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EXPERIMENTAL STUDIES

A BIPHASIC EFFECT OF TUMOR NECROSIS FACTOR-α ON RPMI 2650 CELL LINE *IN VITRO*

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Tumor necrosis factor-α (TNFα) is a key proinflammatory cytokine; its level increased in inflammatory diseases of the upper respiratory tract. In this study, the dose- and time-dependent effects of TNFα (1–100 ng/ml, 6–48 h) on the RPMI 2650 cell line, a model of nasal epithelium, have been investigated. Short-term exposure (6 h) caused activation of NF-κB and an increase in the levels of the intercellular contact proteins E-cadherin and ZO-1, without a significant effect on cell viability. Long-term exposure (24–48 h) led to an increase in the level of pro-IL-1β, activation of apoptosis, and a decrease in cell viability. At the same time, a decrease in the level of intercellular contact proteins was noted. Thus, short-term exposure to TNFα can exert a protective effect by increasing the density of intercellular contacts, while during prolonged exposure it triggers apoptosis and reduces the density of intercellular contacts, which can contribute to increased permeability of the cell layer.

Keywords: tumor necrosis factor; RPMI 2650; apoptosis; intercellular contact proteins

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INTRODUCTION

Tumor necrosis factor-α (TNFα) is a central cytokine involved in the regulation of the immune response, inflammation, and cell death [1]. It can act as a mediator, activating the expression of secondary proinflammatory cytokines, such as IL-1, and adhesion molecules through NF-κB, thus enhancing the inflammatory response [2]. TNFα also triggers apoptosis by activating effector caspases and disrupting the balance of Bcl-2 proteins [3].

Intranasal administration is a promising route for drug delivery to the central nervous system (CNS), bypassing the blood-brain barrier (BBB), and into the systemic circulation [4]. Any intranasally administered drug initially contacts the nasal epithelium and must cross it to enter the systemic circulation or be transported along the nerves to the CNS. The condition of epithelial cells and the tight junctions between them are among the main factors determining the bioavailability of drugs [5]. On the other hand, the nasal mucosa constantly contacts with the environment and is regularly exposed to infectious and non-infectious agents, which can lead to the development of a local inflammatory response. To predict the effectiveness of intranasal drug administration, it is necessary to understand how developing inflammation affects the condition of the nasal epithelium and the intercellular junctions it forms.

Cultured RPMI 2650 cells, derived from human nasal septal carcinoma, are capable of polar differentiation. They form intercellular contacts characteristic of the nasal epithelium, microvilli on the apical surface, and begin to produce mucoid material on the cell surface [6]. Due to these properties, these cells are widely used in studies of toxicity, permeability of the nasal mucosa, metabolism, and drug transport [7, 8].

The aim of this study was to evaluate the dose- and time-dependent effects of TNFα on the condition of the RPMI 2650 cell line.

MATERIALS AND METHODS

Cell Cultivation

The RPMI 2650 cell line was purchased from the Institute of Cytology, Russian Academy of Sciences (St. Petersburg, Russia). RPMI 2650 cells were cultured in MEM medium (PanEco, Russia) supplemented with 10% FBS (fetal bovine serum), 2 mM L-glutamine, 100 U/ml penicillin, and 100 mg/ml streptomycin (all components from PanEco) at 37°C and 5% CO_2 in a WS-189C incubator (World Science, Korea). The cells were cultured in flasks (25 cm^2) and 6-well plates (Corning, USA) for 14 days after the formation of a monolayer in 6-well plates (Corning) [9], then the inflammation inducer TNFα (Cloud-Clone Corp., China) was added to the nutrient medium at concentrations of 1 ng/ml, 5 ng/ml, 10 ng/ml, 50 ng/ml, and 100 ng/ml for 6 h, 24 h, and 48 h. To maintain the concentration of the test substance, the medium was changed every 12 h.

Evaluation of the Proportion of the Living Cell Population by Flow Cytometry

The proportion of the living and dead cells was assessed by flow cytometry using the DNA-binding dye DAPI (4′,6-diamidino-2-phenylindole; Sigma-Aldrich, USA). After incubation with different concentrations of TNFα for certain periods, RPMI 2650 cells were trypsinized and washed twice with phosphate-buffered saline (PBS, PanEco). The cell suspension was incubated with 0.5 μg/ml DAPI for 10 min at room temperature in the dark. Samples were analyzed immediately after staining on a SinoCyteClaster flow cytometer (SinoCyte, China) using a 405 nm laser to excite DAPI fluorescence; signal detection was performed in the channel corresponding to a wavelength of 450±50 nm. At least 10,000 events were recorded for each sample. Cells with increased DAPI fluorescence intensity, indicating membrane integrity disruption and dye penetration into the nucleus, were identified as nonviable (DAPI positive). Data were analyzed using software provided by the cytometer manufacturer.

Western Blot Analysis of the Relative Amounts of Pro-IL1β, Cleaved Caspase-3, Bcl-2, NF-κB, ZO-1, and E-Cadherin

Protein levels were assessed by Western blot. At the end of the experiment, RPMI 2650 cells were removed from the wells of a 6-well plate with trypsin-EDTA solution (0.25% trypsin and 0.2% EDTA, Sigma-Aldrich, Germany) and washed three times with phosphate buffer solution (PanEco). To determine pro-IL1β, cleaved Caspase-3, Bcl-2, ZO-1, and E-cadherin, cells were lysed in RIPA Cell Lysis Buffer Thermo (Thermo Fisher Scientific, USA) supplemented with a proteinase inhibitor mixture (Sigma-Aldrich, Germany) (10^7 cells per 100 μl of buffer) for 30 min at 4°C with constant stirring. The resulting lysate was centrifuged at 5000 *g* (CM-50, Eppendorf, Germany). The clear supernatant was used for Western blot analysis.

To determine NF-κB levels in the nuclear fraction, cells were lysed using the ReadyPrep Protein Extraction Kit (Bio-Rad, USA) with protease inhibitors (Sigma Aldrich, USA).

Thirty micrograms of total lysate proteins (pro-IL1β, cleaved Caspase-3, Bcl-2, ZO-1, E-cadherin) and nuclear fraction (NF-κB) were electrophoresed using a 7.5% TGX Stain-Free FastCast Acrylamide Kit (Bio-Rad) in Laemmli buffer (Bio-Rad). Samples were mixed with Laemmli buffer (Bio-Rad) containing 50 mM β-mercaptoethanol (BioRad) at a 1:2 ratio and incubated for 10 min at 70°C.

