improving its anticancer efficiency [5]. One of the approaches involves looking for natural agents with chemoprevention that can be coupled with CIS. Lovastatin is an inhibitor of β -Hydroxy- β -methylglutaryl-CoA reductase (HMG-CoA reductase) that reduces cholesterol biosynthesis by altering the formation of mevalonate. HMG-CoA reductase inhibitors inhibit cellular proliferation and induce cell death [6-10], might make them potentially antineoplastic compound. However, using HMG-CoA reductase inhibitors in the protocols of cancer treatment, especially solid tumors, has not been established due to the relation between the high dose which is effective in inhibition of proliferation and increase in apoptosis may be associated with significant toxicity. So the goal of our research directed to assess whether lovastatin may enhance the cytotoxic effects of CIS against the growth of colorectal HCT-116 cells. The possible mechanism of interaction between CIS and lovastatin were also explored, regarding CIS uptake in presence and absence of Lovastatin and P-glycoprotein activity after combination treatment.

2. MATERIALS AND METHODS

2.1 Drugs and Chemicals

Cisplatin "Ebewe" and lovastatin were obtained from Aldrich Chem. Corp., USA). CIS (0.5 mg/ml)

is an aqueous isotonic solution for intravenous use and stored at room temperature (not exceeding 25°C) and protected from light and diluted in PBS, while lovastatin supplied as White powder and stored at -20 C. RPMI media, 0.25% trypsin w/v and 0.02% EDTA w/v, Phosphate buffer saline (PBS), Acetic acid, Penicillin G and streptomycin, Trizma base, SulphoRhodamine-B (SRB), were purchased from Aldrich Chem. Corp., USA.

2.2 Cells and Cell Cultures

Colorectal cancer cell line HCT-116, has been used in this study. It has been obtained from National Cancer Institute, Cairo University, Egypt. All cells were grown as monolayer culture and maintained in RPMI-1640 tissue culture medium (supplemented with antibiotics and 10% fetal bovine serum) at 37°C in humidified 5% CO₂ atmosphere and were sub-cultured every 5 days.

2.3 METHODS

2.3.1 Determination of cytotoxic activity

Cytotoxic activity of CIS and CIS plus lovastatin was assessed using sulforhodamine-B method as previously mentioned by Skehan et al. [11].

Cells were cultured in microtiter plates (96 wells) at a concentration of 40×10^3 cells/well in RPMI 1640 medium. The cells kept to attach for 24h then the cells incubated with various concentration of CIS and lovastatin for 48 hours in the following doses (1.25, 2.5 - 20 μ g/ml) for CIS and 10 and 30 μ g/ml for lovastatin (3 wells for each concentration). Drugs were added in simultaneous way. After 48 hours, Cells were fixed by adding 50 μ l of 50% cold TCA for 1 hour at 4 C. The supernatant was then removed, plates were rinsed five times with bidistilled water, air dried, stained with 100 μ l of (SRB) solution 0.4% (w/v) in 1% acetic acid for 30 min and then unbound dye was discarded by rinsing 5 times with 1% acetic acid and plates were air dried. Solubilize the stain with 10 mM Tris base (100 μ l/well, PH 10.5) for 10 minute. The optical density (OD) was read in microplate reader (BioTek, U.S.A.) at wavelength of (490-530) nm.

Surviving fraction = optical density of treated cells / Optical density of control cells
 IC_{50} (the concentration of CIS necessary to produce 50% inhibition of cell growth) was calculated from linear regression equation of the survival fraction curve

$$Y = MX + b$$

Where:

Y=0.5 (the surviving fraction when there is a 50% inhibition of cell growth).

M=the slope

X=dose of CIS showed 50% inhibition.

b=the y-intercept.

2.3.2 Detection of P-glycoprotein activity using rhodamine 123 (RD-123)

P-glycoprotein activity in colorectal cancer cells was assessed after 48 hours of cisplatin treatment according to ludescher et al.[12].The method depends on the properties of fluorescence emitted by RD-123 dye which is transported by the membrane efflux pump P-gp. In brief, Cells were cultured in microplate (6 well plates) at density of 2×10^6 cells/well in RPMI 1640 medium and culture as described before. The cells incubated in CO₂ incubator at 37 °C for at least 24 hrs before treatment. After that the cells incubated with CIS concentration (20 µg/ml) alone or with lovastatin 10 and 30 µg/ml for 48 hrs (3 wells for each concentration), cells were detached from the wells by trypsinization, washed with phosphate buffered saline and suspended again in 2 ml RPMI media with 200 ng/ml RD-123 and incubated for one hour at 37°C. Then cells washed with cold PBS and divided into 2 parts. For accumulation study, the first part of HCT-116 cells were resuspended in 2 ml cold PBS and the fluorescence intensity was quantified by spectrofluorometer (SFM 25 kontron, Italy) at 470 and 590 excitation and emission wave lengths, respectively. For efflux/retention study, the second part of the cells was resuspended in 2 ml RD-123- free RPMI medium for additional 30 min at 37°C. Thereafter, RD-123 efflux/retention was determined as previously mentioned in the accumulation experiment. The function of P-gp was then estimated as the amount of effluxed RD-123 per 30 min using the following formula:

$$\text{Function of P-gp (\%)} = \frac{\text{RD-123 accumulation} - \text{RD-123 retention}}{\text{RD-123 accumulation}}$$

