In vitro apoptotic activity of UVB light in Klebsiella Pneumoniae

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Abstract

Aims: Klebsiella pneumoniae is an opportunistic pathogenic bacterium which may lead to colonization and infection of burn wounds. The objective of this survey was to examine the triggering effect of UVB light for probable induction of programmed cell death (apoptosis) in irradiated colonies of Klebsiella pneumoniae.
Methods: The samples were categorized into control and three irradiated groups. The well grown colonies of Klebsiella pneumoniae were irradiated by UVB beams during ten minutes at the wavelength of 302 nm. 10µl of purified total genomic DNA belonging to control and radiated colonies were extracted by DNP kit and were running on 1% agarose gel electrophoresis up to 30 minutes.
Results: The pattern of DNA bands including both control and UV-irradiated colonies was completely normal. Neither deformity nor unusual patterns like laddering band nor smear was observed.
Conclusion: There are different genetic and biophysical mechanisms which may inhibit the process of apoptosis in irradiated colonies of Klebsiella pneumoniae. That is why the current UV-irradiation protocol is not able to induce the apoptotic reaction in irradiated colonies.

Keywords: apoptosis, in vitro, klebsiella pneumoniae, wound.

Introduction

Klebsiella pneumoniae is a gram negative, non-motile bacterium which causes an expanded spectrum of nosocomial infections from urinary tract and pulmonary infections to life-threatening burn infections (1-4).

The healthy human skin is a natural barrier that prevents invasion of various pathogenic micro-organisms. But, any wounds or lesions like burn wounds may lead to breach in the skin and reduce the immunity of human body. Burn wounds provide susceptible condition for colonization and invasion of endogenous and exogenous opportunistic pathogenic bacteria including Staphylococcus aureus, Pseudomonas aeruginosa and Klebsiella pneumoniae (5-7).

Klebsiella pneumoniae has the ability of adaption to different environmental conditions; for this reason, the use of antibacterial substances like antibiotics has led to increase the prevalence of multidrug resistant microorganisms such as Klebsiella pneumoniae in burn wound infections (4,6,7).

The dramatic condition of rising super-resistant strains has made an urgent necessity for searching other alternatives for treating and controlling microbial burn wound infections. For example, the use of bacteriophages can be a practical choice to antibiotic-resistant strains as an effective therapeutic method in treatment of microbial burn wound infections (6,7).

Another choice for treating bacterial wound infections is the use of phototherapy. Ultraviolet (UV) therapy is a simple, sharp and suitable alternative for treating microbial wound infections in a short time. However, a useful irradiation protocol is needed.

Thus, in the present study we evaluate the efficacy of designed UV-irradation protocol for probable induction of apoptosis in irradiated bacterial cells of Klebsiella pneumoniae.

Methods

Klebsiella pneumoniae was obtained from the maintained microbial collections of microbiology laboratory, Islamic Azad University, Shahr-e-Qods branch. The bacterial samples were identified on the basis of Gram stain, morphological and biochemical characteristics (1).

The bacteria were divided into two categories. One as control case and the other as irradiated group. Therefore, the equal amounts of bacterial cells were inoculated into four plates containing Nutrient Agar (Merck KGaA, Darmstadt, Germany) and were incubated for 72 hours at 37ºC. After observation of well-grown colonies, one plate was selected as control sample and the left three plates were irradiated for 10 minutes by UV-transilluminator (Upland, CA, U.S.A.) with the wavelength of 302 nm (UVB) from the height of 8 centimeters. The UV source was set above the colonies with maximum quantity of light and the minimum quantity of heat. In the following of irradiation, the UV-exposed colonies were placed inside a dark chamber respectively for 1, 24 and 72 hours (5,8,9).

The process of DNA extraction was achieved via a DNP kit (50T, CinnaGen Inc.). Total genomic DNA belonging to all colonies was separately harvested according to the kit protocol.

First, 5µl of protease was added to 100µl of Klebsiella pneumoniae suspensions and placed in 55ºC for 30 minutes within incubator. In continuation stage, respectively 400µl of Lysis Solution and 300µl of Precipitation Solution were added to each bacterial suspension and then microtubes were shaked. After shaking, each suspension was stored refrigerated at -20ºC for 20 minutes. Then, each microtube was centrifuged at 12,000 g for 10 minutes and obtained supernatants were decanted. The left pellet of each microtube was mixed with 1ml Wash Buffer, shaken and then samples were microfuged at 12,000 g for 5 minutes. Again, the supernatants were decanted and the tubes were incubated at 65ºC during 5 minutes for drying the pellets. The dried pellets were then dissolved in 50 µl Solvent Buffer, shaked and kept at 65ºC for 5 minutes. Finally, each microtube was microfuged at 12,000 g in 30 seconds to obtain insoluble compounds as precipitations and the soluble

total genomic DNA as supernatant in each sample (5,8,9).

