放射性标记药物在我国新药研发中的应用进展

刁星星*, 余京华, 钟大放*

(中国科学院上海药物研究所, 上海 201203)

摘要: 放射性标记药物代谢研究在新药研发中发挥着重要作用。它提供药物的吸收、代谢、组织分布和排泄信息, 在新药的代谢物安全性评价和物质平衡研究中, 具有不可替代的作用。美国FDA近期发布的关于放射性标记药物临床试验的新指导原则草案, 提出了更高的标准, 得到业界的广泛关注。国内近年来在新药研发中, 采用^{14}C标记药物开展临床代谢试验, 克服了关键技术瓶颈, 积累了经验。本文综述上述研究进展, 分析存在的问题, 并初步展望未来的技术发展和应用。

关键词: 放射性标记药物; 药物代谢; 物质平衡; 新药研发

中图分类号: R917 **文献标识码**: A **文章编号**: 0513-4870(2023)02-0313-07

Application progress of radiolabeled drugs in new drug research and development in China

DIAO Xing-xing*, YU Jing-hua, ZHONG Da-fang*

(*Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China*)

Abstract: The metabolism study of radiolabeled drugs plays an important role in the development of new drugs. It provides information on drug absorption, metabolism, tissue distribution and excretion, and plays an irreplaceable role in the metabolite safety evaluation and mass balance of new drugs. The new guidance draft on clinical trials of radiolabeled drugs recently released by the US FDA puts forward higher standards and has been widely concerned by the industry. In recent years, in the research and development of new drugs in China, ^{14}C labeled drugs have been used to carry out clinical metabolism studies, which has overcome key technical bottlenecks and accumulated experience. This paper summarizes the above research progress, analyzes the existing problems, and preliminarily looks forward to the future technological development and application.

Key words: radiolabeled drug; drug metabolism; mass balance; drug research and development

随着我国新药创制的加速发展, 放射性标记药物试验日益引发关注。2012年, 边诣聪等[1]综述了放射性标记药物在吸收、分布、代谢、排泄研究中的应用。2016年, 斯琴朝克图等[2]综述了放射性标记药物在药物代谢与处置研究中的应用。这两篇文章介绍了放射性同位素实验相关理论和国外一些研究成果。

近年来, 马晟等[3]综述了放射性标记药物物质平衡及生物转化临床研究进展, 孙鲁宁等[4]综述了新药人体物质平衡与代谢研究同位素示踪技术平台建设的实践与思考。这两篇文章总结了我国近年来放射性标记药物临床研究技术平台的建设情况。2020年, 缪丽燕等[5]发表了“放射性同位素标记药物物质平衡及生物转化临床研究伦理审查专家共识”, 专家来自11家单位, 标志着我国开展放射性标记药物临床试验平台已经成熟。

本文根据文献报道, 总结我国近年来新药研发中开展的放射性标记药物试验进展, 包括放射性标记药物合成、放射性分析技术、临床物质平衡试验及在药物

收稿日期: 2022-09-16; 修回日期: 2022-11-29.
基金项目: 国家自然科学基金资助项目 (81521005).
*通讯作者 E-mail: xxdiao@simm.ac.cn; dfzhong@simm.ac.cn
DOI: 10.16438/j.0513-4870.2022-1061

代谢产物鉴定、血浆蛋白共价结合、代谢物追踪等方面的应用。

1 法规指导原则

美国FDA于2022年5月发布了“人体放射性标记物质平衡研究临床药理学考量”指导原则草案[6]。认为人体放射性标记(通常为^{14}C或^{3}H)物质平衡研究是获得药物在人体内吸收、分布、代谢和排泄(ADME)的定量及全面信息最直接的方法。建议在一般情况下,应该对所有新的分子实体开展物质平衡研究。一般来说,物质平衡研究可以在健康成年受试者中进行。如果因安全问题排除健康受试者入组,则可以在受益患者群体中开展物质平衡研究。物质平衡研究应包括至少6名受试者。要求尿液和粪便的放射性物质总回收率应至少为90%,当回收率低于90%时,应提供充分的理由。

欧洲EMA在“药物相互作用研究指导原则”(2012年6月)中指出,物质平衡试验通过给予放射性标记药物来追踪母体药物和代谢产物,提供代谢物的系统暴露信息以及用于估计主要清除途径的数据资料[7]。

中国国家药品监督管理局药品审评中心在《药物非临床药代动力学研究技术指导原则》(2014年5月)和《创新药临床药理学研究技术指导原则》(2021年12月)中明确,临床前和临床早期阶段,特别是毒性剂量和有效治疗剂量范围确定的情况下运用放射性标记化合物,可通过收集动物和人体粪、尿及胆汁以研究药物的物质平衡。这些研究能够获得化合物的排泄途径和排泄速率等信息,而且有助于代谢产物的性质鉴定,并通过有限的数据比较它们的体内吸收和分布特点[8,9]。

