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Research article

In-vitro safety assessment of meropenem on human retinal pigment epithelium (RPE)

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ABSTRACT

Purpose: Endophthalmitis is a severe infection accompanied by inflammation that affects the anterior and posterior parts of the eye. It is typically treated with a combination of antibiotics that cover various microorganisms. However, retinal pigment epithelium (RPE) cells are highly susceptible to damage from intravitreal injection therapy. This study aimed to investigate the impact of clinically relevant concentrations of meropenem (alone or in combination with vancomycin) on the viability and inflammation of RPE cells.

Design: In-vitro Study.

Methods: RPE cells from passages 5–7 were treated with different concentrations of meropenem (1/4x, x, and 4x; [x = 16 mg/L]), vancomycin (30 mg/L), and meropenem (x) plus vancomycin for 24 h. The morphology assessment and MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay were performed to evaluate cytotoxicity due to drug treatment. Real-time PCR was used to measure the relative expression of apoptotic genes (BCL-2 and BAX) and inflammation biomarkers (IL-1b and IL-6).

Results: Meropenem (alone or in combination with vancomycin) did not have any significant effect on RPE cell morphology, density, and viability. Gene expression analysis confirmed these results, showing no significant changes in the BCL-2/BAX ratio in drug-treated RPE cells compared to controls. Treatment with meropenem significantly induced the expression of IL-1b at all studied concentrations. Additionally, at concentrations of x and 4x, it also significantly increased the expression of IL-6, which was dose-dependent. However, this effect was not observed with vancomycin alone or in combination with meropenem.

Conclusions: The results of this study suggest that meropenem, either alone or in combination with vancomycin, does not induce RPE cytotoxicity. There was an upregulation of IL-1b and IL-6 in meropenem monotherapy, the clinical implication of which should be elucidated in future in-vivo or clinical studies.

1. Introduction

Endophthalmitis, a severe infection accompanied by inflammation that affects both the anterior and posterior parts of the eye, often arises from infectious agents such as bacteria, fungi, or parasites [1,2]. Microorganisms of exogenous origin typically infiltrate the eye during ocular surgery, intravitreal injections, or open globe injuries. The severity and progression of infectious endophthalmitis are

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influenced by the virulence of the infectious agent, delay in diagnosis, and the patient's immune status [3].

The infectious cascade of endophthalmitis begins with the introduction of a critical microbial load into the eye, followed by a phase of proliferation and subsequent infiltration of neutrophils. This leads to an accelerated immune response characterized by the accumulation of macrophages and lymphocytes in the vitreous cavity [4].

Despite the immune response, the vitreous lacks vasculature, hindering efficient microbial control and potentially resulting in tissue destruction if left untreated [5]. Prompt intervention with intravitreal antibacterials becomes imperative to halt the progression of infection [6].

Standard treatment for bacterial endophthalmitis typically involves simultaneous administration of vancomycin and ceftazidime, although incompatibilities between these agents have been noted [7,8]. Moreover, the widespread use of antibiotics may contribute to resistance, necessitating exploration of alternative antimicrobial agents.

Meropenem, a broad-spectrum carbapenem, holds FDA approval for treating various infections, including those caused by Gram-positive and Gram-negative pathogens. This makes it a promising candidate for managing endophthalmitis [9,10]. However, its safety and efficacy specifically in endophthalmitis treatment warrant investigation.

The retinal pigment epithelium (RPE) is the outermost cellular layer of the retina and constitutes the outer blood-retinal barrier. In contrast to the neural retina, the RPE has proliferative capacity and is highly vulnerable to drug-induced damage [11,12]. Despite the therapeutic potential of meropenem, its impact on RPE cells and its utility in endophthalmitis treatment remains unexplored. Therefore, this study aims to evaluate the in-vitro safety of meropenem, alone or in combination with vancomycin, on RPE cells through different approaches. The focus will be on apoptosis and inflammation biomarkers such as BCL-2, BAX, interleukin (IL)-1b, and IL-6 gene expression.

