Research Article

Effect of teicoplanin on the expression of c-myc and c-fos proto-oncogenes in MCF-7 breast cancer cell line

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Abstract

Background: Teicoplanin is a member of vancomycin-ristocetin family of glycopeptide antibiotics. It mediated wound healing by increasing neovascularization possibly through activation of MAP kinase signaling pathway. The aim of this study is an evaluation of c-myc and c-fos genes expression after treatment of cells by teicoplanin and determines whether this glycopeptide antibiotic exerts its proliferation effects by influencing the expression of these genes. Hence, this study was designed to elucidate one possible mechanism underlying teicoplanin effects on cell proliferation using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay.

Materials and Methods: Breast cancer cell line, MCF-7, was cultured, and three different concentrations of teicoplanin were added to the plates. We measured the cell proliferation rate by MTT assay. After cell harvesting, total RNA was extracted to synthesize single-stranded cDNA. Real-time polymerase chain reaction was performed, and the data were analyzed.

Results: It was observed that the level of c-fos and c-myc genes' expressions was decreased at all three different concentrations of teicoplanin.

Conclusion: it could be concluded that although teicoplanin is considered as an enhancing cell growth and proliferation, but probably its effect is not through MAP kinase signaling pathway or perhaps even has inhibitory effect on the expression of some genes such as c-myc and c-fos in this pathway. Hence, the mechanism of action of teicoplanin for increasing cell propagation, through cell signaling pathways or chromosomal abnormalities, remains unclear, and further studies should be conducted.

Key Words: Breast cancer, c-fos, c-myc, MCF-7, teicoplanin

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INTRODUCTION

Teicoplanin is an antibiotic produced by actinoplanes teichomyceticus and a member of vancomycin-ristocetin family of glycopeptide antibiotics. Teicoplanin is mainly used to treat bacterial infections (caused by Gram-positive pathogens) which are resistant to frequently used antibiotics.[1]

It was observed that teicoplanin mediated wound healing by inhibiting bacterial growth and accelerating

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repairing process. In a study, the effect of vancomycin on renal proximal tubule epithelial cells was assessed. It was observed that exposure of these cells to vancomycin leads to an increase in cell proliferation by increasing the number of cells, total protein, and DNA synthesis. It was shown that vancomycin induces its proliferation effect by activation of MAP kinase (mitogen-activated protein kinase) signaling pathway. When cells pretreated by an MAP kinase inhibitor, these cells do not enter into the cell cycle even when treated with vancomycin.[2] MAP kinase signaling pathway intensifies and conveys signals from the wide range of stimuli, and causes a physiological response such as cell proliferation, development, differentiation, apoptosis, and inflammation in mammals. MAP kinase mediates cellular responses by protein phosphorylation and activation of the transcription of different genes, including genes encoding nuclear transcription factors such as c-myc and c-fos.[3]

The c-myc proto-oncogene is cellular homologous of the viral v-myc gene and a member of myc family. It encodes a transcription factor which is a necessary part of normal cell proliferation machinery. In normal cells, mitogenic stimuli induce c-myc gene expression which is required for normal cell propagation and preventing cell differentiation. Increased expression of short-lived c-myc protein makes it oncogenic which subsequently will be involved in the progression of various cancers.[4-6]

C-fos is also a proto-oncogene that has a viral homologous, v-fos. C-fos is a member of Fos family which includes transcription factors such as FosB, Fra-1, Fra-2, FosB2, and ΔFosB.[7] This gene is located on 14q21-31 and encodes c-fos protein, a 62 kDa protein that makes a heterodimer with c-jun (a transcription factor of Jun family) and forms AP-1 complex. This complex binds to DNA at its specific sites in promoters and enhancer regions and causes changes in gene expression patterns. Normally, c-fos protein plays a major role in several cellular functions, and its overexpression has been observed in many neoplasias.[8]

As teicoplanin and vancomycin belong to the same antibiotic family (vancomycin-ristocetin), this hypothesis is proposed that teicoplanin acts via the same mechanism as vancomycin that is through MAP kinase signaling pathway. The aim of this study is to evaluate the expression of c-myc and c-fos genes after treating cells by teicoplanin and determines whether this glycopeptide antibiotic exerts its proliferation effects through affecting the expression of these genes. Hence, this study was designed to elucidate one possible mechanism underlying teicoplanin effects on cell proliferation. This may be applicable for therapeutic purposes.

