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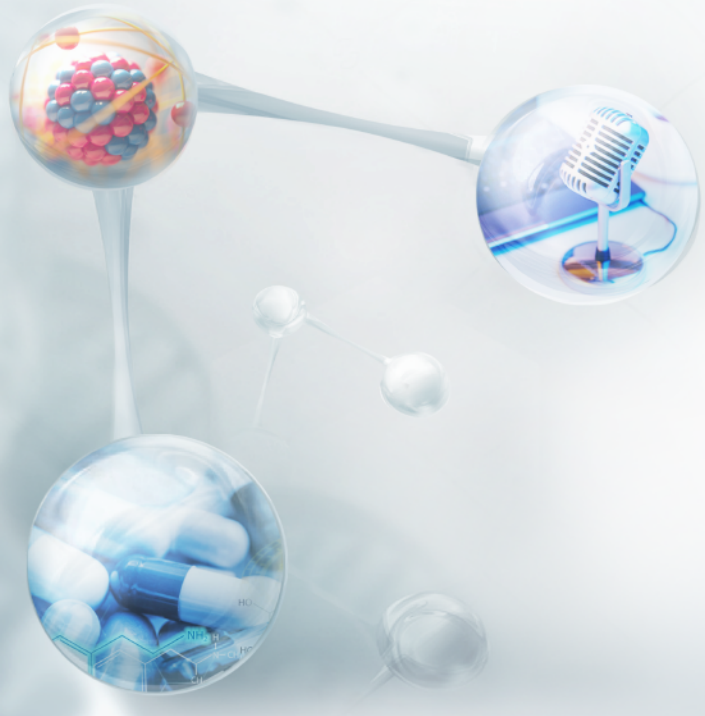

智汇科研宝典

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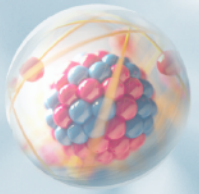
药物代谢和药物动力学研究贯穿药物研究始终，随着当前药学、医学乃至生命学科日新月异的发展，药物代谢领域在新形势下的定位和作用以及与其他学科的关系，需要更深入和深刻的思考。



“赛默飞智汇科研直播间”是赛默飞创设的一个学术交流高端平台，每期设置不同科研领域的热点应用主题，邀请该领域领军讲者，分享最新研究成果以及专业技术应用，致力于打造互动对话与学习交流的共享平台，激发启迪科研思路，发掘创新潜能，促进专业领域的长足发展。

