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ORIGINAL ARTICLE

Klebsiella Pneumoniae Infection in Alveolar Type II Epithelial Cells; Antibiotic-Vitamin Combination Therapeutic Effect

Alveolar Tip II Epitel Hücrelerinde Klebsiella Pneumoniae Enfeksiyonu; Antibiyotik-Vitamin Kombinasyonunun Terapötik Etkisi

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ABSTRACT

Aim: In this study, it was aimed to establish an infection model with Klebsiella pneumoniae on A549 Lung cancer cell line and to evaluate the effect of additional vitamins on the antibacterial effect of ampicillin sulbactam in the treatment. Cell culture and pathology results were determined in parallel with microbial analysis.
Methods: Minimal inhibitory concentration (MIC), fractional inhibitory concentration (FIC), biofilm optical density of ampicillin sulbactam, and vitamins E, K1, and P on Klebsiella pneumoniae ATCC 700603 strain were determined. Cytotoxic activity on A549 cancer cell line in parallel with microbial analysis and pathology results were determined.
Results: Ampicillin Sulbactam MIC dose range used in the treatment of Klebsiella pneumoniae infection is 16 mg/L. Looking at the FIC dose ranges, Vitamin K1+Ampicillin sulbactam, Vitamin P+Ampicillin sulbactam 2.5mg/ml+0.5µg/ml, Vitamin E+Ampicillin sulbactam 5mg/ml+0.5µg/ml, K1+P+E+Ampicillin sulbactam 2,5mg/ml+2.5mg/ml+5mg/ml+0.5µg/ml it was determined that it had a synergistic effect in combination with vitamins K1 and P antibiotics. In addition, in the presence of biofilm below 570 nm, the OD values of K1, P, E, and K1+P+E+ antibiotic combinations were found as 1.006, 0.969, 1.096, and 1.015, respectively. As a result of cell culture studies and evaluation of pathology results, it was determined that increasing the dose of Ampicillin sulbactam to 8 µg/ml increased the antibacterial effect, and the vitamin combination was more effective.
Conclusions: The antibiotic and vitamin combination was found more effective in Klebsiella pneumoniae infection in our study. This situation contributes to the search for alternative antimicrobials.

Keywords: Lung cancer, Antibacterial activity, Antibiotic resistance, Biofilm, Synergistic effect, Vitamin

ÖZ

Amaç: Bu çalışmada A549 Akciğer kanseri hücre hattı üzerinde Klebsiella pneumoniae ile enfeksiyon modeli oluşturup tedavide ampisilin sulbaktam antibakteriyel etkisine ek vitaminlerin etkisini değerlendirmesi amaçlandı. Mikrobiyal analiz ile paralel olarak hücre kültürü ve patoloji sonuçları belirlendi.
Yöntemler: Klebisella pneumoniae ATCC 700603 suşu üzerinde ampisilin sulbaktam, E, K1 ve P vitamininin minimal inhibitör konsantrasyonu (MİK), franksiyonel inhibitör konsantrasyonu (FİK), etkinlik gösterdiği biyofilm optik dansitesi belirlendi. Mikrobiyal analiz ile paralel olarak A549 kanser hücre hattı üzerinde sitotoksik aktivitesi ve patoloji sonuçları belirlendi.
Bulgular: Klebsiella pneumoniae enfeksiyonunun tedavisinde kullanılan Ampisilin Sulbaktam MİK doz aralığı 16 mg/L'dir. FİK doz aralıklarına bakıldığında Vitamin K1+Ampisilin sulbaktam, Vitamin P+Ampisilin sulbaktam 2.5mg/ml+0.5µg/ml, Vitamin E+Ampisilin sulbaktam 5mg/ml+0,5µg/ml, K1+P+E+Ampisilin sulbaktam 2,5mg/ml+2,5mg/ml+5mg/ml+0,5µg/ml olduğu belirlendi. Vitamin K1 ve P antibiyotik ile kombinasyon halinde sinerjistik etki gösterdiği belirlendi. Ayrıca 570 nm altında biyofilm varlığında K1,P,E ve K1+P+E+ antibiyotik kombinasyonlarının OD değerleri sırasıyla 1.006, 0.969, 1.096 ve 1.015 olarak bulundu. Hücre kültürü çalışmaları ve patoloji sonuçlarının değerlendirilmesi sonucunda Ampisilin sulbaktam dozunun 8 µg/ml'ye çıkarılması antibakteriyel etkiyi arttırdığı ve vitamin kombinasyonun daha etkili olduğu belirlendi.
Sonuç: Çalışmamızda oluşturulan Klebsiella pneumoniae enfeksiyonunda antibiyotik ve vitamin kombinasyonun daha etkili olduğu bulundu. Bu durum alternatif antimikrobiyal arayışlarına katkı sağlamaktadır.