2 放射性标记药物的合成与分析

我国科学家近年来使用放射性标记药物开展新药临床物质平衡研究,迄今为止,在药品审评中心临床试验平台登记有64项放射性标记药物人体物质平衡试验,并在国际学术期刊发表了多篇论文。其中,安罗替尼(anlotinib)[10]、吡咯替尼(pyrotinib)[11]、呋喹替尼(fruquintinib)[12]、阿美替尼(almonertinib)[13]、恩沙替尼(ensartinib)[14]、环泊酚(ciprofol)[15]、氟唑帕利(fuzuloparib)[16]、索凡替尼(surufatinib)[17]、伏美替尼(furmonertinib)[18]、康替唑胺(contezolid)[19]先后获批上市;艾维替尼(abivertinib)[20]、维卡格雷(vicagrel)[21]、YY-20394[22]、HR011303[23]、TPN171[24]尚处于临床III期研究阶段。

典型的试验流程包括:① 设计并合成放射性标记药物;② 大鼠体内ADME实验;③ 确定临床试验方案,包括剂量、受试者、采样周期等,经伦理委员会批准;④ 开展临床试验,通过单次给予受试者放射性标记药物,采集血浆、全血、尿、粪样品并监控受试者安全性;⑤ 生物样品分析,得到血浆、全血、尿、粪代谢物谱,根据尿、粪排泄测试结果,获得放射性回收率;⑥ 结合LC-MS/MS分析等,鉴定代谢产物并获得药动学曲线。

笔者实验室已经报道的放射性标记药物动物实验或体外实验还包括TPN729[25]、莫非赛定(morphothiadin)[26]、20(*S*)-原人参二醇(20(*S*)-protopanaxadiol)[27]、雷公藤甲素(triptolide)[28]、鱼腥草素(houttuynin)[29]、丁苯酞(3-*n*-butylphthalide)[30]。

2.1 放射性标记药物合成 在设计放射性标记合成路线时,首选^{14}C标记,但是对部分天然药物,由于合成路线的复杂性,可以采用^{3}H标记。应在前期已有的药物合成路线基础上,优化合成路径,尽可能在合成的靠后步骤中引入含^{14}C或^{3}H的片段,最大程度减少放射性废弃物的产生。特别需要注意,考察标记位点应在生物体内不易代谢丢失,否则失去了标记的意义。放射性标记药物的化学结构分别列于图1(已上市药物)和图2(临床试验中的药物)。这些药物均采用了^{14}C标记,其中仅阿美替尼的标记位点未知。

图3列出了几种放射性标记的化学药物和天然药物结构,它们尚未报道开展人体内物质平衡试验。其中既有^{14}C标记(TPN729、莫非赛定、鱼腥草素、丁苯酞),也有^{3}H标记(20(*S*)-原人参二醇、雷公藤甲素)。

2.2 放射性分析技术 在药物代谢研究中,使用的放射性检测方法包括液体闪烁计数仪(liquid scintillation counting, LSC)、动物全身放射自显影技术(quantitative whole-body autoradiography, QWBA)、流动闪烁分析仪、固体闪烁计数仪等。其中,固体闪烁计数仪与LC-MS/MS联合使用,灵敏度高,可用于确定循环中的主要代谢产物。

液体闪烁计数仪(LSC)是一种通过液体闪烁探测器测定液态样品中放射性活度的设备,设备内直接内置^{133}Ba源,通过淬灭曲线校正测定放射性活度的绝对值。液体闪烁计数仪操作简单:将含放射性的澄清液体样品(血浆、尿、胆汁等,全血和粪匀浆淬灭严重,需氧化燃烧后进行LSC测试)与闪烁液混合均匀,即可检测,已经广泛应用于排泄、组织分布、吸收等实验,成为测定液体样品中放射性活度的首选仪器。

定量全身放射自显影(QWBA)技术是将给药后的动物整体冷冻和切片,利用放射性同位素发射的射线,使感光材料感光,显出影像后进行放射性标记物的定位和定量测量的技术。流动闪烁分析仪一般与HPLC联用,样品经HPLC色谱分离后,流分立即被流动闪烁分析仪检测,适合于分析含高放射性活度的体外样品或来源于动物的生物样品。因为可以实时观察到检测结果,非常适合于放射性的色谱方法开发。

