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Micro-Raman spectroscopy study of the effect of Mid-Ultraviolet radiation on erythrocyte membrane

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ABSTRACT

Mid-Ultraviolet (UVB) has a significant influence on human health. In this study, human erythrocytes were exposed to UVB to investigate the effects of UVB radiation on erythrocytes membrane. And Micro-Raman spectroscopy was employed to detect the damage. Principal component analysis (PCA) was used to classify the control erythrocytes and the irradiated erythrocytes. Results showed that the erythrocytes membrane was damaged by Mid-Ultraviolet (UVB) radiation. The intensity of the Raman peaks at 1126 cm^{-1} and 1082 cm^{-1} were used to calculate the Longitudinal Order-Parameters in Chains (S_{trans}) which can present the liquidity and ionic permeability of erythrocyte membrane. After UVB radiation for 30 min, both the liquidity and ionic permeability decreased. At the same time, the intensity of the peaks at 1302 cm^{-1} (α-helix), 1254 cm^{-1} (random coil), 1452 cm^{-1} and 1430 cm^{-1} (CH_2/CH_3 stretch) have also changed which indicated the membrane protein also been damaged by UVB. In the whole process of radiation, the more UVB radiation dose the more damage on the erythrocyte membrane.

1. Introduction

In addition to infrared and visible light, solar radiation contains about 9% ultraviolet (UV) light. A proper UV radiation is good for treatment of certain diseases, such as eosinophilic fasciitis, cystic fibrosis and short bowel syndrome. However, too much UV radiation is detrimental to human health. The reduction of ozone in the stratosphere has caused fears that more and more UV will reach the ground and lead to skin cancer and immune response. These responses include the increasing content of reactive oxygen species (ROSs), superoxide, singlet oxygen, hydrogen peroxide (H_2O_2) and hydroxyl radicals [1]. All these products play an important role in UV radiation, which can induce cellular physiological and pathological responses.

Over 50 years of UV research, a number of authors have examined the effects of chronic and/or repeated UV exposures. However, only a limited number of reports described the effects of UV on non-skin (internal) tissues. Although skin is the barrier between body and environment, it cannot entirely prevent UV radiation from penetrating into the blood supply systems, and erythrocytes also can be affected by UV. As a result, the cell membrane is the main target of UV radiation in erythrocytes. Erythrocytes play a key role in human metabolism, and it is necessary to fully understand the impact of UV radiation on them [2]. Some papers have mentioned the research on the damage of UV radiation on PLT [3–6], lymphocyte [7–9] and erythrocytes [10–12]. These studies of the damage on erythrocytes only remain on detecting the level of peroxidation by thiobarbituric acid (TBA) [1]. By this method, the sample is difficult to prepare and the results contain little information. In other words, this method does not good enough to study the damage on erythrocytes by UV radiation.

Micro-Raman spectroscopy, which has been widely used in the field of biomedical science [13–15], is a particularly potent tool to probe the biochemical composition of cells. With high quality and function of "fingerprints" [16], the Micro-Raman spectroscopy has become an ideal method to detect the damage on erythrocyte membrane.

In this study, the normal erythrocytes were irradiated for 5 min, 10 min, 20 min and 30 min by BB-UVB light. After radiation, the erythrocyte samples were scanned for Raman spectrum. And then we extracted the erythrocyte membrane for Raman scanning to study the structural damage on erythrocyte membrane in molecular level.

2. Materials and methods

2.1. Preparation of erythrocytes and erythrocyte membrane

Fresh blood was obtained from healthy adult by venipuncture and then collected in a lithium-heparinized anticoagulant tube. The erythrocytes were obtained from the anticoagulated blood

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samples by centrifugation at 3000 rpm for 5 min at 4 °C. The buffy coat was removed and then erythrocytes were washed three times with phosphate-buffered saline (PBS).

Erythrocyte membrane was prepared according to the method of Dodge et al. [17]. The washed erythrocytes were hemolyzed in 40 volumes hypotonic Tris-HCl buffer (10 mmol/L, pH7.4, 4 °C) and placed at 4 °C for 2–3 h to make sure the erythrocytes have been completely hemolysis. Then the haemolytic erythrocytes were centrifuged at 15000 r/min for 30 min at 4 °C. The erythrocytes membrane was washed for three times.

2.2. Sample irradiation

BB-UVB light was purchased from the PHILIPS Company, type of PL-S 9 W/12. Adjusting the distance between the samples and the UVB light to obtain different radiation dose rate, and we also can adjust the exposure time to achieve the different radiation doses.

The normal erythrocytes suspensions were put in a culture dish and the diameter is 3 cm. The thickness of the erythrocyte samples in culture dish was about 2 mm. Adjust the distance between UVB light and samples to be 16.3 cm. At this distance, the UVB radiation does rate is 8.27×10^{-4} W/cm^2. Irradiated the samples respectively for 5 min, 10 min, 20 min, 30 min to make sure that the UVB radiation does were 2.481 J/cm^2, 4.962 J/cm^2, 9.924 J/cm^2, 14.886 J/cm^2. In south China, the average UVB radiation does in summer is about 11.484 J/cm^2 after exposure to the sun for 1–2 h [18–20]. The impediment of skin is taking into account, so the UVB radiation dose was selected as described above.