Gels were electrophoresed at 100 V for 90 min. Proteins were transferred to a nitrocellulose membrane (Trans-Blot Turbo Mini-Size nitrocellulose, Bio-Rad) using a Mini Trans-Blot (Bio-Rad). Software was selected based on the molecular weight of the protein.

Proteins on the membrane were blocked with 1% EveryBlot Blocking Buffer (Bio-Rad) containing 0.1% Tween for 30 min at room temperature.

Protein detection was performed using primary rabbit polyclonal antibodies to pro-IL1β, cleaved Caspase-3, Bcl-2, E-cadherin, ZO-1 (cytoplasmic lysate fraction), and NF-κB (Antibody, Affinity, China) (nuclear lysate fraction) at a concentration of 1:500 (diluted in blocking solution) for 2 h at 37°C.

Visualization of primary antibodies was performed using secondary goat antibodies (Goat-anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, HRP, Invitrogen, USA) at a dilution of 1:4000 and incubation for 1 h at room temperature. Chemiluminescence was detected using ChemiDocXRS+ (Bio-Rad). The intensity of the resulting bands was analyzed densitometrically using ImageLab software (Bio-Rad).

Molecular mass was confirmed by comparison with molecular mass markers (Precision plus protein standards Dual Color, Bio-Rad).

Cytoplasmic protein content was assessed using glyceraldehyde-3-phosphate dehydrogenase (GAPDH, primary antibody GAPDH, Affinity, diluted 1:1000), and nuclear protein content was assessed using laminin B1 (Affinity, rabbit secondary antibody — Rabbit-anti-Mouse IgG (H+L) Secondary Antibody, HRP (Invitrogen), diluted 1:4000. Optimization and validation of the Western blot method was performed for each protein.

Each experiment was performed in triplicate. Results were analyzed using GraphPad Prism Version 9.5.0. ANOVA was used to assess statistical significance, and pairwise comparisons were assessed using the Dunnett's test. Differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Treatment with TNFα increased NF-κB levels in the nuclear fraction of cell lysates after 6 h exposure to 1 ng/ml, 10 ng/ml, 50 ng/ml, and 100 ng/ml TNFα by 25.0% ($p = 0.02$), 62.3% ($p < 0.0001$), 45.5% ($p = 0.0002$), and 34.2% ($p = 0.0025$), respectively. Importantly, the changes were dose-dependent between concentrations of 1 ng/ml and 10 ng/ml, 10 ng/ml and 100 ng/ml, and between 50 ng/ml and 100 ng/ml (Fig. 1). During 24 h incubation, an increase in NF-κB levels was observed at TNFα concentrations of 10 ng/ml, 50 ng/ml, and 100 ng/ml by 137.8% ($p = 0.001$), 78.5% ($p = 0.03$), and 79.5% ($p = 0.03$), respectively; dose-dependent differences were recorded only between concentrations of 1 ng/ml and 10 ng/ml by 93.9% ($p = 0.004$) (Fig. 1). After 48 h of incubation, NF-κB levels increased in the presence of 10 ng/ml, 50 ng/ml, and 100 ng/ml TNFα by 32.0% ($p = 0.02$), 33.2% ($p = 0.02$), and 29.0% ($p = 0.03$), respectively, with no dose-dependent differences observed.

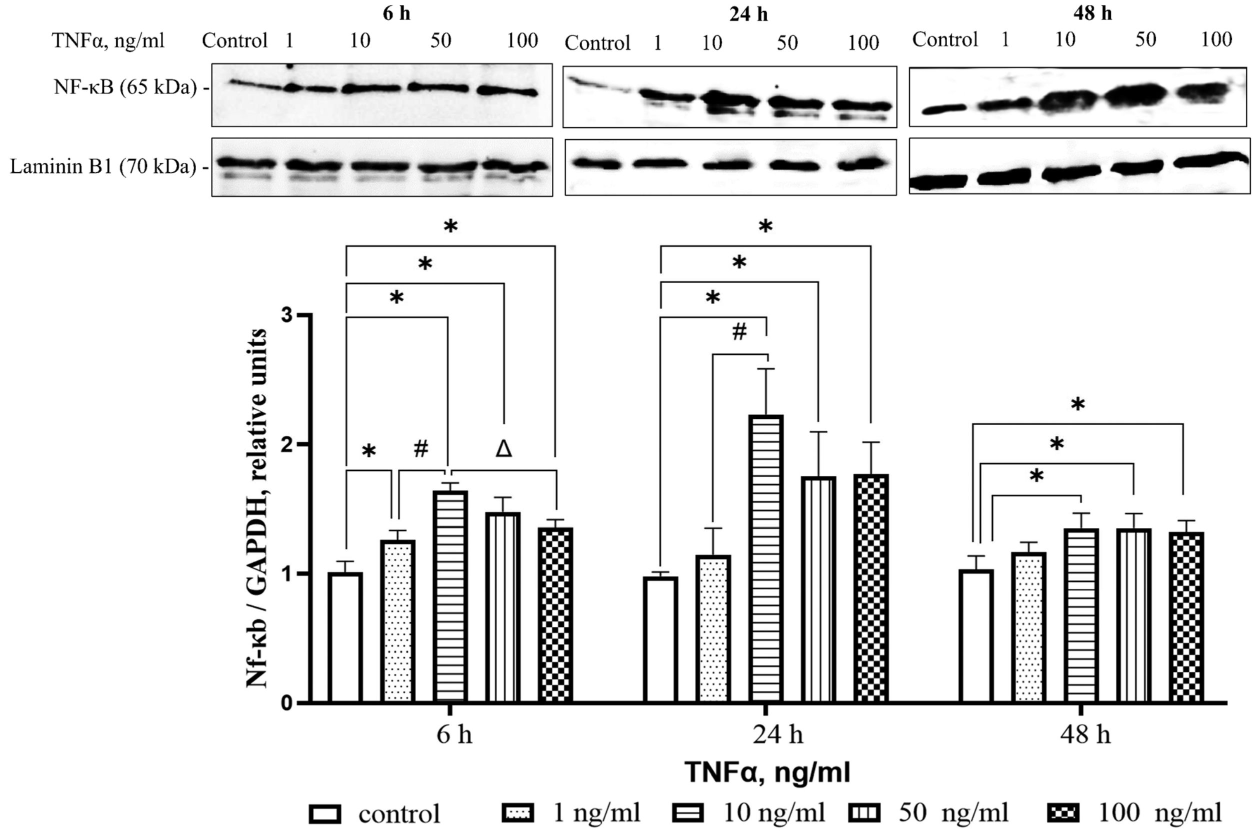

Figure 1. Relative NF-κB levels in RPMI 2650 cells treated with 1 ng/ml, 10 ng/ml, 50 ng/ml, and 100 ng/ml TNFα for 6 h, 24 h, and 48 h. Statistically significant differences: * – vs control; # – vs cells treated with 1 ng/ml TNFα; Δ – vs cells treated with 10 ng/ml TNFα.

