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·综 述·

液质联用技术在中药代谢物及代谢组学研究中的应用分析

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[摘要] 中药和天然产物在生物体内通常发生广泛代谢,因其代谢途径的复杂性以及多组分、多靶点、整体调节作用的特点,现已将代谢组学应用于中药代谢产物及其药效机制的研究中。但是,由于代谢组学研究要求获得生物体液中大量外源性与内源性代谢物信息,这就对分析技术提出了很高的要求。本文综述了中药代谢物和代谢组学研究中常用的液质联用技术,并对PubMed检索到的23篇相关文献进行数据归纳与比较分析,重点讨论不同种类液质联用技术在中药代谢组学研究应用中的优缺点,指出UPLC-Q-TOF/MS的优势,以期为中药代谢组学研究提供新思路。

[关键词] 中草药;代谢产物;代谢组学;液质联用
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Application of LC-MS technique in metabolite and metabonomic research of traditional Chinese medicine

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[**Abstract**] Traditional Chinese medicine (TCM) and natural products are widely metabolized in organism. Due to the complexity of their metabolic pathways and "multiple components, multiple targets and the overall regulation functions" of TCM and natural products in organism, metabonomics have been widely applied in researches on TCM metabolites and pharmacodynamic mechanism. However, metabonomics study needs the information of large amount of exogenous and endogenous metabolites in the biological fluids, which requires more powerful analytical techniques. This paper reviewed liquid chromatography coupled with mass spectrometry (LC-MS) techniques commonly applied in TCM metabolite and metabonomics researches. We summarized and comparatively analyzed the data of 23 literatures we retrieved from Pubmed database. We focused on the advantages and disadvantages of various LC-MS techniques in TCM metabonomics studies; the advantages of UPLC-Q-TOF/MS were introduced in a detained manner, hoping to cast new lights on TCM metabonomics researches.

[**Key words**] Chinese herbal drugs; metabolites; metabonomics; liquidchromatography-mass spectrometry
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代谢组学(metabonomics)是通过考察生物体系受刺激或扰动后,研究其代谢物质组随内源代谢、遗传变异、环境变化乃至各种物质进入代谢系统的特征和影响的学科,是研究生物体系代谢途径的一种新技术[1],是继基因组学、蛋白质组学、转录组学后出现的新兴"组学",现已成为系统生物学研究的重要组成部分[2]。与其他组学相比,代谢组学具有以下优势[3]:一是代谢物的数目远小于基因和蛋白质,从而降低了分析的复杂性;二是代谢组学通过对代谢物的高通量分析,较其他组学价廉、快速;三是研究所采用的技术对于不同对象具有良好的适用性。

传统中医药和天然产物在我国创新药物研发中占据十分重要的战略地位。但由于中药和天然产物药效物质基础的复杂性以及多组分协同、多靶点发挥整体药效作用等特殊性,给中药现代化研究带来了诸多困难。代谢组学的诞生为中医现代化研究与发展带来了新的契机[4]。应用代谢组学来理解疾病过程,与中医药理论的"整体观、动态观、辩证观"及中药的"多组分、多靶点、整体调节作用"不谋而合[5]。因此,代谢组学是目前研究中药整体药效机制和发现潜在活性成分的最佳选择之一。然而,中药化学物质组成复杂,有效成分不明确且可能含量较低、口服生物利用度低、体内的代谢途径复杂;加之生物样品内源性基质干扰,有些化合物稳定性差,这就对代谢组学的分析技术提出了很高的要求。

目前代谢组学的技术平台主要由核磁共振技术(NMR)和质谱(MS)及其联用技术组成。其中NMR用于代谢组学的研究已比较成熟。NMR分析快速、选择性好,其样品处理简单且不造成破坏,只要是含氢的代谢物都可被检测出来[6]。然而,利用NMR进行药物代谢的研究要求待测物分子含有具核磁矩的核素,且必须满足药物给药剂量较高、给药后代谢时间较短、代谢物浓度较高等条件才能达到检测限的要求[7-8]。因此,生物样品中的痕量成分可能会因含量低