2. Materials and methods

2.1. RPE cell culture

The study used human eye globes obtained from two male cadavers, aged 25 and 32, who had consented to organ donation. The globes were obtained from the Central Eye Bank of Iran and transferred to the laboratory under sterile conditions. The globes used for the RPE explant culture remained viable for up to 48 h post-mortem. The lateral tissues were removed, and a small incision was made in the pars plana to evacuate the vitreous. The globe was then cut from the peripheral edges and opened to access the internal tissues. After thoroughly rinsing the globe with sterile PBS, the neural retina was removed, and the pigmented layer (indicating the RPE cells) was detached from the globe. The pigmented RPE monolayer was then cut into approximately 2 $\times$ 2 mm pieces and seeded as an explant culture onto the plate surface. The explants were incubated at 37 °C in a CO2 incubator with complete culture medium composed of DMEM/F12, fetal bovine serum (FBS 10 %), penicillin (120 g/mL), and streptomycin (220 g/mL) added to the plate. RPE cells began to grow from the periphery of the explants approximately 10 days after culturing and were passaged once a week. The RPE cells from the first passage were confirmed to express RPE65 (Rabbit anti-human RPE65 polyclonal antibody, Santa Cruz) as a marker of RPE cells using immunocytochemistry testing [13–15]. RPE cells from passages 5–7 were used for drug treatment and subsequent analysis.

2.2. Drug preparation

Previous studies have reported clinical concentrations of vancomycin as 30 mg/L [16]. The dose of meropenem was selected according to the MIC of this drug. Minimum Inhibitory Concentration, abbreviated as MIC, refers to the smallest amount of an antimicrobial substance required to prevent the visible growth of a microorganism in a lab setting. This crucial measurement is utilized to assess the effectiveness of an antimicrobial agent in halting the growth of particular bacterial or fungal species. Based on the maximum MIC of meropenem for the most important ocular pathogens, the x concentration of meropenem was considered as 16 mg/L [17], and the effect of 4x, x, and 1/4x concentrations of meropenem (Meroxan®, Dana Pharma Co., Iran) were used for RPE treatment. The study also assessed the effects of vancomycin (Vancomax®, 30 mg/L, Dana Pharma Co., Iran) and the combination of vancomycin + meropenem (vancomycin: 30 mg/L and meropenem: 16 mg/L) on RPE cells in terms of cell toxicity and gene expression.

The incubation time with the various concentrations of meropenem (either alone or in combination with vancomycin) in all experiments (microscopic analysis, MTT assay, and gene expression analysis) was 24 h.

2.3. Microscopic analysis

In this study, we investigated the impact of meropenem on RPE cell density using inverted microscopic imaging (at a magnification of 100x) under various treatments. These treatments included 24 h exposure to different concentrations of meropenem (at 1/4x, x, and 4x), vancomycin, and a combination of meropenem and vancomycin. Additionally, we explored the effect of meropenem on RPE cell proliferation using microscopic video capture. This method allowed us to track the RPE cells as they underwent cell division. We conducted real-time observations of treated cells over a 75-min period, capturing videos every 10 min for 5 min each time, in order to assess the influence of drug treatment on cell dynamics and proliferation by spotting cells in the division phase.

2.4. MTT assay

To analyze the toxicity of meropenem alone or in combination with vancomycin on RPE cell viability, an MTT assay was performed following the manufacturer's protocol. RPE cells were cultured at a concentration of 10^4 cells/well within 96 well microplates at 37 °C. After 24 h, the previous medium was removed, and drug-containing medium (meropenem (1/4x), (x), (4x), vancomycin, and meropenem plus vancomycin) was added to the cells as treatment. The control group received no drugs under the same conditions. The incubation lasted another 24 h. Next, 10 μL of MTT (5 mg/mL, GoldBio) was added to each well and incubated for 4 h at the same temperature. Then, 100 μL of lysis buffer (DMSO, Parstous) was added to the wells and incubated for 1 h. The optical density of each well was evaluated using a microplate reader (BMG LABTECH), and cell viability was assessed in drug-treated RPE cells compared to the control.

2.5. RNA extraction, cDNA synthesis, and real-time PCR

RPE cells were seeded at a concentration of 10^6 cells/10 cm^2 and treated with meropenem (1/4x), (x), (4x), vancomycin, and meropenem plus vancomycin for 24 h at 37 °C. The control group received no drugs under the same conditions. Total RNA was extracted using the RNeasy kit (Parstous) according to the recommended protocol, and cDNA was synthesized using the Easy cDNA Synthesis Kit (Parstous). Primer sequences for BAX, BCL-2, and β-actin were designed using the open-source NCBI primer designing tool (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) and verified for efficiency rate using AllelID software (v.7.5, Premier Biosoft International, Palo Alto, CA, USA). Table 1 shows the primer sequences used in this study.