固体闪烁计数仪一般也与HPLC联用,样品经

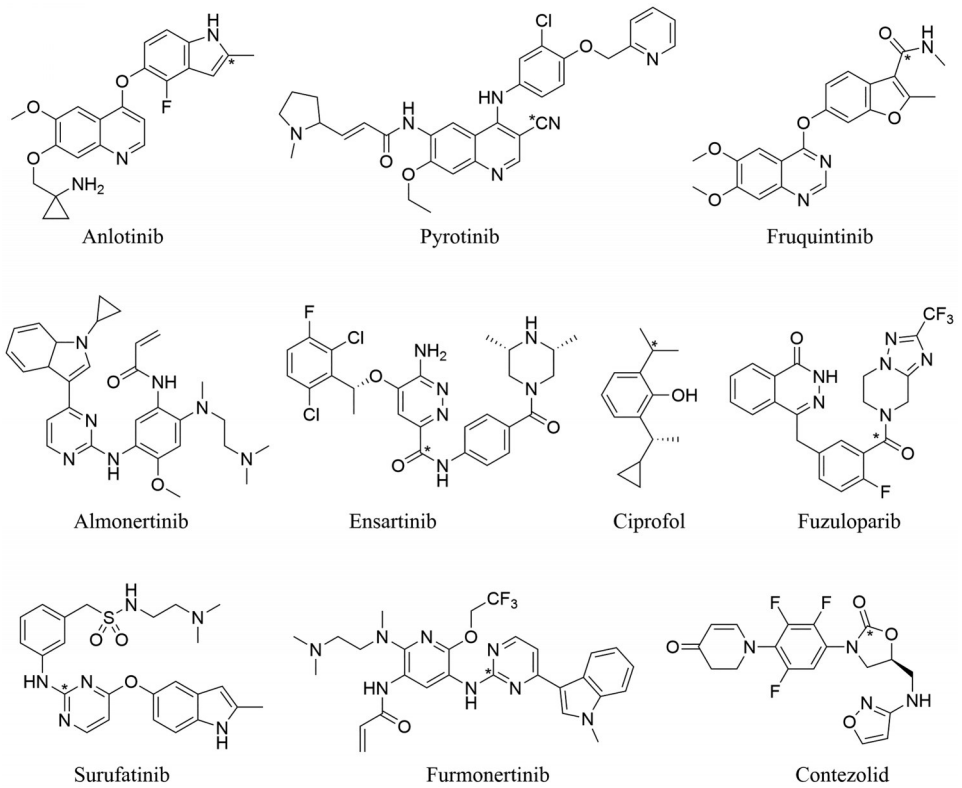

Figure 1 Structures of radiolabeled drugs (approved for marketing). *: ^{14}C radiolabeled site

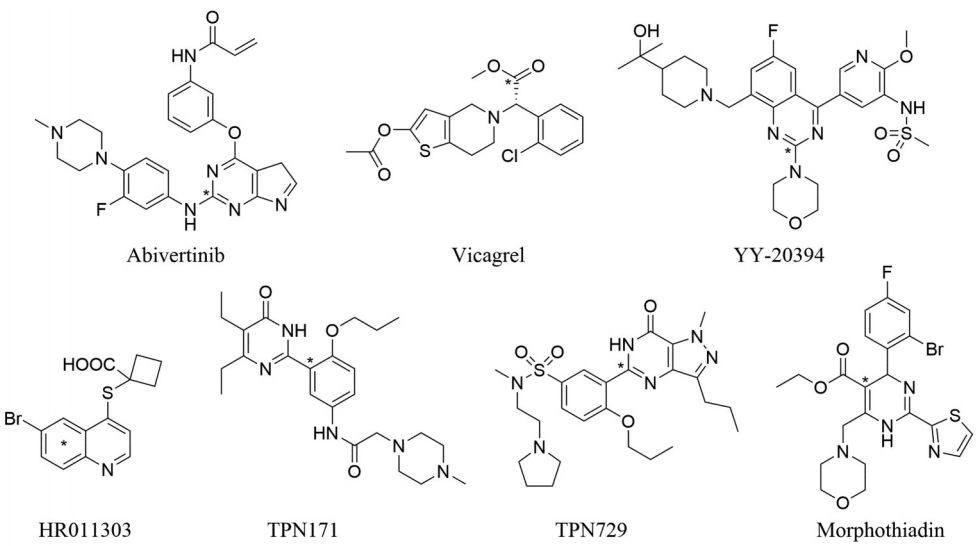

Figure 2 Structures of radiolabeled drugs (in clinical trials). *: ^{14}C radiolabeled site

HPLC 色谱分离后, 流分每一定时间 (如每 10～15 s) 收集在 96 孔板的孔中, 挥干后, 即可由固体闪烁计数仪来测定每个孔中的放射性活度, 此方法适合于含低放射性活度的样品, 比如来源于人的生物样品。图 4 展示了放射性检测 ^{14}C-康替唑胺在人血浆中代谢产物的色谱图[18]。

3 临床物质平衡试验

表 1 列出了我国近年来公开发表的 15 种放射性标记药物的临床物质平衡试验信息[10-24]。苏州大学附属第一医院、南京医科大学附属第一医院等建立了高标准的放射性标记药物临床试验平台, 开展了多项临床试验。

3.1 放射性安全剂量 人体放射性试验开展前, 需要评估受试者单次口服放射剂量药物后, 全身受到的有效辐射剂量。人体全身有效辐射剂量需要根据大鼠放射性组织分布试验的结果来估算, 与监管机构规定的单次人体试验有效辐射剂量限度 (30 mSv) 比较[31]。我

