Iranian Journal of Microbiology

Volume 16 Number 5 (October 2024) 614-623

ORIGINAL ARTICLE

Effect of chitosan nanogels loaded with vancomycin and gamma interferon on TNF-α gene expression in macrophage cell line activated with methicillin-resistant *Staphylococcus aureus* (MRSA)

Sahar Arkiya[1], Ardeshir Hesampour[1], Ali Esrafili[2], Javad Arasteh[1*]

[1]Department of Biology, Faculty of Science, Central Tehran Branch, Islamic Azad University, Tehran, Iran
[2]Department of Environmental Health Engineering, School of Public Health, Iran University of Medical Sciences, Tehran, Iran

Received: January 2024**, Accepted:** August 2024

ABSTRACT

Background and Objectives: *Staphylococcus aureus* is an opportunistic pathogen that frequently leads to asymptomatic infections. Methicillin-resistant strains (MRSA) pose a significant threat as they are resistant to most commonly used antibiotics, complicating treatment efforts. This study aimed to develop chitosan nanogels loaded with vancomycin and IFN-γ and to assess the expression of the TNF-α gene in a cell line infected with MRSA.

Materials and Methods: Following the synthesis and confirmation of the chitosan nanogels, vancomycin and IFN-γ were incorporated into these nanogels. The synthesis was validated using DLS, FTIR, TEM, and SEM. Subsequently, the antibacterial efficacy of the nanogels was assessed. Finally, four groups of cell lines were designed: control, MRSA, chitosan nanogels and IFN-γ-vancomycin chitosan nanogels. After infection of the groups (except control) with MRSA, 5 µg/mL of nanogels, and nanogels (drug and IFN-γ) were added to groups 3 and 4, respectively. Then the expression of TNF-α gene in each group was analyzed by RT-PCR at 6 and 24 hours.

Results: At pH 6.5 and 7.4, the MIC of 1 µg/mL was obtained for free vancomycin, whereas that of IFN-γ-vancomycin nanogels at both pHs was respectively 8 and 64 µg/mL. The IC50 of chitosan nanogels and nanogels loaded with vancomycin-IFN-γ on RAW264.7 cells were 2.37 and 4.15 µg/mL in 24 hours, respectively. In group 4 in comparison to the MRSA group, TNF-α expression decreased significantly following 24 hours.

Conclusion: Loading of vancomycin and IFN-γ in the chitosan nanogel can reduce TNF-α gene expression on MRSA infected cell lines.

Keywords: Tumor necrosis factor (TNF-α); Methicillin-resistant *Staphylococcus aureus* (MRSA); Vancomycin; Interferon-gamma (IFN-γ) chitosan nanogels

INTRODUCTION

The resistance of bacteria against antibiotics is increasing, this issue has caused researchers to think of replacing effective antimicrobial agents with less side effects (1). The development of antibiotic resistance mechanisms by pathogenic microorganisms has become a serious concern (1).

These resistance mechanisms are due to several factors, including enzymes and genetic mutations in pathogenic factors that cause infectious diseases. These factors have encouraged researchers to design

*Corresponding author: Javad Arasteh, Ph.D, Department of Biology, Faculty of Science, Central Tehran Branch, Islamic Azad University, Tehran, Iran. Tel: +98-2144600184 Fax: +98-2144600184 Email: j.arasteh@iauctb.ac.ir

new antimicrobial agents against pathogenic agents to control infections (1, 2). *Staphylococcus aureus* is a gram-positive pathogen that is commonly found in the respiratory tract, wounds, urinary tracts, and other sites. Among the many microorganisms that develop resistant strains, *Staphylococcus aureus* is a serious challenge (3).

This bacterium is a dangerous pathogen due to the potential of resistance to antibiotics (4). For example, acquiring the mecA gene and producing a special protein called penicillin-binding protein (PBP2a), strengthens *Staphylococcus aureus* and makes it resistant to penicillinase-resistant beta-lactams (methicillin or oxacillin) (5). Methicillin-resistant *Staphylococcus aureus* (MRSA) is resistant against several beta-lactam antibiotics, like oxacillin and methicillin, and also other major antibiotic classes, like macrolides and some fluoroquinolones. MRSA leads to chronic wound infections and is the main cause of soft tissue and skin infections (6). Vancomycin is still widely used to combat the increasing number of MRSA infections (7). Although vancomycin has been used for more than 40 years, it is still the standard treatment for infections caused by MRSA. Reports indicate that clinical treatments involving vancomycin have been unsuccessful due to the emergence of *S. aureus* strains with decreased susceptibility to the drug (7). Currently, the use of cytokines has become a significant and effective therapeutic approach for stimulating the immune system and initiating protective responses that influence various aspects of immune function (8, 9). IFN-γ due to its vital functions and its multiple immune protection, is one of the powerful cytokines that can be administered systemically or locally (10). This cytokine is mainly produced by natural killer (NK) and T cells and regulates a wide range of pathological responses (11). IFN-γ is a potent proinflammatory cytokine during innate and acquired immune responses in microbial infections (12). This cytokine has become an attractive therapeutic alternative due to its immune modulating abilities (10). Currently, antibiotic delivery systems using nanoparticles are one of the effective strategies to improve the antimicrobial potential of antibiotics (13, 14). Additionally, antibiotic delivery systems that utilize nanoparticles offer numerous benefits, such as enhancing the solubility of poorly water-soluble drugs, prolonging the drug's half-life and systemic circulation time, and reducing the required drug doses, which in turn minimizes side effects and improves patient outcomes (15). Chitosan is a widely available natural biopolymer sourced from the exoskeletons of crustaceans. It has been effectively utilized in nanomedicine as a delivery system for drugs and proteins due to its remarkable biocompatibility, biodegradability, non-toxicity, and inherent antimicrobial properties, as well as in gene therapy (16, 17). In sepsis, the activation of pro-inflammatory mechanisms is a common occurrence, often indicated by changes in the expression of specific biomarkers (18). Circulating cytokines, particularly TNF-α and certain interleukins, are closely linked to the onset of sepsis and septic shock (18, 19). Consequently, this study aims to examine the impact of chitosan nanogels loaded with vancomycin and gamma interferon on the expression of the TNF-α gene in a macrophage cell line infected with MRSA.