Thus, TNFα triggers activation of the transcription factor NF-κB, a key regulatory protein that controls the expression of genes involved in cell survival, proliferation, and the inflammatory response [10]. In its inactive state, NF-κB is located in the cytoplasm in a complex with inhibitory IκB proteins. Activation of the TNFα-mediated signaling cascade in this study results in nuclear translocation of NF-κB, as confirmed by Western blot analysis. It is now known that phosphorylation and subsequent degradation of IκB underlie this process [2]. Released NF-κB translocates to the nucleus, where it binds to specific DNA sequences and regulates the transcription of target genes.

In the nucleus, NF-κB binds to the promoters of genes encoding numerous proinflammatory cytokines, including IL-1β [11].

Western blot analysis of the cytoplasmic fraction of cell lysates revealed an immunoreactive band with a molecular mass of approximately 31 kDa, corresponding to the inactive precursor form of interleukin-1β, pro-IL-1β [12].

The relative amount of pro-IL-1β did not change during 6 h exposure to TNFα. Prolonged incubation with TNFα for 24 h was accompanied by a relative increase in the pro-IL-1β level versus control at TNFα concentrations of 10 ng/ml, 50 ng/ml, 100 ng/ml by 39.0% ($p = 0.001$), 63.3% ($p < 0.0001$), and 73.7% ($p < 0.0001$), respectively. Dose-dependent changes were observed relative to the concentration of 1 ng/ml at 50 ng/ml and 100 ng/ml by 35.8% ($p = 0.0002$) and 44.2% ($p < 0.0001$), respectively. The dose-dependent changes relative to the concentration of 10 ng/ml were also observed at 50 ng/ml and 100 ng/ml by 17.3% ($p = 0.02$) and 24.4% ($p = 0.02$), respectively (Fig. 2).

Incubation for 48 h was accompanied by the dose-dependent increase relative to the control observed at TNFα concentrations of 10 ng/ml, 50 ng/ml, and 100 ng/ml by 341.0% ($p < 0.0001$), 213.0% ($p = 0.0012$), and 230.0% ($p = 0.0006$), respectively. Relative to the TNFα concentration of 1 ng/ml, statistically significant differences of 210.6% ($p < 0.0001$), 120.4% (p = 0.005), and 132.6% ($p = 0.003$) were obtained at 10 ng/ml, 50 ng/ml, and 100 ng/ml TNFα, and between concentrations of 10 ng/ml and 50 ng/ml by 29.0% ($p = 0.03$) (Fig. 2).

NF-κB is known to regulate *IL-1β* gene transcription and induce pro-IL-1β production in cells [13]. Thus, pro-IL-1β accumulation in cells serves as a direct marker of TNFα-induced NF-κB transcriptional activity and confirms activation of this signaling pathway.

To assess the functional consequences of the NF-κB–pro-IL-1β signaling pathway activation, cell viability was determined. Treatment of RPMI 2650 cells with 1 ng/ml, 10 ng/ml, 50 ng/ml,

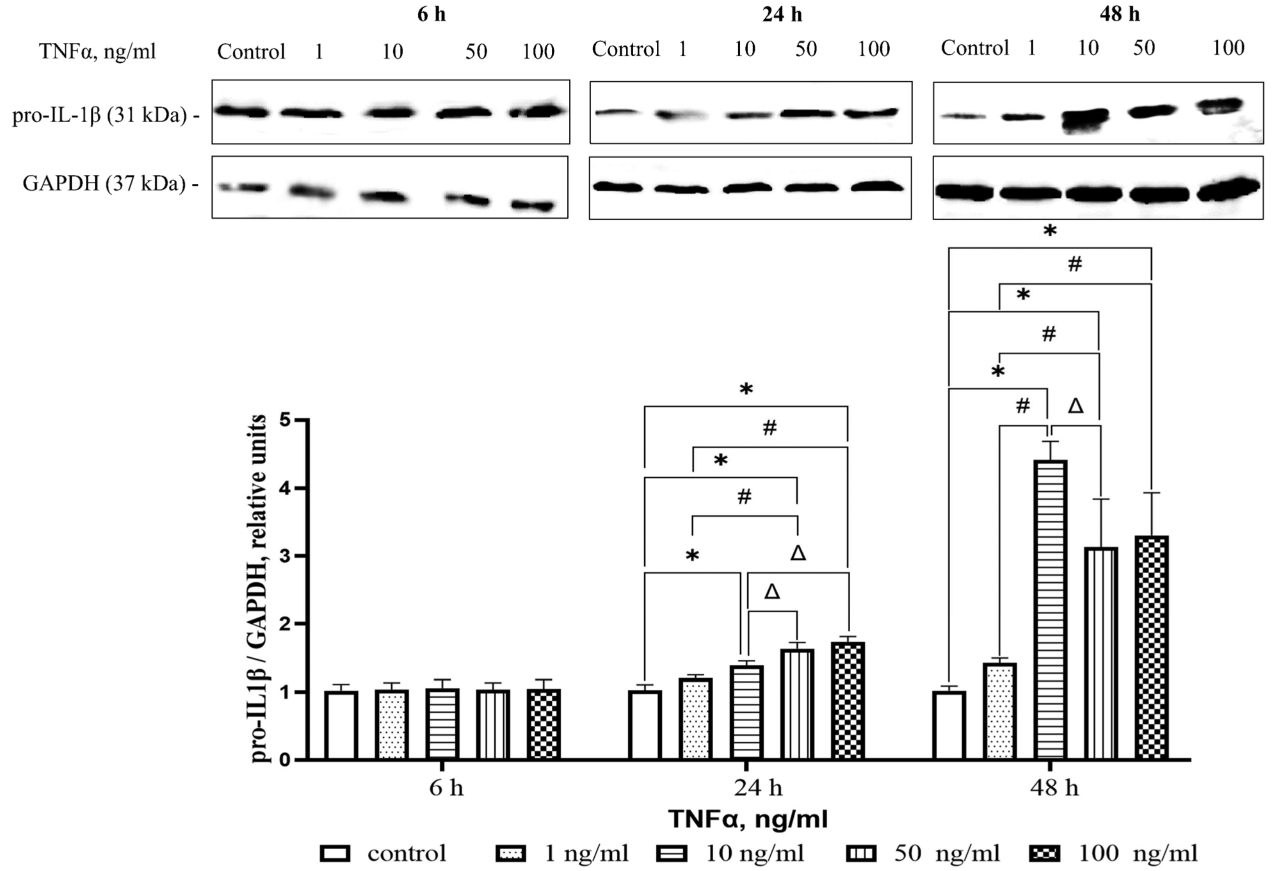

Figure 2. Relative pro-IL-1β levels in RPMI 2650 cells treated with 1 ng/ml, 10 ng/ml, 50 ng/ml, and 100 ng/ml TNFα for 6 h, 24 h, and 48 h. Statistically significant differences: * – vs control; # – vs cells treated with 1 ng/ml TNFα; Δ – vs cells treated with 10 ng/ml TNFα.

and 100 ng/ml TNFα for 6 h, 24 h, and 48 h showed a dose- and time-dependent decrease in the proportion of viable cells.