To quantify the relative expression of the genes, real-time PCR was conducted using the Magnetic Induction Cycler (Mic qPCR, Bio Molecular Systems) and RealQ Plus Master Mix Green (Ampliqon, Denmark). Each reaction included 5 μL of master mix, 1 μL of F and R primer-mix, and 4 μL of DNA for a total volume of 10 μL. The thermal profile used included 40 cycles of 95 °C hold phase (15 min), 95 °C denaturation phase (10 s), and 61 °C annealing and extension phase (45 s). Finally, the relative mRNA expression was calculated using the $2^{-\Delta\Delta Ct}$ method.

2.6. Statistical analysis

All experiments were performed in triplicate, and the data obtained were analyzed using the SPSS software (version 22, SPSS Inc. Chicago, IL, USA). The normality of the data was assessed using the Kolmogorov-Smirnov test. Statistical analysis was conducted using one-way analysis of variance (ANOVA) or the Kruskal-Wallis test for normally and non-normally distributed data, respectively, with a significance level of $P < 0.05$. The Bonferroni correction was used for pairwise comparisons.

3. Results

Morphological Resilience and Proliferative Continuity of RPE Cells in Response to Antibiotic Treatments

RPE cells began to proliferate from the margin of the retinal explants approximately one week after the start of culture. The cells exhibited the typical morphology of highly pigmented RPE cells in the early passages (Fig. 1A). However, as the cells were repeatedly cultured, the pigmentation gradually disappeared, and the cells exhibited a spindle-shaped morphology (Fig. 1B). The primary cultures of RPE cells were confirmed to express the RPE65 marker using the ICC test (Fig. 1C). The fields (Fig. 1D) & (Fig. 1E) show the secondary antibody control confirming the lack of fluorescence in cultures without the secondary antibody of RPE65.

Fig. 2 represents the cell following treatment with different concentrations of meropenem, vancomycin, and a combination of meropenem and vancomycin. The results indicate that RPE cell morphology, distribution and density were not affected by the various treatments, suggesting the safety of meropenem, whether administered alone or in combination with vancomycin. Additionally, we observed RPE cells undergoing cell division under the various treatments applied in the study (supplementary video). These findings suggest that meropenem does not interfere with cell proliferation.

3.1. Nontoxic effect of meropenem on RPE cells

The MTT assay showed that meropenem, whether used alone or in combination with vancomycin, did not have any toxic effects on RPE cells (Fig. 3; top left panel).

Table 1
The primer sequences of the genes under study.

Gene	Sense primer	Anti-sense primer	Product size
BCL-2	CCCGCGACTCCTGATTCATT	CAGTCTACTTCCTCTGTGATGTTGT	167 bp
BAX	TTCTGACGGCAACTTCAACTGG	CACAGGGCCTTGAGCACC	78 bp
IL-1b	AGCAACAAGTGGTGTTCTCC	TGGGATCTACACTCTCCAGC	153 bp
IL-6	TCCTTCTCCACAAGCGCC	ATGCCGTCGAGGATGTACC	185 bp
β-ACT	GCCTCGCCTTTGCCGAT	CATGCCGGAGCCGTTGT	98 bp

