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An *in vitro* Survey on the Apoptotic Effects of UVB Ray in *Bacillus anthracis*

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ABSTRACT

Objectives: Bacillus anthracis *as the bacterial agent of anthrax, is spread in different geographical zones around the world.The purpose of this survey was to observe eventual apoptotic reactions in total genomic DNA of UVB irradiated colonies of* Bacillus anthracis.

Materials and Methods: *The colonies of* Bacillus anthracis *were exposed to UVB ray for 10 minutes; then, the DNA molecules of control and irradiated colonies were extracted. Finally, the DNA samples mixed in loading dye, were run in 1% agarose gel containing fluorescent dye of ethidium bromide to produce visible DNA bands.*

Outcomes: *Neither smear nor DNA laddering band were detected upon the agarose gel.*

Conclusions: *According to the present protocol, the UVB ray can not induce apoptosis feature in colonies of* Bacillus anthracis. *It seems that the recovery and protection systems in* Bacillus anthracis *can resist against eventual UVB disorders.*

Keywords: *Bacillus anthracis*, DNA, Apoptosis, Gel electrophoresis

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BACKGROUND

Bacillus anthracis, a widespread gram positive spore-forming bacterium which is ethiologic agent of anthrax. The anthrax, is one of the old known bacterial infectious diseases. A wide range of herbivorus animals including domestic and wild, even humans are susceptible to bacterial infection of anthrax. The anthrax is known as an ancient infectious disease in Middle East countries like IRAN (1-3).

Anthrax, may cause three forms of disease including Cutaneous anthrax, Gastrointestinal anthrax and Inhalation anthrax (4, 5).

Cutaneous anthrax is the most common form of anthrax involving skin and mucousal membranes. The infection occurs through contamination of wounded skin by spores. According to different studies, the incidence of cutaneous anthrax is higher than 95% around the world (1, 5, 6).

The bacterial agent of *Bacillus anthracis* can be isolated from the papules, black eschar and other lesions, in the cutaneous form of anthrax (4). Although, the cutaneous anthrax can be treated by antibiotic therapy (5), it may be helpful to use a low-dose of ultraviolet (UV) irradiation simultaneously for shortennig the treatment period. Hence, in this study we tried to detect the feature of apoptosis in total genomic DNA of UVB light exposed *Bacillus anthracis*. ❑

MATERIALS AND METHODS

The anthrax bacillus was isolated from bacterial collection of microbiology laboratory of Islamic Azad University, Shahr-e-Qods branch. The accuracy of the sample was confirmed by standard diagnostic methods, such as microscopic evaluation, Gram staining and biochemical tests.

The bacteria were inoculated into four plates onto Nutrient Agar (Merck KGaA, Darmstadt, Germany). After 72 hours incubation at 37°C, one plate was chosen as controland the others were irradiated by UV transilluminator (Upland, CA, U.S.A.) for 10 minutes. During the irradiation, the UV source was placed at a fixed hight of 8 centimeters above the colonies. The wavelength of UV light was 302 nm with maximum quantity and the minimum quantity of heat.

Then, the UVB exposured colonies were categorized into three groups and were put within a dark chamber respectively for 1, 24 and 72 hours.

Thereafter, according to the DNA Extraction kit (DNP kit 50T, CinnaGen Inc.) protocol, the DNA molecules of irradiated and control colonies were extracted (only vegetative cells of *Bacillus anthracis* were considered as target cells for DNA extraction).

In brief, 20 µl of lysozyme (100 mg/ml) was added to 100 µl of *Bacillus anthracis* suspension and incubated in 37°C for 30 minutes. Then, 5 µl of protease was added and reincubated in 55°C for 30 minutes. By preparing the samples, 100 µl of each suspension was mixed with 400 µl Lysis Solution. Then, 300 µl of Precipitation Solution was added and the microtubes were shaked. It was followed by placing the samples at -20°C for 20 minutes and then the microtubes including samples were centrifuged at 12,000 g for 10 minutes.

According to the protocol, the supernatant was decanted and 1 ml Wash Buffer was added to the pellet. After shaking, the samples were centrifuged at 12,000 g for 5 minutes and again the supernatant was decanted. Then, the pellet was dried at 65°C for 5 minutes. The dried pellet was suspended in 50 µl Solvent Buffer and after shaking, it was placed at 65°C for 5 minutes. Finally, the unsolved materials were precipitated through centrifugation at 12,000 g for 30 seconds.

The purified DNA was isolated from supernatant phase of each sample. For separating DNA fragments and making the bands visible in different lanes, 10µl of total genomic DNA of each group (irradiated and control samples) was loaded onto 1% agarose gel containing 1µl ethidium bromide and compared with DNA weight marker III of CinnaGen company, (Figure nr. 1).

The level of RNA contamination was insignificant and no shear was occurred in DNA through the extraction processes (7), (Figure nr. 1).

The lanes of total genomic DNA belonging to control and irradiated colonies were compared for observing the evental apoptotic patterns (8-10). ❑

OUTCOMES

The running of total genomic DNA of control and 10-minute-UVB irradiated colonies through 1% agarose gel indicated neither smear

(necrosis) nor DNA laddering (apoptosis) band. As, the figure shows, no abnormality was observed in DNA bands. ❑

DISCUSSION

The consequence of apoptosis in cells is fundamental physiological alternations including membrane belbbing, cell shrinkage and cleavage of genomic DNA. The DNA fragments of apoptotic cell, creat a pattern of DNA laddering band upon agarose gel electrophoresis (8-10). There are many other new articles on this subject.