Anahtar Kelimeler: Akciğer kanseri, Antibakteriyel aktivite, Antibiyotik direnci, Biyofilm, Sinerjistik etki, Vitamin

Introduction

Lung cancer is the predominant cause of cancer-related deaths worldwide. Infection is the most common cause of death in lung cancer patients because tumor hemorrhage, bronchial obstruction, radiation, chemotherapy, and transplantation can lead to immunodeficiency (1). The most common types of infections in this patient population are pneumonia and septicemia, and gram-negative bacilli are the most common pathogens (1,2). Klebsiella spp. causes most of these infections. Klebsiella pneumoniae remains a major cause of bloodstream infections (BSIs) and directly threatens the health and life of cancer patients (2). Also, in the last few years, an increasing incidence of multidrug-resistant (MDR) Klebsiella pneumoniae has been frequently reported (3-5). Carbapenems, such as imipenem and meropenem, were the first antibiotics recommended for the treatment of MDR Klebsiella pneumoniae infections. Due to the significant increase

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in extended-spectrum beta-lactamase-producing Klebsiella pneumoniae, carbapenem has often been used as the common drug of choice, resulting in the emergence of carbapenem-resistant Klebsiella pneumoniae (6,7). Polymyxins are "last resort" antibiotics for the treatment of MDR Gram-negative bacterial infections, including carbapenem-resistant Klebsiella pneumoniae although treatments with polymyxins can cause nephrotoxicity and neurotoxicity (8,9). With the increasing use of polymyxins in recent years, polymyxin resistance has also been reported, mainly being attributed to chromosomal mutations leading to altered lipopolysaccharide composition, formation of the polysaccharide capsule, or efflux pump function. Vitamins, especially vitamins E, K, and P, have remarkable antimicrobial activity in vitro (10,11). Recent studies have investigated the immunomodulatory and antibacterial properties of vitamin E (a-tocopherol) and acetate and phosphate esters against Gram-positive and Gram-negative bacteria (12). Some studies have found that vitamin E is most effective against gram-positive strains such as Staphylococcus aureus (13), but not against Gram-negative bacterial strains such as Escherichia coli due to the additional presence of an outer lipopolysaccharide layer. a-tocopherol is a fat-soluble vitamin that is characterized by antioxidative and anti-inflammatory properties (14). Vitamin K, is effective against Gram-negative bacteria strains, such as the bacterium because menadione (vitamin K) increases the inhibition by increasing the permeability of the membrane to antibacterial drugs. However, vitamin K was found ineffective against other Gram-negative bacteria strains (15). Pyridoxine or vitamin B6 is a highly water-soluble vitamin. Its main biologically active form is a phosphate ester of its aldehyde form, pyridoxal 5-phosphate (P5P). It plays a major role in many biological pathways, allowing the proper functioning of over 60 enzymes, especially those involved in amino acid metabolism including decarboxylation, desamination, transamination, and transsulfuration (16). Vitamin P plays an important role in maintaining the permeability and structure of capillaries. It is water soluble and has properties quite similar to Vitamin C (17).