After short-term exposure (6 h), no significant decrease in viability was observed at any of the tested concentrations compared to the control group (cells not treated with TNFα). The proportion of viable cells remained at > 85.13±11.02% ($p > 0.05$) in all experimental groups, thus indicating the absence of an immediate cytotoxic effect of TNFα at this time interval. Importantly, in the control, the cell population was shifted to the left (DAPI-negative response), while the TNFα treated the cell population had the right shift, thus indicating a cell viability decrease (Fig. 3).

After 24 h exposure to 1 ng/ml, 10 ng/ml, and 50 ng/ml TNFα, the proportion of viable cells decreased slightly to 81.63±5.23% ($p > 0.05$), 77.32±8.12% ($p > 0.05$), and 73.65±10.05% ($p > 0.05$), respectively. At 100 ng/ml TNFα, a statistically significant decrease in the proportion of viable cells to 70.48±6.05% ($p = 0.03$) was observed, with the cell population shifting to the right toward an increased signal (DAPI-positive reaction) (Fig. 3).

The most significant effect was observed after 48 h incubation with TNFα. The cytotoxic effect was more pronounced: 1 ng/ml TNFα decreased the proportion of viable cells to 79.64±3.23% ($p = 0.045$), 10 ng/ml TNFα decreased to 67.64±7.43% ($p = 0.03$), and 50 ng/ml TNFα and 100 ng/ml TNFα decreased to 60.21±4.32% ($p = 0.03$) and 59.0±5.15% ($p = 0.01$), respectively. Flow cytometry analysis demonstrated a significant population shift toward DAPI-positive cells (Fig. 3).

Thus, a low DAPI signal observed in the control (DAPI-negative cells) suggests maintenance of the integrity of the nuclear membrane. Treatment with TNFα increased the number of DAPI-positive cells, indicating increased nuclear membrane permeability usually observed during the initiation of cell death. NF-κB plays a crucial role in the regulation of cell survival, regulating genes involved in cell survival and apoptosis [14].

To clarify the contribution of this mechanism, the relative amount of the anti-apoptotic protein Bcl-2 in RPMI 2650 cells was assessed.

After 6 h exposure to 1 ng/ml, 10 ng/ml, and 50 ng/ml TNFα, the Bcl-2 level in RPMI 2650 cells increased by 32.7% ($p = 0.003$), 61.4% ($p < 0.0001$), and 40.6% ($p = 0.0005$), respectively. The changes were dose-dependent: the effects induced by 1 ng/ml TNFα and 10 ng/ml TNFα differed by 21.6% ($p = 0.007$), 1 ng/ml TNFα and 100 ng/ml TNFα by 18.6%

