Raman spectroscopic investigations on the interactions of gastric cancer cells with 5-fluorouracil

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To study the efficacy and side effects of antitumor drug by the method of Raman spectroscopy, the cancerous (SGC-7901) and normal (GES-1) gastric cells were treated with 0, 25-, 100-, and 200-mg/L 5-fluorouracil (5-Fu) for 24 h, respectively, then Raman spectra of cells were recorded. The excitation wavelength was 514.5 nm and the Raman spectra in the region of $500-1800$ cm^{-1} were recorded. For the gastric cancer cells, as the concentration of 5-Fu increases, the band at 1094 cm^{-1}attributed to the symmetric stretching vibration mode of PO_2^- in the DNA backbone gradually decreases, and the intensity ratio of the band at 1315 cm^{-1} to that at 1340 cm^{-1} (I_{1315}/I_{1340}) shows the ascending trend, and the ratio of the band area at 1655 cm^{-1} to that at 1450 cm^{-1} (A_{1655}/A_{1450}) shows the slight ascending trend. For the normal gastric cells, these peaks also appear changes, however, the changes are weaker than those for the cancer cells. In SGC-7901 cells, 5-Fu can interfere with the DNA synthesis and result in the reduction of the DNA content. Besides, it can affect the unsaturation degree of the hydrocarbon chains and alter the external environment of guanine and adenine residues in cancer cells. The changes of Raman spectra for normal gastric cells reveal the side effect of 5-Fu.

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Gastric cancer is common in some parts of the world, such as Japan, Korea, China, parts of Eastern Europe and Latin America. In fact, it is one of the leading causes of cancer death in China[1]. Treatment for gastric cancer depends on the location of the cancer, how far it has advanced, and the patient's general health. The treatment may include a combination of surgery, chemotherapy, and radiation therapy. However, for treatment of the terminal cancer patients, chemotherapy is the most effective method[2]. So, it is imperative to enhance the efficacy of drugs and reduce the side effects. 5-fluorouracil (5-Fu) was developed in 1957 and has been widely used in the treatment of cancer including colon and gastric cancers[3−5]. It belongs to the general group of drugs known as antimetabolites, and it can interfere with the synthesis of nucleic acids, and thus disrupt the growth of cancer cells.

Raman spectroscopy is a high-sensitive and information-rich technique and has been extensively applied on the biological analysis, especially in solution and in situ[6−8]. It has been widely employed as a useful tool to investigate the interactions of drugs with DNA[9] and proteins[10], and there is much improvement in recent years[11−13]. However, the investigations on the interactions of the cells with the drugs by Raman spectroscopy are few. In this paper, we employ Raman spectroscopy to explore the spectroscopic evidence of the interactions of the human gastric cells with the anticancer drug 5-Fu and expect to attain an insight into their interaction mechanisms.

The normal (GES-1) and malignant (SGC-7901) gastric cells were purchased from Chinese Fourth Military Medical University and the Institute of Biochemistry and Cell Biology in China, respectively. Cells were cultured at 37 °C in a 5% CO_2 humidified incubator using a RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum, 50-ml HEPES, 100-μg/ml penicillin, and 100-mg/ml streptomycin. 5-Fu was dissolved in RPMI-1640 medium, and then the cells were cultured at 37 °C in the 5% CO_2 humidified incubator. After 24 hours, the gastric cells were centrifuged at the speed of 1500 rpm for 3 min and harvested, and then washed twice in distilled water, and resuspended in distilled water for Raman spectroscopy.

Raman spectra were recorded by a confocal micro-Raman spectrometer (T64000, Jobin-Yvon), equipped with a 50-mW argon-krypton laser excitation source at 514 nm, a microscope (IX 81, Olympus, Japan), a holographic notch filter to reject Rayleigh scattering and a liquid nitrogen cooled charge coupled device (CCD) detector (CCD-3000V, Edison N.J., USA). A 60× microscope water objective was used to focus laser and collect Raman scattering on the cells. The spectrometer was calibrated with the silicon phonon mode at 520 cm^{-1}. Raman spectra in the region of $500-1800$ cm^{-1} were recorded with an integration time of 60 s and were dealt with Labspec 6.0 software.

