Development of Electron Paramagnetic Resonance Spin Probes for Brain Imaging

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学位記番号	生博第２７１号
学位授与年月日	平成２６年３月２６日
学位授与の要件	学位規則第４条第１項該当
研究科，専攻	東北大学大学院生命科学研究科 （博士課程）分子生命科学専攻
論文題目	Development of Electron Paramagnetic Resonance Spin Probes for Brain Imaging （脳イメージングのための電子常磁性共鳴スピンプローブ開発）
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論文内容の要旨

Chapter 1: Introduction

Electron Paramagnetic Resonance (EPR) method has been developed for many decades. With the development, EPR spectrometers are capable of detecting paramagnetic species in living animals in real time and now EPR imaging is an alternative technology that can, like magnetic resonance imaging (MRI), be used to obtain many biologically important information, such as tissue redox status, pO_2, and pH. In resent years, the EPR imaging of the brain becomes a promising area of research because PET requires introduction of a radioactive label into the body with a drawback of exposure to ionizing radiation. However, the challenge has been the synthesis of available stable spin probes that can provide important biological information.

Nitroxide radicals have wide range of applications base on their paramagnetic properties and redox properties. However, fast *in vivo* reduction of nitroxide radical to corresponding hydroxylamines by antioxidants, such as ascorbic acid (AsA) or enzymatic systems, limits their application in biological studies. Since this kind of bioreducton is especially fast in brain, development of EPR spin probes for brain imaging is a big challenge. In fact, there are very few nitroxides are blood-brain-barrier (BBB) permeable that can be used for brain imaging. Moreover, synthesis of spin probes for selective imaging of specific receptors in brain is even a bigger challenge because they must be not only stable and BBB permeable, but also able to bind to target receptors that ligands or pharmaceutical drugs bind to. So far there was no report of a spin probe can localized in a specific area of brain. Therefore, design and synthesis of spin probes for brain imaging is an urgent need for EPR in biological studies.

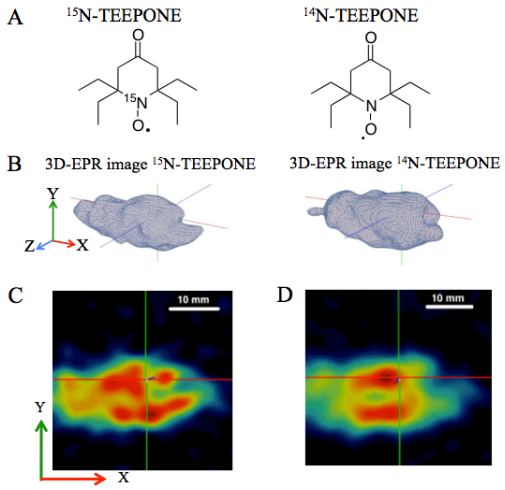

Fig.1 EPR imaging of mouse heads with injected ^{15}N-TEEPONE and ^{14}N-TEEPONE. (A) Chemical structures of ^{15}N-TEEPONE and ^{14}N-TEEPONE (B) 3D-EPR images of mouse heads with injected ^{15}N-TEEPONE and ^{14}N-TEEPONE. (C) Slice-selective EPR image of a mouse head after data accumulation for 3 min following injection of ^{15}N-TEEPONE. (D) Slice-selective EPR image of a mouse head after data accumulation for 3 min following injection of ^{14}N-TEEPONE.

Chapter 2: Isotope Labeled Nitroxide Radical Probes for EPR Imaging

This chapter focused on the synthesis of

stable spin probes for brain imaging by EPR. Thus, I presented a developed scalable synthesis of ^{15}N-4-oxo-2,2,6,6-tetraethylpiperidine nitroxide (^{15}N-TEEPONE), and its first use for the brain imaging of mice. A comparison of ^{15}N-TEEPONE and ^{14}N-TEEPONE was performed in mouse brain imaging and 2D-slice-selective images are depicted in Fig.1 C and D. These images showed that TEEPONE was distributed in the mouse brain, and that the *in vivo* half lives of ^{15}N-TEEPONE and ^{14}N-TEEPONE were both approximately 80 min. These results were consistent with a previous study of ^{14}N-TEEPONE.[1,2] ^{15}N-TEEPONE showed a finer image because of its signal intensity was 50% greater then ^{14}N-TEEPONE. The higher signal intensity of the ^{15}N-probe has significant advantages in brain imaging studies. In addition, ^{15}N-TEEPONE in combination with ^{14}N-TEEPONE has great potential as a spin label precursor for use in simultaneous EPR imaging techniques.