2.3.3 Assessment of CIS cellular uptake

Cells were cultured in microplate (6 well plates) at

density of 10^5 - 10^6 cells/well in RPMI 1640 supplemented medium and culture as described before. The cells incubated in CO₂ incubator at 37 °C for at least 24 hrs before treatment. After that the cells incubated with CIS concentrations (5 and 20 µg/ml) alone or with lovastatin 30 µg/ml for 48 hrs (3 wells for each concentration). Following trypsinization, cells washed with phosphate buffered saline and counted. For CIS uptake analysis, one million cells were suspended in 1% HNO₃ for 24 hrs at 70 °C. Lysed cells were analyzed by inductively coupled plasma mass spectrometry (ICP-MS, Thermoscientific, ICP 6000 series, USA).

2.4 Statistical Analysis

Statistical analysis of data was calculated by using statgraphic computer package (Excel, 2010) and computer program package (SPSS, version 18). All data expressed as mean with their standard deviation (SD) of three separate experiments, each one in triplicate. One way analysis of variance (ANOVA) was used to test for difference between experimental groups. It was followed by the least significance difference (LSD) test. However, two-sample t-test and its P-value to analyze the significance of the difference in the sample means. Differences were considered significant at P <0.05.

3. RESULTS

3.1 Effect of Lovastatin Treatment on the Cytocidal Activity of Cisplatin.

The Cytotoxic activity of CIS against the colorectal cancer cells HCT-116 was expressed as the surviving fraction resulted after treatment in compare to the untreated control (Fig. 1 and Table 1).The HCT-116 cells treated with several concentration of CIS had an IC₅₀ value of 18 µg/ml, simultaneous treatment with lovastatin 10 and 30 µg/ml resulted in IC₅₀ of 7.3 and 5.4 µg/ml, respectively. Lovastatin treatment showed no cytotoxic activity either at 10 or 20 µg/ml (data not shown).

3.2 Effect of CIS and/or Lovastatin on P-gp Function of HTC-116 Cells

Fig. 2 showed that Cisplatin treated cells clearly accumulated less amount of RD-123 compared with those treated with CIS + lovastatin ($16 \text{ ng}/10^8$ cells compared to $35 \text{ ng}/10^8$ cells, respectively).

3.3 Effect of Lovastatin on the Uptake of CIS in HTC-116 Cells

Treatment of HTC-116 cells with CIS (5 µg/ml) showed CIS cellular uptake of 4.3 ng/10⁶ cells, while simultaneous addition of Lovastatin (30 µg/ml) showed CIS uptake of 16.2 ng/10⁶ cells, (Table 2). Moreover, using CIS at a dose of 20 µg/ml showed cellular level of 72.7 ng/10⁶ cells, compare to 110.8 after addition of Lovastatin.

Function of P-glycoprotein (%) = $\frac{RD}{RD_{accumulation}} \times \frac{100}{RD_{retention}}$

The growing cells were treated with Cisplatin (20µg/ml) and/or Lovastatin (30 µg/ml) given simultaneously for 48 h, then the cells washed once with PBS and the cells harvested, and counted and digested by using 1% nitric acid.

Determination of cisplatin uptake by inductively coupled plasma mass spectrophotometry (ICP-MS).

4. DISCUSSION

Cisplatin represents a unique and important drug, with broad effects in numerous human malignancies, either alone or in combination with other cytotoxic compounds. However, its uses have been hampered by severe side effects including renal toxicity [3,4]. To decrease the dose and consequently the side effects, various approaches have been attempted. One of these approaches is the search for some agents with high ability to potentiate the anticancer activity of the cytotoxic drugs. Therefore, in the present study, the effects of Lovastatin (lipid lowering drugs) on cytotoxic activity of CIS against the growth of colorectal cancer HCT - 116

Table 1. Effect of cisplatin and/or lovastatin on growth of the human colorectal cancer cells HCT-116

Treatment	IC50 (µg/ml)
CIS	18 ± 4.2
CIS + Lovastatin (10µg/ml)	7.3± 0.52a
CIS + Lovastatin (30 µg/ml)	5.4 ± 0,35a,b