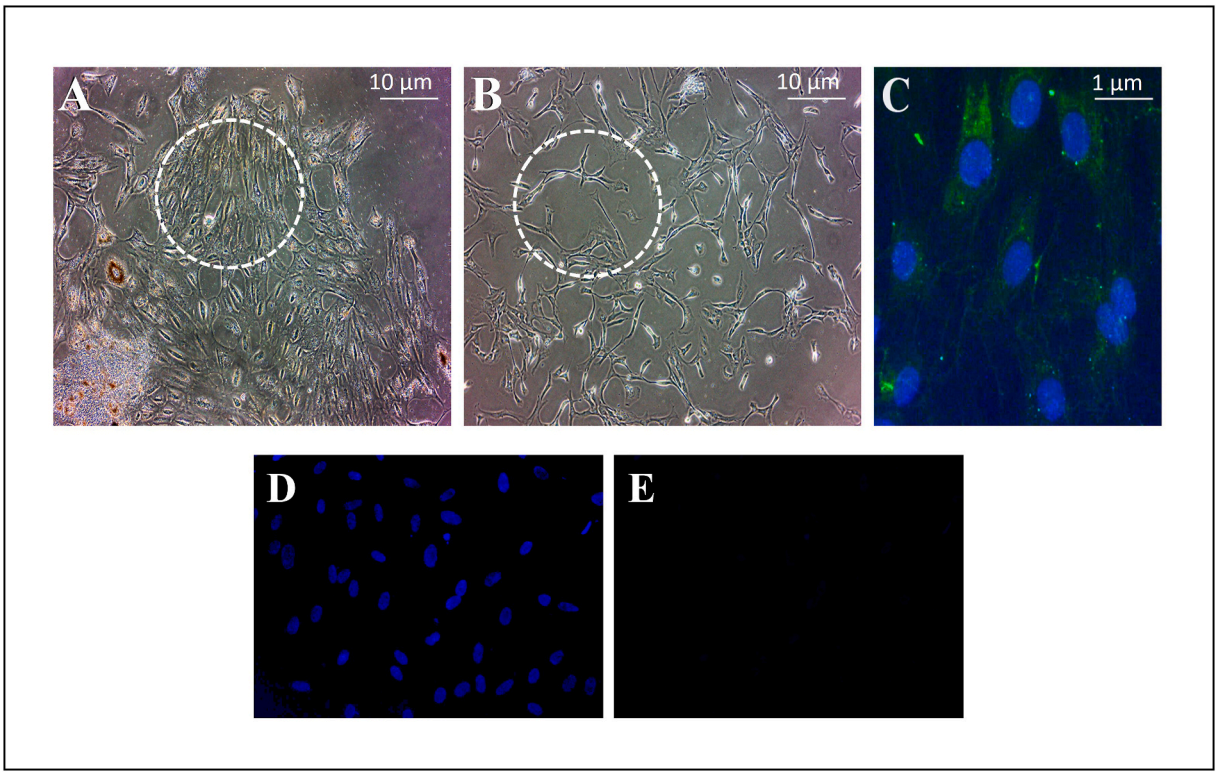

Fig. 1. RPE characterization; (A) Representative image showing proliferating RPE cells with highly pigmented morphology at early passages, observed approximately one week after the initiation of culture. **(B)** Representative image demonstrating changes in cell morphology as pigmentation gradually diminishes and cells adopt a spindle-shaped appearance with repeated passages. **(C)** Immunocytochemistry (ICC) analysis confirming expression of the RPE65 marker in primary cultures of RPE cells. (D) & (E) fields show the secondary antibody control confirming the lack of fluorescence in cultures without the secondary antibody of RPE65.

3.2. Meropenem modulates gene expression without altering apoptotic balance in RPE cells

The analysis of gene expression showed that BAX gene expression was significantly increased in meropenem-treated RPE cells (at 1/4x, x, and 4x concentrations) compared to the control group. However, BAX expression was not significantly altered in vancomycin and vancomycin plus meropenem-treated RPE cells. The BCL-2 gene expression was significantly upregulated in RPE cells treated with the 1/4x concentration of meropenem (Fig. 3; bottom left panel). The BCL-2/BAX ratio, which is an index of apoptosis, was also assessed in our study (Fig. 3; top right panel). The data showed that there was no significant alteration in the BCL-2/BAX ratio from 1:1, indicating the in-vitro safety of meropenem (alone at all studied concentrations, or in combination with vancomycin) on RPE cells.

We also observed that treatment of RPE cells with meropenem at all concentrations significantly induced the expression of IL-1b, and also increased the expression of IL-6 gene at x and 4x concentrations (Fig. 3; bottom right panel). However, this effect was not observed in vancomycin and vancomycin plus meropenem-treated RPE cells.

To investigate whether the potential toxic effect of meropenem was dose-dependent or not, we focused on the pairwise comparison of different concentrations of meropenem. We found no difference in the expression of BCL-2, BAX, BCL-2/BAX ratio, and IL-1b. However, we observed a higher expression of the IL-6 gene at the 4x concentration compared to the 1/4x and x concentrations ($P < 0.001$ and $P = 0.005$, respectively). There was no significant difference between the 1/4x and x concentrations ($P = 0.553$).