In the light of all this information, we aimed to determine the effectiveness of the combination of antibiotics and E and K1, and P vitamins in the Klebsiella pneumoniae infection model created in the A549 lung cancer cell line.

Materials and Methods

Chemicals and Reagents

Tryptic Soy Broth, glucose, Vitamin P, Vitamin K1, Vitamin E (a-Tocopheryl acetate) (≥96%, 0.95 g/ml, 9% Isotonic Sodium Chloride Solution, Blood Agar, 96 well cell culture plate flat bottom (Orange Scientific), NaCl, methanol solution (for HPLC, ≥99.9%), glacial acetic acid (≥99.85%), 2% crystal violet solution, Dulbecco Modified Eagles Medium (DMEM), Fetal calf serum (FBS), phosphate buffer solution (PBS), antibiotic antimitotic solution (100 x), L glutamine, and trypsin-EDTA obtained from Sigma. (St. Louis, MO, USA).

Bacterial Strains

Klebsiella pneumoniae (ATCC 700603) strain was used in the study.

Preparation of Antibiotic Stock Solution

Ampicillin sulbactam was dissolved in 19.5 ml phosphate buffer by weighing 0.1 g on a precision balance. Ampicillin sulbactam was determined as 0.25-64 µg/ml.

Determination of Minimal Inhibition Concentration (MIC)

MIC values of E and K1, P vitamins, and Ampicillin sulbactam against Klebsiella pneumoniae were determined using the microdilution method. Vitamin E (10, 5, 2.5, 1.25 and 0.625 mg/ml), vitamin K1 (5, 2.5, 1.25, 0.625 and 0.312 mg/ml), Vitamin P (10, 5, 2.5, 1.25 and 0.625 mg/ml) and ampicillin-sulbactam (16, 8, 4, 2, 1, 0 0.5 and 0.25 µg/ml) dose ranges. Tryptic Soy Broth (TSB) medium was inoculated into 96-well plates with 180 µl of each dilution. Then, 20 µl of Klebsiella pneumoniae (106 CFU/ml) was added to each well and incubated at 37 °C for 24 hours (18-21).

Determination of Biofilm

MIC or 1/2 MIC of E and K1, P vitamins with TSB medium was inoculated into a flat-bottomed 96-well plate with 180 µl. TSB medium was used as negative control and Klebsiella pneumoniae strain inoculated into TSB medium was used as positive control. Then 20 µl (106 CFU/ml) Klebsiella pneumoniae strain was inoculated into each well except the negative well. It was incubated for 48 hours at 37 °C. The intensity of the red color at the end of the resulting test was considered an indicator of viable cell number and was measured at 570 nm. Results were compared with controls. And the test was applied in 3 repetitions (18-21).

Fractional Inhibitor Concentration (FIC)

When the effectiveness of antibiotic combinations was evaluated according to the Clinical and Laboratory Standards Institute (CLSI) and European Committee for Antimicrobial Susceptibility Test (EUCAST) standards, the checkerboard method was used to determine the effect of the antibiotic and vitamin combination. This test is one of the microdilution synergy tests. Combination activity was determined on a 96-well microplate. First, Mueller Hinton Broth was added to the wells. Gradual dilutions were then prepared from the indicated concentrations of the substances. Amoxicillin was placed vertically on the clavulanic acid plate and vitamins were placed in the horizontal plane from right to left. Bacterial inoculation 0.5 McFarland (1x108 CFU/mL) was prepared in sterile 0.9% saline solution according to standard concentration. The final bacterial concentration in the pores was added to each well at a rate of 5 x 105 CFU/mL. Microdilution plates were incubated at 37°C for 24 hours. The effect of vitamin and antibiotic combination interactions on Klebsiella pneumoniae was evaluated with the following formula. Σ FIC index ≤ 0.5: synergy, Σ FIC index >0.5 and <1: additive, Σ FIC index ≥1 and ≤ 4: indifference, Σ FIC index >4: antagonism (18,19).