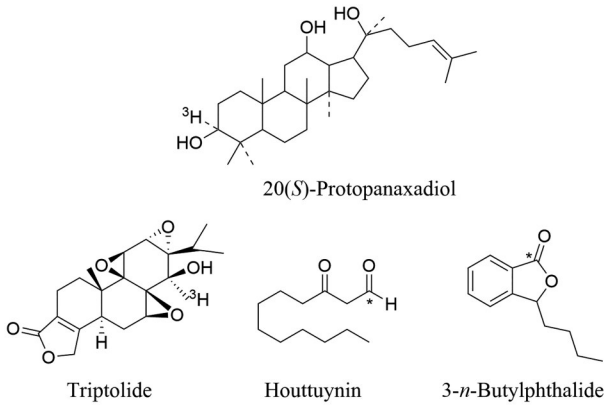

Figure 3 Structures of radiolabeled drugs (non-clinical study). *: ^{14}C radiolabeled site

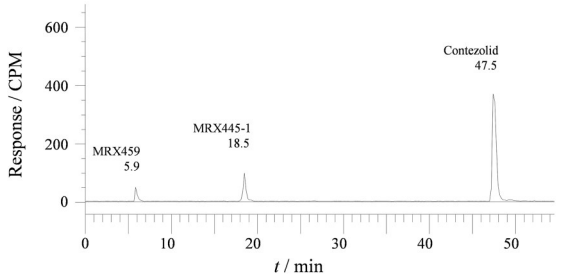

Figure 4 Representative radiochromatogram of pooled human plasma from human subjects administered 99.1 μCi/602 mg [^{14}C] contezolid. Unchanged contezolid, MRX445-1, and MRX459 are the main circulating drug-related materials

国临床医院的伦理委员会一般要求单次人体放射性试验的全身有效辐射剂量小于1 mSv。我国学者目前发表的相关论文中, 放射性给药剂量在50～150 μCi内。

3.2 受试者选择 一般选择健康男性志愿者。在评估对健康受试者可能有潜在风险的情况下, 选择治疗获益的患者。在表1的15项临床试验中, 有3项抗肿瘤药物试验 (安罗替尼、氟唑帕利、艾维替尼) 选择了肿瘤患者, 其中氟唑帕利 (该药物主要用于治疗卵巢癌) 主要选择女性患者。多数试验录入6名受试者。

3.3 采样周期与放射性回收率 多数试验 (10项) 的采样周期在14天以内, 且放射性回收率超过了90%。但是有4项试验涉及靶向抗肿瘤药物 (安罗替尼、阿美替尼、伏美替尼、艾维替尼), 它们的放射性回收率低于90%。对于阿美替尼、伏美替尼和艾维替尼, 其主要原因是该类药物结构中的丙烯酰胺基团可以和人血清白蛋白形成共价结合物, 故消除非常缓慢[13,18,20]。安罗替尼排泄极为缓慢, 110天的放射性回收率仅为62%, 其原因尚不清楚[10]。多数放射性标记药物的排泄可在7天左右超过90%, 例如维卡格雷[21], 但有些不可逆靶向抗肿瘤药放射性排泄缓慢, 例如伏美替尼[18]。

4 药物代谢产物鉴定与追踪

4.1 药物代谢产物鉴定 维卡格雷是抗凝血新药, 与临床一线药物氯吡格雷有相同的活性代谢产物, 但是在体内的活化机制不同。将放射性检测与LC-MS/MS检测相结合, 在受试者口服维卡格雷后的血浆和尿中共检测到43个代谢物, 通过合成对照品, 确定了主要代谢产物的结构。氯吡格雷的代谢物覆盖了维卡格雷的全部代谢物。维卡格雷的活性巯基代谢产物H4在人体内的生成效率远高于氯吡格雷, 临床药动学试验表明, 维卡格雷口服剂量仅为氯吡格雷口服剂量1/15, 就可以获得同样高的活性代谢物血浆浓度, 并且*CYP2C19*基因多态性对维卡格雷的影响较小, 验证了维卡格雷的设计思想[21,32-38]。图5是维卡格雷和氯吡格雷的代谢途径对比[21]。

Table 1 List of clinical mass balance studies of radiolabeled drugs in China. Nanjing: The First Affiliated Hospital of Nanjing Medical University; Suzhou: The First Affiliated Hospital of Soochow University; Huashan: Huashan Hospital Affiliated to Fudan University; Xuhui: Shanghai Xuhui District Central Hospital

