

CUKUROVA 12TH INTERNATIONAL SCIENTIFIC RESEARCHES CONFERENCE

March 9-12, 2024 ADANA



ABSTRACT BOOK

EDITORS Dr. Ethem İlhan ŞAHİN Merve KIDIRYÜZ

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CUKUROVA 12th INTERNATIONAL SCIENTIFIC RESEARCHES CONFERENCE

March 9-12, 2024 Adana / TURKIYE



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CONFERENCE ID

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09.03.2024 Saturday- H1



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PROLIFERATIVE AND CYTOXIC EFFECTS OF AURORA KINASE B INHIBITOR TOZASERTİB AND VITAMIN-D3 ON ACUTE MYELOID LEUKEMIA (AML) CELL LINE (HL-60)

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ÖZET

Hematopoetik malignite özelliği taşıyan AML önemli ve ölümcül bir kan kanseri türüdür. Günümüzde pekçok kanser gibi AML'nin tedavisinde de yüksek yan etkilere sahip kemoterapötik ajanlar kullanılmaktadır. Kemoterapötik ajanların düşük dozda kullanılmasını ve yan etkilerinin azaltılmasını sağlayacak sinerji çalışmaları oldukça önemlidir. İnsan hematopoietik lösemi hücre hatlarında (HL-60, K562 ve U937 gibi) Aurora kinaz B gen ifadesinin arttığı belirlenmiştir. Bu doğrultuda kemik iliğinde ve ekstra medullar alanlarda hematopoetik hücrelerin blast formunda birikimi ile karakterize edilen Akut Miyeloyid Lösemi (AML)'nin moleküler patogenezinde rol oynayan Aurora-B kinazın inhibitörü tozasertib ve vitamin-D3'ün HL-60 hücre hattı üzerinde proliferatif ve sitotoksik etkilerinin belirlenmesi amaçlanmıştır. Bu amaçla oldukça hassas ölçüm sağlayan Hücre Sayım Kiti-8 (WST-8) kiti kullanılmıştır. Radyoaktif olmayan WST-8, hücre proliferasyonu ve sitotoksisite analizlerinde canlı hücre sayısının belirlenmesi için hassas kolorimetrik analizlere olanak tanır. WST-8, hücrelerdeki dehidrojenazlar tarafından indirgenerek doku kültürü ortamında çözünebilen sarı renkli bir ürün (formazan) verir. Hücrelerdeki dehidrojenazların aktivitesi sonucu oluşan formazan boyasının miktarı canlı hücre sayısıyla doğru orantılıdır. CCK-8'in tespit hassasiyeti MTT, XTT, MTS veya WST-1 gibi diğer tetrazolyum tuzlarından daha yüksektir. HL-60 hücrelerine farklı konsantrasyonlarda Tozasertib (0, 1, 5 ve 10 μM) ve Vitamin D3 (0, 1, 10 ve 100 nM) 24, 48, 72 ve 96 saat boyunca uygulanmıştır. WST-8 kiti uygulanarak Elisa mikropleyt okuyucuda spektrofotometrik okuma gerceklestirilmistir. Elde edilen verile GraphPad Prism programı ile analiz edilmiştir. Vitamin D3'ün HL-60 hücreleri üzerinde IC₅₀ değerleri her saat için sırasıyla 4.226, 6.871, 156.6 ve 1.320 nM olarak belirlenmiştir. HL-60 hücrelerinde Tozasertib'in IC₅₀ değerleri sırasıyla 9.561, 4.669, 5.657 ve 0.1306 μM olarak belirlenmiştir.

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Bu çalışmanın sonucunda elde edilen verilerin Akut miyeloid lösemi hücrelerinin proliferasyon ve blast formunda aktif olan Aurora kinaz yolaklarındaki inhibitör etkisi *in-vitro* olarak araştırılacak olan tozasertib ile vitamin-D3 kombinasyonlarının uygulanacağı *in vitro* ve *in vivo* çalışmalara temel oluşturacağı düşünülmektedir.

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Anahtar kelimeler: Akut miyeloid lösemi, Tozasertib, Vitamin D3, WST-8.

ABSTRACT

AML, which is a hematopoietic malignancy, is an important and fatal type of blood cancer. Today, like many cancers, chemotherapeutic agents with high side effects are used in the treatment of AML. Synergy studies that will enable the use of low doses of chemotherapeutic agents and reduce their side effects are very important. It has been determined that Aurora kinase B gene expression is increased in human hematopoietic leukemia cell lines (such as HL-60, K562 and U937). In this regard, the proliferative and cytotoxic effects of tozasertib and vitamin-D3, inhibitors of Aurora-B kinase, which play a role in the molecular pathogenesis of Acute Myeloid Leukemia (AML), which is characterized by the accumulation of hematopoietic cells in blast form in the bone marrow and extra-medullary areas, on the HL-60 cell line. is intended to be determined. For this purpose, the Cell Counting Kit-8 (WST-8) kit, which provides very sensitive measurements, was used. Non-radioactive WST-8 allows sensitive colorimetric analyzes for determining the number of viable cells in cell proliferation and cytotoxicity assays. WST-8 is reduced by dehydrogenases in cells, yielding a yellow product (formazan) that is soluble in tissue culture media. The amount of formazan dye formed as a result of the activity of dehydrogenases in cells is directly proportional to the number of living cells. The detection sensitivity of CCK-8 is higher than other tetrazolium salts such as MTT, XTT, MTS or WST-1. Different concentrations of Tozasertib (0, 1, 5, and 10 µM) and Vitamin D3 (0, 1, 10, and 100 nM) were applied to HL-60 cells for 24, 48, 72, and 96 h. Spectrophotometric reading was performed on the Elisa microplate reader by applying the WST-8 kit. The data obtained was analyzed with the GraphPad Prism program. The IC50 values of Vitamin D3 on HL-60 cells were determined as 4.226, 6.871, 156.6 and 1.320 nM per hour, respectively. IC50 values of Tozasertib in HL-60 cells were determined as 9.561, 4.669, 5.657 and 0.1306 µM, respectively.

The experimental stages of this study, which was carried out within the scope of Mersin University Scientific Research Unit project number 2016-1-TP2-1415, were carried out at Mersin University Advanced Technology Education, Research and Application Center (MEİTAM).

It is thought that the data obtained as a result of this study will form the basis for in vitro and in vivo studies in which tozasertib and vitamin-D3 combinations will be applied, the inhibitory effect of which will be investigated in vitro on the Aurora kinase pathways, which are active in the proliferation and blast form of acute myeloid leukemia cells.

Key words: Acute myeloid leukemia, Tozasertib, Vitamin D3, WST-8.