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于检测限而无法检测到。

代谢组学要求分析生物体系中所有的代谢产物,单一的分析技术难以满足这一要求,故联用技术越来越受到科学家们的重视[9]。气质联用技术(GC-MS)具有灵敏度高、重复性好、有化合物数据库鉴定已知物等优点,其局限性是样品必须气化,不能分析大分子、难挥发性物质和热不稳定性物质[10]。液相色谱质谱联用技术(LC-MS)具有速度快、选择性强、检测限低、定性和定量能力强等优点,是代谢组学研究的理想工具,具有广阔的应用前景[11]。

本文综述了中药代谢物和代谢组学研究中常用的液质联用技术和相关文献,重点讨论不同种类液质联用技术在中药代谢组学研究应用中的特点,以期为代谢组学研究提供新思路。

1　中药代谢组学研究中常用的液质联用技术

1.1　液相分离技术　常规反相液相色谱(RPLC)在分离复杂生物样品时存在明显不足,如分离度较低、分析时间较长等。目前有两种解决方式:一是通过使用更小粒径的色谱柱填料来增加峰容量和分离度;二是增加柱压[11]。为此,具有更高分离能力和更高峰容量的超高效液相色谱,也称快速液相色谱、快速分离色谱(UPLC,RRLC,fast HPLC)得到广泛应用。UPLC 作为一种理想的技术,能在等度和梯度模式下实现复杂样品的快速分离。这种超高压色谱系统使用粒径小于 2 μm 的小微粒填充柱,能够在高压(15 000 psi,1 psi=6 894.8 Pa)下操作,UPLC 系统有着高分离能力的同时还具有较宽的线速度,从而缩短了分析时间[12]。这种高分辨能力使峰宽变窄、信噪比增强,从而提高了生物样品分析的选择性和效率。因此,与传统 RPLC 相比,UPLC 能获得更多代谢物的信息,对于复杂生物样品的分离具有显著优势[13]。

1.2　质谱技术　离子阱(ion trap, IT)质谱是一种低-中分辨率的质谱仪,研究级离子阱可以得到超高分辨率的质谱图[8]。由于离子阱质量分析器的动态范围不高,故离子阱质谱不适合做定量分析。但是,由于离子阱质谱具有成本低,相对较高的灵敏度及多级质谱(MS^n)能力,它仍较适用于代谢组学研究中生物标志物的鉴定[11]。四级杆(quadrupole, Q)质谱的主要优势是性能稳定可靠且成本较低,可同时提供优质的定性和定量结果,其不足之处是只能得到低分辨率质谱数据[14]。四级杆质谱可以获得准确质量,但要求样品有相对较高的纯度,且背景不能存在无法辨别的杂质干扰[15],故在代谢组学研究中应用较少。三重四级杆(triple quadrupole, TQ)质谱的优势在于灵敏度高,可对已知化合物进行定量分析,也可得到相对分子质量和二级碎片等信息。由于使用方便,所以经常用作代谢物筛选和鉴定。然而,它的高检测灵敏度只在多级反应监测模式(MRM)下才被保留,无法阐释样品中未知代谢物的结构信息[16]。飞行时间(time of flight, TOF)质谱全扫描模式下具有高灵敏度,拥有精确相对分子质量测定功能,在一定的线性范围内质量准确度可小于 2×10^{-6},从而可以给出母离子和碎片离子的元素组成,用于鉴定未知化合物和同分异构体的区分[17-18]。四级杆飞行时间(quadrupole-time of flight, Q-TOF)质谱由四级杆质谱和飞行时间质谱串联组成,四级杆在 MS 模式下有离子导向作用,在 MS/MS 状态下有质量分选功能,能同时在 MS 和 MS^2 模式下分析,提供母离子和碎片离子的精确质量,相比较三重四级杆,它具有更高的分辨率、更高效的质量鉴定和更高的选择性[19],在中药代谢组学研究中是一项强而有力的技术,具有广阔的前景[20-21]。线性离子阱(quadrupole-ion trap, Q-IT)质谱由四级杆质谱和离子阱质谱串联组成,具有较单/三重四级杆质谱更高灵敏度,同时四级杆质谱的离子导向作用克服了三重四级杆"1/3 效应"、碰撞效率低、定量能力差等缺点,具有强大的同时定性定量分析能力,尤其适用于药物代谢小分子研究,由于价格昂贵,其应用还未得到推广。