The UV spectrum is classified into three categories including UVA (320-400 nm) which has indirect damage effects on DNA, UVB (280-320 nm) and UVC (100-280 nm) which both have indirect and direct damage effects on DNA molecule. UVB is an apoptosis inducer in cells, which was used in this study (11, 12).

With attention to the importance of anthrax in the Middle East and Central Asia, we tried to study apoptosis feature in *Bacillus anthracis*. In the present study, the UVB light at the wavelength of 302 nm from the distance of 8 centimeters was exposed to the colonies of Bacillus anthracis for 10 minutes and after DNA extraction, the total genomic DNA was run through Agarose gel electrophoresis (9, 10, 13).

The DNA molecules have a strong absorption of UVB radiation and resulting cyclobutane pyrimidine dimmers which are the most important apoptosis inducer factors.

UVB is a carcinogenic ray; the important factor of skin cancer in humans. Besides, UVB ray increases the production of reactive oxygen and nitrogen species; so, it damages proteins, DNA, RNA and lipids in UV exposed cells (11, 14, 15).

Bacteria have different repair mechanisms including dark repair and photoreactivation (11, 14). For this reason, the irradiated colonies were kept in a dark chamber respectively 1, 24 and 72 hours.

According to different studies, the predominant clinical manifestation of anthrax is the cutaneous form. The antibiotic therapy is the main treatment against anthrax (1-6, 13). In our investigation we tried to use UVB light as an apoptotic inducing factor to study the hypothesis of an eventually shortening of the treatment duration. Obviously, the use of UVB ray is dangerous for human skin (15). That is why, the

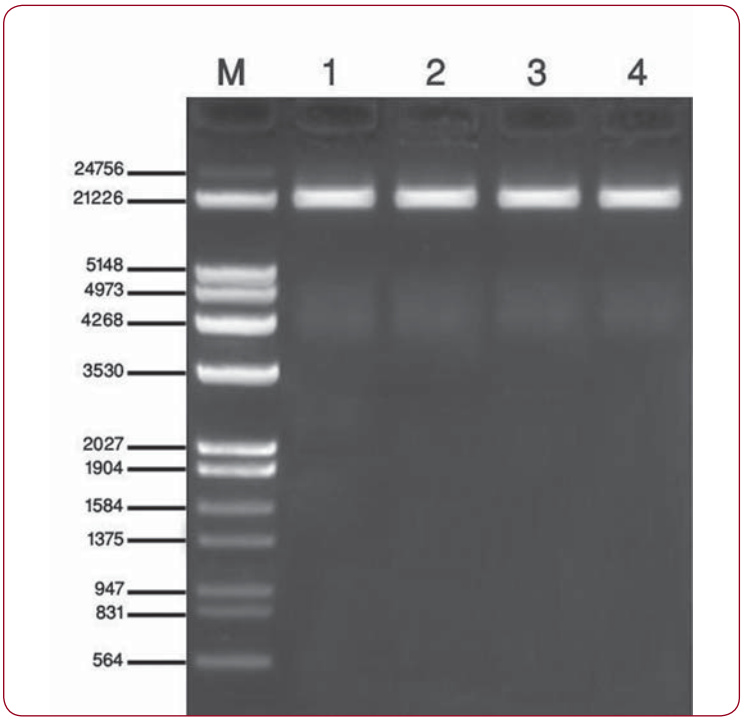

FIGURE 1. The bands belonging to extracted DNA molecules from control and UVB exposed colonies of Bacillus anthracis, which have been run through 1% agarose gel.

Lane M: DNA weight marker III of CinnaGen company. According to the size marker the DNA bands are around 21000 bp.
Lane 1: Extracted DNA belonging to control colonies of Bacillus anthracis.
Lane 2: Extracted DNA belonging to 10 min-irradiated Bacillus anthracis colonies, kept for 1 hour in a dark chamber after UVB exposure.
Lane 3: Extracted DNA belonging to 10 min-irradiated Bacillus anthracis colonies, kept for 24 hours in a dark chamber after UVB exposure.
Lane 4: Extracted DNA belonging to 10 min-irradiated Bacillus anthracis colonies, kept for 72 hours in a dark chamber after UVB exposure.

examination was done *in vitro* and the irradiation time was limited. ❑

CONCLUSION

As the results indicated, neither smear (necrosis) nor DNA laddering (apoptosis) band was observed in total genomic DNA of vegetative cells of anthrax bacilli according to the mentioned protocol in this study (Figure 1). It seems that the recovery and protection systems in *Bacillus anthracis* can resist against UVB disorders.

Besides, as we know the long-term and high dosage of UVB, has harmful effects on human's skin (8). Nevertheless, according to the present UV-irradiation protocol, if we could induce the apoptosis feature in *Bacillus anthracis,* we should continue the present study as *in vivo* experimental investigation; because, the final

goal of these types of studies is to find cheap and safe methods for shortening the antibiotic therapies of microbial infectious diseases. ❑

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