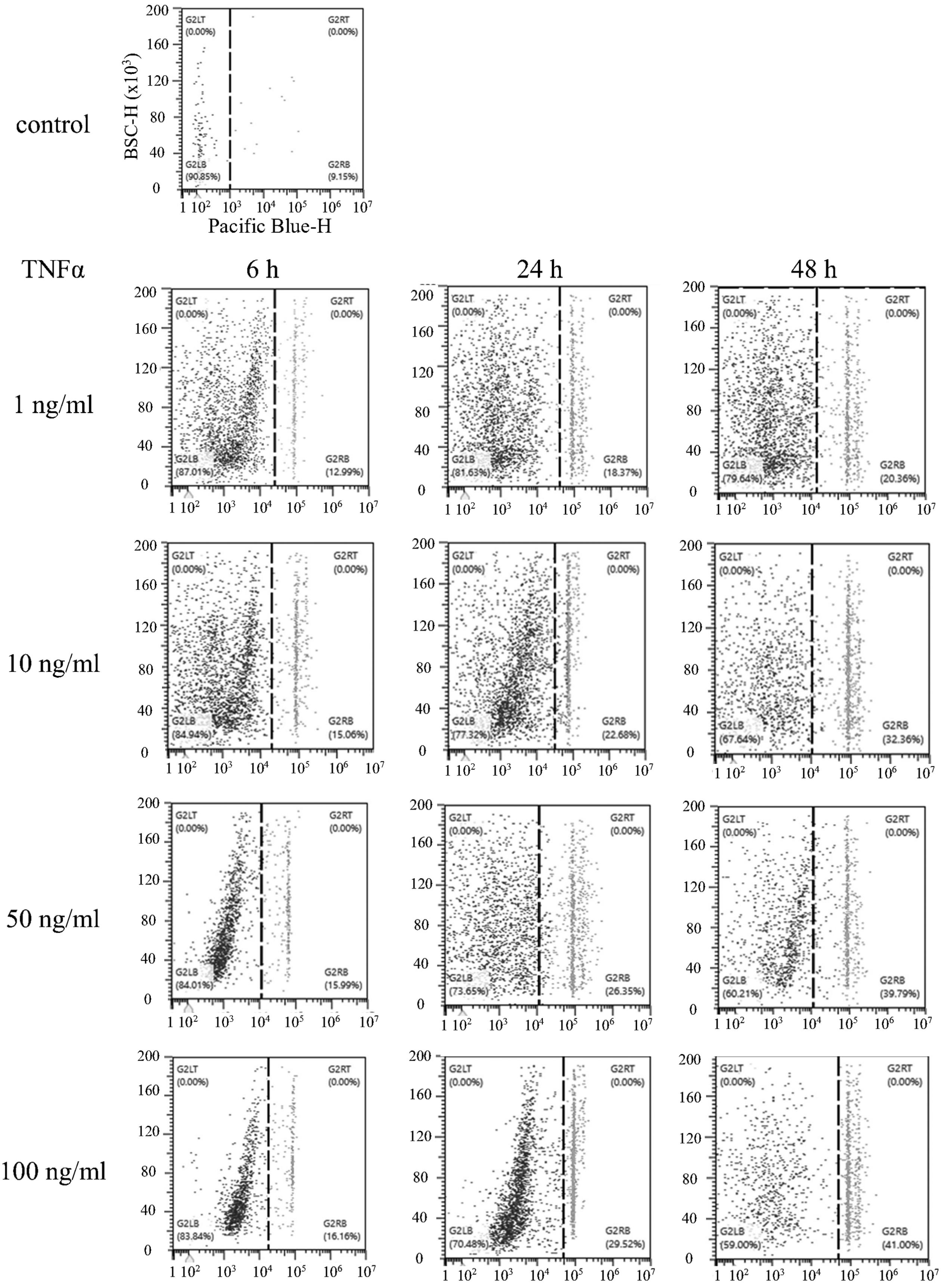

Figure 3. Assessment of DAPI-positive and DAPI-negative cell populations in the RPMI 2650 cell line exposed to TNFα for 6 h, 24 h, and 48 h. BSC-H is a physical parameter characterizing light scattering; Pacific Blue-H is the fluorescence intensity in the blue range of the spectrum.

(p = 0.02), 10 ng/ml TNFα and 100 ng/ml TNFα by 33.1% (p < 0.0001), 50 ng/ml TNFα and 100 ng/ml TNFα by 23.2% (p = 0.003). After 24 h exposure to 1 ng/ml, 10 ng/ml, and 50 ng/ml TNFα, Bcl-2 levels increased in RPMI 2650 cells by 29.0% (p = 0.0008), 33.6% (p < 0.0001), and 33.7% (p = 0.0002), respectively. Changes were dose-dependent: 23.8% (p = 0.0005) between 1 ng/ml TNFα and 10 ng/ml TNFα, 16.1% (p = 0.002) between 10 ng/ml TNFα and 50 ng/ml TNFα, 27.3% (p < 0.0001) between 10 ng/ml TNFα and 100 ng/ml TNFα, and 14.0% (p = 0.02) between 50 ng/ml TNFα and 100 ng/ml TNFα. No statistically significant changes were observed during 48 h incubation (Fig. 4).

Caspase-3 is present in cells in both inactive (32 kDa) and active forms; detection of the cleaved form suggests cell death induction. Detection of the large subunit with a molecular mass of ~17 kDa by Western blotting is the most specific method for confirming Caspase-3 activation [15].

Treatment of cells with TNFα for 6 h and 24 h did not cause significant changes in the relative amount of cleaved Caspase-3. The TNFα exposure increased the relative amount of the cleaved form of Caspase-3 in RPMI 2650 cells only after incubation period of 48 h and at TNFα concentrations of 1 ng/ml, 10 ng/ml, 50 ng/ml, and 100 ng/ml by 25.0% (p = 0.02), 33.7% (p = 0.002), 43.3% (p = 0.0002), and 65.3% (p < 0.0001), respectively. The changes were dose-dependent relative to TNFα concentrations of 100 ng/ml and 1 ng/ml, 10 ng/ml, and 50 ng/ml with the increase of 24.2% (p = 0.0003), 19.3% (p = 0.002), and 13.3% (p = 0.02), respectively (Fig. 5).

Thus, loss of cell viability is accompanied by an increase in the level of cleaved Caspase-3; this may indicate the initiation of cell death processes under conditions of prolonged TNFα action.

The relative amount of E-cadherin increased by 43.7% (p = 0.0012) compared to the control values when cells were incubated with 10 ng/ml TNFα for 6 h (Fig. 6). The changes were dose-dependent: between TNFα concentrations of 1 ng/ml and 10 ng/ml (the level of E-cadherin increased by 33.3%; p = 0.0084), between 10 ng/ml and 50 ng/ml (it decreased by 55.0%; p = 0.0002), and between 10 ng/ml and 100 ng/ml (it decreased by 50.0%; p = 0.0004) (Fig. 6). In the case of the exposure time of 24 h, there was a relative increase versus control in the relative amount of E-cadherin by 83.6% (p = 0.0001),