Ari 等[20]就灵敏度、选择性等方面对以上几种质谱进行了比较,具体见表 1。

表 1　几种质谱参数的比较

Tab 1　Comparison of different MS parameters

Index	Time of flight	Quadrupole-time of flight	Ion trap	Triple quadrupole	Quadrupole-ion trap
Sensitivity (Full scan mode)	High	High	Moderate-high	Low	High
Selectivity	Low	High	Low	Low	High
Accuracy	High	High	Moderate-high	High	High
Dynamic range	Low	Moderate-high	Moderate-high	High	Moderate-high
MS^n	No	Yes	Yes	Yes	Yes
Data acquisition speed	High	High	Low/moderate (3D trap/linear trap)	Low/high (depending on scan mode)	Moderate-high
Function characteristics	Accurate mass and high sensitivity	Accurate mass and high selectivity	MS^n	Neutral loss	Neutral loss and good product ion scanning
Price	Moderate-high	High	Moderate-high	Moderate-high	High

2　液质联用技术在中药代谢及代谢组学中的应用

用 PubMed 数据库检索近年来液质联用技术在中药代谢物及代谢组学中的相关文献,并从研究对象、生物体液类型、液相色谱类型、质谱检测器类型、消耗时间和检测物质这几方面对检索到的 23 篇文献列表分析比较,具体见表 2。

由表 2 可见,液相色谱使用 UPLC 的文献共 8 篇,占 34.8%;使用 HPLC 的文献共 15 篇,占 65.2%。由此可见,由

于 HPLC 对于仪器要求较低，方法适用性较好，目前使用仍然居多，但 UPLC 呈现出强大的快速分离能力。例如文献[23]应用 HPLC 在 40 min 内鉴定中药复方 PHY906 在血浆中 57 种化合物和 27 种代谢产物，而文献[29]则应用 UPLC 在20 min 内鉴定了活血丹属在血浆和尿液中 9 种母体化合物和 80 种代谢产物，表明 UPLC 能在短时间内实现甚至超越 HPLC 长时间所达到的分离效果，随着快速液相色谱系统的发展和普及，UPLC 的应用将在不远的将来占据主导地位。

