PHARMACIA

Pharmacia 71: 1–9
DOI 10.3897/pharmacia.71.e136191

Research Article

Synergistic effect of venetoclax and teglicar in multiple myeloma cell lines

Belal A. Al-Husein[1], Jood Hashem[2], Sara S. Alawi[2], Mohammad A. Y. Alqudah[1], Ahmad Al-Azayzih[1]

1 *Department of Clinical Pharmacy, Faculty of Pharmacy, Jordan University of Science and Technology, P.O. Box 3030, Irbid 22110, Jordan*
2 *Faculty of Applied Medical Sciences, Jordan University of Science and Technology, P.O. Box 3030, Irbid 22110, Jordan*

Corresponding author: Belal A. Al-Husein (belalhusein@just.edu.jo)

Received 3 September 2024 ♦ **Accepted** 7 October 2024 ♦ **Published** 24 October 2024

Citation: Al-Husein BA, Hashem J, Alawi SS, Alqudah MAY, Al-Azayzih A (2024) Synergistic effect of venetoclax and teglicar in multiple myeloma cell lines. Pharmacia 71: 1–9. https://doi.org/10.3897/pharmacia.71.e136191

Abstract

Multiple myeloma is a hematological malignancy characterized by the clonal proliferation of plasma cells. Recent advancements in treatment strategies have led to a significant shift in the management of this malignancy. Venetoclax, an apoptosis inducer, has emerged as a promising therapeutic option for multiple myeloma. Fatty acid oxidation (FAO) is one of the metabolic reprogramming events that occurs because of mutations in certain genes and thus plays a crucial role in cancer progression. Teglicar, a reversible inhibitor of FAO, targets carnitine palmitoyl transferase 1 (CPT1) and has shown potential in cancer treatment due to its interaction with apoptotic regulators such as Bcl-2. In this study we investigated the *in vitro* anti-cancer activity of teglicar, in myeloma cell lines (RPMI 8226 and U266B1). Our findings revealed a significant inhibition of RPMI 8226 cell viability with an IC_{50} = 50 μM, but at a higher concentration for U266B1 cells. Notably, the combination of teglicar with venetoclax showed a synergistic effect. Although both cell lines were known to be resistant to venetoclax, teglicar significantly reduced IC_{50} of venetoclax by 5.33 and 1.7 folds in RPMI 8226 and U266B1 respectively. In addition, single agent and combination treatment were associated with a significant increase in apoptosis in both cell lines. However, the combination therapy had no significant effect on reactive oxygen species (ROS) levels in either cell lines. Our results suggest that the combination of teglicar and venetoclax holds promise in multiple myeloma through induction of apoptosis. This study sheds light on the potential therapeutic implications of targeting FAO, specifically through CPT1 inhibition, in conjunction with apoptosis inducers for enhanced anticancer effects in multiple myeloma.

Keywords

Venetoclax, teglicar, multiple myeloma, synergism, apoptosis

Introduction

Multiple myeloma (MM) is a hematological malignancy which affects terminally differentiated B cells (Harmening 2009). It is characterized by the abnormal proliferation of plasma cells with overproduction of monoclonal immunoglobulins and lytic bone disease (Harmening 2009). Worldwide, the age-standardized incidence rate (ASR) of MM was 1.8/100,000 persons, while the morality was 1.1/100,000 persons (Ferlay et al. 2024). The 5-year overall survival of MM sharply declines with the incidence of distal involvement (American Cancer Society 2023). Over the last decades, new drugs have been introduced, dramatically changing the treatment landscape of this disease (Moreau et al. 2017). The treatment of MM typically involves a combination of three drugs: a proteasome

inhibitor, an immunomodulatory drug, and dexamethasone (DiPiro et al. 2023). These regimens have led to better response rates and outcomes compared to traditional chemotherapy. While the therapy is not curative, ongoing research aims to develop new treatment options for the disease.

Although a variety of treatment regimens are available for MM, the majority of patients will experience relapse during the course of the disease (Moreau et al. 2021; van de Donk et al. 2021). Relapse, coupled with a decreased duration of response has been attributed to the development of resistance (Kumar et al. 2004). Therefore, there is an ongoing need for a continued search for additional therapeutic options.

The oncogenic transformation of normal cells into tumor cells involves multiple mechanisms, among which altered energy metabolism has been well-established as a hallmark of cancer (Hanahan and Weinberg 2011; Zhao et al. 2013). The best known abnormal metabolic activity in cancer cells is the Warburg effect, characterized by increased glucose-to-lactate metabolism despite the presence of sufficient oxygen in the tumor microenvironment (Warburg 1956; Bensinger and Christofk 2012). Although aerobic glycolysis is 18-fold less efficient in producing ATP than oxidative phosphorylation, it is faster in generating energy. Tumor cells have been observed to overcome metabolite limitations by engaging in endogenous fatty acid catabolism through β-oxidation (Ortega et al. 2009; Carracedo et al. 2013).

The essential role of fatty acid oxidation (FAO) in generating antioxidant defenses (Carracedo et al. 2013) and antagonizing the oligomerization of Bax and Bak in response to apoptotic stimuli has been established (Maher et al. 2018). This protective function shields cancer cells from apoptosis. In addition, FAO contributes to the survival of leukemia stem cells by supporting mitochondrial oxidative metabolism and elevating the threshold for activating the intrinsic apoptotic pathway (Samudio et al. 2010). The rate-limiting step in FAO is governed by the carnitine palmitoyl transferase 1a (CPT1a) enzyme, responsible for catalyzing the entry of fatty acids into the mitochondria. The interaction between CPT1a and apoptosis mediators tBid and Bcl-2 has been reported, with the inhibition of CPT1a initiating apoptosis (Paumen et al. 1997; Giordano et al. 2005). Another consequence of CPT1a inhibition involves the accumulation of palmitate, a toxic metabolite to cells, leading to mitochondrial damage and cell death (Paumen et al. 1997; Giordano et al. 2005).

