In vivo Raman measurement of levofloxacin lactate in blood using a nanoparticle-coated optical fiber probe

Shupeng Liu,1,3 Ming Rong,1 Heng Zhang,1 Na Chen,1,4 Fufei Pang,1 Zhenyi Chen,1 Tingyun Wang,1 and Jianshe Yan2,5

1Key Laboratory of Specialty Fiber Optics and Optical Access Networks, School of Communication and Information, Engineering, Shanghai University, 333 Nanchen Road, Shanghai 200444, China
2Shanghai Institute of Immunology, Shanghai Jiao Tong University, School of Medicine, Shanghai 200025, China
3liusp@shu.edu.cn
4na.chen@shu.edu.cn
5yanjianshe@sjtu.edu.cn

Abstract: Monitoring drug concentrations in vivo is very useful for adjusting a drug dosage during treatment and for drug research. Specifically, cutting-edge “on-line” drug research relies on knowing how drugs are metabolized or how they interact with the blood in real-time. Thus, this study explored performing in vivo Raman measurements of the model drug levofloxacin lactate in the blood using a nanoparticle-coated optical fiber probe (optical fiber nano-probe). The results show that we were able to measure real-time changes in the blood concentration of levofloxacin lactate, suggesting that this technique could be helpful for performing drug analyses and drug monitoring in a clinical setting without repeatedly withdrawing blood from patients.

©2016 Optical Society of America

OCIS codes: (170.5660) Raman spectroscopy; (170.1470) Blood or tissue constituent monitoring; (290.5880) Scattering, rough surfaces.

References and links

1. E. Eliasson, J. D. Lindh, R. E. Malmström, O. Beck, and M. L. Dahl, “Therapeutic drug monitoring for tomorrow,” Eur. J. Clin. Pharmacol. 69(Suppl 1), 25–32 (2013).
2. Y. Li, G. Du, W. Cai, and X. Shao, “Classification and quantitative analysis of Azithromycin tablets by Raman spectroscopy and chemometrics,” Am. J. Anal. Chem. 2(02), 135–141 (2011).
3. I. Pecorelli, R. Bibi, L. Fioroni, and R. Galarini, “Validation of a confirmatory method for the determination of sulphonamides in muscle according to the European Union regulation 2002/657/EC,” J. Chromatogr. A 1032(1-2), 23–29 (2004).
4. T. A. M. Msagati and M. M. Nindi, “Multiresidue determination of sulfonamides in a variety of biological matrices by supported liquid chromatography-electrospray mass spectrometry detection,” Talanta 64, 87–100 (2004).
5. P. N. Patsalos and D. J. Berry, “Therapeutic drug monitoring of antiepileptic drugs by use of saliva,” Ther. Drug Monit. 35(1), 4–29 (2013).
6. R. J. Meesters and G. P. Hooff, “State-of-the-art dried blood spot analysis: an overview of recent advances and future trends,” Bioanalysis 5(17), 2187–2208 (2013).
7. I. Taneja, M. Erukala, K. S. Raju, S. P. Singh, and Wahajuddin, “Dried blood spots in bioanalysis of antimalarials: relevance and challenges in quantitative assessment of antimalarial drugs,” Bioanalysis 5(17), 2171–2186 (2013).
8. K. S. Raju, I. Taneja, S. P. Singh, and Wahajuddin, “Utility of noninvasive biomarkers in pharmacokinetic studies,” Biomed. Chromatogr. 27(10), 1354–1366 (2013).
9. E. Eliasson, J. D. Lindh, R. E. Malmström, O. Beck, and M. L. Dahl, “Therapeutic drug monitoring for tomorrow,” Eur. J. Clin. Pharmacol. 69(S1 Suppl 1), 25–32 (2013).
10. J. de Leon, E. Spina, and F. J. Diaz, “Clobazam therapeutic drug monitoring: a comprehensive review of the literature with proposals to improve future studies,” Ther. Drug Monit. 35(1), 30–47 (2013).
11. Y. Xu, Y. Du, Q. Li, X. Wang, Y. Pan, H. Zhang, T. Wu, and H. Hu, “Ultrasonselective Detection of Enrofloxacin in Chicken Muscles by Surface-Enhanced Raman Spectroscopy Using Amino-Modified Glycidyl Methacrylate-Ethyene Dimethacrylate (GMA-EDMA) Powdered Porous Material,” Food Anal. Methods 7, 1–10 (2013).
12. C. Yu, E. Gestl, K. Eckert, D. Allara, and J. Irudayaraj, “Characterization of human breast epithelial cells by confocal Raman microspectroscopy,” Cancer Detect. Prev. 30(6), 515–522 (2006).
14. J. Shao, M. Lin, Y. Li, X. Li, J. Liu, J. Liang, and H. Yao, “In vivo blood glucose quantification using Raman spectroscopy,” PlOS One 7(10), e48127 (2012).