4. Discussion

Despite significant advances in medical knowledge, endophthalmitis remains a leading cause of blindness and vision impairment. Acute bacterial endophthalmitis poses a serious threat to vision and requires urgent management. The clinical outcome depends on both the virulence of the infecting organism and the prompt initiation of appropriate treatment. Intravitreal antibiotic therapy is the cornerstone of endophthalmitis treatment, as it ensures adequate antibiotic levels within the eye. Empirical therapy often involves a combination of vancomycin with ceftazidime or amikacin. However, these regimens face challenges such as antibiotic resistance and cytotoxicity, limiting their effectiveness and safety.

Given the progressive rise in bacterial resistance to existing antibiotics, there is a constant need to explore new therapeutic options. Studies have shown meropenem to be effective against many prevalent and virulent pathogens causing bacterial ocular infections [17].

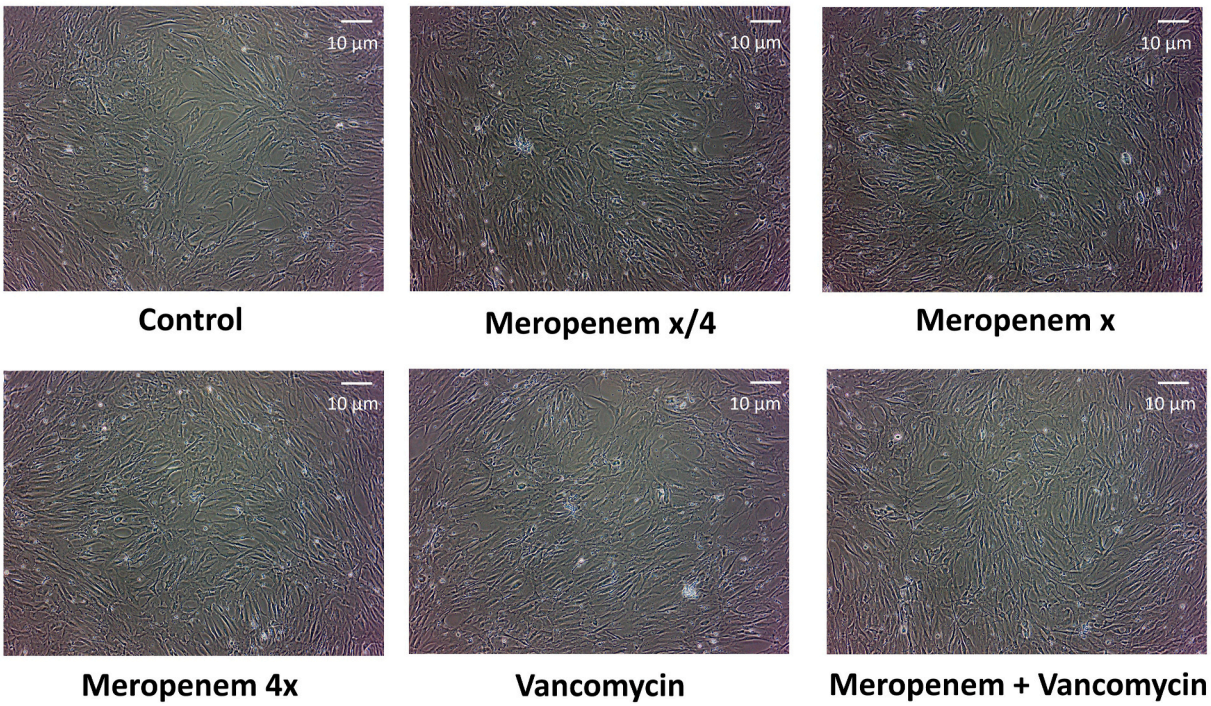

Fig. 2. RPE cell morphology under varying treatments of meropenem, vancomycin, and their combination (invert microscopy, Mag: 100x). The data showed consistent RPE cell distribution and density across treatment with meropenem (1/4x, x, and 4x; [x = 16 mg/L]), vancomycin (30 mg/L), and meropenem (x) plus vancomycin, in comparison with controls, indicating the safety of meropenem alone or in combination with vancomycin. The incubation time with the various concentrations of meropenem (either alone or in combination with vancomycin) was 24 h.

In addition meropenem exhibits favorable intraocular penetration when administered systemically [18].