MATERIALS AND METHODS

Synthesis of chitosan nanogels. First, chitosan (Fluka Biochimica Darmstadt, Germany) was prepared at a ratio of 0.3% (w/v) in an aqueous solution of 1% (v/v) acetic acid and stirred overnight, and its pH was adjusted to 4.6 using 10N NaOH. Then, tripolyphosphate (TPP) (Merck Darmstadt, Germany) was also dissolved in distillated water (0.3% (w/v)) and then added to the chitosan solution with a ratio of 5:1. In order to separate the chitosan nanogels, the chitosan-TPP solution was centrifuged (12000 rpm / 15 minutes) and the supernatant was discarded. The solution was again sonicated in the range of 35 KHZ for 2 minutes and the centrifugation step was repeated. Finally, the sediment was frozen and dried at a temperature of -80 (14).

Characterization of nanogels: Dynamic light scattering (DLS). DLS is a physical technique employed to assess the distribution of particles within solutions and suspensions. This rapid and non-invasive method is utilized to measure particle sizes ranging from a few nanometers to microns. To measure the diameter of nanoparticles and their dispersion, after dissolving the nanogels in water, the resulting mixture was dispersed in a water bath by ultrasonic waves and analyzed by DLS (Malvern instrument Ltd., UK).

Fourier transform infrared spectroscopy (FTIR). In FTIR, each functional group has one or

more specific peaks in different wave numbers. To prepare the samples for FTIR analysis, a very small amount of the nanogel (about 1 mg or less) was mixed with dry KBr at a ratio of 1:100 and finally analyzed by the JASCO FTIR-410 device in the mid-IR spectrum region (400-4000 cm^{-1}) (20).

Scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Surface morphology and structure of the IFN-γ-vancomycin nanogels were analyzed by SEM (XL 30, Philips, USA) and TEM (Leo 906, Zeiss100KV model, Germany). SEM images of nanogels were obtained after coating with a thin film of gold (60 mA current, 25 kV; 40 s). TEM images were also taken by deposition of nanogels onto carbon film grid directly and observed.

Loading of vancomycin and interferon gamma on chitosan nanogel. A total of 100 mg of chitosan nanogel was mixed with 10 cc of deionized water that contained 1 cc of vancomycin (Sigma-Aldrich GmbH, Germany) at a concentration of 2 mg/mL. This mixture was then placed on a magnetic stirrer at room temperature for 24 hours. Then the solution was poured into a falcon and centrifuged twice at 14000 RPM and 24°C. The supernatant solution was collected to measure the amount of drug loading and the resulting sediment containing nanodrug was also analyzed by fluorescence spectrophotometer. For interferon gamma loading, 100 microliters of interferon gamma (R&D System, USA) (200 µg/mL) were added to 10 cc of chitosan nanogel containing vancomycin, and after centrifugation (14,000 RPM), the precipitate and the supernatant were analyzed by by IFN-γ ELISA DuoSet kit (R&D System, USA) at 405 nm and measured using an ELISA reader (TECAN, USA) (14, 21).

***In vitro* evaluation of antimicrobial effect of nanogels.** The antimicrobial activity of nanogels was evaluated using the MIC through the microdilution method. To conduct this study, a suspension of MRSA (ATCC 33591) was prepared at a concentration of 5×10^5 CFU/mL, sourced from the Pasteur Institute of Iran. This bacterial suspension was then treated with vancomycin and nanogels at varying concentrations (0.5 to 1024) and at two different pH levels, 6.5 and 7.4. The MIC was defined as the lowest concentration of the drug and nanogels that effectively inhibited bacterial growth (14, 16).

Macrophage cell line culture. The mouse macrophage cell line RAW264.7 was sourced from the Pasteur Institute of Iran. The cells were cultured in DMEM medium enriched with 10% fetal bovine serum (FBS; Gibco, USA), along with 100 U/mL penicillin (Sigma) and 100 µg/mL streptomycin (Sigma). They were incubated at 37°C in a humidified environment containing 5% CO_2 (21, 22).

Cytotoxicity assay. Initially, the nanogels were sterilized using UV irradiation for 30 minutes, then the *in vitro* toxicity was assessed against RAW264.7 macrophages. In summary, cells were cultured at a density of 1.5 × 10^4 per well in a 96-well tissue culture plate using DMEM supplemented with 10% FBS and incubated at 37°C in a 5% CO_2 environment for 24 hours. The cells were then treated with nanoparticles at various concentrations (0.1, 1, 5, 10, and 15 µg/mL) for an additional 24 hours. Following this, 10 µL of MTT (5 mg/mL) was added to each well and incubated for 4 hours. After incubation, the cells in each well were washed with PBS buffer, and 100 µL of DMSO was added to each well. The absorbance was subsequently measured at 490 nm using a microplate reader (21, 22).