15. Y. Huang and Q. Kang, “Synthesis of conjugates of β-cyclodextrin with polyamidoamine dendrimers and their molecular inclusion interaction with levofloxacin lactate,” J. Incl. Phenom. Macrocycl. Chem. 72(1–2), 55–61 (2012).

16. Y. D. Hu, H. Q. Zhang, and D. M. Cao, “Synthesis of Fe3O4 Nanocrystals and Application in Photocatalytic Degradation of Levofloxacin Lactate,” Mater. Sci. Forum 688, 376–382 (2011).

17. S. Liu, J. Huang, Z. Chen, N. Chen, F. Pang, T. Wang, and L. Hu, “Raman spectroscopy measurement of levofloxacin lactate in blood using an optical fiber nano-probe,” J. Raman Spectrosc. 46(2), 197–201 (2015).

18. Z. Chen, Z. Dai, N. Chen, S. Liu, F. Pang, B. Lu, and T. Wang, “Gold Nanoparticles-modified Tapered Fiber Nanoprobe for Remote SERS Detection,” IEEE Photonics Technol. Lett. 26(8), 777–780 (2014).

19. N. Seedher and P. Agarwal, “Competitive binding of fluoroquinolone antibiotics and some other drugs to human serum albumin: a luminescence spectroscopic study,” Luminescence 28(4), 562–568 (2013).

20. C. Wang, J. Wang, and L. Deng, “Evaluating interaction forces between BSA and rabbit anti-BSA in sulphathiazole sodium, tylosin and levofloxacin solution by AFM,” Nanoscale Res. Lett. 6(1), 579 (2011).

21. W. Yi and X. Luo, Progress and Application of Optical Fibre Probes for in vivo Raman Spectroscopy. IEEE Photonics and Optoelectronics (SOPO) 2012.

22. C. Matthäus, R. Cicchi, S. Dochow, C. Kraft, A. Lattermann, B. R. Brehm, F. Pavone, and J. Popp, “Characterization of Atherosclerotic Plaque Deposits in vivo by Fiber-Optic Raman Spectroscopy and ex vivo by FTIR. Raman and Non-Linear Imaging Techniques,” Biomed. Tech. (Berl.) 57(Suppl. 1), 337 (2012).

23. N. Gandra and S. Singamaneni, “Surface-enhanced Raman scattering for in vivo imaging: the future looks BRIGHT!” Nanomedicine (Lond.) 8(3), 317–320 (2013).

24. N. Gandra and S. Singamaneni, “Bi-layered Raman-intensive gold nanostructures with hidden tags (BRIGHTs) for high-resolution bioimaging,” Adv. Mater. 25(7), 1022–1027 (2013).

25. J. F. Brennan, Y. Wang, R. R. Dasari, and M. S. Feld, “Near-infrared Raman spectrometer systems for human tissue studies,” Appl. Spectrosc. 51(2), 201–208 (1997).

26. J. P. Salenius, J. F. Brennan 3rd, A. Miller, Y. Wang, T. Aretz, B. Sacks, R. R. Dasari, and M. S. Feld, “Biochemical composition of human peripheral arteries examined with near-infrared Raman spectroscopy,” J. Surg. Res. 72(4), 710–719 (1998).

27. E. B. Hanlon, R. Manoharan, T.-W. Koo, K. E. Shafer, J. T. Motz, M. Fitzmaurice, J. R. Kramer, I. Itzkan, R. R. Dasari, and M. S. Feld, “Prospects for in vivo Raman spectroscopy,” Phys. Med. Biol. 45(2), R1–R59 (2000).

