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A Spectral Comparison Study of PDT Drugs – ALA and ALA-Hexyl ester

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5-aminolevulinic acid (ALA) has been used to stimulate endogenous protoporphyrin IX (PpIX) in tumor and then initiate PDT. Recently, ALA-Hexyl ester (He-ALA) was found much effective than ALA on producing PpIX in cancer cells. To clarify the transportation mechanism of ALA and He-ALA, the detection of them is the important step. ALA and its derivatives all don't emit fluorescence, so the Raman spectroscopy was used here for the direct detection of ALA and He-ALA. The results showed that ALA and He-ALA have the common strong Raman peaks at 2930, 2950 CM^{-1}, due to the CH_2 vibration. The peak 3050 CM^{-1} appeared in ALA spectrum can be attributed to OH vibration, while the peaks of 2860, 2900 CM^{-1} in He-ALA spectrum were assigned as the modes of CH_3 . This Raman spectral characteristic is consistence with the structure difference of He-ALA and ALA. Thus, Raman spectroscopy provides a new way to detect and distinguish ALA and He-ALA, and could be explored further in biology system.

Key words: He-ALA, photosensitization, Raman spectrum

INTRODUCTION

Photosensitizers initiate the photodynamic reaction (PDT) to destroy the cancer when irradiated with a suitable wavelength light. Generally, sensitizers emit fluorescence when excited; the transportation course of the drugs can be detected with the fluorescence method [1]. 5-aminolevulinic acid (ALA), a precursor of haemoglobin in the haem biosynthetic pathway, was found to stimulate endogenous protoporphyrin (PpIX) production in cancer cells [2]. PpIX, an effective sensitizer, can carry the PDT accordingly to form so-called "ALA-PDT". In ALA-PDT, the key factor is the PpIX formation in cancer cells. Recently, it was found that ALA-hexyl ester (He-ALA) is more effective in producing PpIX in cancer cells than ALA, so as to improve the PDT efficiency [3-4]. The PpIX formation is closely concerned with transportation of ALA or He-ALA, so the ALA and He-ALA detection is an important step for mechanism studies. However both ALA and He-ALA don't emit fluorescence, that how to measure them becomes a problem. So far no good simple way was reported. Here, the Raman spectroscopy method was used to measure the ALA and He-ALA, and the results showed it's a simple way to detect and distinguish them according to their Raman spectral characteristic.

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MATERIALS AND METHODS

Chemicals. Both ALA and HE-ALA were obtained from

Photocure Company, Norway. Their molecular weight is 168 and 252.

Raman measurements. ISA-T6400 (Jobin-Yvon) Raman Spectrometer was used in experiments. The excitation was 100 mW 488 nm laser beam from Ar^+ laser. The scattering Raman signal was recorded by attached CCD detector (1024 pixels), which was cooled to 77 K^0 by liquid nitrogen. The grating used was 1800 /mm, so the recorded spectral region was about 600 cm^{-1}。 The microscopic system was employed in measurements, putting the sample powder under the object of microscope and then recording the signal in pre-determined integrating time. The slit width was fixed in 0.2 mm, which means the resolution of recorded spectrum was around 3.5 cm^{-1} accordingly.

RESULTS AND DISCUSSION

Fig 1A and Fig 1B are the Raman spectra of ALA and He-ALA in different wavelength regions. From Fig 1A, the typical symmetric and asymmetric stretch vibration modes of CH_2 are clearly shown in 2930 and 2950 cm^{-1}, and the OH vibration mode is shown at 3005 cm^{-1} for ALA. While for He-ALA, besides the CH_2 vibration peaks (2930 and 2950 cm^{-1}) another two peaks appear in 2860 and 2900 cm^{-1}, which can be attributed as CH_3 stretch vibration modes (sym. and asym.), and the 3005 cm^{-1} OH peak disappear correspondingly since O-H band did not exist in He-ALA. ALA molecules tend to form a dimmer state, because the OH band of one molecule like to couple with the C=O band of another molecule forming OH—O=C hydrogen band, which make the C=O vibration peak weak in ALA Raman spectrum (Fig 1B). In the case of He-ALA, the molecules exist in singularity so as the C=O peak is stronger than that of ALA. The Raman spectra show different spectral characteristic of ALA and He-ALA.