Activation of cell lines with MRSA. Four groups of cell lines were designed: control, MRSA, chitosan nanogel and IFN-γ-vancomycin chitosan nanogel groups. In each group 10^4 cells were seeded in the wells of a 96-well plate. Then groups 2 to 4 were infected with MRSA at a ratio of 100:1 (bacteria:cell) and were incubated for 1h at 37°C in 5% CO_2. Nanogels were diluted with DMEM containing 10% FBS and 5 µg/mL of chitosan nanogels and IFN-γ-vancomycin chitosan nanogels were added to groups 3 and 4, respectively. After 24 hours of incubation, cells were harvested for real time PCR (22).

cDNA synthesis and RNA extraction. The RNA of the treated cells was extracted using RNSol solution (Roge Technology Co., Iran) and according to the company's instructions. The optical absorbance of RNA at a wavelength of 260 to 280 nm was calculated between 1.2 and 1.9 µg/mL by the Nanoellsdrop device (Thermo scientific - USA), which indicated the purity of the extracted RNA. Then, cDNA generation was performed by RT-ROSET kit (Roge Technology Co., Iran).

Evaluation of TNF-α gene expression by Real-time PCR approach. Real-Time PCR was done using Takara SYBR Premix Ex TaqTM II kit TNF-α and β-actin (reference gene) primers. The sequences of TNF-α and β-actin genes were obtained from the NCBI website and their forward and reverse primers were designed and synthesized (Table 1). Real Time PCR program was as follows: 95°C (1 min), 95°C (15 s), 60°C (60 s) for 60 cycles. All reactions were conducted three times and gene expression alterations were determined by the $2^{-\Delta\Delta CT}$ method (21).

Statistical analysis. Statistical analysis was conducted using GraphPad Prism 9 software, employing one-way analysis of variance and T-tests, with a significance level set at $P < 0.05$, indicating a meaningful difference.

RESULTS

DLS results. As mentioned, this method determined the size of particles. In this research, (Fig. 1a) shows the DLS of chitosan nanogel, whose particle distribution is estimated to be 67.75 nm. In the case of nanogel loaded with vancomycin and interferon gamma, the particle distribution was calculated to be 338.38 nm (Fig. 1b).

FTIR analysis. The structural characteristics of chitosan nanogels and IFN-γ-vancomycin nanogels are shown in Fig. 2. Investigations showed that the presence of a peak at the wavelength of 3454 indicates the presence of the hydroxyl agent (OH) and also the presence of a peak at the wavelength of 2923 indicates the CH stretching vibration of methyl and methylene groups in chitosan nanogel (Fig. 2a). In the case of vancomycin, the presence of 1665 and 1226 peaks corresponding to C=O and phenolic hydroxyl groups, respectively, indicates the loading of this drug in chitosan nanogel. Also, the amide bonds (amides I- III) and carboxyl group in FTIR indicates the existence of protein. Therefore, the presence of 1365 and 1779 peaks related to the amide III group and the carboxyl group indicates the loading of interferon in the nanogel (Fig. 2b).

SEM and TEM analysis. The size and morphology of nanogels were determined using TEM and SEM, and the images showed that these nanogels are mostly spherical in shape, and in some places, the accumulation of particles and the agglomeration of nanoparticles were observed (Figs. 3a and 3b).

Vancomycin and IFN-γ release results. The quantities of vancomycin and IFN-γ loaded into the system were measured using a fluorescence spectrophotometer. The initial concentration of vancomycin for loading was set at 600 ppm, with the unloaded concentration calculated to be 78.23 ppm. Consequently, the amount of vancomycin incorporated into the chitosan nanogel was 526.34 ppm, resulting in a loading efficiency of 87.66%. For IFN-γ, the initial and unloaded concentrations were 350 ppm and 93.34 ppm, respectively, leading to a loading of 236.44 ppm and a loading efficiency of 67.42%. The release of vancomycin and IFN-γ from the chitosan nanogels at a pH of 7.4 was assessed over 24 hours using a fluorescence spectrophotometer and the IFN-γ ELISA DuoSet kit, respectively. The findings indicated that as the nanogel remained in the environment for a longer duration, the release of both vancomycin and IFN-γ increased (Fig. 4).

MIC test result. The MIC of vancomycin against MRSA strain was 1 μg/mL, which indicates that these strains are sensitive to vancomycin. Antibacterial activity of chitosan nanogel and IFN-γ-vancomycin chitosan nanogels against MRSA strain was tested using microdilution method. In the case of chitosan nanogel, MIC was 1024 μg/mL at both pH 6.5 and 7.4. In the case of IFN-γ-vancomycin chitosan nanogels at pH 6.5 and 7.4, the MIC was respectively 8 and 64 μg/mL (Table 2).