1. Introduction

Therapeutic drug monitoring could help clinicians individualize drug treatment and guide dosages such that systemic drug concentrations optimally reach levels associated with therapeutic efficacy, which in turn would reduce the risk of concentration-dependent adverse effects [1]. In pharmacokinetics, clinical pharmacology, and biopharmaceutical research, drug concentrations are mainly measured by chromatography, immunoassays, isotope labeling, and microbiological assays. Chromatography techniques mainly include colorimetric and fluorescence analysis, high performance liquid chromatography (HPLC) [2,3], and chromatography techniques that combine mass spectrometry [4,5]. Chromatography techniques that employ ultraviolet detectors are simple and fast, but they show poor selectivity and sensitivity, making them unsuitable for measuring low drug concentrations. By contrast, immunoassays, isotope labeling, and microbiological assays are accurate, but these techniques are time-consuming and laborious [5–10].

One technique that has considerable potential for rapid and sensitive detection is surface-enhanced Raman scattering (SERS) [11], which provides detailed molecular-level information [12]. SERS is able to measure drug concentrations of furazolidone and malachite green at concentrations of 1 μg.g-1 and 200 ng.g-1, respectively [13], enrofloxacin at 0.01 mg.kg-1 [11], and blood glucose at 5 mmol/l [14]. In addition, SERS technology has been applied in biomedical imaging.

Thus, SERS technology could potentially be very beneficial for biopharmaceutical analyses, which require large amounts of data for clinical pharmacological analysis as well as fast, accurate, and highly selective and sensitive analysis methods. In addition, analysis methods that can be done in vivo and on-line are even more advantageous, as they offer the potential for real-time monitoring of drug concentrations. Therefore, this study investigated a SERS-based drug analysis method that can be performed in vivo and in real-time. Specifically, we measured concentrations of the model drug levofloxacin lactate (a
fluoroquinolone that inhibits bacterial DNA replication, often used to prevent infectious diseases [15,16]) in vivo and on-line using a gold nanoparticle–coated optical fiber (optical fiber nano-probe). Resulting analyses of the Raman spectrum intensities corresponding to the in vivo drug concentrations indicate that these techniques provides useful information for pharmaceutical detection, and might help advance personalized drug treatment strategies.

2. Materials and methods

2.1 Preparation of the optical fiber nano-probe

The optical fiber nano-probe was fabricated for Raman spectra measurements [17]. The optical fiber was pulled to form a tapered shape and etched with hydrofluoric acid (HF) to make the tip size less than 100 nm. Then, gold nanoparticles were coated onto the surface of the optical fiber tip using an electrostatic self-assembly technology. The fiber was cleaned with sulfuric acid and hydrogen peroxide for 30 min, then soaked in the mixture solution with 5%v/v deionized water, 5%v/v (3-Aminopropyl) trimethoxysilane (APTMS, 97%) and 90%v/v ethanol for 30 min, and then incubated at 90° for 30 min. The fiber was immersed in the gold sol for 48h after the fiber washed thoroughly with deionized water and ethanol [18]. The optical fiber nano-probe was inserted into the needle and situated near the needle tip for protection (Fig. 1).

![Image of the optical fiber nano-probe within the needle.](image-url)

2.2 Preparation of the in vivo experiments

The SD rat, about 326g weight, were obtained from Laboratory of Neuropharmacology and Neurotoxicology, Shanghai University. The rat was housed with temperature range from 20°C to 25 ° C. The rat was fed with commercial aseptic food and tap water ad libitum throughout the experimental period. The rat was anaesthetized with chloral hydrate. The optical fiber nano-probe (~12-cm long), protected by the syringe needle, was inserted into the tail vein of an anaesthetized rat; the other end of the fiber was connected to the confocal Raman spectrometer and then coupled to the 10 × objective lens. The model drug levofloxacin lactate (1 ml 0.1 g/ml) was injected into the rat by intraperitoneal injection and the concentration of the drug in blood was measured through the optical fiber.