Teglicar (ST1326) is an aminocarnitine derivative, serving as a selective inhibitor of the CPT1a isoform and displaying favorable characteristics in comparison to etomoxir, a parent CPT1a inhibitor. The clinical development of etomoxir as an antileukemic agent has been hindered by its high cost and potential off-target toxicity, particularly in skeletal and cardiac muscles. Previous studies have demonstrated that the pharmacological inhibition of FAO by teglicar resulted in impaired cancer cell survival and the inhibition of tumor cell viability in *in vitro* and *in vivo* models of Burkett's lymphoma and leukemia (Pacilli et al. 2013; Ricciardi et al. 2015). It has been suggested that inhibiting FAO with etomoxir directly induces abnormalities in the Bcl-2 family of proteins, potentially explaining the enhanced apoptosis seen with the Bcl-2 inhibitor ABT-737. This aligns with the concept that mitochondrial oxidative metabolism supports the survival of leukemia cell (Samudio et al. 2010). Venetoclax (ABT-199), a clinical derivative of ABT-737, has recently gained approval for treating relapsed/refractory chronic lymphocytic leukemia (CLL) with 17p deletion (Cang et al. 2015). Based on that, we hypothesize that pharmacological FAO inhibition with teglicar could further enhance the efficacy of Bcl-2 inhibition in MM cells. It has been reported that human MM cell lines exhibit varying sensitivities to venetoclax (Touzeau et al. 2014), and limited data are available regarding teglicar's ability to induce oxidative stress comparable to etomoxir.

In this research, we assessed the synergistic effect of the combination of teglicar and venetoclax on two MM cell lines, namely RPMI 8226 and U266B1. The assessment of treatment effects on the viability of myeloma cell lines was conducted using the MTT assay. Furthermore, we explored the treatment's capability to induce apoptosis by quantifying the concentration of pro-caspase-3. Lastly, we investigated the influence of treatment on reactive oxygen species (ROS) accumulation and its inhibitory effect on FAO in myeloma cells.

Materials and methods

Cell lines

Two cell lines were used and were purchased from the American Type Culture Collection (ATCC), RPMI 8226 (ATCC CCL-155), and U266B1 [U266] (ATCC TIB-196). Cell lines were activated according to the manufacturer's recommendation. Cells were cultured according to the manufacturer's recommendation. RPMI 8226 cells and U-266B1 were cultured in complete RPMI-1640 media, supplemented with 10% and 15% fetal bovine serum (FBS), respectively. Growth conditions in the incubator were: 37 °C temperature, and 5% CO_2. Trypan blue viability assays and media replacement were performed every two to three days.

Drugs preparation

Teglicar (cat#870853, Avanti lipid) 10 mM stock solution and venetoclax (cat# ab217298, abcam) 10 mM stock solution was prepared using dimethyl sulfoxide (DMSO). Both stock solutions were aliquoted and stored at -80 °C. For both drugs, working dilutions for treatment of cells were prepared using RPMI-1640 media.

MTT cell viability assay

A total of 2×10^4 cells/100 µL per well of RPMI 8226 and U266B1 cells were suspended in complete RPMI-1640 media and seeded into 96-well plate, by dispensing

100 µL in each well. Cells were then separately treated with 100 µL of different venetoclax concentrations (final concentrations of 1.125, 2.5, 5, 10, 20 and 40 µM), and 100 µL of different teglicar concentrations (final concentrations of 3.125, 6.25, 12.5, 25 and 50 µM). Following 48 hrs of treatment, 20 µL of ready-to-use MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) reagent (abcam; Cat# ab211091) was added to each well, and the plate was incubated for 4 hrs at 37 °C. After that, the plate was centrifuged at 1500 RPM for 5 min, and the supernatant was removed carefully without disrupting the formazan precipitate. Formazan was then solubilized by adding 200 µL DMSO to each well. Plate was then placed on a shaker till formazan was completely dissolved, and absorbance was measured at 570 nm using an EZ Read Biochrom 400 microplate reader (Biochrom, Cambridge, UK).

Apoptosis assay

Sample preparation

Cells were seeded at a density of 5×10^4 cell/well into a 96 well plate, by suspending the cells in 50 µL of media. Cells were treated by adding 50 µL of each drug. RPMI 8226 cells were treated with final concentrations of 20 µM of teglicar and 1.5 µM of venetoclax alone and in combination. U266B1 cells were treated with 20 µM of teglicar and 20 µM of venetoclax, alone and in combination. Plates were incubated for 4 and 18 hrs. Cell lysates were prepared by adding equal volume (equal to the volume of culture media) of cell Extraction Buffer into each well. Plates were incubated on ice for 20 min and placed on a shaker at 300 rpm. After that, samples were aliquoted and stored at -80 °C.

Pro caspase measurement assay procedure

All reagents were prepared according to Pro Caspase-3 Human SimpleStep ELISA® (cat# ab192146). Before performing any test, all materials and reagents were equilibrated to room temperature. Standards and controls were run in duplicates with the recommended dilutions.

A total of 50 µL of samples and standards were added to each well. This was followed by the addition of 50 µL of the Antibody Cocktail to each well. The plate was sealed and incubated for 1 hr at room temperature on a plate shaker at 400 rpm. Then, wells were washed three times with 350 µL 1X Wash Buffer PT. Washing was done by aspirating from wells then dispensing 350 µL 1X Wash Buffer PT into each well. All liquid was completely withdrawn in each step. After washing, the plate was inverted and blot against a clean paper towel to remove excess liquid. A total of 100 µL of TMB substrate was then added to each well and was incubated for 10 minutes in the dark on a plate shaker at 400 rpm till blue color developed. Finally, 100 µL of Stop Solution was added to each well and plate was placed on shaker for 1 minute to be well mixed. Absorbance was measured at 450 nm using EZ Read Biochrom 400 microplate reader (Biochrom, Cambridge, UK).

ROS detection

Sample preparation

Cell-based ROS/Superoxide Detection Assay Kit (abcam, Cat# ab139476) was used in this part. Cells were collected by centrifugation at 400 × g for 5 minutes using a swing-out buckets, then resuspend in the appropriate cell culture medium at a density of 1×10^6 cells/well. Cells were aliquoted in 100 µL of media into a black 96 well plate and incubated in a CO_2 incubator at 37 °C.

