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MEDICAL SCIENCES / DAHİLİ TIP BİLİMLERİ

Investigation of Cytotoxic Effects due to Antibiotic Containing Teicoplanin Active Ingredient on Human Pancreatic Cells by MTT Test

İnsan Pankreas Hücrelerinde Teikoplanin Aktif Madde İçeren Antibiyotiğe Bağlı Sitotoksik Etkilerin MTT Testi ile Değerlendirilmesi

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Abstract

Objectives: Antibiotics are a group of drugs used in the treatment of infectious diseases caused by bacteria. They provide powerful treatment against infections, but they are not without side effects. These side effects are not always clinically detectable and their damage at the cellular level may contribute to the development of new disorders. Teicoplanin is one of the glycopeptide antibiotics and is used as a potent agent in the treatment of serious infections caused by Gram-positive bacteria. It may have side effects at cellular level.

Materials and Methods: In this study, the cytotoxicity of the antibiotic teicoplanin in human pancreatic cell (hTERT-HPNE) was investigated. Cytotoxicity was determined using the MTT test. hTERT-HPNE cells were exposed to drug containing the active ingredient teicoplanin. The experimental group concentration was prepared in the range of 0.054 mM, 0.109 mM, 0.218 mM, 0.437 mM, 0.875 mM, 1.75 mM, 3.5 mM, 7 mM.

Results: The active ingredient teicoplanin caused a change in the morphology of the cells in the MTT test. Dose-related decreases in cell viability were observed with the effect of teicoplanin and they were found to be statistically significant compared to the negative control ($p<0.05$). As a result of the experiment, it was determined that teicoplanin caused cytotoxicity on hTERT-HPNE.

Conclusion: Our study supports the hypothesis that teicoplanin-based antibiotic may have cytotoxic effects on human pancreatic cells and draws attention to the side effects of antibiotics at the cellular level.

Key Words: Teicoplanin, Glycopeptide Antibiotic, MTT Test, Human Pancreatic Cell

Öz

Amaç: Antibiyotikler, bakterilerin neden olduğu bulaşıcı hastalıkların tedavisinde kullanılan bir grup ilaçtır. Enfeksiyonlara karşı güçlü tedavi sağlarlar, ancak yan etkisiz değillerdir. Bu yan etkiler her zaman klinik olarak saptanmayabilir ve hücresel düzeyde gelişebilir. Hücresel düzeydeki hasarlar yeni bozukluklara yolaçabilir. Teikoplanin, glikopeptid antibiyotiklerden biridir ve Gram-pozitif bakterilerin neden olduğu ciddi enfeksiyonların tedavisinde güçlü bir ajan olarak kullanılır. Hücresel düzeyde yan etkileri olabilir.

Gereç ve Yöntem: Bu çalışmada, teikoplanin antibiyotiğinin insan pankreas hücresinde (hTERT-HPNE) sitotoksisitesi araştırıldı. Sitotoksisite MTT testi kullanılarak belirlendi. hTERT-HPNE hücreleri, teikoplanin etken maddesi içeren ilaca maruz bırakıldı. Deney grubu konsantrasyonu, 0,054 mM, 0,109 mM, 0,218 mM, 0,437 mM, 0,875 mM, 1,75 mM, 3,5 mM, 7 mM aralığında hazırlandı.

Bulgular: Teikoplanin, MTT testinde hücrelerin morfolojisinde değişikliğe neden oldu. Teikoplanin dozuna bağlı olarak hücre canlılığında azalmalar gözlendi ve negatif kontrole kıyasla istatistiksel olarak anlamlı bulundu ($p<0,05$). Deney sonucunda teikoplaninin hTERT-HPNE üzerinde sitotoksisiteye neden olduğu belirlendi.

Sonuç: Çalışmamız teikoplanin bazlı antibiyotiğin insan pankreas hücreleri üzerinde sitotoksik etkileri olabileceği hipotezini desteklemekte ve antibiyotiklerin hücresel düzeyde yan etkilerine dikkat çekmektedir.