MATERIALS AND METHODS

Cell culture

Breast cancer cell line, MCF-7, was cultured in RPMI-1640 culture medium (Invitrogen) supplemented with 100 units/mL penicillin G, 100 μg/mL streptomycin, and 10% fetal bovine serum. Cells were incubated at 37°C in a humidified atmosphere supplemented with 5% CO_2. After cells reached to 70–80% confluence, teicoplanin was added to the plates at concentrations of 130, 520, and 890 μg/ml. After incubating for 68 h, cells were harvested. The concentrations and time were selected according to the previous study.[9]

RNA extraction and real-time polymerase chain reaction

Total RNA was extracted by RNX-Plus kit (Cinaclon, Iran) according to the manufacturer's protocol. Then, total RNA was treated with DNase I before cDNA synthesis to avoid DNA contamination. One microgram of total RNA was used to generate single-stranded cDNA using RevertAid™ First Strand cDNA Synthesis Kit and random hexamer primers (Thermo Fisher Scientific, Germany) according to the manufacturer's protocol.

The real-time polymerase chain reaction (RT-PCR) was performed using SYBR Green/Rox quantitative PCR (qPCR) Mastermix (Thermo Fisher Scientific, Germany) and the StepOne Plus™ qRT-PCR detection System (Applied Biosystems, USA). The reactions were conducted in 20 μl with 10 μl SYBR Green/Rox qPCR Mastermix, 200 μM forward and reverse primers and 1.5 μl cDNA. The following PCR cycling program was used: Primary denaturation in 95° for 10 min, denaturation in 95° for 15 s, annealing and extension in 60° for 1 min for 40 cycles and finally melt curve (increment 0.3°C, 60°C→95°C) analysis. All experiments were performed in triplicates for each sample. The genes of interest were normalized against the reference gene glyceraldehyde-3-phosphate dehydrogenase. The sequences of specific primers used for RT-PCR are given in Table 1.

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide assay

Tetrazolium colorimetric assay was carried out using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), to determine whether teicoplanin had any effect on inhibition of MCF-7 cell proliferation or not. So that, 200 μl medium containing 20 μl MTT reagent (5 mg/mL, Sigma-Aldrich, Saint

Table 1: Sequences of primers used in this study

Primer	Sequence	Length (bp)
c-MYC-F	5′-GTAGTGGAAAACCAGCAGCC-3′	20
c-MYC-R	5′-AGAAATACGGCTGCACCGAG-3′	20
c-FOS-F	5′-GGGGCAAGGTGGAACAGTTA-3′	20
c-FOS -R	5′-AGTTGGTCTGTCTCCGCTTG-3′	20
GAPDH-F	5′-AAGCTCATTTCCTGGTATG-3′	19
GAPDH -R	5′-CTTCCTCTTGTGCTCTTG-3′	18

GAPDH: Glyceraldehyde-3-phosphate dehydrogenase

Louis, USA) was added to the wells and incubated for 3 h at 37°C; the medium was replaced with 200 μL DMSO for dissolving Formazan crystals. The absorbance was determined at 570 nm (A_{570}) using a 96-well plate reader (MRX, Dynex, USA).

Analysis of data

The SPSS version 20 (IBM, NY, USA) was utilized for statistical analyses and $P < 0.05$ was considered as statistically significant. The comparative Ct (ΔΔCt) method was used to calculate fold changes in gene expression. In addition, the results were statistically analyzed using independent sample *t*-test.