Data expressed as the mean± S.D. of three separate experiments, each one in triplicate. IC50 the concentration of CIS necessary to produce 50% inhibition in the growth of the cells.^aP≤ 0.01 as compared with the corresponding CIS treatment alone ^bP<0.05, as compared with the CIS + Lovastatin (10 µg/ml)

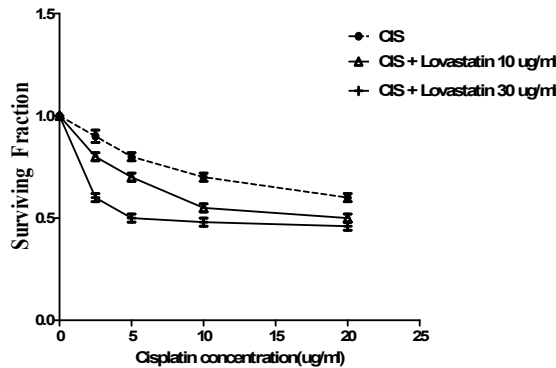


Fig. 1. Effect of cisplatin (CIS) and/or lovastatin treatment on the growth of HCT-116 human colorectal cancer cells

Data expressed as the mean± S.D. of three separate experiments, each one in triplicate

Table 2. Cisplatin uptake by colorectal cancer cells HCT-116 in presence of lovastatin

CIS concentration (µg/ml)	CIS concentration in HCT-116 cells (ng/10 ⁶ cells)	
	CIS	CIS + Lovastatin (30 µg/ml)
5	4.36 ± 1.34	16.2± 3.3 a
20	72.7 ± 12.9	110.8 ± 24 b

Data expressed as mean ± S.D of three separate experiments .a, b significantly different from corresponding CIS at ^aP-value < 0.05, ^bP-value < 0.01)

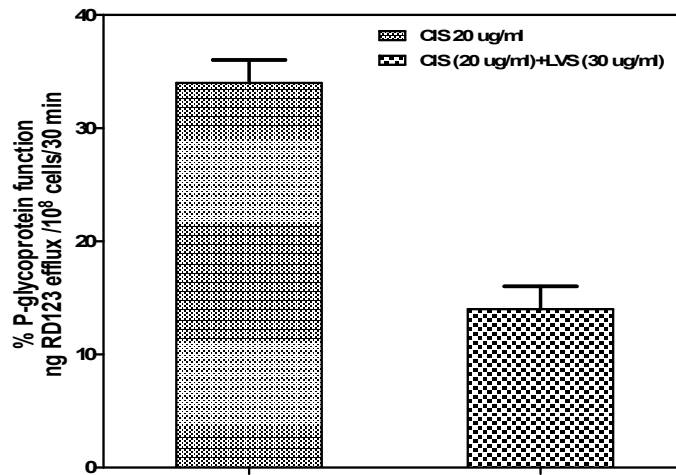


Fig. 2. Effect of cisplatin and cisplatin + lovastatin (LVS) on P-glycoprotein function in colorectal cancer cells

Data expressed as mean \pm S.D. ^a Significant different from CIS at $p \leq 0.001$

cells were determined. Different concentrations of CIS were shown to be cytotoxic to the HCT-116 cell line.

It is currently known that platinum analogs destroy cancer in all stages of cell cycle and interacts with DNA by intra- and inter-strand cross links, leading to inhibition of DNA synthesis and function [13,14]. Decreasing intracellular CIS accumulation has been identified as a major cause of acquired resistance, and may be due to either inhibited drug uptake or increased drug efflux [15]. The present study showed more CIS uptake in the HCT-116 cells by Lovastatin (Fig. 1 and Table 2). It has been reported that Lovastatin pretreatment induced significant apoptosis induced by 5-fluorouracil (5-FU) or cisplatin in four colon cancer cell lines. Moreover, treatment with Lovastatin resulted in decreased expression of the anti-apoptotic protein bcl-2 and increased the expression of the proapoptotic protein, bax [16]. The increased in CIS uptake into HCT-116 cells after treatment with Lovastatin has been explained due to decrease in P-gp expression [17,18]. In our study simultaneous treatment of CIS + Lovastatin showed a decrease in P-gp function (about 2 fold decrease) which indicate that lovastatin has the ability to inhibit the CIS efflux from HCT-116 cells, So increase its cellular accumulation and its cytotoxic activity. Furthermore, future work may also look at lovastatin in an APC mutant colorectal cancer cell line.

5. CONCLUSION

In conclusion, lovastatin treatment increased cytotoxic activity of CIS against the growth of colorectal cancer cells through inhibition of P-gp pump and so increased its cellular uptake and its cytotoxic activity.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All studies approved by the ethical research committee unit of College of Medicine, King Abdulaziz University (Reference 223-19).

ACKNOWLEDGEMENTS

The present study was funded by a grant from the Deanship of Scientific Research, King Abdulaziz University, Jeddah, Saudi Arabia.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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