Table 1. Sequence of primers used in Real-time PCR

Gene	Primer sequence
TNF-α	Forward: 5'- CCAGGAGAAAGTCAGCCTCCT -3'
	Revers: 5'- TCATACCAGGGCTTGAGCTCA -3'
B-actin	Forward: 5’- AGAGCTATGAGCTGCCTGACG -3’
(Reference gene)	Revers: 5’- CTGCATCCGGTCAGCGATAC -3’

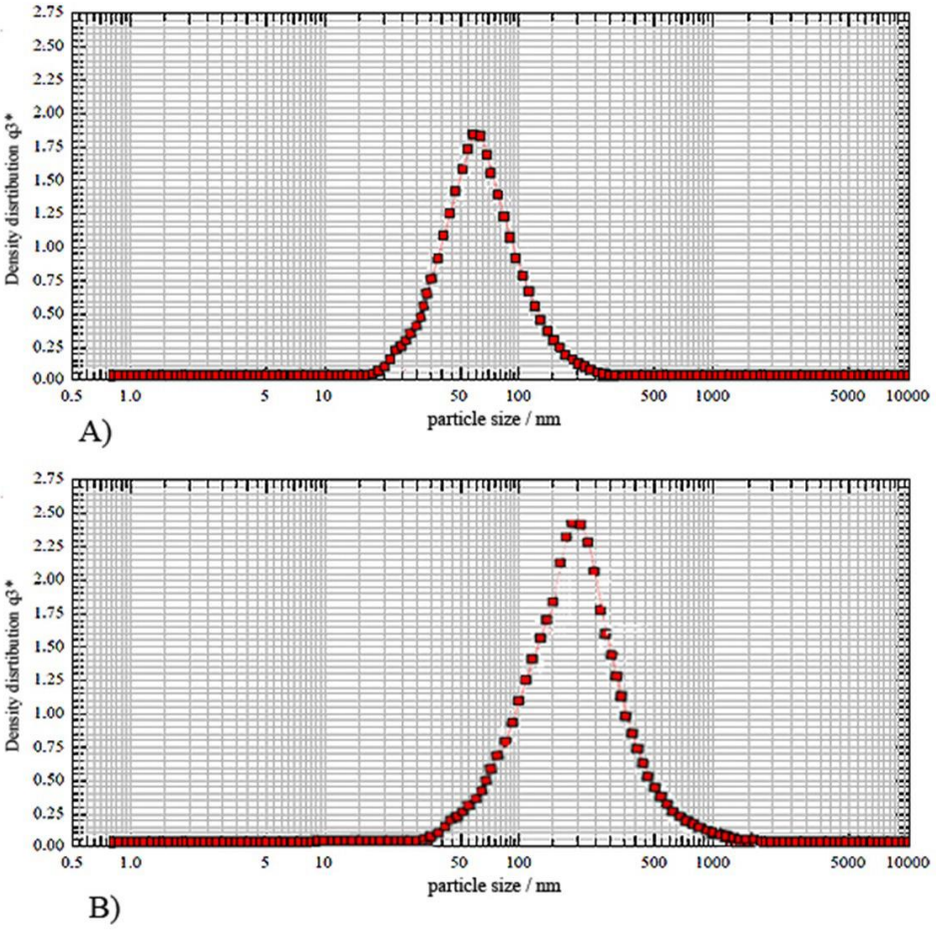

Fig. 1. DLS results of nanoparticles (a) chitosan nanogel (b) IFN-γ-vancomycin nanogels