药物代谢和药代动力学论文精华摘要

Number, Author	Article	Organization	Journal
01 Yanqing Liu,[1*] Xiaoli Zheng,[1*] Qinqin Yu,[1] Hua Wang,[2] Fuqing Tan,[3] Qianying Zhu,[1] Lingmin Yuan,[1] Huidi Jiang,[1] Lushan Yu,[1†‡] Su Zeng[1†‡]	Epigenetic activation of the drug transporter OCT2 sensitizes renal cell carcinoma to oxaliplatin	[1]Institute of Drug Metabolism and Pharmaceutical Analysis, Zhejiang Province Key Laboratory of Anti-Cancer Drug Research, College of Pharmaceutical Sciences, Zhejiang University. [2]Department of Urology, Cancer Hospital of Zhejiang Province. [3]Department of Urology, The First Affiliated Hospital, School of Medicine, Zhejiang University.	Magazine: Science Translational Medicine, 2016, Vol. 8, Issue 348
02 Lu Chen[1*], Zeyang Wang[1*], Qingwen Xu[1], Yuxi Liu[1], Le Chen[1], Suhang Guo[1], Hua Wang[2], Kui Zeng[1],Junqing Liu[3], Su Zeng[1] and Lushan Yu[1]	The failure of DAC to induce OCT2 expression and its remission by hemoglobin-based nanocarriers under hypoxia in renal cell carcinoma	1. Institute of Drug Metabolism and Pharmaceutical Analysis, Zhejiang Province Key Laboratory of Anti-Cancer Drug Research, College of Pharmaceutical Sciences, Zhejiang University. 2. Department of Urology, Cancer Hospital of Zhejiang Province. 3. The First Affiliated Hospital, School of Medicine, Zhejiang University.	Magazine: Theranostics 2020, Vol.10, Issus 8
03 Lu Chen[1], Le Chen[1], Zhiyuan Qin[1], Jinxiu Lei[1], Sheng Ye[2], Kui Zeng[1],Hua Wang[3], Meidan Ying[1], Jianqing Gao[1], Su Zeng[1], Lushan Yu[1,*]	Upregulation of miR-489-3p and miR-630 inhibits oxaliplatin uptake in renal cell carcinoma by targeting OCT2	[1]Institute of Drug Metabolism and Pharmaceutical Analysis, Zhejiang Province Key Laboratory of Anti-Cancer Drug Research, College of Pharmaceutical Sciences, Zhejiang University, China [2]Paediatric Intensive Care Unit, the Children's Hospital, Zhejiang University School of Medicine, China	Magazine: Acta Pharmaceutica Sinica B 2019; 9(5):1008-1020
04 Chaonan Ye[1,*], Kun Han[1,*], Jinxiu Lei[1], Kui Zeng[1], Su Zeng[1], Haixing Ju[2] and Lushan Yu[1]	Inhibition of histone deacetylase 7 reverses concentrative nucleoside transporter 2 repression in colorectal cancer by up-regulating histone acetylation state	[1]College of Pharmaceutical Sciences, Zhejiang University, and [2]Department of Colorectal Surgery, Zhejiang Cancer Hospital.	Magazine: British Journal of Pharmacology (2018) 175 4209-4217
05 Nan Hu, Shanshan Xie, Li Liu, Xinting Wang, Xian Pan, Guanming Chen, Lulu Zhang,Haiyan Liu, Xiang Liu, Xiaodong Liu, Lin Xie, and Guangji Wang	Opposite Effect of Diabetes Mellitus Induced by Streptozotocin on Oral and Intravenous Pharmacokinetics of Verapamil in Rats	Key Laboratory of Drug Metabolism and Pharmacokinetics, China Pharmaceutical University, Nanjing, China (N.H., S.X., L.L.,X.W., X.P., G.C., L.Z., H.L., Xian.L., Xiao.L., L.X., G.W.); and the Second Affiliated Hospital of Nanchang University, (S.X.)	Magazine: Drug Metabolism and Disposition, 2011, 39:419
06 Haiyan Liu, Li Liu, Jia Li, Dan Mei, Ru Duan, Nan Hu, Haifang Guo, Zeyu Zhong,and Xiaodong Liu	Combined Contributions of Impaired Hepatic CYP2C11 and Intestinal Breast Cancer Resistance Protein Activities and Expression to Increased Oral Glibenclamide Exposure in Rats with Streptozotocin-Induced Diabetes Mellitus	Key Laboratory of Drug Metabolism and Pharmacokinetics, China Pharmaceutical University.	Magazine: Drug Metabolism and Disposition, 2012, 40:1104-1112
07 Nan Shu[1*], Mengyue Hu[1*], Can Liu[2], Mian Zhang[1], Zhaoli Ling[1], Ji Zhang[3], Ping Xu[1], Zeyu Zhong[1], Yang Chen[1],Li Liu[1], and Xiaodong Liu[1]	Decreased exposure of atorvastatin in diabetic rats partly due to induction of hepatic Cyp3a and Oatp2	[1]Center of Drug Metabolism and Pharmacokinetics, China Pharmaceutical University. [2]Department of Biochemistry and Molecular Biology, Miller School of Medicine, University of Miami, and [3]Department of Pharmacy, the First Affiliated Hospital, Zhengzhou University.	Magazine: Xenobiotica, 2016, Vol. 46, Issue 10
08 Zhongjian Wang, Hanyu Yang, Jiong Xu, Kaijing Zhao, Yang Chen, Limin Liang, Ping Li,Nan Chen, Donghao Geng, Xiangping Zhang, Xiaodong Liu, and Li Liu	Prediction of Atorvastatin Pharmacokinetics in High-Fat Diet and Low-Dose Streptozotocin-Induced Diabetic Rats Using a Semiphysiologically Based Pharmacokinetic Model Involving Both Enzymes and Transporters	Center of Drug Metabolism and Pharmacokinetics, School of Pharmacy, China Pharmaceutical University.	Magazine: Drug Metabolism and Disposition, 2019, 47:1066-1079
09 Dan XU[1, 2], Feng LI[1], Mian ZHANG[1], Ji ZHANG[1], Can LIU[1], Meng-yue HU[1], Ze-yu ZHONG[1], Ling-ling JIA[1], Da-wei WANG[2],Jie WU[2], Li LIU[1, *], Xiao-dong LIU[1, *]	Decreased exposure of simvastatin and simvastatin acid in a rat model of type 2 diabetes	[1]Key Laboratory of Drug Metabolism and Pharmacokinetics, China Pharmaceutical University; [2]Jiangsu Provincial Institute of Traditional Chinese Medicine.	Magazine: Acta Phaemacologica Sinica (2014) 35:1215-1225
10 Feng Xu, Liang Zhu, Chaoqun Qian, Junjie Zhou, Donghao Geng, Ping Li, Wenjing Xuan,Fangge Wu, Kaijing Zhao, Weimin Kong, Yuanyuan Qin, Limin Liang, Li Liu,[1] and Xiaodong Liu[1]	Impairment of Intestinal Monocarboxylate Transporter 6 Function and Expression in Diabetic Rats Induced by Combination of High-Fat Diet and Low Dose of Streptozocin: Involvement of Butyrate–Peroxisome Proliferator-Activated Receptor-γ Activation	Center of Drug Metabolism and Pharmacokinetics, College of Pharmacy, China Pharmaceutical University.	Magazine: Drug Metabolism and Disposition, 2019, 47:556-566

Contents

Epigenetic activation of the drug transporter OCT2 sensitizes renal cell carcinoma to oxaliplatin

Yanqing Liu,[1*] Xiaoli Zheng,[1*] Qinqin Yu,[1] Hua Wang,[2] Fuqing Tan,[3] Qianying Zhu,[1] Lingmin Yuan,[1] Huidi Jiang,[1] Lushan Yu,[1†‡] Su Zeng[1†‡]

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ABSTRACT

Renal cell carcinoma (RCC) is known for its multidrug resistance. Using data obtained from the cancer transcriptome database Oncomine and the proteome database The Human Protein Atlas, we identified the repression of organic cation transporter OCT2 as a potential factor contributing to oxaliplatin resistance in RCC. By analyzing OCT2 expression in collected patient tissues and commercial tissue microarray specimens, we demonstrated OCT2 repression in RCC at both transcription and protein levels. Epigenetic analysis revealed that the repressed OCT2 promoter in RCC is characterized by hypermethylated CpG islands and the absence of H3K4 methylation. Further mechanistic studies showed thatDNAhypermethylation blockedMYCactivation of OCT2 by disrupting its interaction with the E-Box motif,which prevented MYC from recruiting MLL1 to catalyze H3K4me3 at the OCT2 promoter and resulted in repressed OCT2 transcription. Targeting thismechanism, we designed a sequential combination therapy and demonstrated that epigenetic activation of OCT2 by decitabine sensitizes RCC cells to oxaliplatin both in vitro and in xenografts. Our study highlights the potential of translating “omics” data into the development of targeted therapies.