The structure difference of He-ALA and ALA is that, For He-ALA the $(CH_2)_5CH_3$ group replaces the H position in O-H band of ALA molecule. Here in Raman

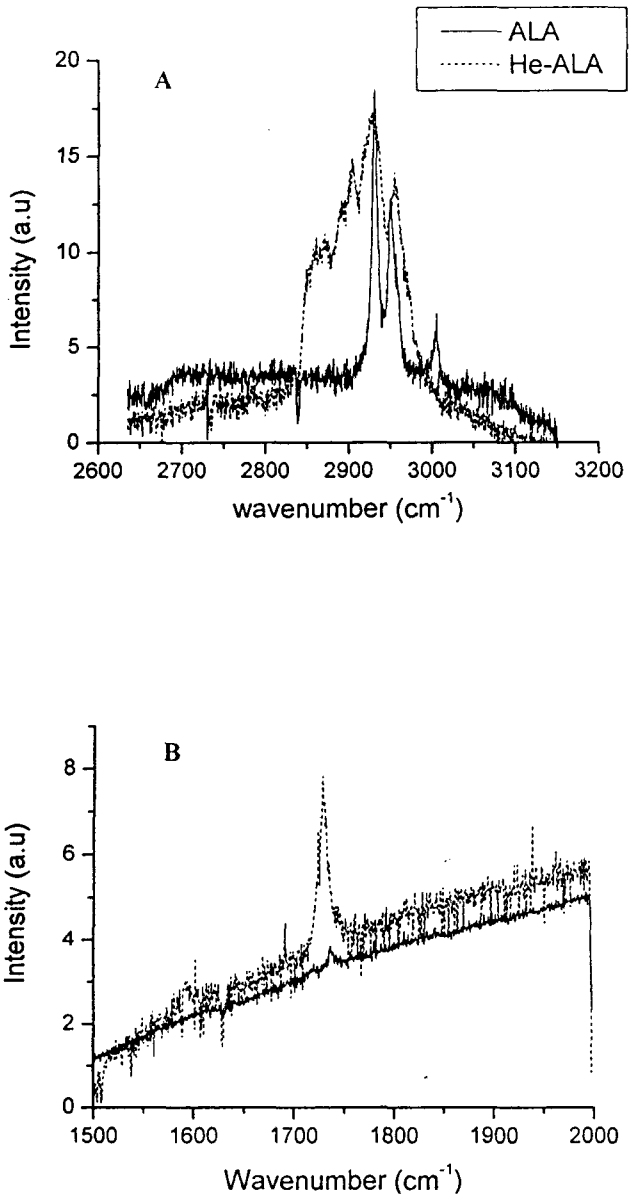

Fig 1. The Raman spectra of ALA (solid line) and He-ALA (dash line) in the region 2600-3200 cm^{-1} (A) and 1500-2000 cm^{-1} (B). Excitation: 100 mW laser beam (488 nm).

spectra, the OH peak appear in ALA case and disappear in He-ALA case, CH_3 peaks are shown in He-ALA case but do not exist in ALA case. The Raman spectral characteristic is consistence with the structure difference of ALA and He-ALA, showing that Raman spectrum is a good tool for detecting and distinguishing the ALA and He-ALA.

In ALA-PDT the cell inactivation is determined by two factors. One factor is the irradiation light dose; another is the PpIX amount in cells induced by ALA or He-ALA. The transportation and kinetics of ALA and He-ALA in cells is directly concerned to the PpIX formation [5]. So the direct detection of ALA and He-ALA is very important for mechanism study of ALA-PpIX course in cells. He-ALA was found much more effective for PpIX formation than ALA, which was believed that ester side of He-ALA has stronger affinity to plasma membrane of cells and thus He-ALA is easier to enter the cells than ALA. However, that when penetrating into cells He-ALA still remain its structure or turn to ALA by losing $(CH_2)_5CH_3$ group, is no literature reports, which may due to the reason of no simple detecting way to distinguish ALA and He-ALA. Here, the Raman spectroscopy provides a simple way to detect and distinguish them, which will help the mechanism study of ALA and He-ALA photosensitization.

In conclusion, the Raman spectroscopy can be successfully used in ALA and He-ALA detection. This method could be explored further in living cells to study the related ALA-PDT mechanism.

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