No.	Drug	Hospital	Subject	Number & sex	Dose /μCi	Duration	Total recovery
1	Anlotinib	Nanjing	Cancer patients	6 m	80/120	110 d	62.03%
2	Pyrotinib	Suzhou	Healthy volunteers	6 m	150	10 d	92.60%
3	Fruquintinib	Nanjing	Healthy volunteers	6 m	100	14 d	90.11%
4	Almonertinib	Nanjing	Healthy volunteers	4 m	50	36 d	86.15%
5	Ensartinib	Nanjing	Healthy volunteers	6 m	100	10 d	101.20%
6	Ciprofol	Suzhou	Healthy volunteers	6 m	47−57	10 d	87.3%
7	Fuzuloparib	Suzhou	Cancer patients	4 f, 1 m	100	12 d	103.30%
8	Surufatinib	Suzhou	Healthy volunteers	6 m	100	11 d	92.10%
9	Furmonertinib	Suzhou	Healthy volunteers	6 m	97.9	35 d	77.80%
10	Contezolid	Huashan	Healthy volunteers	6 m	99.7	7 d	91.50%
11	Abivertinib	Nanjing	Cancer patients	7 m	83	10 d	82.16%
12	Vicagrel	Suzhou	Healthy volunteers	6 m	120	7 d	96.71%
13	YY-20394	Suzhou	Healthy volunteers	6 m	100	11 d	92.10%
14	HR011303	Suzhou	Healthy volunteers	6 m	80	9 d	91.75%
15	TPN171	Xuhui	Healthy volunteers	6 m	100	9 d	95.21%

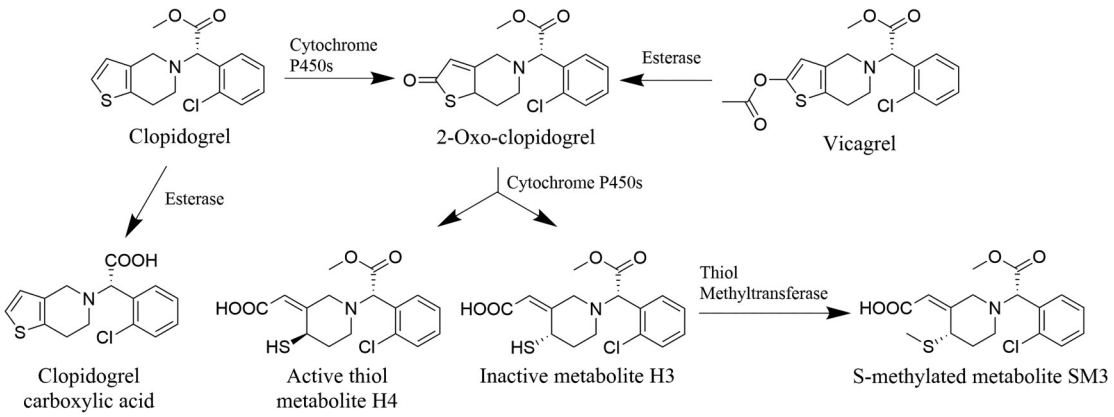

Figure 5 Metabolic pathways of vicagrel and clopidogrel in humans. H4 is the only active isomer *in vivo* in humans

放射性标记药物相关代谢产物鉴定有很多报道, 例如20(*S*)-原人参二醇代谢物[39,40]、丁苯酞代谢物[41-43]、雷公藤甲素代谢物[44-46]、TPN729代谢物[47,48]、莫非赛定代谢物[49]、吡咯替尼代谢物[50]、康替唑胺代谢物[51]等。

4.2 血浆蛋白共价结合 在受试者口服某些放射性标记药物后, 观察到其血浆中总放射性暴露远远高于母体药物和主要代谢物的暴露, 例如吡咯替尼、伏美替尼[18]等。这类靶向抗肿瘤药物结构中含有丙烯酰胺结构, 提示可能发生药物与血浆蛋白的共价结合。进一步研究发现, 这些药物通过丙烯酰胺可缓慢与人血清白蛋白的赖氨酸氨基共价结合, 且部分可逆[52]。

鱼腥草素制剂在人体可以引发过敏性不良反应广为人知。使用^{14}C-鱼腥草素与人血清白蛋白共孵化, 可以观察到明显的共价结合[29], 后续研究确认了该类共价结合反应机制, 被认为是导致药物过敏的原因之一[53]。

这些结果表明, 放射性同位素标记药物的代谢实验, 可以提供独特的线索, 为阐明某些药物代谢机制发挥引导作用。

4.3 代谢物追踪 原人参二醇和雷公藤甲素都是重要的天然药物。由于它们结构中缺乏极性基团, 质谱响应很差, 所以难于通过LC-MS/MS等技术, 追踪其代谢产物。放射性同位素标记提供了有效的手段, 使这类药物的代谢研究得以开展。例如, 对大鼠灌胃给予^{3}H-雷公藤甲素, 在血浆中观察到6种以上的代谢产物, 为后续研究奠定了基础[44]。

丁苯酞又名芹菜甲素, 是一种治疗脑卒中的新药, 2004年批准上市。但是, 丁苯酞在人体和临床前动物中的药物代谢信息空白。笔者实验室与有放射性合成资质的公司合作, 合成了[^{14}C]丁苯酞, 利用放射性技术, 系统研究了丁苯酞在大鼠体内的吸收、分布、代谢、排泄过程[30], 为丁苯酞放射性人体物质平衡试验提供了坚实的基础。