表 2　液质联用技术在中药代谢物及代谢组学中的应用

Tab 2　Application of LC-MS in TCM metabolite and metabonomics researches

Subject	Biological fluids	Liquid chromatography		Mass spectrometry					Run time and detected substances	Literatures
		UPLC	HPLC	Q-TOF	TOF	IT	TQ	Q-IT		
Scopolamine	Rat urine	-	√	-	-	-	-	√	18 metabolites were detected in 8 min	[22]
PHY906	Human plasma	-	√	-	-	-	-	√	57 compounds and 27 metabolites were identified in 40 min	[23]
Morning Glory Seed	Rat urine	√	-	-	-	-	√	-	12 metabolites were detected in 17 min	[24]
Xindi soft capsules	Rat urine	√	-	√	-	-	-	-	7 metabolites were identified in 20 min	[25]
Liu Wei Di Huang Wan	Rat urine	√	-	√	-	-	-	-	20 metabolites were identified in 13 min	[26]
TTE-50	Rat bile	-	√	-	-	√	-	-	16 tanshinones and 17 phase Ⅰ metabolites were identified in 60 min	[27]
Shaofu Zhuyu decoction	Rat plasma	√	-	√	-	-	-	-	12 parent components and 9 metabolites were identified in 20 min	[28]
Glechoma longituba extract	Rat plasma and urine	√	-	√	-	-	-	-	80 metabolites of 9 parent compounds were detected in 20 min	[29]
Decoction of Ginseng	Rat plasma	√	-	√	-	-	-	-	45 major ginsenosides and 21 metabolites were identified in 18 min	[30]
Cryptotanshinone	Rat bile	-	√	-	-	√	-	-	19 phase Ⅰ and 6 phase Ⅱ metabolites were identified in 20 min	[31]
Puerarin	Rat bile and plasma	-	√	-	-	-	√	-	4 metabolites were identified in 10 min	[32]
Salvianolic acid B	Rat bile and feces	-	√	-	-	√	-	-	4 biliary and 5 fecal metabolites were identified in 50 min	[33]
Danshensu, caffeic acid, ferulic acid and isoferulic acid	Rat plasma, bile, urine and feces	-	√	-	-	√	-	-	19 metabolites were detected and identified in 40 min	[34]
Oxymatrine	Rat urine	-	√	-	-	√	-	-	6 phase Ⅰ metabolites and the major metabolite matrine were detected in 15 min	[35]
Ephedrine	Rat urine	-	√	-	-	√	-	-	3 phase Ⅰ metabolites were identified in 20 min	[36]
Tectoridin	Rat bile, feces, intestinal tract and liver	-	√	-	-	√	-	-	6 metabolites of tectoridin in urine, 3 metabolites in faeces, 1 metabolite in intestinal tract and 4 metabolites in liver were identified in 30 min	[37]
Aesculin	Rat urine	-	√	-	-	√	-	-	6 metabolites were identified in 35 min	[38]
Baicalin	Rat plasma, bile, urine and feces	-	√	-	-	√	-	-	3 metabolites were identified in 45 min	[39]
Ligusticum chuanxiong	Hairless mouse urine and feces	√	-	-	-	-	√	-	3 metabolites were identified in 18 min	[40]
Angelica	Rat plasma	√	-	-	√	-	-	-	6 metabolites were identified in 15 min	[41]
Flavones of epimedium	Rat serum	-	√	-	-	√	-	-	25 metabolites were identified in 80 min	[42]
Palmatine	Rat urine	-	√	-	-	-	-	√	6 phase Ⅰ and 2 phase Ⅱ metabolites were identified in 6 min	[43]
Iridoid glycosides of gardenia	Rat plasma	-	√	-	-	-	√	-	7 metabolites were identified in 16 min	[44]

UPLC: Ultra-performance liquid chromatography; HPLC: High-performance liquid chromatography; Q-TOF: Quadrupole-time of flight; TOF: Time of flight; IT: Ion trap; TQ: Triple quadrupole; Q-IT: Quadrupole-ion trap

比较质谱检测器使用 Q-TOF 的文献共 5 篇，占 21.7%；使用 TOF 的文献共 1 篇，占 4.3%；使用 IT 的文献共 10 篇，占 43.5%；使用 TQ 的文献共 4 篇，占 17.4%；使用 Q-IT 的文献共 3 篇，占 13%。可见，目前使用 IT 的居多，原因在于 IT 价格较低且可进行多级质谱分析，从而可对靶标代谢物进行筛选和定性分析，但对于非靶标代谢物，其分析能力明显不足。观察表 2 中使用 IT 的 10 篇文献[27,31,33-39,43]容易发现，其分析代谢物的量均明显少于 Q-TOF 等串联质谱，且分析物质均为明确的靶标化合物，与代谢组学要求尽可能达到的表征“全代谢物”信息的目标相距甚远。Q-TOF、TQ 和 Q-IT 作为串联质谱，价格较高，故应用相对受限。但从表 2 中容易看出串联质谱，尤其是 Q-TOF 和 Q-IT，检测效率明显高于普通质谱，且体现出强大的分析非靶标物质的能力。例如文献[29]在 20 min 内检测了活血丹在血浆和尿液中 9 种母体化合物和 80 种代谢产物，其中多数为非靶标代谢物，均通过 Q-TOF 的获取精确相对分子质量和二级打击质谱功能等表征。文献[23]和[43]应用 Q-IT 对中药复杂体系以及中药活性单体在生物体液中大量非靶标代谢产物进行分析，体现

