様式３

論　文　内　容　の　要　旨

氏　　名　（　　PAN YUE　潘越　　）	
論文題名	Steroid profiling of biological samples by LC-MS/MS (LC-MS/MSによる生体試料中のステロイドのプロファイリング)

論文内容の要旨

Steroid hormones are small molecules, known to have a profound biological impact on target cells or organs. Steroid hormones are endogenous compounds that are synthesized from cholesterol by a series of enzymatic reactions in the adrenal cortex, gonads, and placenta. There are four classes of steroid hormones, which include corticosteroids, androgens, estrogens, and progestogens. Endogenous steroids are involved in many biological processes, such as reproduction, stress management, inflammation, etc. They also play important roles in the development of many diseases such as breast cancer, prostate cancer, and coronary artery disease. Thus, the comprehensive analysis of those steroids in various biological samples has a significant impact on disease diagnosis or health monitoring. However, conventional analytical methods have been difficult to allow multiple steroids to be detected at a time because of the wide range of concentrations (0.006 - 0.690 μM) and diversity in the chemical properties.

The aim of this study is to develop an efficient, sensitive and prompt analytical method for detecting steroid hormones in various biological samples such as blood, urine, tissues, and cerebrospinal fluid. However, detection of steroids, especially, those in urine, has a difficult issue, which owes to the conjugated steroids being involved with high contents. It thus prompted me to establish the analytical platform for profiling urinary steroids. I conducted optimization of sample pretreatment of urine, i.e., enzymatic hydrolysis, which could convert the conjugates to the free forms, removal of proteins, and purification by solid-phase extraction. Free urinary steroids were isolated directly from urine by solid-phase extraction (SPE) with 80% acetonitrile. The total steroids were prepared by enzymatic treatment of urine with a cocktail of sulfatase and glucronidase, protein precipitation, and separation with the above SPE. In order to detect as many steroid types as possible, UPLC-MS/MS (Li method) with Li+ solution added after the column was used for analysis in addition to the conventional method of detecting protonated ions (H method). The 13 3-OH steroids and the remaining 16 steroids were quantified by standard curves prepared using product ion transitions derived from [M+Li]+ and MH+, respectively.

Two groups of human urine, male and female urine, were analyzed. The absolute amount of each steroid was determined based on creatinine levels. 3-OH steroids could be detected with greater sensitivity using the Li method than the conventional method. The differences between the male and female groups are clearly attributable to sex steroids. 7-OH P5 and 7-OH DHEA were, for the first time, quantified in the total steroids of female urine, and the latter was identified in both female and male urine. In this study, by combining UPLC-MS/MS based on lithium ion incorporation with conventional UPLC-MS/MS, a total of 29 steroids were identified in human urine containing two newly found steroids[1].(Figure 1)

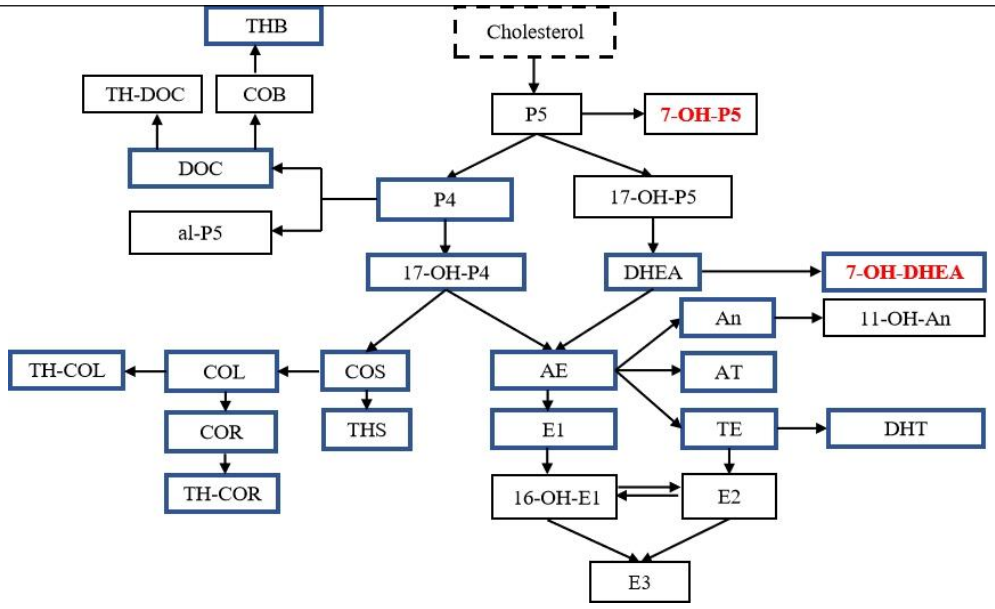

Figure 1. Biosynthesis map of steroid hormones. Steroids (total steroids) identified in this study are boxed with a solid line, and free steroids are boxed with a thick dark blue line. Newly identified steroids are shown in red.

[1] Yue Pan, Qiuyi Wang, et al. Profiling of urinary steroids by lithium ion adduction-based UPLC-MS/MS. Rapid Communications in Mass Spectrometry. November 17, 2023. DOI：10.1002/rcm.9719

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論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

体内ステロイドは、コレステロールから生合成され、３０種類を超える様々な構造異性体を構成し、それぞれは生体ホルモンとして著しいい生理活性を示す。ステロイドの体内プロファイルは生理的状態や様々な病態を反映するため、極めて重要と考えれている。測定対象としては、各組織や体液で、血液は最も頻繁に分析されているが、慢性疾患など長期的な生理的状態の変化を知るために、尿中の抱合ステロイドを対象とする研究も報告されている。第１章では、当該研究室において自ら共同で開発したリチウムイオンを付加イオンとして用いるESI-MS/MS法により尿中の総ステロイドの分析、及び、定量法を確立し、ヒト尿（市販品）に対して２９種類のステロイドの同定と定量に成功している。その内、１種類（7α-OH P5）はこれまで報告例のないものであった。また、7α-OH DHEAの分析においては、これまでの方法に比べ、明瞭に同定できることを示しており、ドーピング検査の分析対象となっている7β-OH DHEA（7α-OH DHEAの構造異性体）の定量に本法が応用できるものと期待できる。第２，３章では、尿よりも多くの夾雑物を含む血中ステロイドを血清から効率的に単離するためにアミノ樹脂を用いた新奇な方法の開発を行っており、実際の血清に本法を応用したところ、４種類の3-ケトステロイドに対して、感度、定量精度において従来法よりも優れた結果を得ている。

以上のように、本論文では、尿中ステロイドの網羅的分析法を確立することにより２９種のステロイドの一斉分析が可能であることを示し、さらに、これまで検出が難しかった１種類のステロイドを尿中から始めて同定している。尿中ステロイドのプロファイルは、将来的に、病態や様々な生理的変化の理解へとつながる重要な知見になるものと考えられる。また、樹脂を使った化学的単離法も新規性があり、血中ステロイドの分析において新たな展開が期待できる。

よって、本論文は博士（理学）の学位論文として十分価値あるものと認める。