Assay procedure

Detection reagents, inducers, inhibitors, and controls were reconstituted according to manufacturer's instructions. Buffers were equilibrated to room temperature prior to use. All controls and samples were run in duplicate. Media was removed by centrifugation of the plate at 400 × g for 5 min. Cells were then washed with 1X Wash Buffer and centrifugation at 400 g for 5 min. Simultaneously, cells were treated with 50 µL of variable reagents and controls: positive control, negative control and desired concentrations of drug treatments. Cells were incubated with treatments for 30 min. After that, cells were treated with the 100 µL of the 2X ROS/Superoxide detection solution.

RPMI 8226 cells were treated with 20 µM teglicar and 1.5 µM venetoclax alone or in combination. U266B1 cells, on the other hand, were treated with 20µM of both teglicar and venetoclax, alone or in combination. Positive control was 500 µM pyocyanin, a ROS inducer. Negative control was 5 mM of N-Acetyl-L-Cysteine, a ROS Inhibitor. Cells were incubated for 60 min at 37 °C in the dark then analyzed using a BioTek Synergy HT Microplate Reader (BioTek Instruments), (Ex = 488 nm, Em = 520 nm).

Statistical analysis

All data were analyzed using GraphPad Prism version 9.5.1 for MacOS, GraphPad Software, Boston, Massachusetts USA, www.graphpad.com". Statistical analyses were carried out using ANOVA (for normally distributed data) or its non-parametric equivalent (for not normally distributed data). P-values of < 0.05 were considered significant. Symbols for significancy used were the one adopted by GraphPad software: *, $P < 0.05$; **, $P < 0.005$: ***, $P < 0.0005$; ****, $P < 0.0001$.

IC_{50} and isobologram calculations

After we analyzed the data of MTT assay, the IC_{50} values were calculated using GraphPad Prism. Isobolograms were used to demonstrate synergism of the IC_{50} of the combination treatments. In addition to that, the dose reduction index (DRI) and the combination index (CI) were calculated using the following equations:

$$DRI = \frac{X}{X_c}$$

where X is the IC_{50} of venetoclax when used as single agent and X_c is the IC_{50} of venetoclax in combination with the fixed concentration of teglicar that was used.

$$CI = \left(\frac{C_c}{C}\right) + \left(\frac{X_c}{X}\right)$$

where C is the IC_{50} of teglicar when used as single agent and C_c is the fixed concentration of teglicar that was used.

Results

Teglicar and venetoclax show a synergistic effect on the viability of myeloma cell lines

Figs 1, 2 show the effect of teglicar and venetoclax, alone or in combination, on viability of RPMI 8226 and U266B1, respectively. Fig. 1A, B demonstrate the sensitivity of RPMI 8226 cells to both drugs, while Fig. 2A, B reveal resistance in U266B1 cells to both drugs, with only a high dose of venetoclax exerting an effect. Figs 1C, 2C, however, reveal a synergistic effect of the drug combination on both cells. Isobolograms, the DRI, and CI for both drugs on both cell lines are presented in Figs 1D, 2D.

Teglicar and venetoclax show a synergistic effect on apoptosis of myeloma cell lines

Fig. 3 illustrates the impact of teglicar and venetoclax, alone and in combination, on pro-caspase 3 levels. Significant reduction in pro-caspase 3 levels was observed in RPMI 8226 cells treated with 1.5 μM of venetoclax. Further enhanced effect was seen in the combination, but it was not significant compared to venetoclax arm. In U266B1 cells, however, treatment with combination of 20 μM venetoclax and 20 μM teglicar, significantly reduced pro-caspase 3 levels compared to individual drug treatments. The reduction in pro-caspase 3 levels, indicates increased activation of apoptosis.

Teglicar and venetoclax had no significant effect on oxidative stress in myeloma cell lines

As shown in Fig. 4, venetoclax treatment led to increased ROS levels in both cell lines. Combination with teglicar, however, led to neutralizing the ROS spike caused by venetoclax.