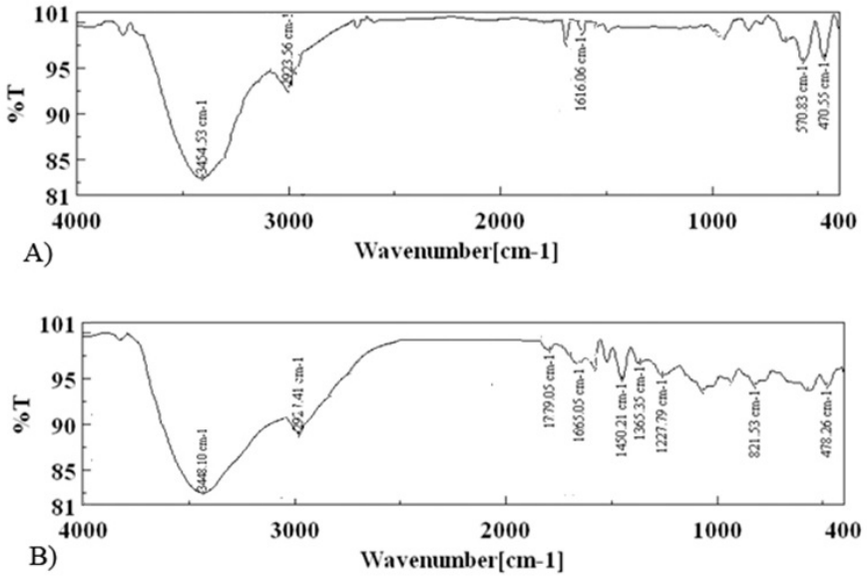

Fig. 2. FTIR of (a) chitosan nanogel (b) IFN-γ-vancomycin nanogels

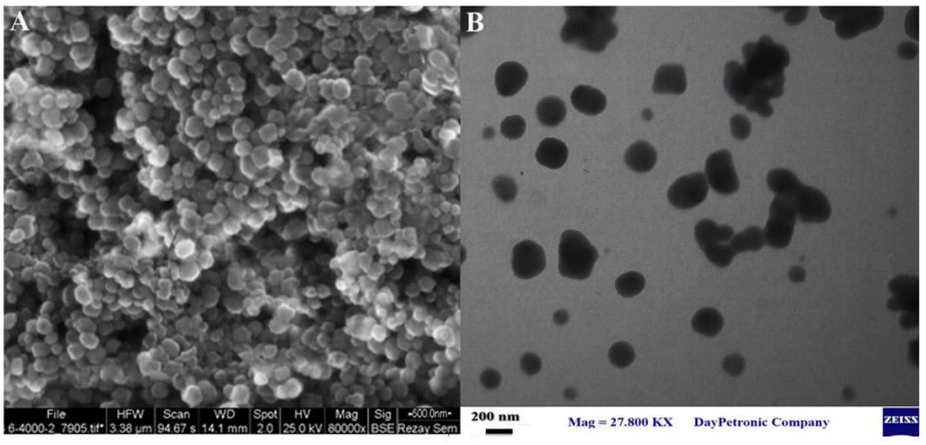

Fig. 3. SEM (a) and TEM (b) analysis of IFN-γ-vancomycin nanogels

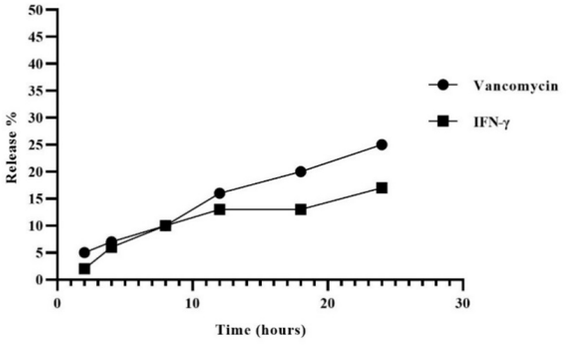

Fig. 4. Release of vancomycin and IFN-γ from nanogel in 24 hours

Table 2. Minimum inhibitory concentration (MIC) of nanogels against MRSA

MIC (µg/mL)	pH= 6.5	pH= 7.4
Vancomycin	1	1
Chitosan nanogel	1024	1024
IFN-γ-vancomycin chitosan nanogel	8	64

Cytotoxicity of nanogels to RAW264.7 cells. Treatment of RAW264.7 cells with chitosan nanogels and IFN-γ-vancomycin chitosan nanogels showed that with increasing concentration of both nanogels, their cytotoxicity on cells also increases. According to (Fig. 5), the results showed that at concentrations of 10 and 15 µg/mL, IFN-γ-vancomycin nanogels have more toxicity on cells in comparison to the untreated group ($p<0.05$). The IC50 of chitosan nanogels and IFN-γ-vancomycin chitosan nanogels against RAW264.7 cells were 2.37 and 4.15 µg/mL in 24 hours, respectively.

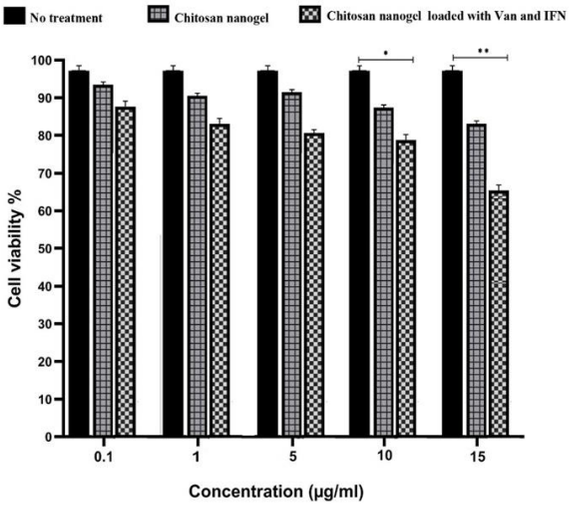

Fig. 5. The viability of RAW264.7 cells with chitosan nanogels and IFN-γ-vancomycin nanogels. *$p < 0.05$, **$p < 0.01$.

Results of TNF-α gene expression by real-time PCR. TNF-α expression in healthy and infected cell lines with MRSA was evaluated at 6 and 24 hours using real-time PCR analysis. Six hours after infecting the cells with MRSA, the level of TNF-α gene expression increased significantly ($p<0.01$) compared to the controls (Fig. 6a). Fig. 6b shows a significant reduction ($p<0.001$) of TNF-α gene expression in the chitosan nanogels and IFN-γ-vancomycin nanogels groups compared to the MRSA group after 24 hours. In the same group that received chitosan nanogel (empty nanogel), a decrease in gene expression was observed compared to the MRSA group, but this decrease was not significant.

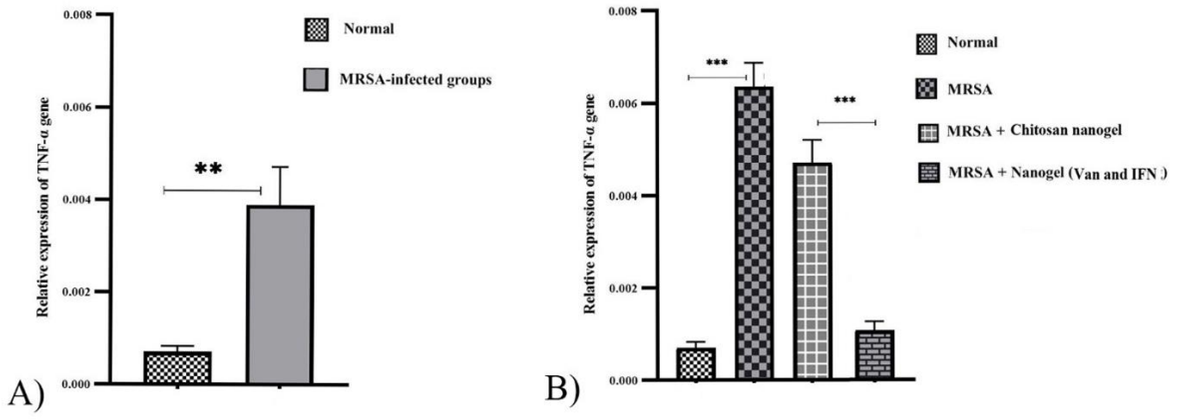

Fig. 6. TNF-α gene expression at different times. (a) 6 hours, (b) 24 hours after infection of cells with MRSA. **p < 0.01, ***p < 0.001