Anahtar Kelimeler: Teikoplanin, Glikopeptid Antibiyotik, MTT Testi, İnsan Pankreas Hücresi

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Introduction

Antibiotics are a group of drugs used in the treatment of infectious diseases caused by bacterias. They provide powerful treatment against infections, but they are not without side effects. These side effects are not always clinically detectable, and their damage by cellular level may contribute to the development of novel disorders. Teicoplanin, which was extracted from *Actinoplanes teichomyceticus* is one of the glycopeptide antibiotics and it is used since 1984 (1). It is a strong agent against the Gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus* and *Enterococcus faecalis.* Its mechanism of action is to inhibit peptidoglycan polymerization, resulting in inhibition of synthesis of Gram-positive bacteria cell walls and consequent cell death (2,3). Teicoplanin is predominantly bound to plasma proteins and eliminated by the kidneys, it has longer serum half-life and has not requiring close monitoring (4). It is not absorbed orally, but intravenous and intramuscular administration is well tolerated. It is approved in many hospitals, clinics, intensive care units, in the treatment of diseases caused by susceptible Gram-positive bacterias, such as pneumonia, endocarditis, complicated urinary tract infections, skin structure infections, bone and joint infections (5). Teicoplanin has low rate of side effects and has no known pancreatic side effects (6). But we don't know what damage it does at the cellular level. Recently, various results have been reported in the literature regarding the side effects of teicoplanin *in vitro* on human macrophage and neutrophil function, but no data are yet available on human pancreatic cell cytotoxicity. Various methods such as staining methods, fluorometric methods, and colorometric methods are used to determine cytotoxicity. Among colorimetric methods, the MTT (3-[4,5-dimethyl-thiazolyl-2,5-diphenyltetrazolium bromide]) test is one of the most widely used methods for the determination of cell viability and cytotoxicity. After application, cell viability is evaluated with spectrophotometric readings using different dyes. The viability of untreated cells is assumed to be 100%, and compared to this, the viability of treated cells is determined as a percentage (%). The method developed by Mosmann (7) in 1983 is called the "gold standard" of cell viability tests. MTT test is particularly preferred because it is a reliable and rapid method, has high reproducibility, determines both cytotoxicity and cell proliferation, and can be used *in vitro* not only in cell but also in tissue cultures (7).

In this study, we investigated whether the drug containing teicoplanin has a cytotoxic effect on human pancreatic cells.

Materials and Methods

Antibiotic and Chemicals

Drug containing teicoplanin was obtained from the local pharmacy in Ordu, Türkiye. The chemical structure of teicoplanin is shown in Figure 1.

Cell Culture

The hTERT-HPNE cell lines were obtained from American Type Culture Collection (Rockville, MD, USA). The hTERT-HPNE cells were cultured in 500 mL Dulbecco's modified Eagle's medium with 55 mL FBS(10%) and 0.1 mL penicillin/streptomycin (1%). The mediums were stored at +4 °C and cells were removed by trypsinization. Cells were incubated in 5% CO_2 and 95% humidity at 37 °C.

MTT

MTT is a yellow formazan salt and proliferating cells convert MTT into purple-colored water-insoluble formazan crystals with increased mitochondrial dehydrogenase enzyme activity. These crystals formed are soluble in dimethyl sulfoxide (DMSO) or isopropyl alcohol (8). For the cytotoxicity determination MTT test was applied in hTERT-HPNE cells which were seeded in a 96-well plate at the density of 1×10^4 cells/well. The experimental group concentration of the drug containing teicoplanin active ingredient was prepared in the range of 0.054 mM, 0.109 mM, 0.218 mM, 0.437 mM, 0.875 mM, 1.75 mM, 3.5 mM, 7 mM. Each of these were given to hTERT-HPNE cells cultivated in certain volumes and incubated for 24, 48 and 72 hours. After the incubation times were over, 20 µL of MTT solution was added to each well, then it was left to incubate again for 3 hours, and after this time, 100 µL of DMSO was added to each well and left for 10 minutes. After all the procedures, the intensity of color created in each plate was measured at 570 nm wavelength.

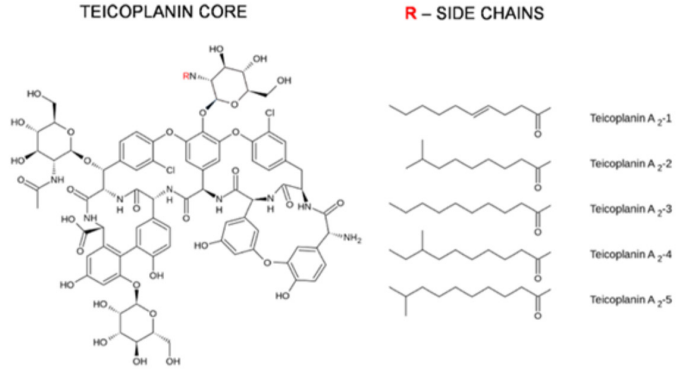

Figure 1: Chemical structure of teicoplanin

Statistical Analysis

Data show the mean of at least 3 independent experiments ± standard error mean. GraphPad Prism 8 software (San Diego, CA, USA) software was used to perform the Student's two-tailed t-test and for 2- way ANOVA analysis. P-values lower than 0.05 were considered statistically significant.