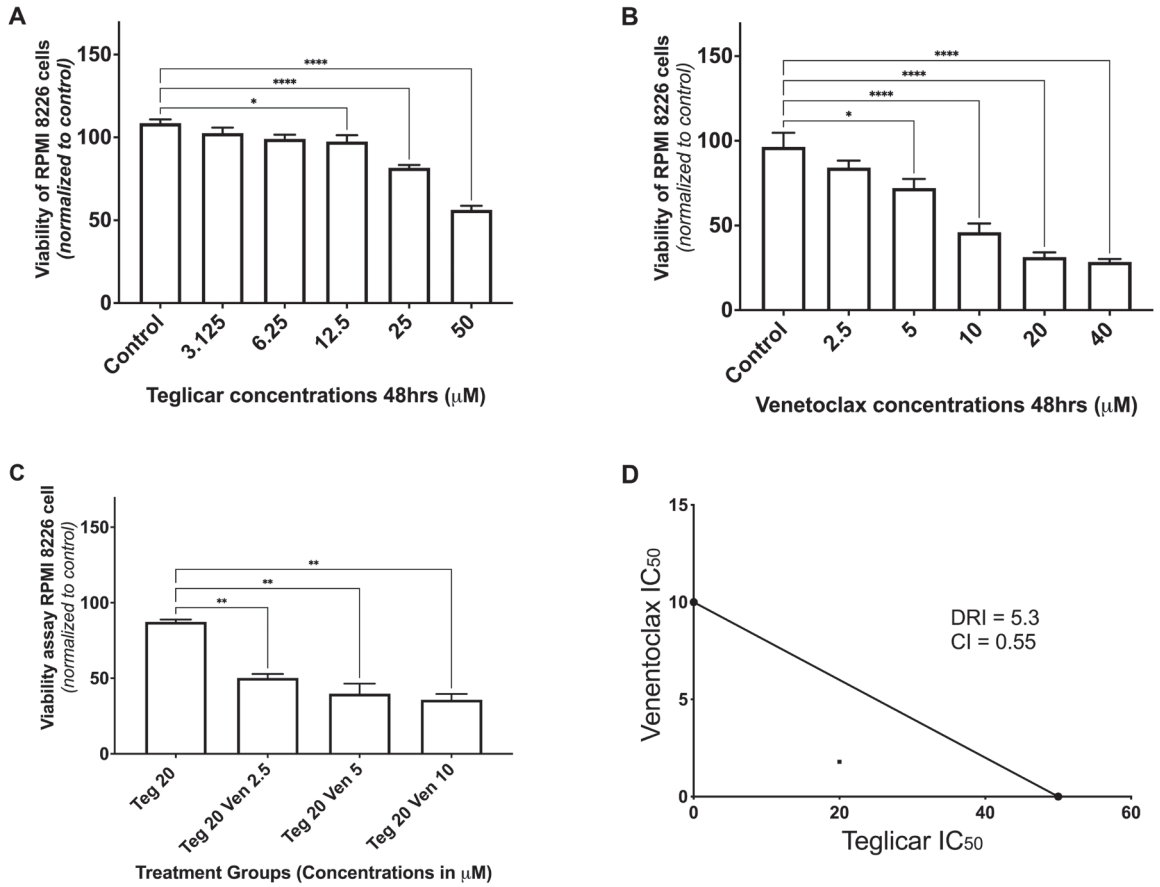

Figure 1. Effect of treatments with teglicar and venetoclax, alone and in combination on viability of RPMI 8226 cells. **A** The effect of serial dilutions teglicar vs. control. **B** The effect of serial dilutions of venetoclax vs. control. **C** Effect of combination treatment. **D** Isobologram showing combination effect on RPMI 8226 cells. (*, P <0.05; **, P < 0.005; ****, P < 0.0001).

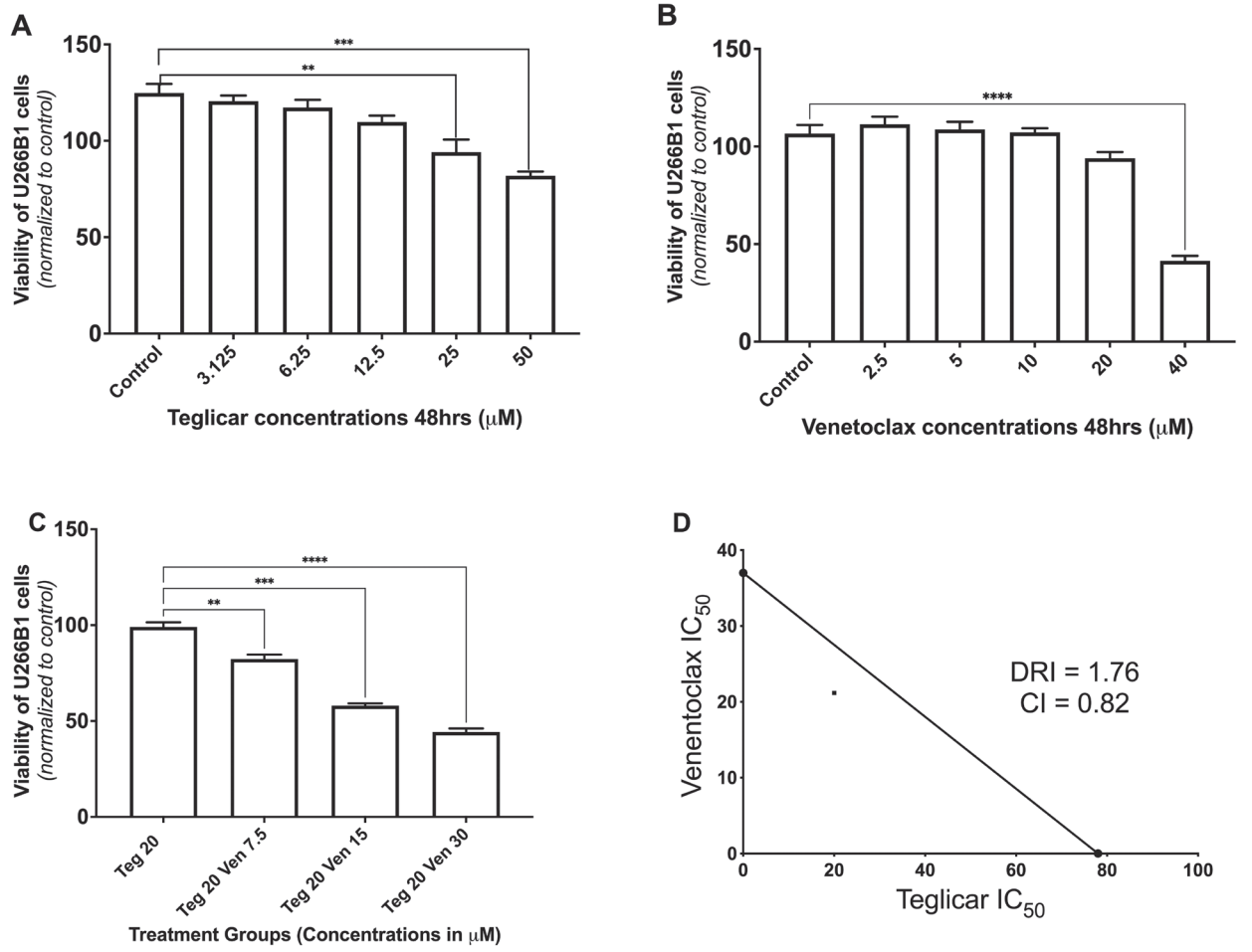

Figure 2. Effect of treatments with teglicar and venetoclax, alone and in combination on viability of U266B1 cells. **A** The effect of serial dilutions teglicar vs. control. **B** The effect of serial dilutions of venetoclax vs. control. **C** Effect of combination treatment. **D** Isobologram showing combination effect on U266B1 cells. (**, P < 0.005: ***, P < 0.0005; ****, P < 0.0001).