The failure of DAC to induce OCT2 expression and its remission by hemoglobin-based nanocarriers under hypoxia in renal cell carcinoma

Lu Chen[1*], Zeyang Wang[1*], Qingwen Xu[1], Yuxi Liu[1], Le Chen[1], Suhang Guo[1], Hua Wang[2], Kui Zeng[1],Junqing Liu[3], Su Zeng[1]and Lushan Yu[1]

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ABSTRACT

Background: Human organic cation transporter 2 (OCT2) is the most abundant and important uptake transporter involved in the renal excretion of cationic drugs. Abnormal hypermethylation- mediated silencing of OCT2 results in oxaliplatin resistance in renal cell carcinoma (RCC). The epigenetic activation of OCT2 by decitabine (DAC) reversed this resistance in normoxic conditions. Given the hypoxic characteristic of RCC, it is still unclear whether hypoxia promotes DAC resistance and is involved in the regulation of OCT2.

Methods: The mRNA and protein expression of OCT2 was determined by qRT-PCR and Western blotting. MSRE-qPCR and BSP were used to examine methylation modifications at the OCT2 promoter.The ChIP-qPCR analysis was performed to detect the abundance of histone modification and HIF-1α. The accumulation of DAC and 5-mC were detected using LC-MS, and the amount of 5-hmC was determined by dot blot analysis. To understand the role of hypoxia in the regulation of equilibrative nucleoside transporter 1 (ENT1) expression, the HIF-1α KO cell model was constructed. The re-emulsion method was used for the construction of H-NPs, an oxygen nanocarrier based on hemoglobin, to alleviate the drug resistance of DAC under hypoxia.

Results: DAC was unable to upregulate OCT2 expression in hypoxic conditions because of the hypermethylation and low H3K4me3 modification in its promoter region. Hypoxia-mediated repression of human ENT1, which was markedly suppressed in RCC, resulted in a decrease in the cellular accumulation of DAC. Besides, hypoxia-induced upregulation of histone deacetylase HDAC9, which impaired the enrichment of H3K27ac modification in the OCT2 promoter, led to the transcriptional repression of OCT2. H-NPs could attenuate the hypoxia-induced loss of DAC activity and sensitize RCC cells to the sequential combination therapy of DAC and oxaliplatin.

Conclusions: Hypoxia-mediated repression of ENT1 led to the inability of DAC to upregulate the expression of OCT2 under hypoxia. H-NPs could alleviate resistance to oxaliplatin and DAC in RCC cells under hypoxia and may have potential clinical applications.

Key words: renal cell carcinoma, hypoxia, OCT2, ENT1, nanoparticles

Upregulation of miR-489-3p and miR-630 inhibits oxaliplatin uptake in renal cell carcinoma by targeting OCT2

Lu Chen[a], Le Chen[a], Zhiyuan Qin[a], Jinxiu Lei[a], Sheng Ye[b], Kui Zeng[a],Hua Wang[c], Meidan Ying[a], Jianqing Gao[a], Su Zeng[a], Lushan Yu[a,*]

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ABSTRACT

Abstract Renal cell carcinoma (RCC) is one of the most common malignant tumors affecting the urogenital system, accounting for 90% of renal malignancies. Traditional chemotherapy options are often the front-line choice of regimen in the treatment of patients with RCC, but responses may be modest or limited due to resistance of the tumor to anticarcinogen. Downregulated expression of organic cation transporter OCT2 is a possible mechanism underlying oxaliplatin resistance in RCC treatment. In this study, we observed that miR-489-3p and miR-630 suppress OCT2 expression by directly binding to the OCT2 30-UTR. Meanwhile, via 786-O-OCT2- miRNAs stable expression cell models, we found that miRNAs could repress the classic substrate 1-methyl-4-phenylpyridinium (MPPt), fluorogenic substrate N,N-dimethyl-4-(2-pyridin-4-ylethenyl) aniline (ASPt), and oxaliplatin uptake by OCT2 both in vitro and in xenografts. In 33 clinical samples, miR-489-3p and miR-630 were significantly upregulated in RCC, negatively correlating with the OCT2 expression level compared to that in adjacent normal tissues, using tissue microarray analysis and qPCR validation. The increased binding of c-Myc to the promoter of pri-miR-630, responsible for the upregulation of miR-630 in RCC, was further evidenced by chromatin immunoprecipitation and dual-luciferase reporter assay. Overall, this study indicated that miR-489-3p and miR-630 function as oncotherapy-obstructing microRNAs by directly targeting OCT2 in RCC.

Key words: OCT2; miRNA; Renal cell carcinoma; Epigenetic regulation; Oxaliplatin

Inhibition of histone deacetylase 7 reverses concentrative nucleoside transporter 2 repression in colorectal cancer by up-regulating histone acetylation state

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ABSTRACT

BACKGROUND AND PURPOSE

The concentrative nucleoside transporter 2 (CNT2) mediates the uptake of both natural nucleosides and nucleoside-derived drugs. Therefore, it is important both physiologically and pharmacologically. However, CNT2 expression is significantly repressed in colorectal cancer (CRC). Here, we have elucidated the mechanism(s) underlying CNT2 repression in CRC.