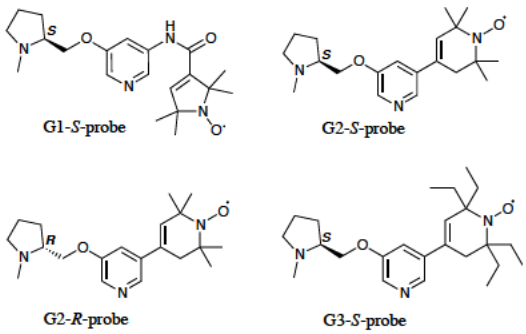

Fig. 2 Chemical structures of synthesized spin probes for EPR imaging of nAChRs

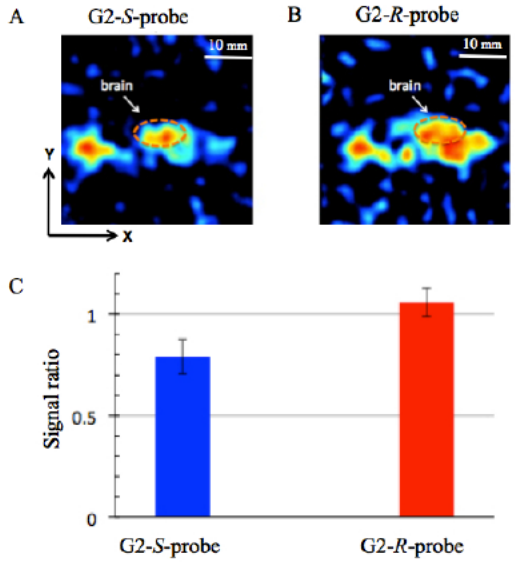

Fig. 3 EPR imaging of mouse heads with injected G2-*S*-probe and G2-*R*-probe. (A) Slice-selective EPR image of a mouse head with G2-*S*-probe; (B) Slice-selective EPR image of a mouse head with G2-*R*-probe (C) The EPR spectral signal ratio of 100 mM (-)-nicotine added samples to non-added samples for G2-*S*-probe or G2-*R*-probe ($N = 4$).

Chapter 3: Design and Synthesis of Brain Imaging Spin Probes for EPR Imaging of Mouse Brain

The nicotine acetylcholine receptors (nAChRs) are widely distributed throughout the central and peripheral nervous systems, where mediate a variety of brain function. Central nervous diseases, such as Alzheimer's disease, have been associated with changes in nAChRs.[3] It suggests that imaging of nAChRs might provide valuable in vivo information in the early course of the disease and on the efficacy of treatment.

Considering the role of nAChR in the

pathophysiology of Alzheimer's disease, it is of great interest to study nAChR in brain of living object. Thus I designed and synthesized a series of spin probes (Fig. 2) for EPR brain imaging by using nAChRs ligands, 3-pyridyl-ether compounds,[4] which generally possess subnanomolar affinity for brain nAChRs. The first generation spin probe (G1-*S*-probe) was not BBB permeable. To overcome this problem, the second generation of spin probes (G2-*S*-probe and G2-*R*-probe), which have lower molecular weight and higher lipophilicity, were synthesized. The results of mouse brain in vivo EPR imaging indicated that G2-*S*-probe and G2-*R*-probe were BBB-permeable, and the highest signal intensities were found in thalamusthe and colliculus regions of mouse brain was similar to that of the radiolabeled trace 5-[^{125}I]-A-85380 for nAChRs by PET.[5] Futhermore, it is essential to illuminate that synthesized spin probes are specifically bound to nAChRs, the difference in binding affinities of two enantiomeric probes was clarified according to inhibit effect by nicotine. This result indicated that G2-*S*-probe was selectively bound to nAChRs acting like a nicotinic agonist, and (-)-nicotine didn't show any inhibit effect to G2-*R*-probe. It implied that G2-*S*-probe has greater specific binding affinity to nAChRs than G2-*R*-probe, which is corresponding with the characteristics of their mother molecules. Although G2-*S*-probe was successfully use as a spin probe for brain imaging, toxicity still a problem for animal experiments. An attempt (G3-*S*-probe) to avoid toxicity was the introduction of TEEPONE to probe instead of TEMPONE, but the broadened linewidth EPR spectrum were observed.