5 总结与展望

国内近年来在新药研发中, 采用^{14}C标记药物开展临床代谢试验, 克服了关键技术瓶颈, 积累了较多经验, 其技术指标基本达到了FDA指导原则的最新要求, 为我国新药研发做出了重要贡献, 并在国际学术期刊发表了几十篇研究论文。放射性标记药物临床试验平台发挥了重要作用, 多个实验室开展了放射性标记药物合成和生物分析。在检测技术方面, 我国从2020年起, 逐渐采用定量整体放射性自显影 (QWBA) 技术进行动物组织分布研究, 并尝试采用微剂量放射性药物进行人体代谢研究。国内正在开始引入加速器质谱高灵敏度检测, 将使微剂量 (给药剂量<1 μCi) 放射性药物临床试验成为可能。

作者贡献: 刁星星、余京华提供资料和修改文章; 钟大放负责文章选题、文献检索、起草和审定文章。

利益冲突: 所有作者均声明不存在利益冲突。

References

[1] Bian YC, Hu HH, Zeng S. Application of radiolabeled drugs in absorption, distribution, metabolism and excretion studies [J]. Chin J Pharm Anal (药物分析杂志), 2012, 32: 906-911.

[2] Harnud S, Huang LL, Yuan ZH. The use of radiolabeled drugs in drug metabolism and disposition studies [J]. Chin J New Drugs (中国新药杂志), 2016, 25: 1475-1484.

[3] Ma S, Yi L, Miao LY. Progress in development of human radiolabeled mass balance and biotransformation studies [J]. Chin Pharm J (中国药学杂志), 2020, 55: 1184-1188.

[4] Sun LN, Li TP, Zhou C, et al. Practice and consideration on the platform construction of isotope tracer technology for human mass balance and metabolism study of new drugs [J]. Chin J New Drugs (中国新药杂志), 2021, 30: 1570-1573.

[5] Miao LY, Wang XQ, Xu YT, et al. Expert consensus in mass

balance and biotransformation studies with radiolabeled drugs [J]. Chin Pharm J (中国药学杂志), 2020, 55: 1649-1652.

[6] US FDA. Considerations for human radiolabeled mass balance studies. Guidance for Industry (draft guidance) [EB/OL]. 2022-05. https://www.fda.gov/media/158178/download.

[7] European Medicines Agency. Guideline on the investigation of drug interactions [EB/OL]. 2012-06-21. https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-investigation-drug-interactions-revision-1_en.pdf.

[8] Center for Drug Evaluation, National Medical Products Administration of China. Technical guidelines for non-clinical pharmacokinetics study of drugs (药物非临床药代动力学研究技术指导原则) [EB/OL]. 2014-05-13. https://www.cde.org.cn/zdyz/downloadAtt?idCODE=afb141e7188a96faeee37722b629219b.

[9] Center for Drug Evaluation, National Medical Products Administration of China. Technical guidelines for clinical pharmacology study of innovative drugs (创新药临床药理学研究技术指导原则) [EB/OL]. 2021-12-20. https://www.cde.org.cn/zdyz/downloadAtt?idCODE=dbbea043751df42c9cea5a430ed1ee43.

[10] Liu YQ, Liu LK, Liu LX, et al. A phase I study investigation of metabolism, and disposition of [^{14}C]anlotinib after an oral administration in patients with advanced refractory solid tumors [J]. Cancer Chemother Pharmacol, 2020, 85: 907-915.

[11] Meng J, Liu XY, Ma S, et al. Metabolism and disposition of pyrotinib in healthy male volunteers: covalent binding with human plasma protein [J]. Acta Pharmacol Sin, 2019, 40: 980-988.

[12] Zhou SF, Shao F, Xu ZQ, et al. A phase I study to investigate the metabolism, excretion, and pharmacokinetics of [^{14}C]fruquintinib, a novel oral selective VEGFR inhibitor, in healthy Chinese male volunteers [J]. Cancer Chemother Pharmacol, 2017, 80: 563-573.

[13] Zhou C, Xie LJ, Liu W, et al. Absorption, metabolism, excretion, and safety of [^{14}C]almonertinib in healthy Chinese subjects [J]. Ann Transl Med, 2021, 9: 867.

[14] Zhou SF, Liu W, Zhou C, et al. Mass balance, metabolic disposition, and pharmacokinetics of [^{14}C]ensartinib, a novel potent anaplastic lymphoma kinase (ALK) inhibitor, in healthy subjects following oral administration [J]. Cancer Chemother Pharmacol, 2020, 86: 719-730.

[15] Bian YC, Zhang H, Ma S, et al. Mass balance, pharmacokinetics and pharmacodynamics of intravenous HSK3486, a novel anesthetic, administered to healthy subjects [J]. Br J Clin Pharmacol, 2021, 87: 93-105.