EXPERIMENTAL APPROACH

Repression of CNT2 in tumour samples from patients with CRC was identified using Western blot and RT-qPCR. The histone acetylation state at the CNT2 promoter region was then evaluated with chromatin immunoprecipitation and trichostatin A (TSA) treatment. To find the key enzyme responsible for hypoacetylation at the CNT2 promoter region, siRNA knockdown and RT-qPCR were used. Effects of combining HDAC inhibitors and cladribine were studied in HCT15 and HT29 cells.

KEY RESULTS

Histone deacetylase 7 was significantly up-regulated in CRC, leading to histone hypoacetylation at the CNT2 promoter region, especially at sites H3K9Ac, H3K18Ac and H4Ac. This hypoacetylation condensed the chromatin structure and reduced CNT2 expression. All these effects were reversed by treatment with TSA, a histone deacetylase inhibitor. In HCT15 and HT29 cells, inhibition of histone deacetylase increased cell uptake and decreased IC50 for cladribine.

CONCLUSIONS AND IMPLICATIONS

Histone hypoacetylation due to increased levels of histone deacetylase 7 results in CNT2 repression in CRC tumour tissue and could lead to decreased uptake of and consequent resistance to nucleoside anti-cancer agents. Such resistance could be overcome by combining inhibitors of histone deacetylase with the nucleoside anti-cancer agent.

Key words: 2-CdA, cladribine; CRC, colorectal cancer; TSA, trichostatin A; HDAC, histone deacetylase; HAT, histone acetyltransferase

Opposite Effect of Diabetes Mellitus Induced by Streptozotocin on Oral and Intravenous Pharmacokinetics of Verapamil in Rats

Nan Hu, Shanshan Xie, Li Liu, Xinting Wang, Xian Pan, Guanming Chen, Lulu Zhang,Haiyan Liu, Xiang Liu, Xiaodong Liu, Lin Xie, and Guangji Wang

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ABSTRACT

The aim of this study was to report the effect of diabetes mellitus on the pharmacokinetics of verapamil in a route-dependent manner.Diabetes in rats was induced by streptozotocin.Plasma concentrations of verapamil and its metabolite, norverapamil, were measured after oral (10 mg/kg) or intravenous (1 mg/kg) administration.The concentrations of verapamil in portal plasma after oral administration were also determined. Norverapamil formation was used for assessing CYP3A activity in hepatic and intestinal microsomes of diabetic rats. The protein levels of CYP3A1 and CYP3A2 in liver and intestine were measured by Western blot. It was found that diabetes significantly increased the plasma concentration of verapamil and norverapamil after oral administration, which resulted in a 74% increase in the area under the concentration-time curve (AUC) of verapamil, but the ratio of $AUC_{(norverapamil)}/AUC_{(verapamil)}$ was significantly decreased by 38%. In contrast, diabetes significantly decreased the AUC of verapamil by 22% after intravenous administration. Diabetes also resulted in increased AUC of verapamil in portal vein by 3.8-fold compared with that in control rats.The absolute bioavailability of verapamil was higher than that of control rats. An in vitro study showed that increased CYP3A activity in the hepatic microsome and decreased CYP3A activity in the intestinal microsome were accompanied by an increase and decrease in the protein expression of CYP3A1/2 in liver and intestine of diabetic rats, respectively. In conclusion, diabetes mellitus revealed a tissue-specific effect on CYP3A activity and expression (induced in liver and inhibited in intestine), resulting in opposite pharmacokinetic behaviors of verapamil after oral and intravenous administration to diabetic rats.

Key words: STZ, streptozotocin; AUC, the area under the concentration-time curve; P-gp, P-glycoprotein; HPLC, high-performance liquid chromatography.

Combined Contributions of Impaired Hepatic CYP2C11 and Intestinal Breast Cancer Resistance Protein Activities and Expression to Increased Oral Glibenclamide Exposure in Rats with Streptozotocin-Induced Diabetes Mellitus

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ABSTRACT

The purpose of this study was to evaluate the contributions of impaired cytochrome P450 and breast cancer resistance protein (BCRP) activity and expression to drug pharmacokinetics under diabetic conditions. Diabetes was induced in rats with the intraperitoneal administration of streptozocin. Glibenclamide (GLB), a substrate of BCRP, served as a model drug. The pharmacokinetics of orally administered GLB (10 mg/kg) were studied. The results showed that diabetes mellitus significantly increased exposure (area under the curve and peak concentration) to GLB after oral administration. Data from hepatic microsomes suggested impairment of GLB metabolism in diabetic rats. GLB metabolism in hepatic microsomes was significantly inhibited by a selective inhibitor (sulfaphenazole) of CYP2C11 and an anti-CYP2C11 antibody.Western blotting further indicated the contribution of impaired CYP2C11 expression to the impairment of GLB metabolism. Excretion data showed that _72% of the orally administered dose was excreted in the feces of normal rats, which indicates an important role for intestinal BCRP. Diabetes significantly decreased the recovery from feces, which was only 40% of the orally administered dose. Results from in situ, single-pass, intestinal perfusion experiments revealed that diabetes significantly increased the apparent effective permeability and decreased the efflux of GLB through the intestine; this suggests impairment of intestinal BCRP function,which may play a role in the increased exposure to orally administered GLB in diabetic rats. Insulin treatment partly or completely reversed the changes in diabetic rats. All results yielded the conclusion that impaired hepatic CYP2C11 and intestinal BCRP expression and activity induced by diabetes contributed to the increased exposure of orally administered GLB.

Key words: P450, cytochrome P450; AUC, area under the concentration-time curve; BCRP, breast cancer resistance protein; C_{max}, peak concentration; GLB, glibenclamide; HPLC, high-performance liquid chromatography; NOV, novobiocin; P_{eff}, apparent effective permeability; P-GP, P-glycoprotein; STZ, streptozotocin; SUL, sulfaphenazole; DM, diabetes mellitus; IN, insulin; CON, control.