The observation of DNA bands was performed by loading 10µl of purified DNA belonging to each sample (UVB-radiated and control colonies) into 1% agarose gel electrophoresis containing 1µl ethidium bromide. Simultaneously, DNA bands were compared with DNA weight marker III of CinnaGen Company (Figure 1).

Figure 1. The DNA molecules harvested from control and UVB- irradiated colonies of *Klebsiella pneumoniae*, running in 1% agarose gel

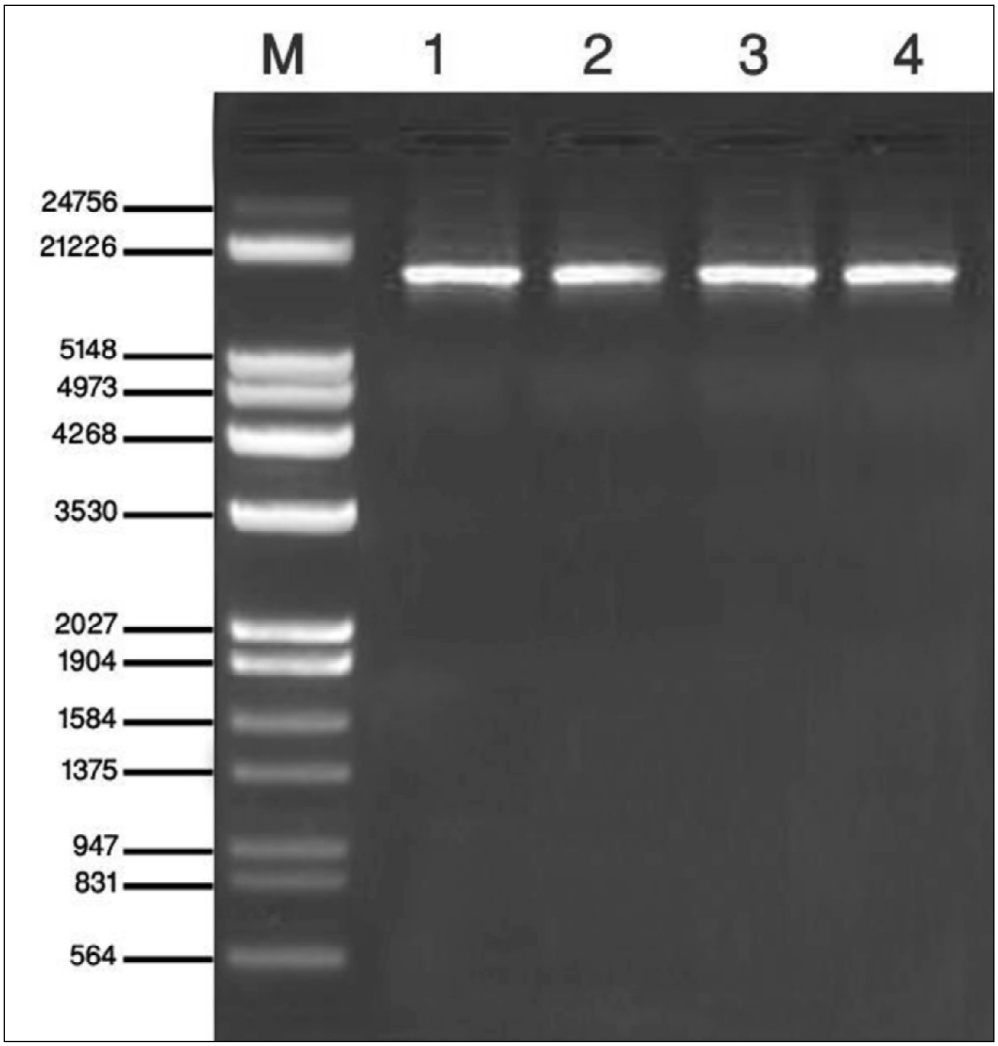

Figure Legend:

Lane M: weight marker III (Size Marker) of CinnaGen Company indicating the DNA bands around 17.5 kbp.

Lane 1: DNA bands of non-irradiated colonies of *Klebsiella pneumoniae*.

Lane 2: DNA bands of 10-minute-irradiated colonies of *Klebsiella pneumoniae*, incubated for 1 hour in a dark chamber after UVR.

Lane 3: DNA bands of 10-minute-irradiated colonies of *Klebsiella pneumoniae*, incubated for 24 hours in a dark chamber after UVR.