2.3 SERS measurement setup

Once the optical fiber nano-probe was in place, a Renishaw Raman microscope employing a 10 × objective and 633-nm excitation was used to obtain all of the spectra. The SERS spectra were acquired with a 10-s integration time and in the range of 900 - 1800 cm⁻¹. A 10 × objective lens (numerical aperture, NA = 0.25) was close to the tip of the multimode optical fiber (core diameter of 50 μm, NA = 0.20). The experimental setup for in vivo experiments is shown in Fig. 2.
3. Results

The Raman spectra measurements of levofloxacin lactate in vivo

The in vivo Raman spectrum of the drug detected by the optical fiber nano-probe is shown in Fig. 3, where curve a represents the raw data, while curve b shows the resulting curve once the baseline is subtracted. The in vivo spectra obtained by the optical fiber nano-probe show Raman peak positions for levofloxacin lactate at 1404 cm$^{-1}$, 1574 cm$^{-1}$, and 1621 cm$^{-1}$. Among them, the 1404 cm$^{-1}$ and 1621 cm$^{-1}$ are the characteristic peaks of levofloxacin lactate [17]. Other characteristic peaks of levofloxacin lactate could have potentially been shifted by an interaction between the drug and the albumin in the blood, which may affect the drug’s molecular conformation [19, 20].

After the intraperitoneal injection of levofloxacin lactate, the Raman spectra were measured in vivo and in real-time using the optical fiber. The resulting Raman signals of levofloxacin lactate are shown in Fig. 4. The Raman intensity of characteristic peak at 1621 cm$^{-1}$ was the strongest at the 21st minute, but then gradually became weaker with time. After
the 1621 cm\(^{-1}\) peak disappeared, the Raman intensity of peak 1604 cm\(^{-1}\) appeared, after which it became stronger and then decreased with time.

By 25 minutes, the peak at 1621 cm\(^{-1}\) was decreasing gradually, but the peak at 1604 cm\(^{-1}\) had been increasing, and reached its maximum. Thus, the Raman peak of levofloxacin lactate in blood seems to have shifted from 1621 cm\(^{-1}\) to 1604 cm\(^{-1}\) over time. This indicates that the drug may happen to interact with blood components such as the proteins. The peak at 1621 cm\(^{-1}\) disappeared by the 25th minute, which indicates that the drug may be cleared from the body’s circulatory system at that time.

The Raman peak at 1404 cm\(^{-1}\) indicates the symmetric stretching vibrations of O-C-O. The stronger levofloxacin lactate peak at 1621 cm\(^{-1}\) is attributed to C = C stretching and C = N stretching.

From Fig. 4, it seems that the optical nano-probe may be associated with some time-delay in the measurements, since the drug peak only appeared at the 10th minute, and the strongest peaks were reached at the 20th minute.

Raman spectroscopy measurements with optical fiber nano-probes could potentially be in vivo as a diagnostic tool, which would be helpful for performing real-time analyses in current clinical procedures and avoiding long time waiting. In vivo Raman spectroscopy may fulfill important roles in a variety of different medical applications [21]; for example, optical fiber probes have been used for Raman measurements of inner arterial plaque depositions [22] and for imaging cancer in vivo, where nanoparticles are used as the Raman tags [23,24]. In addition, a clinical Raman system using an optical fiber probe has been used to perform in vivo Raman spectroscopy of the esophageal and peripheral arteries [25–27]. Thus, Raman spectra can be applied to in vivo imaging using multimode and multifunctional nanoparticles or probes.

4. Conclusions

The concentration of levofloxacin lactate in the blood was measured in vivo and in real-time using an optical fiber nano-probe. In this experiment, the typical levofloxacin lactate peaks changed in intensity over time. Because these results showed that the optical fiber nano-probe was able to detect the typical Raman spectra peaks of levofloxacin lactate, this system may represent a useful tool for measuring drug concentrations in vivo and in real-time and
potentially allowing for rapid analyses. Such rapid analyses would be useful for predicting an individual's drug exposure level and the corresponding clinical effects, and may also be helpful for studying the in vivo interactions that occur between drugs and components of the blood. Overall, this experiment showed that nanoparticle-coated optical fibers have the potential possibility to be used in real-time detection of the model drug levofloxacin lactate, indicating that this technique might represent a new and effective measurement method for pharmaceutical investigation of some drugs with stronger SERS spectra signals in the future.

Acknowledgments

This work was supported by the Natural Science Foundation of China (NSFC) (61107076, 61177088, 61027015, 61475095) and the National Program on Key Basic Research Project (973 Program, 2012CB723405). This project was funded by the Science and Technology Commission of Shanghai Municipality (STCSM) (14511105602, 14DZ1201403, 13NM1401101, 14440500100). And thanks for the support of the Key Laboratory of Specialty Fiber Optics and Optical Access Networks (SKLSF02013-02).