Decreased exposure of atorvastatin in diabetic rats partly due to induction of hepatic Cyp3a and Oatp2

Nan Shu[1*], Mengyue Hu[1*], Can Liu[2], Mian Zhang[1], Zhaoli Ling[1], Ji Zhang[3], Ping Xu[1], Zeyu Zhong[1], Yang Chen[1],Li Liu[1], and Xiaodong Liu[1]

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ABSTRACT

1. Atorvastatin is frequently prescribed for lowering blood cholesterol and for prevention of events associated with cardiovascular disease. The aim of this study was to investigate the pharmacokinetics of atorvastatin in diabetic rats.

2. Diabetes was induced in rats by combination of high-fat diet and low-dose streptozotocin (35 mg/kg). Plasma concentrations of atorvastatin following oral (10 mg/kg) and intravenous (2 mg/kg) administrations to rats were measured by LC-MS. Metabolism and uptake of atorvastatin in primary hepatocytes of experimental rats were assessed. Protein expressions and activities of hepatic Cyp3a and Oatp2 were further investigated.

3. Clearances of atorvastatin in diabetic rats following oral and intravenous administrations were remarkably increased, leading to marked decreases in area-under-the-plasma concentration–time curve (AUC). The estimated oral and systematic clearances of atorvastatin in diabetic rats were 4.5-fold and 2.0-fold of control rats, respectively.Metabolism and uptake of atorvastatin in primary hepatocytes isolated from diabetic rats were significantly increased, which were consistent with the up-regulated protein expressions and activities of hepatic Cyp3a and Oatp2.

4. All these results demonstrated that the plasma exposure of atorvastatin was significantly decreased in diabetic rats, which was partly due to the up-regulated activities and expressions of both hepatic Cyp3a and Oatp2

Key words: Atorvastatin, Cyp3a, diabetes, organic anion transporting polypeptide 2 (Oatp2), pharmacokinetics, rat

Prediction of Atorvastatin Pharmacokinetics in High-Fat Diet and Low-Dose Streptozotocin-Induced Diabetic Rats Using a Semiphysiologically Based Pharmacokinetic Model Involving Both Enzymes and Transporters

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ABSTRACT

Atorvastatin is a substrate of cytochrome P450 3a (CYP3a), organic anion-transporting polypeptides (OATPs), breast cancer–resistance protein (BCRP), and P-glycoprotein (P-gp). We aimed to develop a semiphysiologically based pharmacokinetic (semi-PBPK) model involving both enzyme and transporters for predicting the contributions of altered function and expression of CYP3a and transporters to atorvastatin transport in diabetic rats by combining high-fat diet feeding and low-dose streptozotocin injection. Atorvastatin metabolism and transport parameters comes from in situ intestinal perfusion,primary hepatocytes, and intestinal or hepatic microsomes. We estimated the expressions and functions of these proteins and their contributions. Diabetes increased the expression of hepatic CYP3a,OATP1b2, and P-gp but decreased the expression of intestinal CYP3a, OATP1a5, and P-gp. The expression and function of intestinal BCRP were significantly decreased in 10-day diabetic rats but increased in 22-day diabetic rats. Based on alterations in CYP3a and transporters by diabetes, the developed semi-PBPK model was successfully used to predict atorvastatin pharmacokinetics after oral and intravenous doses to rats. Contributions to oral atorvastatin PK were intestinal OATP1a5 < intestinal P-gp < intestinal CYP3a < hepatic CYP3a < hepatic OATP1b2 < intestinal BRCP. Contribution of decreased expression and function of intestinal CYP3a and P-gp by diabetes to oral atorvastatin plasma exposure were almost attenuated by increased expression and function of hepatic CYP3a and OATP1b2. Opposite alterations in oral plasma atorvastatin exposure in 10- and 22-day diabetic rats may be explained by altered intestinal BCRP. In conclusion, the altered atorvastatin pharmacokinetics by diabetes was the synergistic effects of altered intestinal or hepatic CYP3a and transporters and could be predicted using the developed semi-PBPK

Key words: Ator, atorvastatin; $AUC_{0-\infty}$, area under the concentration-time curve from time zero to infinity; BCRP/Bcrp, breast cancer resistance protein; $CL_{int,up}$, intrinsic uptake clearance; C_{max}, the maximum concentration; CYP3A/Cyp3a, cytochrome P450 3A; f_u, unbound fraction in plasma; HBSS, Hanks' balanced salt solution; k_a and k_b, absorption and efflux rate constant; K_m, Michaelis-Menten constant; LC-MS, liquid chromatography-mass spectrometry; MRT, mean residence time; Nar, naringin; O-O-Ator, ortho-hydroxy atorvastatin; O/P-OH-Ator, ortho/ para-hydroxy atorvastatin; OATPs/Oatps, organic anion transporting polypeptides; PBS, phosphate-buffered saline; PBSF, physiologically based scaling factor; P_{eff}, effective permeability; P-gp, P-glycoprotein; Pra, prazosin; Rho123, rhodamine 123; semi-PBPK model, semiphysiologically based pharmacokinetic model; SC, scaling facror; STZ, streptozotocin; $t_{1/2}$, terminal half-life; TC, triglyceride; TG, total cholesterol; V_{max}, maximum metabolic velocity.

Decreased exposure of simvastatin and simvastatin acid in a rat model of type 2 diabetes

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[1]Key Laboratory of Drug Metabolism and Pharmacokinetics, China Pharmaceutical University; [2]Jiangsu Provincial Institute of Traditional Chinese Medicine.