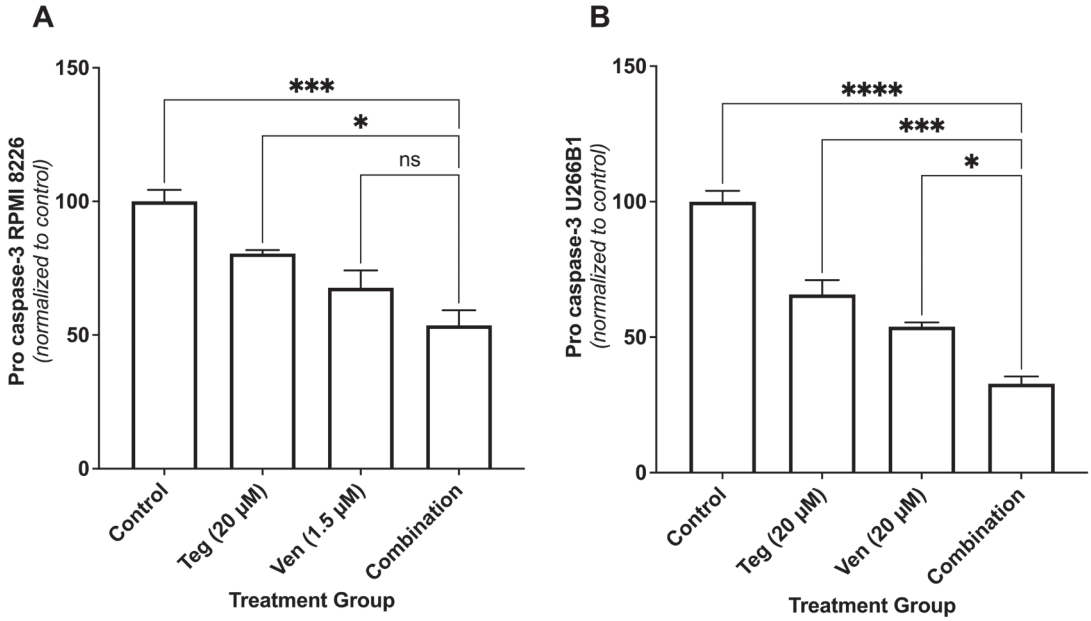

Figure 3. Effect of treatments with teglicar and venetoclax, alone and in combination on apoptosis of RPMI 8226 and U266B1 cells, respectively. **A** Effect of treatments on RPMI 8226. **B** Effect of treatments on U266B1 Cells. (ns, non-significant; *, P <0.05; **, P < 0.005: ***, P < 0.0005; ****, P < 0.0001).

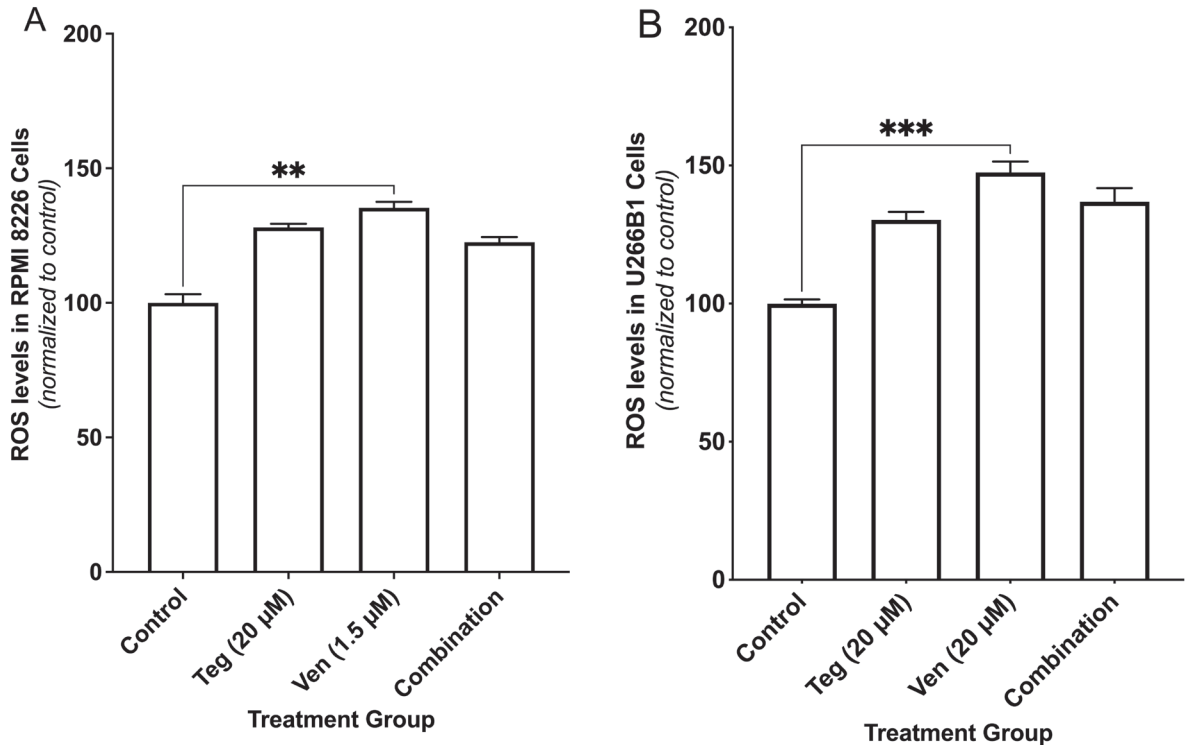

Figure 4. Effect of treatments with teglicar and venetoclax, alone and in combination on ROS formation in **A** Effect of treatments on RPMI 8226 cells. **B** Effect of treatments on U266B1 cells. (**, $P < 0.005$: ***, $P < 0.0005$).

Discussion

In this research, we evaluated the potential of teglicar to enhance the anticancer effects of venetoclax on MM cell lines: U266B1 and RPMI 8266. Despite recent advances in chemotherapy and stem-cell transplantation, MM remains an incurable disease. Since drug resistance is continuously developing, emerging novel therapeutic agents and more effective drug combinations are required. Venetoclax is clinically indicated for the management of MM specifically in patients with t(11,14) translocation (Gong et al. 2016; NCCN 2024). Bcl-2 family proteins have been shown to be crucial regulators of MM cell survival, particularly in those with the t(11;14) translocation (Touzeau et al. 2014). Consequently, MM patients with this translocation exhibit a higher response rate to venetoclax compared to those without this translocation (24% versus 4%) (Kumar et al. 2016).