DISCUSSION

Utilizing cytokines represents a significant and effective approach for enhancing the immune system and triggering protective responses. While the application of cytokines in cancer immunotherapy has significantly increased, their use is often restricted due to potential side effects, as systemic delivery can result in negative outcomes. However, in some patients, cytokine therapy has had many advantages and has obtained a better prognosis (9). On the other hand, the widespread and uncontrollable use of antibiotics causes resistance to many pathogens and is a serious threat to health. For example, in some countries, 25% of dangerous staphylococcal isolates are MRSA (23, 24). Vancomycin is widely used against MRSA infections (7), But now vancomycin doses are increasing for the treatment of MRSA. Furthermore, the use of a high-dose vancomycin regimen against severe infections has little effect on MRSA eradication (25, 26). On the other hand, treatment-related side effects (especially nephrotoxicity) should be considered (25, 26). However, encapsulating drugs and cytokines in nanoparticles can reduce their side effects and also improve their therapeutic effects. Consequently, chitosan, a biodegradable nanopolymer, which plays a crucial role in this area because of its superior encapsulation capabilities, controlled release properties, and low toxicity (20). It seems that chitosan with a positive charge can interact with the cell membrane with a negative charge and open tight junctions between epithelial cells and cause the transfer of macromolecules in the epithelial tissue and the absorption of hydrophilic macromolecule drugs (27). In this research, the therapeutic combination of vancomycin and gamma interferon was used in the chitosan nanogel structure and the release of vancomycin and interferon gamma was investigated. Our results showed that the release rate of this drug from nanoparticles increases with time. In the study of Lee et al. they also showed that the release of vancomycin from Silica xerogel-chitosan hybrids increases with time, so that after 31 days, they observed the highest release rate of this drug from these hybrids (28). Also, in another study, Faraji et al showed that the release of interferon gamma from hyaluronic acid nanogel increases with time in 24 hours (21).

On the other hand, our studies also showed that the MIC of nanogels loaded with vancomycin and interferon gamma is lower at pH 6.5 in comparison to pH 7.4. Karakeçili et al. also loaded vancomycin as an antibiotic into ZIF8 nanocrystals (ZIF8/VAN) for pH-sensitive controlled release. Their results showed that vancomycin loaded in ZIF8 nanocrystals are released from chitosan scaffolds by pH-controlled method. They also showed that about 70% of vancomycin was released over 8 hours at pH 5.4, whereas it was about 55% at pH 7.4, which indicates that the release of vancomycin increases in acidic conditions (29). Therefore, pH appears to be an attractive stimulus for targeted antibiotic delivery, as some bacteria, such as *S. aureus*, MRSA, and bacteria causing dental decay produce acid (14). In this study, the effects of chitosan nanoparticles were also investigated. Different studies have shown that chitosan nanoparticles alone have a microbicidal effect on many microorganisms (30). Also, studies have shown that these nanoparticles can have antibacte-

rial activity as an anti-staphylococcal compound, especially against MRSA (31, 32). The group that received only chitosan nanogel showed a decrease in TNF-α gene expression compared to the MRSA group. On the other hand, it has been shown in a research that peritoneal macrophages stimulated with *Staphylococcus aureus* can produce reactive oxygen species (nitric oxide, superoxide, and hydrogen peroxide) and cytokines like TNF-α and IL-1β (33). The binding of these cytokines to their receptors induces the expression of some cytokines and inflammatory genes (34). Therefore, the decrease or increase in TNF-α gene expression is somehow directly associated with staphylococcal infection. In this study, IFN-γ was also used to increase the efficiency of cell lines infected with MRSA, and the results of the groups that received IFN-γ-vancomycin nanogels showed that this cytokine together with vancomycin can significantly decrease TNF-α gene expression. The reduction of TNF-α gene expression can be related to the function of macrophages as one of the important sources of TNF-α production (35). In our study, the effectiveness of IFN-γ in the group receiving IFN-γ-vancomycin nanogel increased significantly over time compared to the MRSA group. The pharmacological effect of vancomycin is due to its ability to inhibit bacterial cell wall biosynthesis. Vancomycin is non-covalently attached to a precursor in bacterial cell wall biosynthesis and inhibits peptidoglycan synthesis. In addition, vancomycin increases the permeability of the bacterial cell wall and inhibits RNA synthesis (36). On the other hand, IFN-γ is a major activator of macrophages and increases their ability to kill microorganisms by producing mediators such as nitric oxide and superoxide production (37, 38). IFN-γ also activates various efflux systems that remove essential cations from pathogens, thereby inhibiting their growth within the host (39). Conversely, research indicates that chitosan nanoparticles exhibit greater antibacterial activity compared to chitosan itself, attributed to the unique properties of nanoparticles. Polycationic chitosan nanoparticles, which possess a higher surface charge density than chitosan, can adhere to the surfaces of bacteria and diminish the thickness of their cell walls. Reduction in the thickness of the cell wall leads to the release of intracellular potassium and the leakage of intracellular components and causes the destruction of bacteria (16). Therefore, it has been stated that loading vancomycin in the nanostructure is an effective drug delivery system for drug-resistant *Staphylococcus aureus*, which can have high antimicrobial activity (16). Finally, according to the properties of nanoparticles, the antimicrobial activity of vancomycin and the effect of interferon gamma on increasing the activity of macrophages, the results of this research also indicated that the groups which received IFN-γ-vancomycin nanogels compared to other groups showed a marked decrease in TNF-α gene expression, which indicates the synergistic effect of these two compounds on MRSA infected cells.