[16] Bian YC, Meng J, Ma S, et al. Metabolite profiles and mass balance of fuzuloparib, a novel poly (ADP-ribose) polymerase inhibitor, in subjects with advanced solid cancers [J]. Br J Clin Pharmacol, 2022, 88: 3307-3320.

[17] Li K, Ma S, Miao LY, et al. Absorption, metabolism and excretion of surufatinib in rats and humans [J]. Curr Drug Metab, 2020, 21: 357-367.

[18] Meng J, Zhang H, Bao JJ, et al. Metabolic disposition of the EGFR covalent inhibitor furmonertinib in humans [J]. Acta Pharmacol Sin, 2022, 43: 494-503.

[19] Wu XJ, Meng J, Yuan H, et al. Pharmacokinetics and disposition of contezolid in humans: resolution of a disproportionate human metabolite for clinical development [J]. Antimicrob Agents Chemother, 2021, 65: e0040921.

[20] Wang L, Guo L, Wang Y, et al. Metabolic disposition of [^{14}C] abivertinib, an epidermal growth factor receptor tyrosine kinase inhibitor: role of glutathione conjugation [J]. Br J Clin Pharmacol, 2021, 87: 1475-1485.

[21] Zheng YD, Zhang H, Zhan Y, et al. [^{14}C]Vicagrel, a novel irreversible P2Y12 inhibitor, in humans [J]. Acta Pharmacol Sin, 2021, 42: 1535-1546.

[22] Yu JH, Zhang H, Zhang Y, et al. Absorption, metabolism, and excretion of [^{14}C]YY-20394, a highly selective PI3K-Delta inhibitor in humans [J]. Xenobiotica, 2022, 52: 254-264.

[23] Zheng YD, Zhang H, Liu M, et al. Pharmacokinetics, mass balance, and metabolism of the novel URAT1 inhibitor [^{14}C] HR011303 in humans: metabolism is mediated predominantly by UDP-glucuronosyltransferase [J]. Drug Metab Dispos, 2022, 50: 798-808.

[24] He YF, Liu Y, Yu JH, et al. Pharmacokinetics, mass balance, and metabolism of [^{14}C]TPN171, a novel PDE5 inhibitor, in humans for the treatment of pulmonary arterial hypertension [J]. Acta Pharmacol Sin, 2022. DOI: 10.1038/s41401-022-00922-6.

[25] Cheng H, Yu JH, Yang C, et al. Absorption, distribution, metabolism, and excretion of [^{14}C]TPN729 after oral administration to rats [J]. Xenobiotica, 2022, 52: 79-90.

[26] Zhou X, Gao ZW, Meng J, et al. Effects of ketoconazole and rifampicin on the pharmacokinetics of GLS4, a novel anti-hepatitis B virus compound, in dogs [J]. Acta Pharmacol Sin, 2013, 34: 1420-1426.

[27] Meng J, Zhao LZ, Hu YH, et al. Synthesis of [3-^{3}H]20(S)-protopanaxadiol [J]. J Label Compd Radiopharm, 2009, 52: 482-484.

[28] Liu J, Zhou X, Chen XY, et al. Excretion of [^{3}H]triptolide and its metabolites in rats after oral administration [J]. Acta Pharmacol Sin, 2014, 35: 549-554.

[29] Deng ZP, Zhong DF, Meng J, et al. Covalent protein binding and tissue distribution of houttuynin in rats after intravenous administration of sodium houttuyfonate [J]. Acta Pharmacol Sin, 2012, 33: 568-576.

[30] Tian JJ, Lei P, He YF, et al. Absorption, distribution, metabolism, and excretion of [^{14}C]NBP (3-*n*-butylphthalide) in rats [J]. J Chromatogr B, 2021, 1181: 122915.

[31] US FDA. CFR-Code of Federal Regulations Title 21, Part 361. Prescription drugs for human use generally recognized as safe and effective and not misbranded: Drugs used in research [EB/OL]. 2022-3-29. https://www. accessdata. fda. gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=361.1.

[32] Liu C, Chen ZQ, Zhong K, et al. Human liver cytochrome P450 enzymes and microsomal thiol methyltransferase are involved in the stereoselective formation and methylation of the pharmacologically active metabolite of clopidogrel [J]. Drug Metab Dispos, 2015, 43: 1632-1641.

[33] Jiang JF, Chen XY, Zhong DF. Arylacetamide deacetylase is involved in vicagrel bioactivation in humans [J]. Front Pharmacol, 2017, 8: 846.

[34] Li XJ, Liu C, Zhu XX, et al. Evaluation of tolerability, pharmacokinetics and pharmacodynamics of vicagrel, a novel P2Y12 antagonist, in healthy Chinese volunteers [J]. Front Pharmacol, 2018, 9: 643.