In the present study, the x concentration of meropenem was chosen based on the maximum MIC against the most common, virulent, or resistant ocular pathogens, including coagulase-negative staphylococcus, *S. aureus*, streptococci, Enterobacteriaceae, and *P. aeruginosa* [17]. The highest MIC among the aforementioned pathogens was 16 mg/L for *P. aeruginosa*. Additionally, we included two logarithmical proportions (1/4 and 4 times) around the chosen level to create a range of concentrations to be studied. We also assessed the meropenem (x concentration) plus vancomycin (30 mg/L), because this combination has additive bacterial coverage and has the potential to be used as a starting empirical therapy for postoperative bacterial endophthalmitis.

In vitro studies have gained popularity as the initial step to investigate the safety of new drugs on ocular tissue [19–21] and thus provide baseline information for further in vivo or clinical studies. RPE is one of the most sensitive cell types in the retina and though is a suitable initial target for drug safety studies [22]. In this study, we used several approaches to investigate meropenem toxicity on RPE cells, including morphologic assessment with light microscopy (cell shape, density, cell division), MTT assay (mitochondrial apoptosis), and gene expression analysis (apoptotic and inflammatory pathways).

The results from the light microscopy morphologic study, MTT test and the apoptosis index (BCL-2/BAX ratio) indicated that meropenem, administered at concentrations of 1/4x, x, and 4x, as well as in combination with vancomycin, did not exhibit toxic effects on human RPE cells. In our study, although the statistical analysis did not reveal a significant difference between the treated groups and the control, a closer examination of the raw data suggests that lower concentrations of meropenem and vancomycin may indeed promote cell proliferation. The phenomenon where cell viability appears greater than 100% is not uncommon in cell proliferation assays and can be attributed to several factors, including experimental variability and the nature of the assay itself. This observation aligns with the concept of hormesis, where substances can elicit a stimulatory effect at low doses. We hypothesize that this could be a compensatory response of the RPE cells to the antibiotic treatment during the initial exposure period [23]. These findings suggest a favorable safety profile of meropenem on RPE cells, even at doses 4 times the maximum MIC for *P. aeruginosa*.

Mitochondrial apoptosis refers to the programmed cell death induced by mitochondrial processes, and controlled by the BCL-2 family of proteins [24]. BCL-2, an anti-apoptotic protein, and BAX, an apoptotic protein, play important roles in regulating cell death processes. BCL-2, a member of the BCL-2 family, inhibits apoptosis by preventing the release of cytochrome *c* and reactive oxygen species from mitochondria, thereby promoting cell survival [25,26]. On the other hand, BAX, another member of the BCL-2 family, acts as a pro-apoptotic protein, promoting the permeabilization of the mitochondrial membrane and the release of apoptotic signals. The balance between these two proteins, as indicated by the BCL-2/BAX ratio, is crucial in determining the susceptibility threshold to mitochondrial apoptosis. A stable ratio observed under certain conditions can indicate the absence of apoptosis induction and suggest safety [27–30]. Our findings revealed that the BCL-2/BAX ratio remained unchanged across all concentration