CONCLUSION

Nowadays, due to the increase in drug resistance, the use of combined therapy methods is very useful. On the other hand, high doses of drugs and cytokines lead to side effects in humans. Therefore, the use of nanoparticles as a carrier is rapidly expanding. The results of this research showed that loading of vancomycin and gamma interferon in the chitosan structure and its effect on MRSA infected cell lines can reduce TNF-α gene expression.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the Islamic Azad University Central Tehran Branch and Iran University of Medical Sciences.

REFERENCES

1. Reygaert WC. An overview of the antimicrobial resistance mechanisms of bacteria. *AIMS Microbiol* 2018; 4: 482-501.
2. Yah CS, Simate GS. Nanoparticles as potential new generation broad spectrum antimicrobial agents. *Daru* 2015; 23: 43.
3. Lakhundi S, Zhang K. Methicillin-resistant *Staphylococcus aureus:* molecular characterization, evolution, and epidemiology. *Clin Microbiol Rev* 2018; 31(4): e00020-18.
4. Serra R, Grande R, Butrico L, Rossi A, Settimio UF, Caroleo B, et al. Chronic wound infections: The role of *Pseudomonas aeruginosa* and *Staphylococcus aureus. Expert Rev Anti Infect Ther* 2015; 13: 605-613.
5. Shalaby MW, Dokla EME, Serya RAT, Abouzid KAM.

Penicillin binding protein 2a: An overview and a medicinal chemistry perspective. *Eur J Med Chem* 2020; 199: 112312.
6. Lee AS, de Lencastre H, Garau J, Kluytmans J, Malhotra-Kumar S, Peschel A, et al. Methicillin-resistant *Staphylococcus aureus. Nat Rev Dis Primers* 2018; 4: 18033.
7. Friães A, Resina C, Manuel V, Lito L, Ramirez M, Melo-Cristino J. Epidemiological survey of the first case of vancomycin-resistant *Staphylococcus aureus* infection in Europe. *Epidemiol Infect* 2015; 143: 745-748.
8. Bhutiani N, Li Q, Anderson CD, Gallagher HC, De Jesus M, Singh R, et al. Enhanced gut barrier integrity sensitizes colon cancer to immune therapy. *Oncoimmunology* 2018; 7(11): e1498438.
9. Ardolino M, Raulet DH. Cytokine therapy restores antitumor responses of NK cells rendered anergic in MHC I-deficient tumors. *Oncoimmunology* 2015; 5(1): e1002725.
10. Yang P-M, Chou C-J, Tseng S-H, Hung C-F. Bioinformatics and *in vitro* experimental analyses identify the selective therapeutic potential of interferon gamma and apigenin against cervical squamous cell carcinoma and adenocarcinoma. *Oncotarget* 2017; 8: 46145-46162.
11. McNab F, Mayer-Barber K, Sher A, Wack A, O'Garra A. Type I interferons in infectious disease. *Nat Rev Immunol* 2015; 15: 87-103.
12. Swindle EJ, Brown JM, Rådinger M, Deleo FR, Metcalfe DD. Interferon-γ enhances both the anti-bacterial and the pro-inflammatory response of human mast cells to *Staphylococcus aureus. Immunology* 2015; 146: 470-485.
13. Gao W, Chen Y, Zhang Y, Zhang Q, Zhang L. Nanoparticle-based local antimicrobial drug delivery. *Adv Drug Deliv Rev* 2018; 127: 46-57.
14. Kalhapure RS, Jadhav M, Rambharose S, Mocktar C, Singh S, Renukuntla J, et al. pH-responsive chitosan nanoparticles from a novel twin-chain anionic amphiphile for controlled and targeted delivery of vancomycin. *Colloids Surf B Biointerfaces* 2017; 158: 650-657.
15. Honary S, Ebrahimi P, Hadianamrei R. Optimization of particle size and encapsulation efficiency of vancomycin nanoparticles by response surface methodology. *Pharm Dev Technol* 2014; 19: 987-998.
16. Chakraborty SP, Sahu SK, Pramanik P, Roy S. *In vitro* antimicrobial activity of nanoconjugated vancomycin against drug resistant *Staphylococcus aureus. Int J Pharm* 2012; 436: 659-676.
17. Cheung RC, Ng TB, Wong JH, Chan WY. Chitosan: An update on potential biomedical and pharmaceutical applications. *Mar Drugs* 2015; 13: 5156-5186.
18. Spyropoulos V, Chalkias A, Georgiou G, Papalois A, Kouskouni E, Baka S, et al. Initial Immune Response in Escherichia coli, *Staphylococcus aureus*, and *Candida albicans* Bacteremia. *Inflammation* 2020; 43: 179-190.
19. Osuchowski MF, Craciun F, Weixelbaumer KM, Duffy ER, Remick DG. Sepsis chronically in MARS: systemic cytokine responses are always mixed regardless of the outcome, magnitude, or phase of sepsis. *J Immunol* 2012; 189: 4648-4656.
20. Li P, Zhao J, Chen Y, Cheng B, Yu Z, Zhao Y, et al. Preparation and characterization of chitosan physical hydrogels with enhanced mechanical and antibacterial properties. *Carbohydr Polym* 2017; 157: 1383-1392.
21. Faraji N, Esrafili A, Esfandiari B, Abednezhad A, Naghizadeh M, Arasteh J. Synthesis of pH-sensitive hyaluronic acid nanogels loaded with paclitaxel and interferon gamma: Characterization and effect on the A549 lung carcinoma cell line. *Colloids Surf B Biointerfaces* 2021; 205: 111845.
22. Pei Y, Mohamed MF, Seleem MN, Yeo Y. Particle engineering for intracellular delivery of vancomycin to methicillin-resistant *Staphylococcus aureus* (MRSA)-infected macrophages. *J Control Release* 2017; 267: 133-143.
23. Chang VS, Dhaliwal DK, Raju L, Kowalski RP. Antibiotic Resistance in the Treatment of *Staphylococcus aureus* Keratitis: a 20-Year Review. *Cornea* 2015; 34: 698-703.
24. Saeb AT, Alshammari AS, Al-Brahim H, Al-Rubeaan KA. Production of silver nanoparticles with strong and stable antimicrobial activity against highly pathogenic and multidrug resistant bacteria. *ScientificWorldJournal* 2014; 2014: 704708.
25. Tongsai S, Koomanachai P. The safety and efficacy of high versus low vancomycin trough levels in the treatment of patients with infections caused by methicillin-resistant *Staphylococcus aureus:* a meta-analysis. *BMC Res Notes* 2016; 9: 455.
26. Hermsen ED, Hanson M, Sankaranarayanan J, Stoner JA, Florescu MC, Rupp ME. Clinical outcomes and nephrotoxicity associated with vancomycin trough concentrations during treatment of deep-seated infections. *Expert Opin Drug Saf* 2010; 9: 9-14.
27. Yang Z, Liu J, Gao J, Chen S, Huang G. Chitosan coated vancomycin hydrochloride liposomes: Characterizations and evaluation. *Int J Pharm* 2015; 495: 508-515.
28. Lee EJ, Jun SH, Kim HE, Kim HW, Koh YH, Jang JH. Silica xerogel-chitosan nano-hybrids for use as drug eluting bone replacement. *J Mater Sci Mater Med* 2010; 21: 207-214.
29. Karakeçili A, Topuz B, Korpayev S, Erdek M. Metal-organic frameworks for on-demand pH controlled delivery of vancomycin from chitosan scaffolds. *Mater Sci Eng C Mater Biol Appl* 2019; 105: 110098.
30. Chávez de Paz LE, Resin A, Howard KA, Sutherland DS, Wejse PL. Antimicrobial effect of chitosan