Lane 4: DNA bands of 10-minute-irradiated colonies of *Klebsiella pneumoniae*, incubated for 72 hours in a dark chamber after UVR.

Results

The control and irradiated colonies of Klebsiella pneumoniae were precisely checked for probable changes in their sufaces, margins and appearances via x4 microscope. No changes were observed in the mucosal appearance of control and irradiated colonies of Klebsiella pneumoniae which is related to polysaccharide capsule production. Neither pigmentation nor slimy properties were changed at all. All in all, no deformity or changes happened in macroscopic and microscopic properties of irradiated samples. In other word, there was a clear similarity between control and irradiated colonies of Klebsiella pneumoniae.

Then, the bacterial samples were checked for molecular properties. The running DNA bands belonging to control and 10-minute-UVB irradiated colonies showed no unusual patterns such as DNA laddernig bands, deformity or smear through 1% agarose gel (Figure 1). The procedure was repeated twice.

Discussion

The growing rate of transferring plasmids containing extended-spectrum beta lactamase (SEBL) in multidrug-resistant strains of Klebsiella pneumoniae has led to more concern and consideration in the field of burn wound infections. Klebsiella pneumoniae is a member of Enterobacteriaceae and possesses a pool of regulatory mechanisms for surviving in diverse conditions. These properties cause a series of problems in treatment procedures relating to Klebsiella pneumoniae (4,10,11).

UVB light (290-320 nm) is able to trigger programmed cell death reaction in cells and lead to cell shrinkage, membrane blebbing, compression of chromatin and genomic DNA scraps. The apoptosis reaction mediated by UV ray is a complex process in which several pathways are involved (12-14).

So, a suitable UVB irradiation protocol may help us to obtain a safe, cheap and easy UV therapy for treating microbial wound infections caused by multidrug-resistant Klebsiella pneuomoniae.

Bacteria comprise a series of small RNAs (sRNAs) networks in which the Hfq protein is located at the center of them. The Hfq as a critical chaperone is a minute, excessive and ubiquitous RNA-binding protein producing by hfq genes and regulates the physiological compatibility and inherent virulence in several pathogenic bacteria such as Klebsiella pneuomoniae. The major amount of Hfq is concentrated within cytoplasm and a limited group of Hfq binds to nucleoid. The bilateral connections between Hfq proteins, RNA and DNA confer Hfq a structural role as a scaffold molecule in cellular regulatory circuits, transcription and translation. The microarray data reported by some authors, have shown that hfq mutations can lead to virulence attenuation and increasing the sensitivity in Klebsiella pneuomoniae to UV beams (4,15-17).

UVB light is recognized as a super carcinogenic physical stressor that may lead to initiate apoptotic reactions and mutations in the genome of different cells through the creation of cyclobutane pyrimidine dimers (CPDs), pyrimidine(6-4)pyrimidone photoproducts (64PPs), reactive oxygen species (ROS), 8-hydroxyguanine (8OH-G) and 8-Hydroxydeoxyguanosine-triphosphate (8OH-d-GTP) (9,13,14).

Bacteria possess different mechanisms for repairing UV lesions such as photoreactivation and dark repair; for this reason the UVB exposed colonies of Klebsiella pneumoniae were stored within a dark incubation chamber during 1,24 and 72 hours, respectively (5,8,9,13).

The mutation of hfq genes and triggering the apoptosis pathway by a determined UVB irradiation protocol, may lead to find an alternative as a suitable treatment procedure via UV therapy. But in this in vitro survey, the applied UVB irradiation protocol is not able to induce the apoptosis pathway or mutation in hfq genes.

The presence of different genetic and biophysical mechanisms may lead to inhibit the process of apoptosis in irradiated colonies of Klebsiella pneumoniae. Thus, the current UV-irradiation

protocol is not able to induce the apoptotic reaction in irradiated colonies and further investigations are needed to reveal an acceptable UVB exposure protocol to get a favor UV therapy method for replacing chemotherapy against multidrug-resistant strains of Klebsiella pneumoniae.

Acknowledgements

Persian Science & Research Publisher (Persian S&RP) is the financial sponsor of this scientific project. We appreciate Mr. Bahman Qadami (Expert of Microbiology Laboratory of Islamic Azad University, Shahr-e-Qods Branch, Tehran, IRAN) and Mr. Cyrus CHEGINI (Expert of Biochemistry and Genetics Laboratory of Islamic Azad University, Shahr-e-Qods Branch, Tehran, Iran) for providing us microbial strains and lab equipments in this study.

Conflicts of interest: None declared.

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