Three samples of the gastric cancer cells were treated with 0, 25-, 100-, and 200-mg/L 5-Fu, respectively. 24 h later, it is found that the higher the concentration of 5-Fu is, the less the density of the cells is.

Raman spectra for the gastric cancer cells interacted with 5-Fu were recorded in the range of $500-1800$ cm^{-1}, as shown in Fig. 1. The spectra in the range of $500-1200$ cm^{-1} are normalized by the peak at 1003 cm^{-1}, and the spectra in the range of $1200-1800$ cm^{-1} are normalized by the peak at 1450 cm^{-1}. In Fig. 1(A), the band at 1003 cm^{-1} is assigned to the phenylalanine[14]. The band at 1094 cm^{-1} is attributed to the symmetric stretching vibration mode of PO_2^- in the DNA backbone, and it can be used as an internal intensity standard for the DNA content[15,16]. As the concentration of 5-Fu increases, the

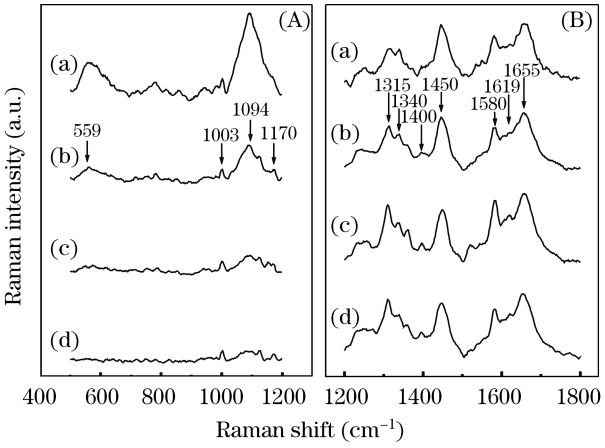

Fig. 1. Mean Raman spectra for the cancer cells interacted with 5-Fu at the concentrations of (a) 0 mg/L, (b) 25 mg/L, (c) 100 mg/L, and (d) 200 mg/L in the region of (A) 500−1200 cm^{-1} and (B) 1200 − 1800 cm^{-1}.

Table 1. Raman Band Intensity and Area, Their Ratios for the Gastric Cancer Cells Interacted with 5-Fu

Concentration of 5-Fu (mg/L)	0	25	100	200
Intensity at 559 cm^{-1} (a.u.)	447	219	73	37
Intensity at 1094 cm^{-1} (a.u.)	1235	612	299	176
I_{1315}/I_{1340}	1.07	1.23	1.45	1.36
A_{1655}/A_{1450}	1.00	1.03	1.15	1.10

band at 1094 cm^{-1} gradually decreases, which indicates the interaction with 5-Fu can result in the reduction of the DNA content in the cancer cells. 5-Fu can interfere with the DNA synthesis by blocking the thymidylate synthase conversion of deoxyuridylic acid to thymidylic acid[17], and the duplication of DNA in the cancer cells can be restrained by 5-Fu, so the interaction with 5-Fu results in the reduction of the DNA content. Besides, the bands at 1315 and 1340 cm^{-1} can be attributed to guanine (G) and adenine (A), respectively. The intensity ratio of the band at 1315 cm^{-1} to that at 1340 cm^{-1} shows the ascending trend with the concentration of 5-Fu increasing, as shown in Table 1, and it suggests that 5-Fu may bring the alteration of external environment of guanine and adenine residues in gastric cancer cells.