Cell culture

A549 cell (CCL-185, ATCC) cultures were obtained from Bilecik Seyh Edebali University Faculty of Medicine Department of Medical Pharmacology (Bilecik, Turkey) for this study. Briefly, cells were resuspended in fresh medium (DMEM), 10% fetal bovine serum (FBS), and 1% antibiotic (penicillin, streptomycin, and amphotericin B) (Sigma Aldrich, St. Louis, MO, USA). Cells were then seeded in 24-well plates (Corning, Inc.) as previously described and stored in an incubator (5% CO2; 37°C). After reaching 85% confluence on a 0.5 McFarland scale, the bacterial suspension was added to the cell culture. After 30 minutes, treatments were administered for 24 hours. The experiments was performed in two many groups: experiment no 1 consist of Control, Klebsiella pneumoniae, Ampicillin Sulbactam 4 µg/ml, Ampicillin Sulbactam 4 µg/ml + K1vit 2.5 mg/kg, Ampicillin Sulbactam 4 µg/ml + Pvit 2.5 mg/ml, Ampicillin Sulbactam 4 µg/ml + Evit 5 mg/ml and Ampicillin Sulbactam 4 µg/ml + K1vit 2.5 mg/ml+ Pvit 2.5 mg/ml + Evit 5mg/ml. Experiment no 2 consists of Control, Klebsiella pneumoniae, Ampicillin Sulbactam 8 µg/ml, Ampicillin Sulbactam 8 µg/ml + K1vit 2.5 mg/kg, Ampicillin Sulbactam 8 µg/ml + Pvit 2.5 mg/ml, Ampicillin Sulbactam 8 µg/ml + Evit 5 mg/ml and Ampicillin Sulbactam 8 µg/ml + K1vit 2.5 mg/ml+ Pvit 2.5 mg/ml + Evit 5mg/ml (18-21)

MTT Analysis

At the end of the experiment (following treatment with Ampicillin Sulbactam and vitamins combinations for 24 hours), 10 µL of MTT solution (Sigma Aldrich, St. Louis, MO, USA) was added to each well plate and the samples were incubated for 4 hours. h; 100 µL of DMSO (Millipore Sigma) was added to all wells to dissolve the Formazan crystals. The optical density of the solutions was read at 570 nm using a Multiskan™ GO microplate spectrophotometer (Thermo Fisher, Porto Salvo, Portugal) (18-21).

Immunofluorescence Analysis

Cells cultivated in cell culture were incubated for 30 minutes in a paraformaldehyde solution. The cells were then incubated in 3% H2O2 for 5 minutes. 0.1% Triton-X solution was dripped onto the cells washed with PBS and left for 15 minutes. After the incubation period, protein blocks were dripped onto the cells and kept in the dark for 5 minutes. Then, the primary antibody (8-OHdG cat no: sc-66036, Dilution Ratio:1/100 US) was dropped and incubated in accordance with the instructions for use. An immunofluorescence secondary antibody was used as a secondary marker (FITC Cat No: ab6785 Diluent Ratio: 1/500, UK) and kept in the dark for 45 minutes. Then, DAPI with mounting medium (Cat no: D1306 Dilution Rate: 1/200 UK) was dripped onto the sections and kept in the dark for 5 minutes, and the sections were closed with a coverslip. The stained sections were examined under a fluorescent microscope (Zeiss Axıo Germany) (18-21).

Statistical analysis

To determine the intensity of positive staining from the pictures obtained as a result of the dyeing; 5 random areas were selected from each image and evaluated in the ZEISS Zen Imaging Software program. Data were statistically defined as mean and standard deviation (mean±SD) for % area. Mann-Whitney U test was performed to compare positive immunoreactive cells and immunopositive stained areas with healthy controls. As a result of the test, an AP value of <0.05 was considered significant and the data were presented as mean ± SD.