[35] Zhu YQ, Romero EL, Ren XD, et al. Clopidogrel as a donor probe and thioenol derivatives as flexible promoieties for enabling H2S biomedicine [J]. Nat Commun, 2018, 9: 3952.

[36] Liu C, Lu YM, Sun HB, et al. Development and validation of a sensitive and rapid UHPLC-MS/MS method for the simultaneous quantification of the common active and inactive metabolites of vicagrel and clopidogrel in human plasma [J]. J Pharm Biomed Anal, 2018, 149: 394-402.

[37] Liu C, Zhang YF, Chen WL, et al. Pharmacokinetics and pharmacokinetic/pharmacodynamic relationship of vicagrel, a novel thienopyridine P2Y12 inhibitor, compared with clopidogrel in healthy Chinese subjects following single oral dosing [J]. Eur J Pharm Sci, 2019, 127: 151-160.

[38] Zhang YF, Zhu XX, Zhan Y, et al. Impacts of CYP2C19 genetic polymorphisms on bioavailability and effect on vicagrel, a novel thienopyridine P2Y12 [J]. Br J Clin Pharmacol, 2020, 86: 1860-1874.

[39] Meng J, Zhao LZ, Chen XY, et al. Metabolic profiling of [^{3}H] 20(*S*) -protopanaxadiol in rat bile [J]. Drug Metab Rev, 2009, 41: 99.

[40] Li L, Chen XY, Li D, et al. Identification of 20(*S*)-protopanaxadiol metabolites in human liver microsomes and human hepatocytes [J]. Drug Metab Dispos, 2011, 39: 472-483.

[41] Diao XX, Pang XY, Xie C, et al. Bioactivation of 3-*n*-butylphthalide via sulfation of its major metabolite 3-hydroxy-NBP: mediated mainly by sulfotransferase 1A1 [J]. Drug Metab Dispos, 2014, 42: 774-781.

[42] Diao XX, Zhong K, Li XL, et al. Isomer-selective distribution of 3-*n*-butylphthalide (NBP) hydroxylated metabolites, 3-hydroxy-NBP and 10-hydroxy-NBP, across the rat blood-brain barrier [J]. Acta Pharmacol Sin, 2015, 36: 1520-1527.

[43] Xue YR, Ren XL, Zhu ZD, et al. Site-specific protein modification by 3-*n*-butylphthalide in primary hepatocytes: covalent protein adducts diminished by glutathione and *N*-acetylcysteine [J]. Life Sci, 2021, 287: 12012.

[44] Liu J, Li L, Zhou X, et al. Metabolite profiling and identification of triptolide in rats [J]. J Chromatogr B, 2013, 939: 51-58.

[45] Xu Y, Zhang YF, Chen XY, et al. CYP3A4 inducer and inhibitor strongly affect the pharmacokinetics of triptolide and its derivative in rats [J]. Acta Pharmacol Sin, 2018, 39: 1386-1392.

[46] Xu Y, Du JB, Feng HJ, et al. Studies on the metabolism of a triptolide derivative (5*R*) -5-hydroxytriptolide *in vitro* [J]. Acta Pharm Sin (药学学报), 2019, 54: 1484-1492.

[47] Zhu YT, Li L, Deng P, et al. Characterization of TPN729 metabolites in humans usingultra-performance liquid chromatography/quadrupole time-of-flight mass spectrometry [J]. J Pharm Biomed Anal, 2016, 117: 217-226.

[48] Tian QQ, Zhu YT, Diao XX, et al. Species differences in CYP3A-catalyzed metabolism of TPN729, a novel PDE5 inhibitor [J]. Acta Pharmacol Sin, 2021, 42: 482-490.

[49] Zhou X, Li L, Deng P, et al. Characterization of metabolites of GLS4 in humans using ultrahigh-performance liquid chromatography/quadrupole time-of-flight mass spectrometry [J]. Rapid Commun Mass Spectrom, 2013, 27: 2483-2492.

[50] Zhu YT, Li L, Zhang G, et al. Metabolic characterization of pyrotinib in humans by ultra-performance liquid chromatography/quadrupole time-of-flight mass spectrometry [J]. J Chromatogr B, 2016, 1033-1034: 117-127.

[51] Meng J, Zhong DF, Li L, et al. Metabolism of MRX-I, a novel antibacterial oxazolidinone, in humans: the oxidative ring-opening of 2, 3-dihydropyrindin-4-one catalyzed by non-P450 enzymes [J]. Drug Metab Dispos, 2015, 43: 646-659.

[52] Liu XY, Feng D, Zheng MY, et al. Characterization of some covalent tyrosine kinase inhibitors covalent binding to human plasma protein [J]. Drug Metab Pharmacokinet, 2020, 35: 456-465.

[53] Deng ZP, Zhong DF, Chen XY. Identification of modification sites on human serum albumin and human hemoglobin adducts with houttuynin using liquid chromatography coupled with mass spectrometry [J]. Biomed Chromatogr, 2012, 26: 1377-1385.