2.3. Raman spectra

After radiation, the erythrocyte samples were put at 4 °C for 2–4 h. Then for each erythrocyte sample, some of the erythrocytes were collected for Raman scanning and the others were used to prepare the erythrocyte membrane according to the method described above. Raman spectra of erythrocytes and erythrocyte membrane were all recorded by a Via + Plus laser Micro-Raman spectroscopy system which was purchased from the Renishaw Company. The resolution of this instrument is 1 cm^{-1}. All the Raman spectra were recorded for 10 s and 5 accumulations. 785 nm laser was chose and 5% laser exposed which was much lower than the safe limit of exposure indicated in previous studies [21,22].

2.4. Data analysis

In order to compare the related spectrum changes, we chose the intensity of phenylalanine band (1002 cm^{-1}) to normalize the spectrum. At least 5 spectra were obtained from each erythrocyte sample and erythrocyte membrane sample. Then the spectra were smoothed, normalized by Origin 7.5 and baseline corrected by the software R 2.8.1.

The spectra recorded from control and irradiated erythrocytes (UVB radiated for 30 min) were analyzed statistically using principal component analysis (PCA) [21,23]. The analysis is oriented toward modeling a variance–covariance structure of a data matrix from which the eigenvalues, corresponding to principal components, are extracted. Each principal component (PC) is a linear combination of the n independent variables $x_1, x_2, x_3, \cdots, x_n$. For example:

$$\mathrm{PC1} = a_1x_1 + a_2x_2 + a_3x_3 + \cdots + a_nx_n$$

The first PC accounts for the greatest variance, and so corresponds to the largest eigenvalue. The second PC is orthogonal to the first, with each successive PC being both orthogonal to all those preceding, and accounting for a decreasing proportion of the variance. In this paper, we choose the first three PC for analysis.

3. Results and discussion

3.1. The Raman spectra of normal erythrocyte

A typical Raman spectrum of normal erythrocyte is shown in Fig. 1. We had known that the Raman peaks of erythrocyte mostly come from intracellular hemoglobin and erythrocyte membrane. But previous works suggest that the contribution of erythrocyte membrane to the Raman spectra is minimum compared with the contribution of hemoglobin [21,24–26]. For the heme group, the spin state marker region appeared at 1546 cm^{-1}, 1565 cm^{-1}, 1583 cm^{-1}, 1605 cm^{-1}. Various pyrrole ring vibration modes of heme were observed at 1399 cm^{-1}, 1375 cm^{-1}, 1341 cm^{-1}. The vinyl in-plane C—H bending mode appeared at 1307 cm^{-1}. The CH_2/CH_3 deformation modes, primarily from amino acid side chains of membrane protein, appeared at 1453 cm^{-1}. The peak at 1002 cm^{-1} is assigned to C—C twist and symmetric C—C stretch of phenylalanine ring. The complete Raman frequency assignment of spectra recorded is presented in Table 1 [21,22,25,27].

3.2. The effect of peroxide on erythrocyte by UVB radiation

Free radicals are the product of metabolism in cells. Under normal circumstances, the free radicals can be used by the immune system to kill the pathogen. However, in some conditions (drug decoys, ultraviolet radiation, high temperature exposure and so on), the content of free radical will increase sharply. This will induce cells in oxidation pressure and then damage cells structure. The Raman spectra of control erythrocyte and the erythrocytes that irradiated for 10 min and 30 min were shown in Fig. 2. From Fig. 2, we see that under oxidative pressure, the s–s makers during the region 550–650 cm^{-1} are increased obviously with the increase of UVB does. This result is consistent with the fact that the oxidative stress leads to the increase of concentration of disulfide bridges within the erythrocytes. Meanwhile, the formation of Protein-ss-Glutathione (PSSG) mixed disulfide is expected to be formed inside the cells as a response to oxidative stress [28]. On the other hand, there is a decrease of the intensity in the high spin (deoxygenated-Hb) marker band at 1609 cm^{-1} and an increased intensity at the peak 1375 cm^{-1} which belong to ν_4 as a sign of concentration of O_2.

The spectra recorded from control and peroxide erythrocytes that irradiated for 30 min were analyzed statistically using principal component analysis (PCA). A total of 44 control cells and 45 peroxide cells were used for analysis and the results are depicted in Fig. 3A and B. Plot between first principal component (PC1) and second principal component (PC2) was shown in Fig. 3A. This plot shows that almost all control erythrocytes (CES) lie in the bottom left while the peroxide erythrocytes (PES) lie in the upper right. Also the PC1 and PC3 are used to distinguish the control

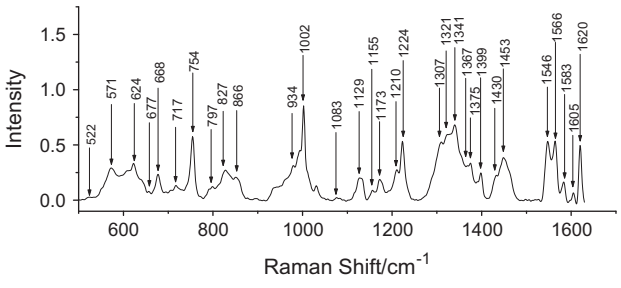

Fig. 1. Raman spectrum of normal erythrocyte which was averaged from five control erythrocytes Raman spectra.