The expression of Bcl-2 family proteins is heterogeneous, and it remains unclear which anti-apoptotic Bcl-2 protein is primarily responsible for the survival of myeloma cell lines. Compared to other MM cell lines, both RPMI 8226 and U266B1 are known to be resistant to venetoclax, having IC_{50} values greater than 1 μmol/L (Punnoose et al. 2016). RPMI 8226 cells exhibited intermediate resistance, while U266B1 were more resistant, with an IC_{50} of 37 μM. One study found that BCL2 was overexpressed in U266B1 cells compared to RPMI8226, which may explain the greater responsiveness of RPMI 8226 cells versus U266B1 (Valdez et al. 2020).

Our research showed different IC_{50} values for both cell lines compared to those reported by others, with IC_{50} values of 5 μM for RPMI 8226 and 24 μM for U266B1. However, the trend observed in our findings is consistent with the previous research, showing relatively similar values (Valdez et al. 2020). In contrast to our findings and previous research, one study reported that RPMI 8226 cells were less responsive to venetoclax than U266B1 cells, with IC_{50} values around 24 μM for RPMI 8226 and 4–8 μM for U266B1 (Cao et al. 2022).

Targeting the metabolic activity of cancer cells is emerging as a novel therapeutic approach. In Burkitt's lymphoma, inhibition of FAO with teglicar was found to impact cancer cell viability, correlating with the inhibition of fatty acid metabolism (Pacilli et al. 2013). Another study demonstrated that FAO inhibition by teglicar could arrest the cell cycle, cause mitochondrial damage, and induce apoptosis (Ricciardi et al. 2015). Additionally, teglicar impaired the upregulation of Mcl-1 and Bcl-xl expression in response to microenvironmental stimulation, leading to increased sensitivity to apoptosis induction by venetoclax in leukemia cells isolated from patients (Cang et al. 2015). All the previous results verified the idea that leukemia cells survival is supported by the mitochondrial oxidative metabolism.

Bcl-2 family proteins have a significant role in regulating apoptosis, and they are overexpressed in MM. It is tempting to suggest that pharmacological inhibition of FAO using teglicar could further enhance the efficacy of Bcl-2 inhibition by venetoclax in myeloma cells. RPMI

8226 and U266B1 MM cell lines are particularly known to be more resistant to venetoclax. In contrast, etomoxir failed to demonstrate the same activity at equivalently higher concentrations (Tirado-Velez et al. 2012). A previous study on several leukemia cell lines showed that teglicar had an effect on MOLT-4 (an acute lymphocytic leukemia cell line), whereas etomoxir failed to show any activity under the same conditions and concentrations (Mirabilii et al. 2012). Earlier research indicated that etomoxir-mediated FAO inhibition was associated with the inhibition of myeloma cell proliferation, though apoptosis remained unchanged (Gugiatti et al. 2018). Teglicar, on the other hand, has been documented to induce dose-dependent FAO inhibition, while also leading to greater viability suppression, mitochondrial damage, and apoptosis induction compared to etomoxir (Pacilli et al. 2013).

The mechanism of cell death was subsequently studied in both cell lines to elucidate the effect of using both drugs. Consumption of pro-caspases was measured to assess apoptosis. Our viability and apoptosis assay results come in agreement with findings from previous research (Souers et al. 2013), demonstrating that the addition of teglicar to venetoclax can enhance apoptosis induction. Another study showed that teglicar induces apoptosis in canine mammary cancer cells (Cacciola et al. 2023). The synergistic effect may be explained by teglicar's role in downregulating Mcl-1 and Bcl-xl expression, thereby increasing the cells' sensitivity to the BCL-2 inhibitor venetoclax (Gugiatti et al. 2018).

Consequently, we studied the potential of both drugs to induce oxidative stress and ROS formation using a ROS detection kit, as both drugs were reported to cause an increase in ROS (Souers et al. 2013; Chong et al. 2018). FAO is an essential source of NADPH, providing the redox power to counteract oxidative stress. Teglicar has been reported to induce the accumulation of ROS, which increases mitochondrial damage and apoptosis. Additionally, Bcl-2 itself possesses antioxidant activity and can regulate cellular antioxidant pathway. When RPMI 8226 cells were treated with 20 μM teglicar, there was a slight increase in ROS level, and treatment with 1.5 μM venetoclax resulted in a more significant increase. However, the outcome of the combination treatment did not align with our theory, as ROS levels remained unchanged. Similarly, U266B1 cells exhibited the same response. Treatment with 20 μM of teglicar did not produce an observable increase in ROS levels, and while 20 μM of venetoclax resulted in a greater increase, that was still not significant. The combination treatment produced results similar to those observed in RPMI 8226 cells.

Conclusion

In conclusion, our study revealed that the addition of teglicar to venetoclax results in a synergistic effect that significantly enhances its anti-proliferative activity compared to venetoclax alone. Both drugs are capable of inducing apoptosis individually, but their combination leads to a greater induction of apoptosis. Teglicar's role in downregulating Mcl-1 and Bcl-xl expression, along with the critical role of the Bcl-2 apoptotic protein in triggering apoptosis in myeloma cells, was evident. Additionally, both drugs can initiate oxidative stress and increase ROS levels, albeit at very low levels. Unfortunately, the combination treatment did not have any significant impact on ROS formation.

Author contributions

Conceptualization: BA, JH; Data curation: BA, SA, MA; Formal Analysis: BA, SA, MA, AA; Funding acquisition: BA; Investigation: BA; Methodology: BA, SA, JH; Resources: BA; Software: BA; Supervision: BA, JH; Writing – original draft: BA, SA, JH, MA, AA; Writing – review & editing: BA, JH, MA, AA.

Acknowledgments

The research was funded by grant number 20180185 to B.A. from the Deanship of Scientific Research of the Jordan University of Science and Technology.