Results

Ampicillin Sulbactam MIC dose range used in the treatment of infection due to Klebsiella pneumoniae was 16 mg/L, and when looking at FIC dose ranges, Vitamin K1+ Ampicillin Sulbactam, Vitamin P+Ampicillin Sulbactam were 2.5mg/ ml+0.5µg/ml, Vitamin E+Ampicillin Sulbactam was 5mg/ml+0.5µg/ml, K1+P+E+ Ampicillin Sullbactam were 2.5mg/ml+2,5mg/ml+5mg/ml+0.5µg/ml respectively. Vitamin K1 and Vitamin P showed a synergistic effect in combination with antibiotics, while Vitamin E and K1+P+E showed additive effects in antibiotic combination. In addition, OD values of K1, P, E, and K1+P+E+ antibiotic combinations were 1.006, 0.969, 1.096, and 1.015, respectively, with the presence of biofilm under 570 nm. These results are shown in Table 1 and Table 2.

Table 1. Results of the checkerboard assay with biofilm and fractional inhibitory concentration indices of agents combinations

Bacteria Strains ATCC No	Agent	Biofilm Highest OD Value	Positive Control	Negative Control	FIC	Dose	Interpretation
	Vitamin K	1.006	0.861	0.33	0.48	2.5mg/ml+0.5µg/ml	Synergy
	Ampicillin Sulbactam						
	Vitamin P	0.969	0.529	0.177	0.39	2.5mg/ml+0.5µg/ml	Synergy
	Ampicillin Sulbactam						
	Vitamin E	1.096	1.138	0.224	0.71	5mg/ml+0.5µg/ml	Additive
***Klebsiella pneumoniae* ATCC 700603**	Ampicillin Sulbactam						
	K+P+E	1.015	1.103	0.182	0.52		
	Ampicillin Sulbactam					2.5mg/ml+2.5mg/ml+5mg/ml+0.5µg/ml	Additive

Table 2. Results of the microdilution assay with biofilm and minimal inhibitory

Agent	Biofilm OD Highest Value	Positive Control	Negative Control	MIC /Dose
Ampicillin Sulbactam	0,163	0.225	0.146	2.well/16 mg/L
P vitamin	0.578	0.707	0.094	1.well/5 mg/ml
K vitamin	0.705	0.717	0.184	5.well/0.312 mg/ml
E vitamin	0.983	1.304	0.123	4.well/0.625 mg/ml

Table 3. Analysis data and statistical analysis of immunofluorescent staining results

	8 OHdG
Control	23.46±2.58[a]
DMSO	24.72±2.06[a]
KLEB	128.59±1.09[b]
SAM4	95.59±2.37[c]
SAM4+K	61.58±3.29[d]
SAM4+P	62.74±3.06[d]
SAM4+E	81.58±2.5[e]
SAM4+COM	45.52±2.59[f]
SAM8	59.78±3.57[d]
SAM8+K	43.5±2.84[f]
SAM8+P	44.82±2.29[f]
SAM8+E	59.59±3.89[d]
SAM8+COM	28.61±1.58[g]

a,b,c,d,e,f,g: Different letters in the same column represent the statistical difference (p<0.05 AP).