As shown in Fig. 1(B), the band at 1400 cm^{-1} is due to the presence of the CH_2 vibration mode in lipids, and the interaction with 5-Fu can result in an increase of the lipid content. Moreover, the bands at 1170, 1619, and 1580 cm^{-1} can be assigned respectively to tyrosine, tryptophan, and heme, and become stronger after the interaction with 5-Fu. The bands at 1655 cm^{-1} and 1450 cm^{-1} can be attributed to the C=C stretching vibration and CH_2 bending mode in the hydrocarbon chains of fatty acids, respectively. Their band area ratio can indicate the unsaturation degree of the hydrocarbon chains. As the unsaturation degree increases, the Raman intensity at 1655 cm^{-1} will increase, whereas that at 1450 cm^{-1} will decrease[18]. In Table 1, the ratio of the band area at 1655 cm^{-1} to that at 1450 cm^{-1} shows the slight ascending trend with the increase of concentration of 5-Fu. So, it can be concluded that the interaction with 5-Fu will result in an increase in the unsaturation degree of the fatty acids in the lipids and membranes of the gastric cancer cells.

Raman spectra for the normal gastric cells interacted with 5-Fu in the range of 500 − 1800 cm^{-1} are shown in Fig. 2. The spectra in the range of 500 − 1200 cm^{-1} are normalized by the peak at 1003 cm^{-1}, and those in the range of 1200 − 1800 cm^{-1} are normalized by the peak at 1450 cm^{-1}. Compared with that in Fig. 1, the Raman spectra for the normal gastric cells show rather few change after the interaction with the anticancer drug of 5-Fu. As shown in Fig. 2, the bands at 1094, 1315, and 1340 cm^{-1} decrease for the interaction of 5-Fu. Compared with the Raman spectra for the gastric cancer cells, the intensity at 1094 cm^{-1} for the normal gastric cells shows less decrease and the intensity ratio of the band at 1315 cm^{-1} to that at 1340 cm^{-1} exhibits irregular alteration, which suggests that 5-Fu shows more profound effects on gastric cancer cells than on normal gastric cells, and has not only the antitumor effect but also the side effect. When the normal cells interact with 5-Fu, there appears no obvious Raman frequency shift for DNA bands. The spectral changes arising from DNA for normal cells imply that 5-Fu may also interact with normal gastric cells through suppressing the activation of thymidylate synthase (TS) and synthesis of DNA, and 5-Fu results in the reduction of the DNA content while not interacts directly with DNA or change the double-stranded structure of DNA in the normal cells. So, it can be inferred that 5-Fu plays as inhibitor on the gastric cancer cells, and has a little side effect on the growth of the normal gastric cell.

The main changes related to proteins can be observed from the bands at 1170, 1619, and 1655 cm^{-1}. When the concentration of 5-Fu increases from 25 to 100 mg/L, a sharp decrease is observed in the magnitude of the band at 1170 cm^{-1}, and the spectral profile change of the band at 1655 cm^{-1} is also observed. However, the area ratio of the band at 1655 cm^{-1} to that at 1450 cm^{-1} in the normal gastric cells appears little change, and it indicates that the interaction of 5-Fu has little effect on the unsaturation degree of the fatty acids in the lipids and membranes of the normal gastric cells.

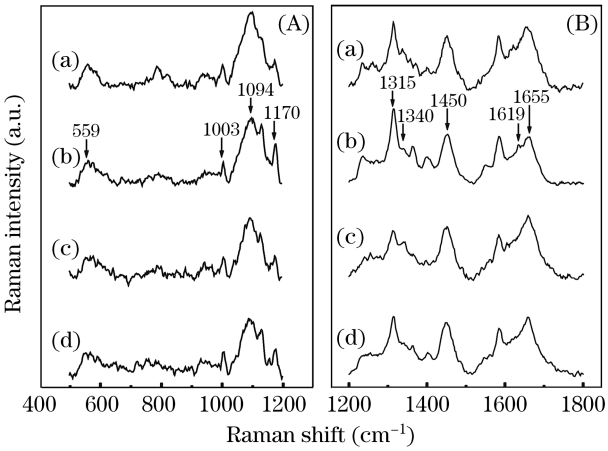

Fig. 2. Mean Raman spectra of the normal cells interacted with 5-Fu with the different concentrations of (a) 0 mg/L, (b) 25 mg/L, (c) 100 mg/L, and (d) 200 mg/L in the region of (A) 500 − 1200 cm^{-1} and (B) 1200 − 1800 cm^{-1}.