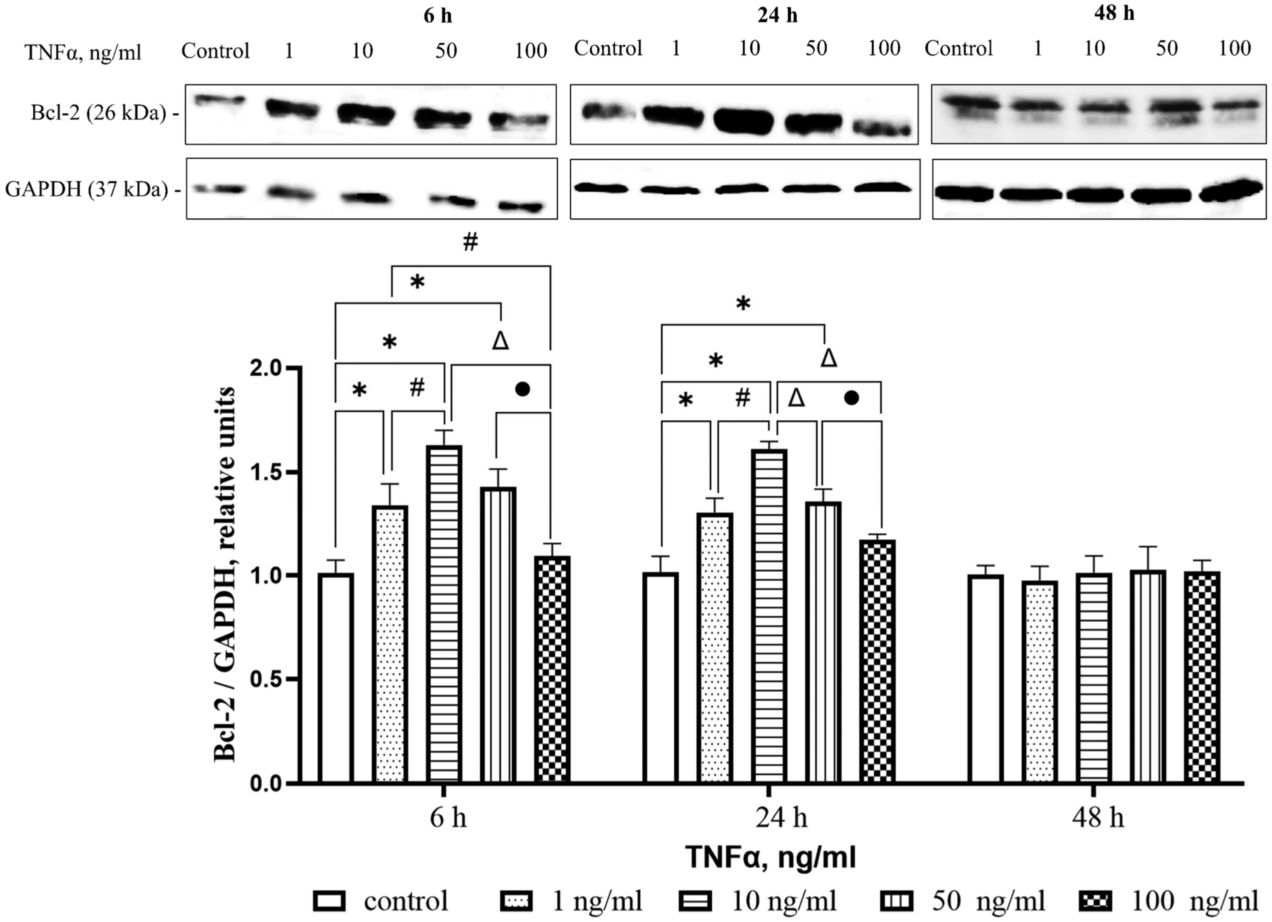

Figure 4. Relative Bcl-2 levels in RPMI 2650 cells treated with 1 ng/ml, 10 ng/ml, 50 ng/ml, and 100 ng/ml TNFα for 6 h, 24 h, and 48 h. Statistically significant differences: * – vs control; # – vs cells treated with 1 ng/ml TNFα; Δ – vs cells treated with 10 ng/ml TNFα; ● – vs. cells treated with 50 ng/ml TNFα.

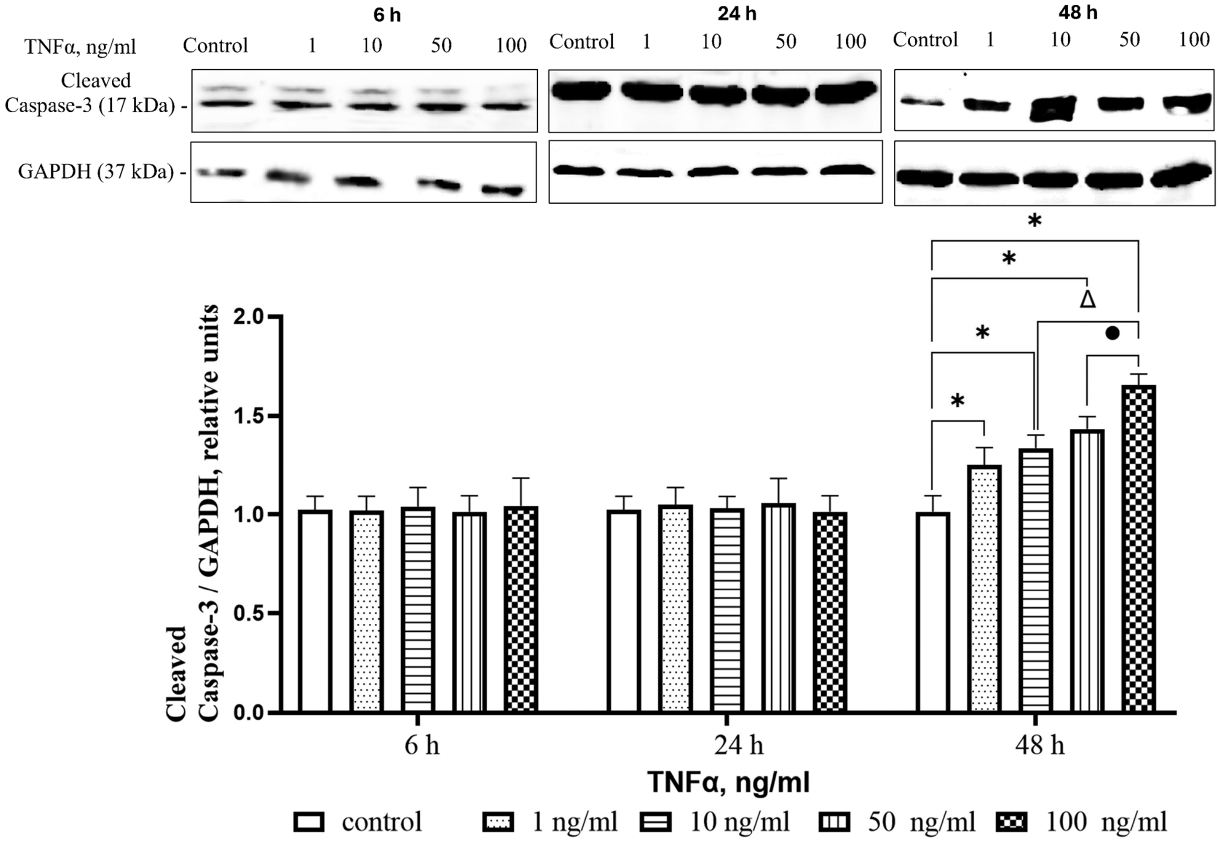

Figure 5. Relative cleaved Caspase-3 levels in RPMI 2650 cells treated with 1 ng/ml, 10 ng/ml, 50 ng/ml, and 100 ng/ml TNFα for 6 h, 24 h, and 48 h. Statistically significant differences: * – vs control; # – vs cells treated with 1 ng/ml TNFα; Δ – vs cells treated with 10 ng/ml TNFα; ● – vs. cells treated with 50 ng/ml TNFα.