Conclusion

I successfully synthesized ^{15}N-TEEPONE and EPR results implied that ^{15}N-TEEPONE was suitable for EPR brain imaging, and its higher sensitivity is an important advantage for brain imaging studies. In order to study nAChRs in brain of living object, a series of spin probes were synthesized. Among them, G2-*S*-probe was successfully used as a spin probe for brain imaging and its specific binding affinity to nAChRs was indicated. The G3-*S*-probe, an attempt to reduce the toxicity for animal experiments didn't work. Thus, the work presented here is still in progress. I suggest that a perdeuterated spin probe derivative would be the next option because deuteration of the nitroxide moiety could narrowing EPR linewidth and therefore make reduced injection dose possible.

References

1. M. Emoto, F. Mito, T. Yamasaki, K. Yamada, H. Sato-Akaba, H. Hirata and H. Fujii, *Free Radical Res*. **2011**, *45*, 1325−1332
2. M. C. Emoto, K. Yamada, M. Yamato and H. G. Fujii, *Neurosci. Lett.* **2013**, *546*, 11−15.
3. W. Sihver, P. G. Gillberg, A. L. Svensson and A. Nordberg, *Neurosci*. **1999**, *94*, 685−696.

4. M. A. Abreo, N. H. Lin, D. S. Garvey, D. E. Gunn, A. M. Hettinger, J. T. Wasicak, P. A. Pavlik, Y. C. Martin, D. L. Donnelly-Robers, D. J. Anderson, J. P. Sullivan, M. Williams, S. P. Arneric and M. W. Holladay, *J. Med. Chem*. **1996**, *39*, 817−825.
5. D. B. Vaupel, A. G. Mukhin, A. S. Kimes, A. G. Horti, A. O. Koren and E. D. London, *NeuroReport* **1998**, *7*, 2311−2317.

論文審査結果の要旨

生体イメージングは，疾患の早期診断や医薬候補化合物の体内動態解析などに有用な方法である。なかでも，EPR イメージングは，放射性同位体を用いないことから今後の発展が期待されている。王**暁**蕾君提出の本論文は，EPR イメージング用プローブの開発について述べた。

まず第一章では，EPR の基礎理論について述べ，次に現在の EPR イメージング装置について，現状と課題を述べた。

第二章では，同位体標識されたニトロキシドラジカルのプローブとしての応用について論じた。14N を含むニトロキシドと 15N を含むニトロキシドは、シグナルの分裂パターンが異なるので，両者を区別して画像化できる。まず in vitro の系において，これを実証した。続いて、15N 標識体が同濃度において 14N 標識体よりも EPR シグナルが大きいことに着目し，in vivo イメージングの画質向上に与える影響を調べた。この研究では TEEPONE をプローブとして用いた。TEEPONE は生体内還元速度が遅いという利点を持つが，スペクトルの線幅が大きいため検出感度はそれほど高くない。実際，マウス脳内のイメージングを行うと測定開始３０分で画像化が困難となった。王君は，15N 標識された TEEPONE を初めて化学合成し，この問題を解決した。すなわち，Meyer-Schuster 転位を鍵反応とする合成経路を立案し，２グラムを超える TEEPONE を得ることが出来た。高価な 15N 標識の導入が，合成の最後段に位置しているため，コストが抑制でき高く評価できる。15N 標識 TEEPONE をマウスに投与し，同様に脳内のイメージングを行うと測定開始１時間後においても積算を行うことなく明瞭な３次元画像を得ることが出来た。15N 標識 TEEPONE は，それ自身がプローブとして有用であるだけでなく，生物活性物質のスピンラベル化剤としても応用が期待される。

第三章では，ニコチン誘導体とニトロキシドを結合させ，マウス脳内での化合物動態可視化に挑戦した。当初は，プローブが血液脳関門を透過しないという問題にぶつかったが，巧みな分子設計によって解決し画像の取得に成功した。今後はプローブの急性毒性を低減させる必要があるが，新たな試みとして評価できる。

以上のように，王**暁**蕾君提出の論文は，各種プローブの開発や，同位体標識の有用性実証など，EPR イメージング研究に大きな進展をもたらした。このことは，同君が自立して研究活動を行うに必要な高度の研究能力と学識を有することを示している。したがって，博士（生命科学）の博士論文として合格と認める。