Results

Cell Viability

By MTT test, it was determined that teicoplanin decreased cell viability in a concentration-dependent manner for all treatment times. For 24 and 48 hours of treatment, cell viability was below 50% at 0.437, 0.875, 1.75, 3.5 and 7 mg/ml concentrations, while for 72 hours treatment, cell viability value was below %50 at 0.218, 0.437, 0.875, 1.75, 3.5 and 7 mg/mL concentrations. The cytotoxicity values of hTERT-HPNE cells exposed to teicoplanin with exposure times of 24, 48 and 72 hours were calculated with the formula; Percentage of cell viability (% cell viability) = (A samples-A blank) / (A control-A blank) x 100). These values were compared statistically and graphed. The reductions in cell viability caused by teicoplanin at all concentrations were significant compared to the negative control ($p<0.05$). The effects of teicoplanin active ingredient on hTERT-HPNE cell viability are shown in Figure 2.

Morphological Changes

Teicoplanin caused morphological changes on hTERT-HPNE cells at the application period as 24, 48 and 72 hours. These changes are shown in Figures 3-5.

Discussion

Antibiotic group drugs are used consciously or unconsciously but excessively in the world and in Türkiye. Teicoplanin is an antibiotic with chemically similar activity to vancomycin, used

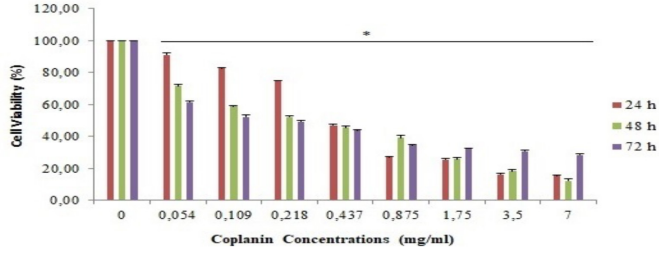

Figure 2: Effects of teikoplanin on cell viability of hTERT-HPNE cells. Data represent mean ± SE for three independent experiments. *$p<0.05$ compared to the control

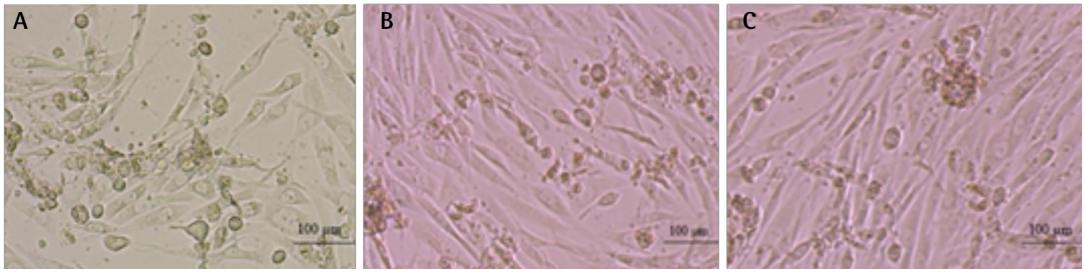

Figure 3: A: Control/Others: Morphological Changes of hTERT-HPNE by 24 h exposure to teicoplanin