Cell culture

The cytotoxic effects of Control, Klebsiella pneumoniae, Ampicillin Sulbactam 4 µg/ml, Ampicillin Sulbactam 4 µg/ml + K1vit 2.5 mg/kg, Ampicillin Sulbactam 4 µg/ml + Pvit 2.5 mg/ml, Ampicillin Sulbactam 4 µg/ml + Evit 5 mg/ml, Ampicillin Sulbactam 4 µg/ml + K1vit 2.5 mg/ml+ Pvit 2.5 mg/ml + Evit 5mg/ml, Ampicillin Sulbactam 8 µg/ml, Ampicillin Sulbactam 8 µg/ml + K1vit 2.5 mg/kg, Ampicillin Sulbactam 8 µg/ml + Pvit 2.5 mg/ml, Ampicillin Sulbactam 8 µg/ml + Evit 5 mg/ml and Ampicillin Sulbactam 8 µg/ml + K1vit 2.5 mg/ml+ Pvit 2.5 mg/ml + Evit 5mg/ml were determined after 24 hours using the MTT method (Figure 1 and 2). The control group and K.pneumoniae was compared and a significant difference was determined (# p<0.001). The viability rate of the treated cell is compared with that of K. pneumoniae. There are no significant differences between Ampicillin Sulbactam 4 µg/ml, Ampicillin Sulbactam 4 µg/ml + K1vit 2.5 mg/kg, Ampicillin Sulbactam 4 µg/ml + Pvit 2.5 mg/ml and K.pneumoniae group. The significant differences were seen in Ampicillin Sulbactam 4 µg/ml + Evit 5 mg/ml (P<0.05) and Ampicillin Sulbactam 4 µg/ml + K1vit 2.5 mg/ml+ Pvit 2.5 mg/ml + Evit 5mg/ml (P<0.001) respectively. Figure 2 result shows the increase in Ampicillin Sulbactam dose to 8 µg/ml increases anti-bacterial effects and also vitamin combination acts more effectively. Significant differences were detected between Ampicillin Sulbactam 8 µg/ml, Ampicillin Sulbactam 8 µg/ml + K1vit 2.5 mg/kg, Ampicillin Sulbactam 8 µg/ml + Pvit 2.5 mg/ml treatment (P<0.05) and K.pneumoniae group. The P<0.001 significant difference was seen in Ampicillin Sulbactam 8 µg/ml + Evit 5 mg/ml and Ampicillin Sulbactam 8 µg/ml + K1vit 2.5 mg/ml+ Pvit 2.5 mg/ml + Evit 5mg/ml.

Immunofluorescence

Control and DMSO group: As a result of the immunofluorescence staining method in cell culture, 8 OHdG expressions in cells were evaluated as negative. Klebsiella group: As a result of the immunofluorescence staining method in cell culture, very severe cytoplasmic 8 OHdG expressions were detected in the cells. SAM4 group: As a result of the immunofluorescence staining method in cell culture, intense intracytoplasmic 8 OHdG expressions were detected in the cells. SAM4+K group: As a result of the immunofluorescence staining method in cell culture, moderate cytoplasmic 8 OHdG expressions were observed in the cells. SAM4+P group: As a result of the immunofluorescence staining method in cell culture, moderate intracytoplasmic 8 OHdG expressions were observed in the cells. SAM4+E group: As a result of the immunofluorescence staining method in cell culture, intense intracytoplasmic 8 OHdG expressions were detected in the cells. SAM4+COM group: As a result of the immunofluorescence staining method in cell culture, mild 8 OHdG expressions were detected in the cells. A significant difference was detected when compared with the Klebsiella group (p<0.05). SAM8 group: As a result of the immunofluorescence staining method in cell culture, moderate intracytoplasmic 8 OHdG expressions were detected in the cells. SAM8+K group: As a result of the immunofluorescence staining method in cell culture, mild intracytoplasmic 8 OHdG expressions were detected in the cells. SAM8+P group: As a result of the immunofluorescence staining method in cell culture, mild intracytoplasmic 8 OHdG expressions were detected in the cells. SAM8+E group: As a result of the immunofluorescence staining method in cell culture, moderate cytoplasmic 8 OHdG expressions were observed in the cells. SAM8+COM group: As a result of the immunofluorescence staining method in cell culture, very mild intracytoplasmic 8 OHdG expressions were detected in the cells. A significant difference was detected when compared with the Klebsiella group (p<0.05). Analysis data and statistical analyzes of immunofluorescent staining findings are presented in Figure 2, Figure 3, and Table 3.