ABSTRACT

Aim: Simvastatin is frequently administered to diabetic patients with hypercholesterolemia. The aim of the study was to investigate the pharmacokinetics of simvastatin and its hydrolysate simvastatin acid in a rat model of type 2 diabetes.

Methods: Diabetes was induced in 4-week-old rats by a treatment of high-fat diet combined with streptozotocin. After the rats received a single dose of simvastatin (20 mg/kg, po, or 2 mg/kg, iv), the plasma concentrations of simvastatin and simvastatin acid were determined. Simvastatin metabolism and cytochrome P4503A (Cyp3a) activity were assessed in hepatic microsomes, and its uptake was studied in freshly isolated hepatocytes. The expression of Cyp3a1, organic anion transporting polypeptide 2 (Oatp2), multidrug resistance-associated protein 2 (Mrp2) and breast cancer resistance protein (Bcrp) in livers was measured using qRT-PCR.

Results: After oral or intravenous administration, the plasma concentrations and areas under concentrations of simvastatin and simvastatin acid were markedly decreased in diabetic rats. Both simvastatin metabolism and Cyp3a activity were markedly increased in hepatocytes of diabetic rats, accompanied by increased expression of hepatic Cyp3a1 mRNA. Furthermore, the uptake of simvastatin by hepatocytes of diabetic rats was markedly increased, which was associated with increased expression of the influx transporter Oatp2, and decreased expression of the efflux transporters Mrp2 and Bcrp.

Conclusion: Diabetes enhances the metabolism of simvastatin and simvastatin acid in rats via up-regulating hepatic Cyp3a activity and expression and increasing hepatic uptake.

Key words: diabetes; hypercholesterolemia; simvastatin; pharmacokinetics; hepatocyte; microsome; Cyp3a; organic anion transporting polypeptide 2; multidrug resistance-associated protein 2; breast cancer resistance protein

Impairment of Intestinal Monocarboxylate Transporter 6 Function and Expression in Diabetic Rats Induced by Combination of High-Fat Diet and Low Dose of Streptozocin: Involvement of Butyrate–Peroxisome Proliferator-Activated Receptor-γ Activation

Feng Xu, Liang Zhu, Chaoqun Qian, Junjie Zhou, Donghao Geng, Ping Li, Wenjing Xuan,Fangge Wu, Kaijing Zhao, Weimin Kong, Yuanyuan Qin, Limin Liang, Li Liu,[1] and Xiaodong Liu[1]

Center of Drug Metabolism and Pharmacokinetics, College of Pharmacy, China Pharmaceutical University.

ABSTRACT

Generally, diabetes remarkably alters the expression and function of intestinal drug transporters. Nateglinide and bumetanide are substrates of monocarboxylate transporter 6 (MCT6). We investigated whether diabetes down-regulated the function and expression of intestinal MCT6 and the possible mechanism in diabetic rats induced by a combination of high-fat diet and low-dose streptozocin.Our results indicated that diabetes significantly decreased the oral plasma exposure of nateglinide. The plasma peak concentration and area under curve in diabetic rats were 16.9% and 28.2% of control rats, respectively. Diabetes significantly decreased the protein and mRNA expressions of intestinal MCT6 and oligopeptide transporter 1 (PEPT1) but up-regulated peroxisome proliferator-activated receptor γ (PPARγ) protein level.Single-pass intestinal perfusion demonstrated that diabetes prominently decreased the absorption of nateglinide and bumetanide.The MCT6 inhibitor bumetanide, but not PEPT1 inhibitor glycylsarcosine, significantly inhibited intestinal absorption of nateglinide in rats. Coadministration with bumetanide remarkably decreased the oral plasma exposure of nateglinide in rats.High concentrations of butyrate were detected in the intestine of diabetic rats. In Caco-2 cells (a human colorectal adenocarcinoma cell line), bumetanide and MCT6 knockdown remarkably inhibited the uptake of nateglinide. Butyrate down-regulated the function and expression of MCT6 in a concentration-dependent manner but increased PPARγ expression. The decreased expressions of MCT6 by PPARγ agonist troglitazone or butyrate were reversed by both PPARγ knockdown and PPARγ antagonist 2-chloro-5-nitro-N-phenylbenzamide (GW9662). Four weeks of butyrate treatment significantly decreased the oral plasmaconcentrations of nateglinide in rats, accompanied by significantly higher intestinal PPARγ and lower MCT6 protein levels. In conclusion, diabetes impaired the expression and function of intestinal MCT6 partly via butyrate-mediated PPARγ activation,decreasing the oral plasma exposure of nateglinide.

Key words: AUC, area under the curve; BCRP, breast cancer resistance protein; Caco-2, human colorectal adenocarcinoma cell line; CL_{int}, intrinsic clearance; CON, control; CYP, cytochrome P450 enzyme; DM, diabetes mellitus; FBG, fasting glucose in blood; FINS, fasting insulin; G-6PDH, glucose-6-phosphate dehydrogenase; G-6-P, D-glucose-6-phosphate; Gly-Sar, glycylsarcosine; GW9662, 2-chloro-5-nitro-Nphenylbenzamide; HBSS, Hanks' balanced salt solution; HFD, high-fat diet; HOMA-IR, homeostasis model assessment - insulin resistance;Ko143(tert-butyl3-[(2S,5S,8S)-14-methoxy-2-(2-methylpropyl)-4,7-dioxo-3,6,17-triazatetracyclo[8.7.0.0^{3,8}.0^{11,16}]heptadeca-1(10),11,13,15- tetraen-5-yl]propanoate); LC-MS, liquid chromatography with mass spectrometry; MCT6, monocarboxylate transporter 6; M1, N-[trans-4-(1- hydroxy-1-methylethyl)-cyclohexanecarbonyl]-D-phenylalanine; MRP4, multidrug resistance protein 4; NKCC, sodium-potassium-2chloride cotransporter; OAT2, organic anion transporter 2; OATPs, organic anion-transporting polypeptides; OCTs, organic cation transporters; P_{eff}, apparent effective permeability; PEPT, oligopeptide transporter; P-GP, P-glycoprotein; PPARγ, peroxisome proliferator-activated receptor g; qRT-PCR, quantitative real-time polymerase chain reaction; RIPA, radioimmunoprecipitation assay; SCFA, short-chain fatty acids; siRNA, small nterfering RNA; SPIP, single-pass intestinal perfusion; STZ, streptozocin; TC, total cholesterol; TG, triglyceride.