In conclusion, the Raman spectroscopy on the interactions between gastric cells and 5-Fu has been investigated. The experimental results show that 5-Fu has not only the antitumor effect on the gastric cancer cells but also the side effect on the normal gastric cells and induces apoptotic death of the gastric cancer cells. The interaction of the normal gastric cells with 5-Fu is rather weak and results in change of their DNA content. However, the interaction of the gastric cancer cells with 5-Fu can restrain the duplication of DNA and induce the decrease of their DNA content, and furthermore it results in the unsaturation degree of the fatty acids in their lipids and membranes.

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References

1. Z. Wang, X. Han, and G. Li, Chin. J. Light Scatt. (in Chinese) **11**, 398 (2000).
2. P. Bonomi, K. Kim, D. Fairclough, D. Cella, J. Kugler, E. Rowinsky, M. Kiroutek, and D. Johnson, J. Clin. Oncol. **18,** 623 (2000).
3. C. L. van der Wilt, K. Smid, G. W. Aherne, P. Noordhuis, and G. J. Peters, Eur. J. Cancer **33,** 471 (1997).
4. Y. M. Chung, S.-H. Park, J.-K. Park, Y.-T. Kim, Y.-K. Kang, and Y. D. Yoo, Cancer Lett. **159,** 95 (2000).
5. J.-S. Park, S. Y. Yoon, J.-M. Kim, Y.-I. Yeom, Y. S. Kim, and N.-S. Kim, Cancer Lett. **214,** 19 (2004).
6. D. Pappas, B. W. Smith, and J.-D. Winefordner, Talanta **51,** 131 (2000).
7. P. V. Huong, Vib. Spectros. **11,** 17 (1996).
8. Y. Li, R. Chen, H. Zeng, Z. Huang, S. Feng, and S. Xie, Chin. Opt. Lett. **5,** 105 (2007).
9. X. Zhao, D. Lu, S. Jiang, C. Mao, Y. Fan, C. An, and Z. Li, Sci. Chem. (Ser. B) (in Chinese) **38,** (5) 555 (1995).
10. A. N. C. Anigbogu, A. C. Williams, B. W. Barry, and H. G. M. Edwards, Int. J. Pharm. **125,** 265 (1995).
11. S. Sánchez-Carrasco, J. G. Delcros, A. A. Moya-García, F. Sánchez-Jiménez, and F. J. Ramírez, Biophys. Chem. **133,** 54 (2008).
12. A. A. Elkordy, R. T. Forbes, and B. W. Barry, Eur. J. Pharm. Sci. **33,** 177 (2008).
13. C. Scalfi-Happ, A. Jauss, W. Ibach, O. Hollricher, S. Fulda, C. Hauser, R. Steiner, and A. RüCK, Medical Laser Application **22,** 157 (2007).
14. X. Yan, Q. Wang, R. Dong, Z. Sun, and P. Xiu. Spectrosc. Spect. Anal. (in Chinese) **23,** 1129 (2003).
15. R. H. Hawi, W. C. Campbell, A. Kajdacsy-Balla, R. Murphy, F. Adar, and K. Nithipatikom, Cancer Lett. **110,** 35 (1996).
16. L. Tang, Z. Sun, J. Guo, and Z. Wang, Chin. Opt. Lett. **4,** 101 (2006).
17. M. Kakimoto, H. Uetake, T. Osanai, Y. Shirota, Y. Takagi, E. Takeshita, Y. Toriya, K. Danenberg, P. V. Danenberg, and K. Sugihara, Cancer Lett. **223,** 103 (2005).
18. A. T. Tu, *Raman Spectroscopy in Biology: Principles and Applications* (John Wiley and Sons, New York, 1982).