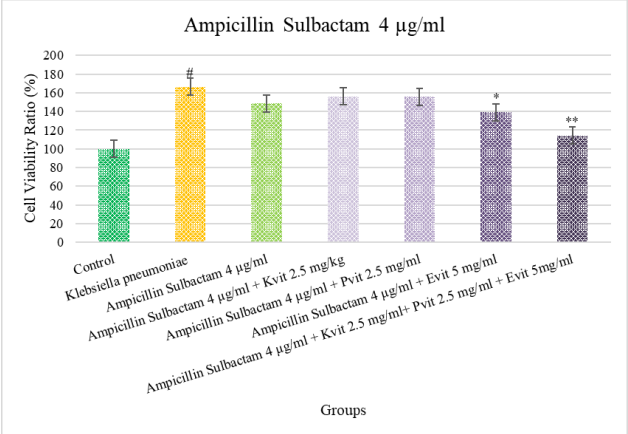

Figure 1. Cell viability ratio of A549 cells after 24 h. The viability ratios of the Control, *Klebsiella pneumoniae*, Ampicillin Sulbactam 4 µg/ml, Ampicillin Sulbactam 4 µg/ml + Kvit 2.5 mg/kg, Ampicillin Sulbactam 4 µg/ml + Pvit 2.5 mg/ml, Ampicillin Sulbactam 4 µg/ml + Evit 5 mg/ml, Ampicillin Sulbactam 4 µg/ml + Kvit 2.5 mg/ml+ Pvit 2.5 mg/ml + Evit 5mg/ml groups were compared with that of the control group (#P<0.001, *P<0.05 and ** P<0.001).

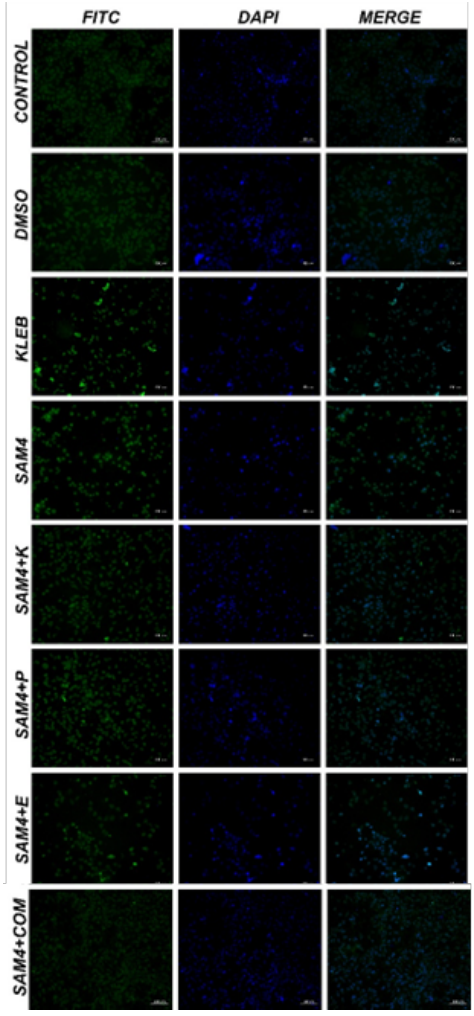

Figure 2. Cell culture, intracytoplasmic 8 OHdG expressions in cells (FITC), IF, Bar:100µm.