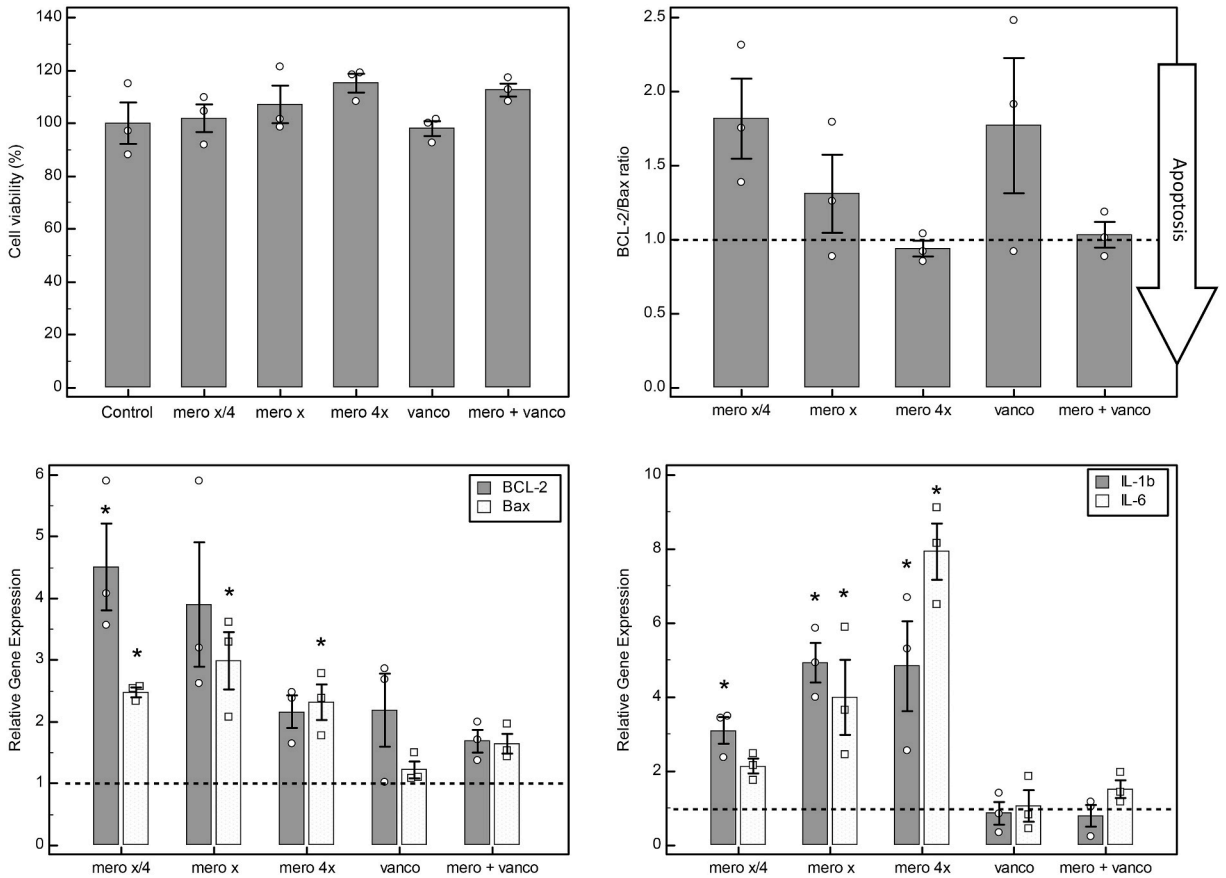

Fig. 3. Top left: MTT assay; **Top right:** BCL-2/BAX ratio; **Bottom left:** Relative gene expression of BCL-2 and BAX; **Bottom right:** Relative gene expression of inflammatory genes (IL-1b, IL-6) in RPE cells treated with meropenem (1/4x, x, and 4x; [x = 16 mg/L]), vancomycin (30 mg/L), and meropenem (x) plus vancomycin, in comparison with controls (the dashed line). The incubation time with the various concentrations of meropenem (either alone or in combination with vancomycin) was 24 h. The mean value of relative expression ± standard error of mean (SEM) is represented in the graph. All experiments were performed in triplicate. The individual data points of independent biological repetitions are provided on the figure. Asterisk signs (*) bold the statistically significant differences ($P < 0.05$).

ranges studied, compared to the control group. This suggests that apoptosis was not induced and indicates that the treatment is safe in this regard.

Although our study primarily focused on BAX and BCL-2 gene expression and their role in apoptosis, the BCL-2 family proteins also play a crucial role in autophagy. Given Bcl-2's ability to inhibit Beclin-1, an important protein in autophagy initiation, the upregulation of BCL-2 at lower concentrations of meropenem (1/4 x) may have the potential to suppress autophagy. However, the process of autophagy is regulated by other important pathways such as autophagic flux, LC3-II and p62 that were not assessed in our study. Therefore, we cannot come up with a valid conclusion about the effect of meropenem on autophagy of RPE cells, and this issue may be the subject of future studies [31,32].We also observed a significant increase in the expression of IL-1b and IL-6 genes following treatment with meropenem at all doses studied, except for IL-6 at the dose of 1/4x. Similar effects have been reported in previous studies involving certain cell types after antibiotic treatment [33,34]. This is important because hemorrhagic occlusive retinal vasculitis (HORV) is a rare but potentially blinding complication of some antibiotics, particularly vancomycin and gentamicin, after intraocular injection [35,36]. Both IL-1b and IL-6, which are being investigated here, have been implicated in the development of retinal vasculitis caused by other, more common factors. The use of their antagonists has proven successful in the treatment of retinal vasculitis [37,38]. However, it is worth noting that the levels of cytokines observed in in-vitro experiments may differ from those observed in in-vivo examinations, and resultant clinical consequences could not be easily anticipated [34]. Nonetheless, these changes should alert us to possible immune-mediated complications, particularly those mediated by IL-6, which also showed a dose-dependent increase.