nanoparticles on Streptococcus mutans biofilms. *Appl Environ Microbiol* 2011; 77: 3892-3895.
31. Divya K, Vijayan S, George TK, Jisha MS. Antimicrobial properties of chitosan nanoparticles: Mode of action and factors affecting activity. *Fibers Polym* 2017; 18: 221-230.
32. Costa EM, Silva S, Vicente S, Neto C, Castro PM, Veiga M, et al. Chitosan nanoparticles as alternative anti-staphylococci agents: Bactericidal, antibiofilm and antiadhesive effects. *Mater Sci Eng C Mater Biol Appl* 2017; 79: 221-226.
33. Dey S, Bishayi B. Killing of *Staphylococcus aureus* in murine macrophages by chloroquine used alone and in combination with ciprofloxacin or azithromycin. *J Inflamm Res* 2015; 8: 29-47.
34. Turner MD, Nedjai B, Hurst T, Pennington DJ. Cytokines and chemokines: At the crossroads of cell signalling and inflammatory disease. *Biochim Biophys Acta* 2014; 1843: 2563-2582.
35. Popa C, Netea MG, van Riel PL, van der Meer JW, Stalenhoef AF. The role of TNF-α in chronic inflammatory conditions, intermediary metabolism, and cardiovascular risk. *J Lipid Res* 2007; 48: 751-762.
36. Dasgupta A. Advances in antibiotic measurement. *Adv Clin Chem* 2012; 56: 75-104.
37. Nalos M, Santner-Nanan B, Parnell G, Tang B, McLean AS, Nanan R. Immune effects of interferon gamma in persistent staphylococcal sepsis. *Am J Respir Crit Care Med* 2012; 185: 110-112.
38. Smith RP, Baltch AL, Ritz WJ, Michelsen PB, Bopp LH. IFN-γ enhances killing of methicillin-resistant *Staphylococcus aureus* by human monocytes more effectively than GM-CSF in the presence of daptomycin and other antibiotics. *Cytokine* 2010; 51: 274-277.
39. Barin JG, Talor MV, Schaub JA, Diny NL, Hou X, Hoyer M, et al. Collaborative Interferon-γ and Interleukin-17 signaling protects the oral mucosa from *Staphylococcus aureus*. *Am J Pathol* 2016; 186: 2337-2352.