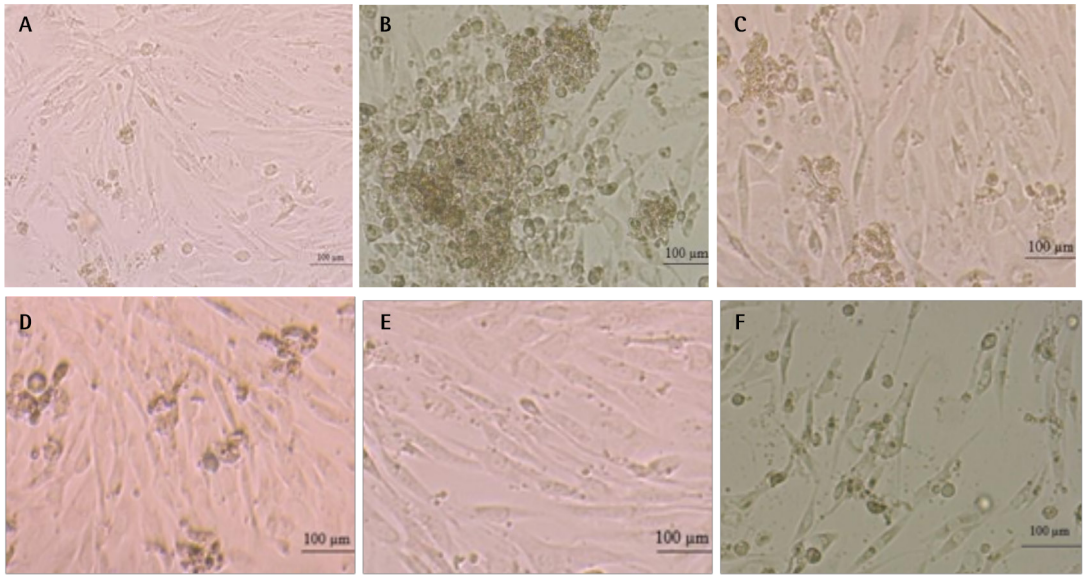

Figure 4: A: Control/Others: Morphological Changes of hTERT-HPNE by 48 h exposure to teicoplanin

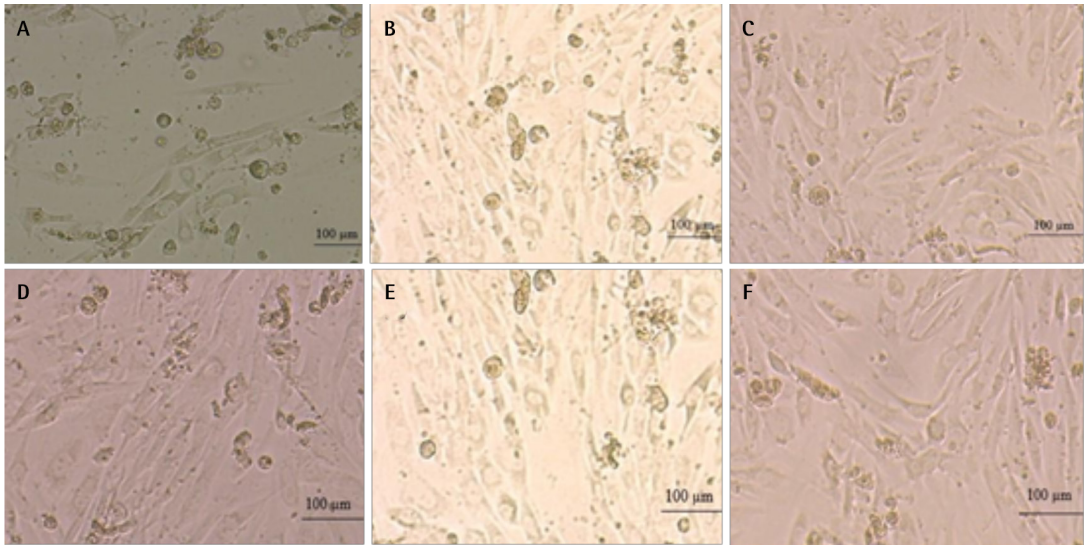

Figure 5: A: Control/Others: Morphological Changes of hTERT-HPNE by 72 h exposure to teicoplanin