While many studies focus on reducing inflammatory cytokines, research suggests that transient increases in cytokine levels may have beneficial effects on cells. For example, IL-1b is a crucial proinflammatory cytokine involved in the response to infection, contributing to host defense mechanisms against infection and injury [39]. It has been shown that a transient inflammatory response mediated by IL-1b is necessary for cell regeneration [40]. In addition, Juel et al. demonstrated that cytokine increases can induce antioxidative stress responses and activate antioxidant pathways in RPE cells, potentially protecting them from oxidative

stress-induced death [41].

Currently, there have been limited cytotoxicity studies conducted on RPE cells, and there is a shortage of research on the effects of drugs on the human retina compared to animal models. One notable study in this field is by Ay et al. They investigated the effects of intravitreal ceftazidime and meropenem in an animal laboratory model of pseudomonal posttraumatic endophthalmitis in rabbits [42]. The study found no significant difference in the vitreous concentrations of three different doses of meropenem (0.5, 1, and 2 g/L) 2 h after treatment. Both meropenem and ceftazidime were found to be effective in the treatment of endophthalmitis, with no discernible difference in therapeutic properties between the two antibiotics. However, the study by Ay et al. did not provide clear information regarding the safety of meropenem on the retina and RPE, creating a gap in understanding the potential retinal toxicity of this drug.

4.1. Power and limitations

This study is the first of its kind to assess the safety of meropenem on human RPE cells in vitro. Since RPE cells are sensitive to environmental changes, the results can serve as a reference for further in vivo or clinical studies, eventually leading to an optimal prophylactic or therapeutic dose for intravitreal injection. We did not include other retinal cell pathways, so the outcomes should not be generalized to overall retinal safety. We used a multidimensional approach to assess RPE safety, including morphology, MTT assay, apoptotic and inflammatory gene expression analysis. However, due to resource constraints, our study was limited to the analysis of a select number of inflammatory cytokines at the transcriptional level, and did not include protein analysis. Nonetheless, we included two of the most important ones that have been implicated in retinal vasculitis [37,38] a potential serious side effect of intravitreal drug injection. It's important to note that the findings of this study cannot be generalized to in vivo and clinical conditions, which involve more complex and intricate interactions at the cellular level.

Another limitation of our study is the 24-h incubation period for antibiotic treatment. The pharmacokinetics of intravitreal injections allow for sustained drug presence, potentially up to several days. However, it is important to consider the dynamic nature of the RPE in vivo. The RPE has a strong regenerative capacity and metabolizes drugs while renewing itself. Ocular blood flow also helps to clear the drugs from the RPE cells over time. In vitro settings present challenges because they do not accurately replicate these conditions. In vitro environments accumulate metabolic byproducts and cellular waste, which can cause cytotoxicity. Also, replacing the culture medium to mimic drug clearance would not accurately reflect the gradual decrease in drug concentration in vivo. Therefore, our study focused on the first 24 h of drug exposure to simulate the early in vivo environment and establish a safe dose range to be tested in future animal studies, which are optimal for understanding the drug's long-term effects.

Finally, the concentration of meropenem in this study was determined using a study on ocular pathogens from corneal keratitis cases [17]. We were unable to find a similar high-quality article on infectious endophthalmitis, but the reported pathogens closely resemble those that are prevalent or important in postoperative endophthalmitis.

5. Conclusions

The results of this study showed that meropenem and the combination of meropenem and vancomycin, at clinically effective doses, have no cytotoxic effect on RPE cells. Therefore, they can be considered as possible candidates in future animal and clinical studies for the prophylaxis or treatment of endophthalmitis. We observed an increase in IL-1b and IL-6 with meropenem monotherapy, while the meropenem/vancomycin combination did not lead to an overproduction of inflammatory cytokines, suggesting a different outcome compared to meropenem alone. Whether the induction of inflammatory cytokines by meropenem could be a clinical concern needs further investigation through future in-vivo studies.

Ethics approval and consent to participate

The protocol used in this study was approved by the Ethics Committee of Shiraz University of Medical Sciences (IR.SUMS.MED.REC.1401.567).

Consent for publication

Not applicable.

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Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Fatemeh Sanie-Jahromi: Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Seyedeh Shahla Hoseini:** Writing – original draft, Visualization, Formal analysis, Conceptualization. **M. Hossein Nowroozzadeh:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e33916.

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