RESULTS

Cell proliferation showed significant difference between cells which were treated with different concentrations of teicoplanin in mock and negative control groups ($P < 0.05$) [Figure 1]. Relative mRNA expression of c-myc in different concentrations of teicoplanin in MCF-7 cells is shown in Figure 2a. Cells were preincubated with 130, 520, 890 μg/ml concentrations of teicoplanin for 68 h. In addition, at all concentrations of teicoplanin the level of mRNA expression of c-myc compared to the reference gene was significantly decreased ($P < 0.05$). Meanwhile, the level of mRNA expression of c-myc after 68 h preincubation of cells with 890 μg/ml of teicoplanin was significantly higher compared to the level of mRNA expression of c-myc after preincubation with concentrations of 130 and 520 μg/ml of teicoplanin ($P < 0.05$). In addition, there is nonsignificant difference between c-myc expression levels at 130 and 520 μg/ml concentrations of teicoplanin ($P < 0.05$).

Figure 2b shows relative mRNA expression of c-fos in different concentrations of teicoplanin in MCF-7 cells. Cells were preincubated with 130, 520, 890 μg/ml concentrations of teicoplanin for 68 h and it was observed that at all three concentrations of teicoplanin the level of mRNA expression of c-fos compared to the reference gene was significantly decreased ($P < 0.05$). The treated MCF-7 cells with 890 μg/ml of teicoplanin exhibited a statistically nonsignificant difference in the level of mRNA expression of c-fos compared to 130 and 520 μg/ml concentrations of teicoplanin ($P > 0.05$). It was shown that preincubition of cells with 520 μg/ml of teicoplanin lead to a significantly lower c-fos mRNA level expression compared to preincubition of cells with 130 μg/ml of teicoplanin ($P < 0.05$).

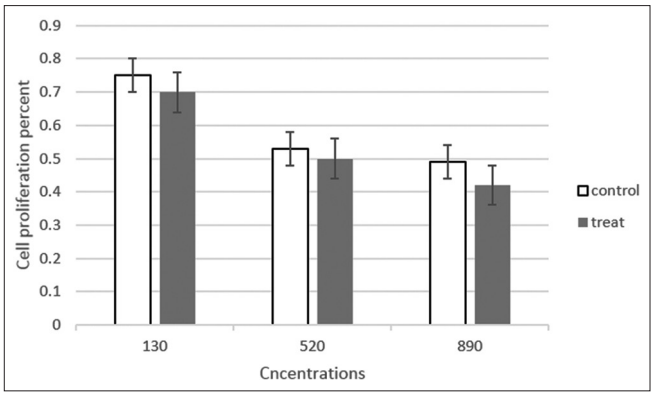

Figure 1: Cell proliferation showed significant difference between cells which were treated with different concentrations (130, 520 and 890 μg/ml) of teicoplanin in mock and negative control groups ($P < 0.05$)

DISCUSSION

Teicoplanin, the glycopeptide antibiotic, acts against Gram-positive bacteria including streptococci, listeria, enterococci, *Clostridium* spp., and staphylococci (including methicillin-resistant strains). The structure of teicoplanin is similar to vancomycin that has a complex structure with five glycopeptides. Because of advantages of teicoplanin than with vancomycin, teicoplanin is selected in identical situations.[10,11] Moreover, teicoplanin is used for treating infections caused by methicillin-resistant bacteria like *Staphylococcus aureus*. In spite of the everyday increasing use of this antibiotic, there is no research conducted on the possible therapeutic purposes. To our knowledge, there is not any available paper on the effect of teicoplanin on gene expression in human breast tumor cell line, MCF-7. Therefore, this study was designed to investigate whether the higher proliferation rate of MCF-7 cells in response to teicoplanin is mediated through induction the expression of c-myc and c-fos genes.