出灵敏、准确、高效的优点。因此,在中药复方、中药单体代谢产物鉴定中,以 Q-TOF 和 Q-IT 为代表的串联质谱技术具有广阔的应用前景,其高选择性和灵敏度适合于复杂生物基质中痕量物质的测定,同时能够提供强大的定量能力,也能提供大量碎片离子信息,达到定性非靶标代谢物的目的[14]。由于代谢物的定性对于代谢途径的寻找和药效机制的判别具有重要意义,所以液质联用技术能很好地适用于中药代谢组学的研究。其中串联质谱,如 Q-TOF 和 Q-IT 能提供丰富的精确相对分子质量和碎片离子信息,是代谢物鉴定的有力工具。

需要指出的是,以 Q-TOF 和 Q-IT 为代表的串联质谱技术灵敏度较高,但不可避免的也提高了其基质效应参数。生物样品质谱分析很难将机制效应完全消除,为了使基质效应对分析结果产生的影响达到最小,可以通过以下方法解决:(1)优化前处理方案,选择效率更高、分离纯化效果更好的提取方式,如固相萃取、基质固相分散萃取、浊点萃取、分子印记、微透析等。(2)优化色谱条件,一般的基质抑制主要是前沿峰里的混合物质,最好通过优化色谱条件使检测物质最大程度分离,同时可以加入一些提高离子强度的物质。(3)优化离子源参数,以实际响应的提高为标准。(4)对于内源性物质基质效应处理相对困难,可以采用标准添加或加入内标的方法尽量消除基质效应的干扰,但此方法仅适用于少量代谢物的分析,对于代谢组学大规模的分析还需辅助统计学软件使各批次结果归一化[45]。

UPLC 快速液相分离系统和 Q-TOF 等串联质谱系统分别有着明显的优势。因此,将两者联用能获得更低的检测限,更高的色谱分离和全面的质谱数据。如文献[29]和[30]均应用了 UPLC-Q-TOF 策略,在 20 min 内完成了大规模代谢物的鉴别分析,在节省分析时间和检测效果上明显优于其他策略。

综上所述,UPLC-Q-TOF 因其独有的优势在诸如化学、生物、环境和药物等许多研究领域应用越来越多。近年来,UPLC-Q-TOF 方法已广泛地应用于农药、药物化合物和药物滥用的分析[12]。因此, UPLC-Q-TOF 在代谢组学研究中的应用也受到了关注。美国 Waters 公司根据代谢组学发展的要求,与代谢组学创始人 Jeremy Nicholson 教授合作,首创全球领先的超高效液相色谱 UPLC 技术,该技术与高分辨质谱技术和计算技术结合,推出了以超高效液相色谱/高分辨质谱联用仪 UPLC-Q-TOF 为代表的代谢组学分析系统,一次可以从尿液样品中快速获取 2 万多个数据点,为从整体上深入把握药物在体内的生理代谢状况、细致入微地刻画和反映人体疾病的发生、发展过程提供了先进有效的工具。

3　展　望

目前,中药代谢组学的研究已取得了喜人的成果,但也应看到,当前代谢组学的研究还处于模式识别和生物标志物鉴定的层次,中药研究和系统生物学的整合仍然缺乏[21]。代谢组学的发展依然落后于基因组学和蛋白质组学,然而,与其他"组学"相比,代谢组学的费用更低,可以先通过代谢组学研究筛选出代谢产物,然后采用更昂贵的基因组学和蛋白质组学的方法对有意义的代谢产物进一步加以研究。因此,代谢组学研究具有重要意义。虽然液质联用技术在中药代谢组学研究中发挥着不可替代的作用,但在液质联用图谱中还存在大量的非靶标化合物。若要将液质联用技术在中药代谢组学中的作用发挥到最大,就要尽可能地鉴定出这些内源性代谢产物并建立数据库[11]。与气质联用技术不同,液质联用技术很难建立在同一条件下的所有代谢物的标准数据库,但我们相信,随着超高效液相系统联合高分辨串联质谱系统应用的日益广泛,这项挑战性的工程将在不远的将来得以实现。

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