in the treatment of serious gram-positive bacterial infections. It is known to have less side-effect profile than vancomycin (9). However, it is not possible to say exactly for any drug" what kind of damage it causes at the cellular level. Cytotoxicity; refers to the rate of toxic effects on living cells. Cytotoxicity tests are cell toxicity and proliferation rate based tests for the evaluation of the substance considered to be toxic in the appropriate cell culture. Cells may die due to events such as apoptosis, necrosis, and autophagy if they are exposed to a cytotoxic substance. In addition, they may lose their proliferation properties due to the arrest or inhibition of cell growth (10). Phagocytes harvested from mice receiving intravenous teicoplanin showed increased phagocytic activity, suggesting that potentiation of host defences can occur *in vivo* (11). Again, teicoplanin caused a dose-dependent inhibition of human PMN chemiluminescence as well as the release of lysozyme/ß-glucoronidase and lactate dehydrogenase, in the absence of any detectable cytotoxic effects. However, the variability amongst individual donors of PMN was large, casting doubts on the significance of these data (12,13). Determining the viability of cells exposed to chemical agents experimentally is an important step in cytotoxicity studies and the amount of live and dead cells can be determined at the end of the study (14). In this study, the cytotoxicity of the drug as an antibiotic with the active ingredient teicoplanin, on human pancreatic cells *in vitro* was investigated. Teicoplanin is administered clinically, i.v. or i.m., usually at a starting dose of 400 mg, followed by 200 or 400 mg every other day (15). In the studies conducted the concentrations of teicoplanin which revealed cell toxicity were much higher than the clinically used concentrations (4,8,16). It might thus be concluded that teicoplanin appears to be a safe drug. However, teicoplanin cytotoxicity studies with MTT are very few in the literature. In this study, we used the MTT analysis method, which is inexpensive, fast, effective, easy to use, reliable and widely used worldwide, to monitor cell viability. Cell viability was evaluated by measuring mitochondrial activity in human pancreatic cell after exposure to the active ingredient teicoplanin. Teicoplanin caused cytotoxicity due to increasing concentrations in hTERT-HPNE cells after 24, 48, 72 hours of exposure. In one study, teikoplanin cytotoxicity on fibroblast was investigated. Nanofibers were able to release teicoplanin up to 12 days for the treatment of infections in orthopedic surgery. It was detected that teicoplanin loaded nanofibers did not show any cytotoxicity to human fibroblast (17). But in another study as similar to our study, the percentage of viability of the cell lines studied after 24-hour treatment with teicoplanin at concentrations ranging from 0 to 11.000 µg/mL, measured by the MTT assay showed that dose dependent manner of cell toxicity occurred (18). Antibiotics are powerful treatment tools against infections and are clinically useful drugs. However, in recent years, studies on cellular effects in antibiotics have yielded different results. In a study of Yavuz (19), the findings obtained showed that quinolone group antibiotics have cytotoxic, genotoxic and chondrotoxic effects by *in vivo*/*in vitro* methods. In another study, triple antibiotic paste and double antibiotic paste, especially in their higher concentrations, induced cytotoxicity and genotoxicity in contrast to calcium hydroxide on human stem cells of the apical papilla (20). Wu et al. (21) evaluated the cytotoxicities of sulfamethoxazole , ciprofloxacin, and tetracycline. All three antibiotics showed cytotoxic effects of on ctenopharyngodon idellus kidney cells. Aminoglycoside antibiotics have been used for treating serious enfections. But, evaluation of the cytotoxicity of dihydrostreptomycin and neomycin *in vitro* on three cell cultures (Syrian golden hamster kidney fibroblast, African green monkey kidney fibroblast and feline embryonic fibroblast cells) demonstrated that these antibiotics decreased cell viability after treatment (22). These studies were carried out in the last one year. Each antibiotic

may have different cytotoxic effects, and different results may be obtained depending on the cell to which the antibiotic is applied. Therefore, a more precise consensus can be reached by conducting multiple studies on different antibiotics in different cells.

Study Limitations

The most important limitation of this study is that it was performed in vitro, not performed on humans. Additionally, it was performed on pancreatic cell, therefore we cannot state that these effects occur in other tissues and cells.

Conclusion

Antibiotics are powerful life-saving molecules, but their possible harm at the cellular level should be kept in mind. Teicoplanin has a cytotoxic effect in human living pancreatic cells. Cytotoxicity studies of teicoplanin with MTT are very few in the literature. New studies on the effects of antibiotics on cell viability can increase our knowledge of their cytotoxicities.

Antibiotics should be used with correct and specific indications, taking into account the damage they may cause on cell viability.

Ethics

Ethics Committee Approval: This study was carried out using a cell line. It does not include human and animal testing. Therefore, ethical committee approval is not required.

Informed Consent: This study was carried out using a cell line. It does not include human and animal testing. Informed consent is not required.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: Ö.Ö., Ö.E. Design: Ö.Ö., Ö.E. Data Collection or Processing: Ö.E.B , Analysis or Interpretation: Ö.E. Literature Search: Ö.E., Ö.Ö. Writing: Ö.Ö.

Conflict of Interest: The authors does not reported any conflict of interest.

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