赛默飞药物代谢和药代动力学部分流程解决方案

主要研究手段

药物转运体研究部分常见模型

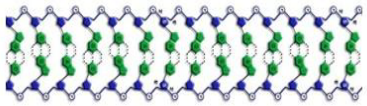
核酸 蛋白 底物 / 代谢物

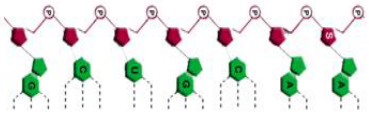
体外模型

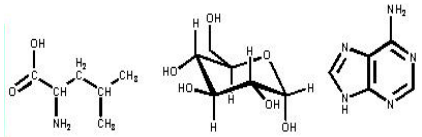
- **体外细胞模型**
  - 不含重组转运体的细胞系(Caco-2.…)
  - 原代细胞
  - 三明治培养原代肝细胞模型
- **基于膜的体外模型**
  - ATP 酶测定法
  - 膜囊泡转运模型
- **肾切片摄取模型**

体内模型

- 基因敲除小鼠
- 抑制剂“敲除”转运体
- 小动物活体成像







细胞培养

分子生物学

浓度分析

动物水平评价

生物学实验室(细胞房)

细胞房必需设备

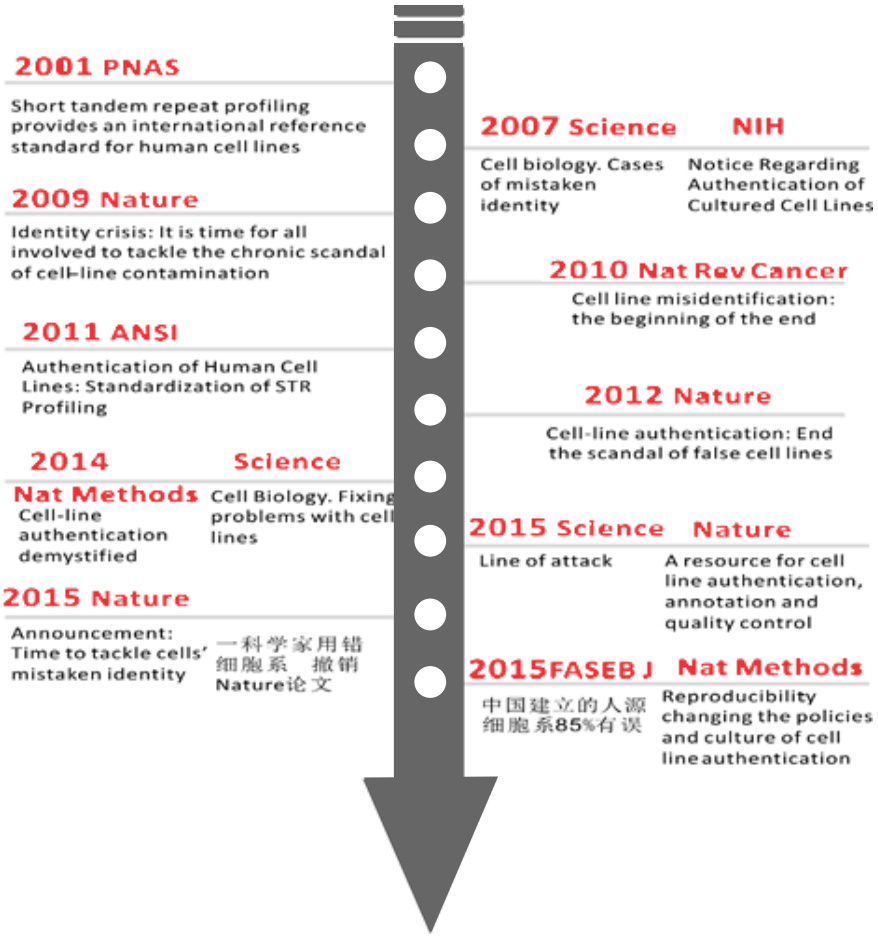
细胞冻存	试剂储存	细胞复苏	细胞培养	细胞处理	细胞离心	细胞观察	细胞处理	配液	细胞冻存	器具处理
液氮罐	4/-20℃冰箱	恒温水浴	CO_2 培养箱	生物安全柜	离心机	显微镜	移液和培养耗材	超纯水机	-86℃冰箱	烘箱
										

Gibco 细胞培养基和血清

细胞培养材料

样本鉴定：细胞系鉴定

关于细胞STR鉴定的重量级报道：



NIH、ATCC 等权威机构呼吁
研究者对细胞进行鉴定！

AACR Journals
- Cancer Discovery
- Cancer Research
- Clinical Cancer Research
- Cancer Epidemiology, Biomarkers & Prevention
- Molecular Cancer Research
- Molecular Cancer Therapeutics
- Cancer Prevention Research