In some previous studies, expressions of c-myc and c-fos genes by different factors like hormones, have been studied in different cell lines including MCF-7,[12-15] Molis *et al*. observed that in estrogen-responsive MCF-7 cell line, the expression of c-myc was increased but c-fos expression was decreased in response to melatonin, a pineal hormone.[16] In another study, Van der Burg *et al*. showed that estrogen induces c-myc and c-fos expression independent of protein synthesis.[17] Moreover, in a study conducted by Hodges *et al*. it was reported that tamoxifen induce the expression of

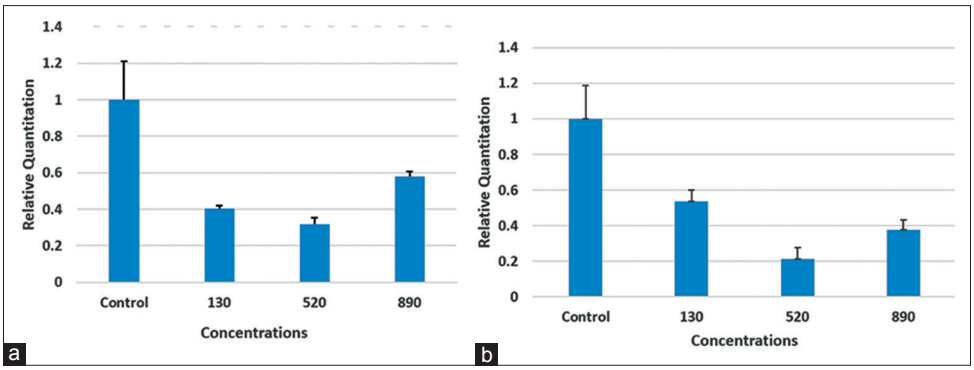

Figure 2: The effects of 68-h preincubation of MCF-7 cells with teicoplanin on mRNA expression of c-myc (a) and c-fos (b) determined by quantitative real-time polymerase chain reaction. The treated cell line is indicated by teicoplanin concentration below the corresponding columns. Data are presented as mean ± standard error mean of three samples. At all concentrations of teicoplanin the level of mRNA expression of c-myc and c-fos genes compared to the reference gene were significantly decreased ($P < 0.05$)

c-myc, c-fos, and some other genes involved in cell cycle progression in MCF-7 cells.[18] A study by Kashkooli Nezhad Koohi *et al*. have been revealed that teicoplanin increase MCF-7 cells proliferation.[9] In addition, the previous data have shown that vancomycin (a member of vancomycin-ristocetin family like teicoplanin) increases cell growth by affecting MAP kinase signaling pathway.[2]

In this study, we found that different concentrations of teicoplanin (130, 520, and 890 μg/ml), reduce the expression of c-myc and c-fos genes. In a study by Wang *et al*., it was observed that *in vitro* and *in vivo* knockdown of c-myc gene by RNAi inhibits MCF-7 cells growth.[19] In addition, Watson *et al*. showed that inhibition of c-myc expression by antisense oligonucleotide inhibit estrogen-induced cell growth significantly.[20] Besides, in a study by Shi *et al*. it was observed that down-regulation of c-fos in MCF-7/adriamycin (ADR) cells, i.e. an ADR selected human breast cancer cell line with the multidrug resistance phenotype, enhances cell apoptosis.[21] Hence, decreased expression of c-myc and c-fos inhibits cell proliferation. From these evidence, it can be concluded that teicoplanin exerts its proliferation effects in a different way than increasing the expression of c-myc and c-fos genes.

In a study by Nezhad Koohi *et al*., it was observed that teicoplanin, in addition to increasing the growth and proliferation rate of CHO cells, caused chromosome abnormalities significantly in 40 out of 100 teicoplanin-treated cells compared to 5 out of 100 control (not treated by teicoplanin) cells.[22]

CONCLUSION

From this study and other studies mentioned above, it could be concluded that although teicoplanin is considered as an enhancing cell growth and proliferation, but probably its effect is not through MAP kinase signaling pathway or perhaps even has inhibitory effect on the expression of some genes such as c-myc and c-fos in this pathway. So, the mechanism of action of teicoplanin for increasing cell propagation, through cell signaling pathways or chromosomal abnormalities, remains unclear, and further studies should be conducted.

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Conflicts of interest

There are no conflicts of interest.

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