References

American Cancer Society (2023) Survival Rates for Multiple Myeloma. https://www.cancer.org/cancer/types/multiple-myeloma/detection-diagnosis-staging/survival-rates.html [accessed June 2nd 2024]

Bensinger SJ, Christofk HR (2012) New aspects of the Warburg effect in cancer cell biology. Seminars in Cell & Developmental Biology 23: 352–361. https://doi.org/10.1016/j.semcdb.2012.02.003

Cacciola NA, Sepe F, Fioriniello S, Petillo O, Margarucci S, Scivicco M, Peluso G, Balestrieri A, Bifulco G, Restucci B, Severino L (2023) The carnitine palmitoyltransferase 1A inhibitor teglicar shows promising antitumour activity against canine mammary cancer cells by inducing apoptosis. Pharmaceuticals 16: 987. https://doi.org/10.3390/ph16070987

Cang S, Iragavarapu C, Savooji J, Song Y, Liu D (2015) ABT-199 (venetoclax) and BCL-2 inhibitors in clinical development. Journal of Hematology & Oncology 8: 129. https://doi.org/10.1186/s13045-015-0224-3

Cao L, Chen Q, Gu H, Li Y, Cao W, Liu Y, Qu J, Hou Y, Chen J, Zhang E, He J, Cai Z (2022) Chidamide and venetoclax synergistically exert cytotoxicity on multiple myeloma by upregulating BIM expression. Clin Epigenetics 14: 84. https://doi.org/10.1186/s13148-022-01306-7

Carracedo A, Cantley LC, Pandolfi PP (2013) Cancer metabolism: fatty acid oxidation in the limelight. Nature Reviews Cancer 13: 227–232. https://doi.org/10.1038/nrc3483

Chong SJF, Lai JXH, Eu JQ, Bellot GL, Pervaiz S (2018) Reactive oxygen species and oncoprotein signaling-A dangerous liaison. Antioxidants & Redox Signaling 29: 1553–1588. https://doi.org/10.1089/ars.2017.7441

DiPiro JT, Yee GC, Haines ST, Nolin TD, Ellingrod VL, Posey LM (2023) In: DiPiro's Pharmacotherapy: A Pathophysiologic Approach, 12th Edition. McGraw Hill, New York, NY.

Ferlay J, Ervik M, Lam F, Laversanne M, Colombet M, Mery L, Piñeros M, Znaor A, Soerjomataram I, Bray F (2024) Multiple Myeloma. Global Cancer Observatory: Cancer Today Lyon, France: International Agency for Research on Cancer, 2 pp.

Giordano A, Calvani M, Petillo O, Grippo P, Tuccillo F, Melone MA, Bonelli P, Calarco A, Peluso G (2005) tBid induces alterations of mitochondrial fatty acid oxidation flux by malonyl-CoA-independent inhibition of carnitine palmitoyltransferase-1. Cell Death & Differentiation 12: 603–613. https://doi.org/10.1038/sj.cdd.4401636

Gong JN, Khong T, Segal D, Yao Y, Riffkin CD, Garnier JM, Khaw SL, Lessene G, Spencer A, Herold MJ, Roberts AW, Huang DCS (2016) Hierarchy for targeting prosurvival BCL2 family proteins in multiple myeloma: pivotal role of MCL1. Blood 128: 1834–1844. https://doi.org/10.1182/blood-2016-03-704908

Gugiatti E, Tenca C, Ravera S, Fabbi M, Ghiotto F, Mazzarello AN, Bagnara D, Reverberi D, Zarcone D, Cutrona G, Ibatici A, Ciccone E, Darzynkiewicz Z, Fais F, Bruno S (2018) A reversible carnitine palmitoyltransferase (CPT1) inhibitor offsets the proliferation of chronic lymphocytic leukemia cells. Haematologica 103: e531–e536. https://doi.org/10.3324/haematol.2017.175414

Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. Cell 144: 646–674. https://doi.org/10.1016/j.cell.2011.02.013

Harmening D (2009) Clinical hematology and fundamentals of hemostasis. F.A. Davis Co., Philadelphia, PA.

Kumar S, Vij R, Kaufman JL, Mikhael J, Facon T, Pegourie B, Benboubker L, Gasparetto C, Amiot M, Moreau P, Diehl S, Alzate S, Ross JA, Dunbar M, Zhu M, Agarwal SK, Leverson J, Maciag PC, Verdugo ME, Touzeau C (2016) Phase I venetoclax monotherapy for relapsed/refractory multiple myeloma. Journal of Clinical Oncology 34: 8032–8032. https://doi.org/10.1200/JCO.2016.34.15_suppl.8032

Kumar SK, Therneau TM, Gertz MA, Lacy MQ, Dispenzieri A, Rajkumar SV, Fonseca R, Witzig TE, Lust JA, Larson DR, Kyle RA, Greipp PR (2004) Clinical course of patients with relapsed multiple myeloma. Mayo Clinic Proceedings 79: 867–874. https://doi.org/10.4065/79.7.867

Maher M, Diesch J, Casquero R, Buschbeck M (2018) Epigenetic-transcriptional regulation of fatty acid metabolism and its alterations in leukaemia. Frontiers in Genetics 9: 405. https://doi.org/10.3389/fgene.2018.00405

Mirabilii S, Ricciardi MR, Allegretti M, Licchetta R, Iacovelli S, Bergamo P, Petrucci MT, Nicolai R, Peluso G, Foà R, Alimena G, Tafuri A (2012) Targeting metabolic pathways for leukemia treatment. Blood 120: 1371–1371. https://doi.org/10.1182/blood.V120.21.1371.1371