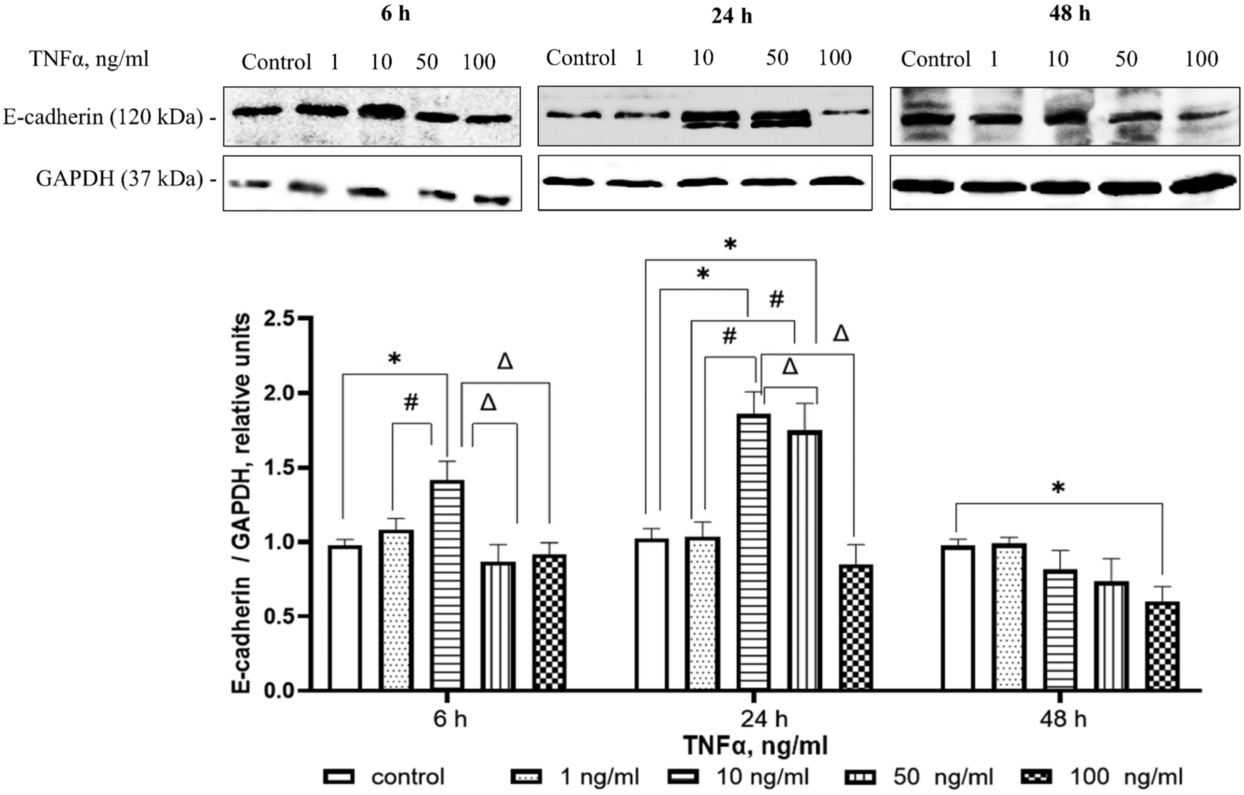

Figure 6. Relative E-cadherin levels in RPMI 2650 cells treated with 1 ng/ml, 10 ng/ml, 50 ng/ml, and 100 ng/ml TNFα for 6 h, 24 h, and 48 h. Statistically significant differences: * – vs control; # – vs cells treated with 1 ng/ml TNFα; Δ – vs cells treated with 10 ng/ml TNFα.

72.7% ($p = 0.0004$) observed when cells were incubated with 10 ng/ml and 50 ng/ml TNFα, respectively (Fig. 6). The changes depended on the TNFα concentration: between 1 ng/ml and 10 ng/ml (82.7%; $p = 0.0001$), between 1 ng/ml and 50 ng/ml (71.7%; $p = 0.0004$); between 10 ng/ml and 50 ng/ml (101.0%; $p = 0.0001$), between 10 ng/ml and 100 ng/ml (90.0%; $p < 0.0001$) (Fig. 6). After incubation with TNFα for 48 h, a change of 41.3% ($p = 0.048$) was noted in the 100 ng/ml group versus control.

The relative amount of ZO-1 (versus control) increased after 6 h exposure to 10 ng/ml, 50 ng/ml, and 100 ng/ml TNFα by 39.2% ($p = 0.0043$), 49.7% ($p = 0.0008$), and 69.1% ($p < 0.0001$), respectively (Fig. 7). The changes were dose-dependent: between 1 ng/ml and 10 ng/ml TNFα (30.0%; $p = 0.02$), between 10 ng/ml and 50 ng/ml TNFα (40.3%; $p = 0.036$), and between 1 ng/ml and 100 ng/ml TNFα (60.7%; $p = 0.0001$); between 10 ng/ml and 100 ng/ml TNFα (30.8%; $p = 0.023$).

Cell treatment with 50 ng/ml and 100 ng/ml TNFα for 24 h and increased the relative levels of ZO-1 (versus control) by 23.3% ($p = 0.046$) and 23.8% ($p = 0.04$), respectively. Dose-dependent differences were 24.7% ($p = 0.034$) between TNFα concentrations of 1 ng/ml and 50 ng/ml, and 20.5% ($p = 0.035$) between 1 ng/ml and 100 ng/ml TNFα concentrations (Fig. 7).

No changes in the levels of the intercellular junction protein ZO-1 were observed after 48 h exposure to TNFα (Fig. 7).

The most significant result of our study was the observed biphasic dynamics of changes in key markers of barrier function and cell fate. Contrary to the initial assumption of a direct disruptive effect of inflammation on the epithelial barrier, we recorded a significant increase in the levels of the intercellular junction proteins E-cadherin and ZO-1 during the first 24 h of TNFα exposure. This effect can be considered a compensatory response of the epithelium, aimed at strengthening the barrier in response to an inflammatory stimulus to prevent further pathogen invasion. Similar protective mechanisms have been described for intestinal epithelium, where certain cytokines can temporarily enhance the barrier function at early stages [16, 17]. At the same time, during this phase, cells showed signs of activation of anti-apoptotic mechanisms, as evidenced by an increase in Bcl-2 protein levels. This is consistent with the known role of the NF-κB pathway in the induction of survival genes [14] and explains the absence of a significant loss of cell viability in the first 24 h, despite active inflammation.