Discussion

Klebsiella pneumoniae has recently become a notorious lethal factor due to the increase in the number of seriously infected patients. Considering the evolutionary diversity of clinical strains, many infection models are studied including pneumonia, liver abscess, and gastrointestinal intestinal colonization (1). In this study, we tried to determine the effectiveness of the combination of Ampicillin sulbactam and E and K1, P vitamins in the Klebsiella pneumoniae ınfection model that we created in the A549 Lung cancer cell line. Shahzad et al. (17) In their study on the evaluation of the synergistic antimicrobial effect of vitamins (A, B1, B2, B6, B12, C, D, E, and K) against antibiotic-resistant bacterial strains, K and E vitamins with piperacillin/tazobactam, imipenem, and doripenem. A. baumannii. reported that vitamins B1, B2, and B12 showed remarkable synergistic activity with linezolid against MRSA. Vitamin B1 has also been determined to have better synergy with oxacillin, tetracycline, rifampicin, and linezolid against MRSA. While fat-soluble vitamins E and K represent good synergism against Gram-negative A. baumannii, water-soluble vitamins B1, B2, and B12 were found effective against MRSA, but not against A. baumannii. Riberio et al. reported that vitamin C

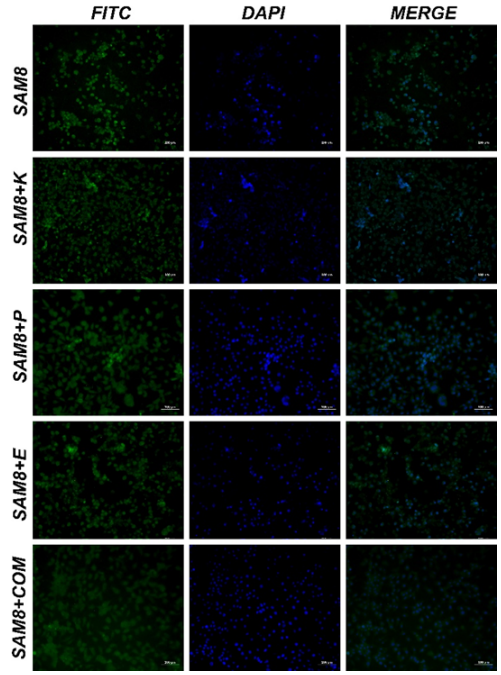

Figure 3. Cell culture, intracytoplasmic 8 OHdG expressions in cells (FITC), IF, Bar:100µm.

caused a decrease in the transcription of genes that are effective in biofilm formation (22). In a study by Pandit et al. (23) it has been reported that vitamin C inhibits EPS production and prevents biofilm formation. Bidossi et al. (12) stated in their study (200 mg/ml) that although vitamin E did not show strong antimicrobial activity against all strains tested, it could still reduce the biofilm production capacity with a variable efficacy between strains without any correlation. Naguib et al. (24) reported that the antimicrobial activity of vitamin E alone was very low when compared to its combinations with antibiotics. In a study by Celebi et al. (2023), the antibacterial and anti-biofilm activities of boron compounds were evaluated in the infection model created by Klebsiella pneumoniae in the liver cell line. In the study, minimum inhibitor concentration, fractional inhibitor concentration and biofilm optical density levels were determined. It has been determined that boron compounds are effective and their synergistic effects increase when used together (18). Another study investigated the in vitro antimicrobial activity of deferoxamine alone or in combination with ascorbic acid against 10 clinical S. aureus isolates using the liquid dilution test and time-kill method. It has been determined that the antimicrobial effect of deferoxamine can be eliminated by adding large amounts of ferric citrate to saturation of deferoxamine with iron (25).

Considering the results of our study, an additive effect was detected in the combination of E+ampicillin sulbactam. The biofilm formation inhibitory effect of vitamin E alone or in combination with antibiotics could not be determined. While the combination of K, P, and E ampicillin sulbactam shows a more significant synergistic effect than the efficacy of the antibiotic alone, the combination of E and K + P + E + ampicillin

sulbactam has the same effect as the antibiotic alone, that is, additive effect.

Conclusions

In our study, synergistic effects were found mostly in antibiotic and vitamin combinations. This situation contributes to the search for alternative antimicrobials. However, we believe that a wider study at the in vivo level and a more detailed examination of the mechanisms of action of vitamins will open up different horizons for the studies.

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