Moreau P, Kumar SK, San Miguel J, Davies F, Zamagni E, Bahlis N, Ludwig H, Mikhael J, Terpos E, Schjesvold F, Martin T, Yong K, Durie BGM, Facon T, Jurczyszyn A, Sidana S, Raje N, van de Donk N, Lonial S, Cavo M, Kristinsson SY, Lentzsch S, Hajek R, Anderson KC, João C, Einsele H, Sonneveld P, Engelhardt M, Fonseca R, Vangsted A, Weisel K, Baz R, Hungria V, Berdeja JG, Leal da Costa F, Maiolino A, Waage A, Vesole DH, Ocio EM, Quach H, Driessen C, Bladé J, Leleu X, Riva E, Bergsagel PL, Hou J, Chng WJ, Mellqvist UH, Dytfeld D, Harousseau JL, Goldschmidt H, Laubach J, Munshi NC, Gay F, Beksac M, Costa LJ, Kaiser M, Hari P, Boccadoro M, Usmani SZ, Zweegman S, Holstein S, Sezer O, Harrison S, Nahi H, Cook G, Mateos MV, Rajkumar SV, Dimopoulos MA, Richardson PG (2021) Treatment of relapsed and refractory multiple myeloma: recommendations from the International Myeloma Working Group. The Lancet Oncology 22: e105–e118. https://doi.org/10.1016/S1470-2045(20)30756-7

Moreau P, San Miguel J, Sonneveld P, Mateos MV, Zamagni E, Avet-Loiseau H, Hajek R, Dimopoulos MA, Ludwig H, Einsele H, Zweegman S, Facon T, Cavo M, Terpos E, Goldschmidt H, Attal M, Buske C, Committee EG (2017) Multiple myeloma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Annals of Oncology 28: iv52–iv61. https://doi.org/10.1093/annonc/mdx096

Ortega AD, Sanchez-Arago M, Giner-Sanchez D, Sanchez-Cenizo L, Willers I, Cuezva JM (2009) Glucose avidity of carcinomas. Cancer Letters 276: 125–135. https://doi.org/10.1016/j.canlet.2008.08.007

Pacilli A, Calienni M, Margarucci S, D'Apolito M, Petillo O, Rocchi L, Pasquinelli G, Nicolai R, Koverech A, Calvani M, Peluso G, Montanaro L (2013) Carnitine-acyltransferase system inhibition, cancer cell death, and prevention of myc-induced lymphomagenesis. Journal of the National Cancer Institute 105: 489–498. https://doi.org/10.1093/jnci/djt030

Paumen MB, Ishida Y, Han H, Muramatsu M, Eguchi Y, Tsujimoto Y, Honjo T (1997) Direct interaction of the mitochondrial membrane protein carnitine palmitoyltransferase I with Bcl-2. Biochemical and Biophysical Research Communications 231: 523–525. https://doi.org/10.1006/bbrc.1997.6089

Punnoose EA, Leverson JD, Peale F, Boghaert ER, Belmont LD, Tan N, Young A, Mitten M, Ingalla E, Darbonne WC, Oleksijew A, Tapang P, Yue P, Oeh J, Lee L, Maiga S, Fairbrother WJ, Amiot M, Souers AJ, Sampath D (2016) Expression profile of BCL-2, BCL-XL, and MCL-1 predicts pharmacological response to the BCL-2 selective antagonist venetoclax in multiple myeloma models. Molecular Cancer Therapeutics 15: 1132–1144. https://doi.org/10.1158/1535-7163.MCT-15-0730

Ricciardi MR, Mirabilii S, Allegretti M, Licchetta R, Calarco A, Torrisi MR, Foa R, Nicolai R, Peluso G, Tafuri A (2015) Targeting the leukemia cell metabolism by the CPT1a inhibition: functional preclinical effects in leukemias. Blood 126: 1925–1929. https://doi.org/10.1182/blood-2014-12-617498

Samudio I, Harmancey R, Fiegl M, Kantarjian H, Konopleva M, Korchin B, Kaluarachchi K, Bornmann W, Duvvuri S, Taegtmeyer H, Andreeff M (2010) Pharmacologic inhibition of fatty acid oxidation sensitizes human leukemia cells to apoptosis induction. Journal of Clinical Investigation120: 142–156. https://doi.org/10.1172/JCI38942

Souers AJ, Leverson JD, Boghaert ER, Ackler SL, Catron ND, Chen J, Dayton BD, Ding H, Enschede SH, Fairbrother WJ, Huang DC, Hymowitz SG, Jin S, Khaw SL, Kovar PJ, Lam LT, Lee J, Maecker HL, Marsh KC, Mason KD, Mitten MJ, Nimmer PM, Oleksijew A, Park CH, Park CM, Phillips DC, Roberts AW, Sampath D, Seymour JF, Smith ML, Sullivan GM, Tahir SK, Tse C, Wendt MD, Xiao Y, Xue JC, Zhang H, Humerickhouse RA, Rosenberg SH, Elmore SW (2013) ABT-199, a potent and selective BCL-2 inhibitor, achieves antitumor activity while sparing platelets. Nature Medicine 19: 202–208. https://doi.org/10.1038/nm.3048

Tirado-Velez JM, Joumady I, Saez-Benito A, Cozar-Castellano I, Perdomo G (2012) Inhibition of fatty acid metabolism reduces human myeloma cells proliferation. PloS one 7: e46484. https://doi.org/10.1371/journal.pone.0046484

Touzeau C, Dousset C, Le Gouill S, Sampath D, Leverson JD, Souers AJ, Maiga S, Bene MC, Moreau P, Pellat-Deceunynck C, Amiot M (2014)

The Bcl-2 specific BH3 mimetic ABT-199: a promising targeted therapy for t(11;14) multiple myeloma. Leukemia 28: 210–212. https://doi.org/10.1038/leu.2013.216

Valdez BC, Li Y, Murray D, Liu Y, Nieto Y, Bashir Q, Qazilbash MH, Andersson BS (2020) Panobinostat and venetoclax enhance the cytotoxicity of gemcitabine, busulfan, and melphalan in multiple myeloma cells. Experimental Hematology 81: 32–41. https://doi.org/10.1016/j.exphem.2020.01.003

van de Donk N, Pawlyn C, Yong KL (2021) Multiple myeloma. Lancet 397: 410–427. https://doi.org/10.1016/S0140-6736(21)00135-5

Warburg O (1956) On the origin of cancer cells. Science 123: 309–314. https://doi.org/10.1126/science.123.3191.309

Zhao Y, Butler EB, Tan M (2013) Targeting cellular metabolism to improve cancer therapeutics. Cell Death & Disease 4: e532. https://doi.org/10.1038/cddis.2013.60