However, during prolonged exposure (48 h), the situation changed dramatically. The compensatory capacity of cells was apparently exhausted and

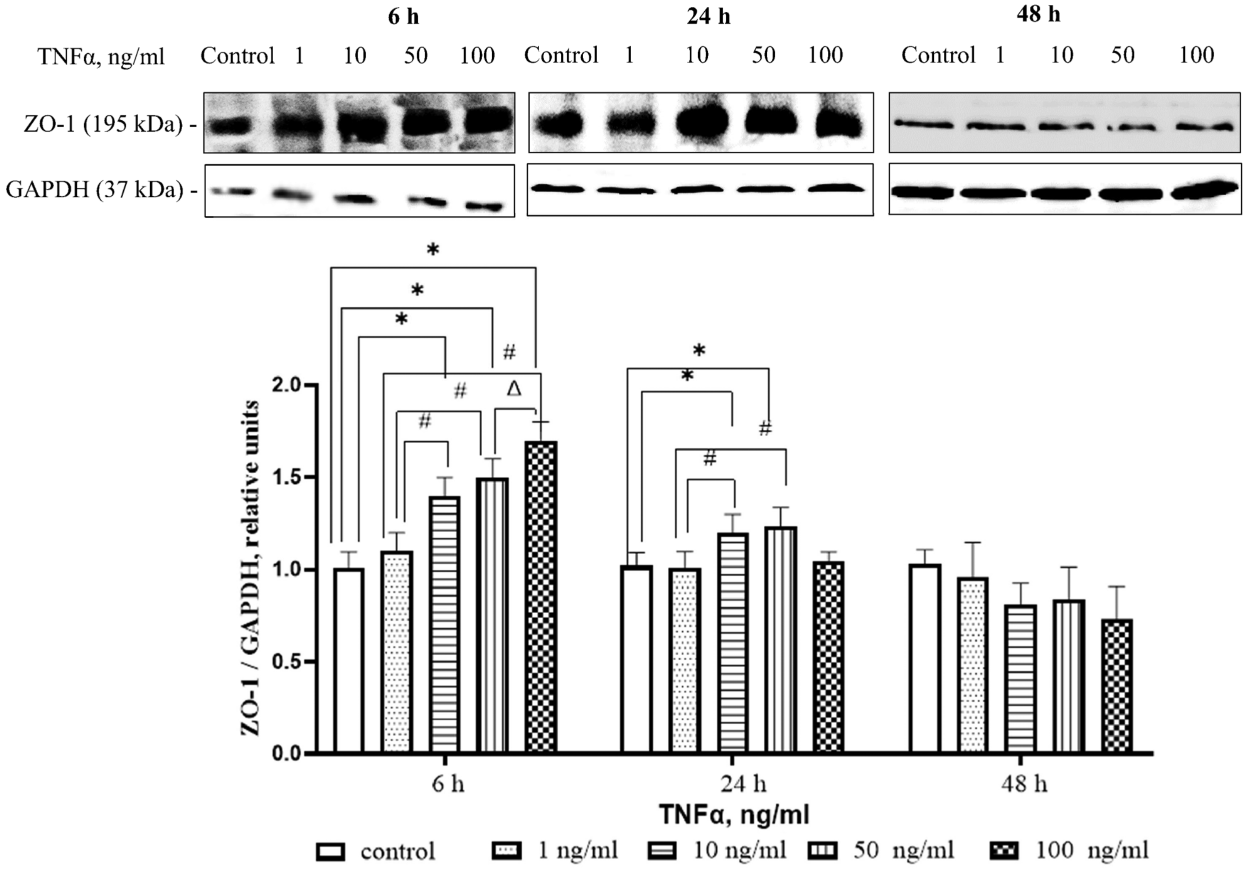

Figure 7. Relative ZO-1 levels in RPMI 2650 cells treated with 1 ng/ml, 10 ng/ml, 50 ng/ml, and 100 ng/ml TNFα for 6 h, 24 h, and 48 h. Statistically significant differences: * – vs control; # – vs cells treated with 1 ng/ml TNFα; Δ – vs cells treated with 10 ng/ml TNFα.

this resulted in a sharp decrease in their viability. This decrease correlated with significant activation of effector Caspase-3 (cleaved Caspase-3), clearly indicating the initiation of apoptotic cell death [15]. At the same time, the level of key proteins of intercellular contacts returned to baseline values; in combination with massive cell death, the latter inevitably leads to disruption of the epithelial layer integrity. This late phase corresponds to classical concepts of the damaging role of chronic inflammation, leading to degradation of the tissue barrier [18].

From a practical perspective, the identified biphasic nature has direct implications for intranasal drug delivery. A temporary enhancement of barrier function in the early stages of rhinitis could theoretically reduce the bioavailability of intranasal drugs. Conversely, during a protracted inflammatory process, massive cell death and disruption of junctional contacts can lead to an uncontrolled increase in permeability. On the one hand, this increases systemic absorption of the drug, but on the other hand, it can contribute to the development of undesirable side effects.

Limitations of the study. The main limitation of our study is that conclusions about barrier function were drawn based on indirect data (cell junction protein levels and cell viability). To directly confirm changes in permeability, transepithelial endothelial resistance (TEER) and mannitol permeability have been assessed in separate experiments. The results will be published in a separate article.

CONCLUSIONS

Thus, this study revealed a biphasic effect of TNFα on RPMI 2650 nasal epithelial cells. Short-term exposure (6–24 h) leads to activation of the NF-κB signaling pathway and increases the levels of intercellular junction proteins without significantly changes in cell viability. This phase can be considered as a compensatory response aimed at limiting the penetration of inflammatory agents through the cell monolayer. Prolonged exposure (48 h) results in activation of apoptosis, decreased cell viability, and a decrease in the levels of intercellular junction proteins, which should be accompanied by increased permeability of the nasal epithelial layer. These results should be taken into account during intranasal drug administration, as increased monolayer permeability may increase the systemic bioavailability of the administered pharmacological agents and lead to the development of adverse drug reactions.

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COMPLIANCE WITH ETHICAL STANDARDS

This article does not contain any research involving humans or the use of animals as objects.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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ДВУХФАЗНОЕ ВЛИЯНИЕ ФАКТОРА НЕКРОЗА ОПУХОЛИ-α НА КЛЕТКИ ЛИНИИ RPMI 2650 *IN VITRO*

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Фактор некроза опухоли-α (TNFα) — ключевой провоспалительный цитокин, повышение уровня которого наблюдается при воспалительных заболеваниях верхних дыхательных путей. В работе исследовано дозо- и времязависимое влияние TNFα (1–100 нг/мл, 6–48 ч) на линию клеток RPMI 2650 — модели назального эпителия. Кратковременное воздействие (6 ч) вызывало активацию NF-κB и повышение уровня белков межклеточных контактов E-кадгерина и ZO-1 без существенного влияния на жизнеспособность. Продолжительная экспозиция (24–48 ч) приводила к увеличению уровня про-IL-1β, активации апоптоза и снижению жизнеспособности клеток. При этом отмечалось снижение уровня белков межклеточных контактов. Таким образом, при кратковременном воздействии TNFα может оказывать защитное действие, повышая плотность межклеточных контактов, а при увеличении длительности экспозиции он запускает процессы апоптоза и снижает плотность межклеточных контактов, что может способствовать повышению проницаемости клеточного слоя.

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Ключевые слова: фактор некроза опухоли; RPMI 2650; апоптоз; белки межклеточных контактов

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