Endocrine Society Journals
- Endocrinology
- Endocrine Reviews
- Journal of Clinical Endocrinology & Metabolism
- Molecular Endocrinology
- Hormones and Cancer

Society for Endocrinology Journals
- Journal of Endocrinology
- Journal of Molecular Endocrinology
- Endocrine-Related Cancer

Carcinogenesis

Cell Biochemistry and Biophysics

Cell Biology International

International Journal of Cancer

In Vitro Cellular & Developmental Biology – Animal

Journal of Molecular Biology

Journal of the National Cancer Institute

Molecular Vision

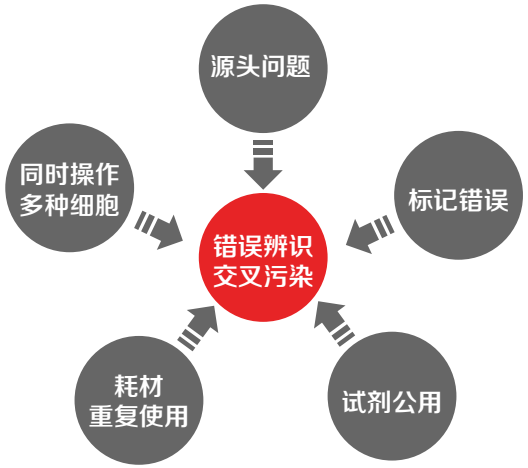
Nature Publishing Group
- Nature Reviews Molecular Cell Biology
- Nature
- Nature Genetics
- Nature Reviews Immunology
- Nature Reviews Cancer
- Nature Reviews Neuroscience
- Nature Biotechnology
- Nature Methods

Neuro-Oncology

Placenta AACR Journals

2015 年有文献报道称：国内建立的人源细胞系错误率竟高达 **85.51%**！

警惕！以下原因均可导致所用细胞错误辨识或交叉污染



何时需做细胞STR鉴定：

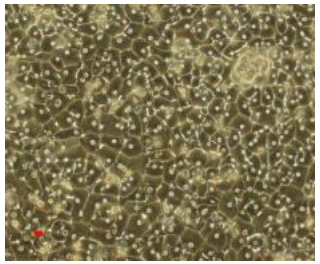
1、发表文章或申请课题经费前
2、使用细胞进行临床治疗（试验）前
3、准备冻存保种或已冻存多年的细胞
4、一个涉及到细胞试验项目开始 / 结束时
5、新得到的细胞或实验室培养 5 代以上的细胞
6、细胞系表现不稳定或结果与预期差别较大
7、异体细胞移植后嵌合情况检查

A

B

ADME 研究解决方案

肝细胞获取



原代肝细胞

- 悬浮和贴壁系统
- 经转运体验证
- 经诱导验证
- 经代谢验证
- 经3D培养验证

肝细胞系、非实质类

- HepaRG™ 细胞系
- Kupffer 细胞
- Stellate 细胞

细胞培养

复苏 & 洗涤 & 铺板 & 培养

- Recovery 复苏培养基
- Washing 试剂
- Williams'E 培养基
- HepExtend 复苏添加剂

细胞外基质

- 2D & 3D
- Geltrex 基质蛋白
- 大鼠胶原

亚组分和转运蛋白

肝亚细胞组分

- 肝微粒体
- S9组分
- 胞浆组分

CYP 及transporter蛋白

- Transporter囊泡和过表达细胞株
- Baculosome CYP蛋白
- CYP Vivid检测试剂盒及试剂

功能检测

检测

- 核受体
- TaqMan® Assays

仪器

- EVOS FL Auto Cell Imaging System

mRNA表达检测

经典方案：

RNA提取 → **RNA 逆转录** → **mRNA 表达检测** → **数据分析**

单管离心柱法：细胞培养液上清

PureLink™ Viral RNA/DNA Mini Kit(12280050)

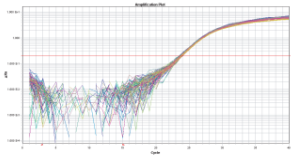
SuperScript IV VILO 逆转录预混液

SuperScript IV VILO 逆转录预混液

PCR仪

TaqMan Gene expression Assay

QuantStudio 6 和 7 Pro 实时荧光定量 PCR 系统



Amplification plot

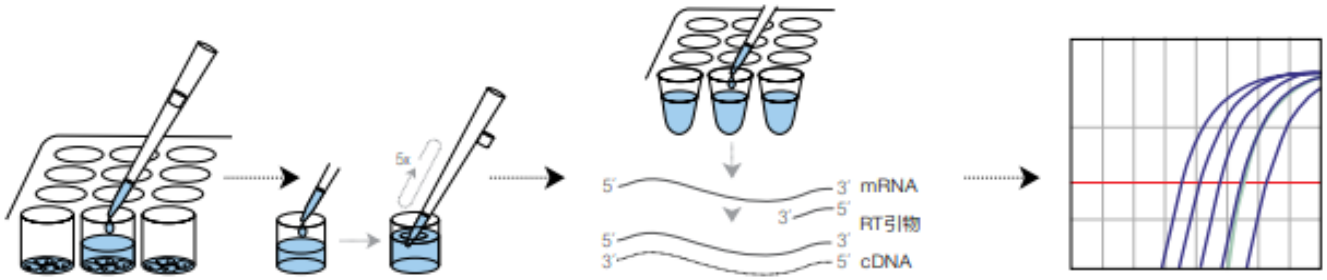
快速方案：Fast Advanced Cells-to-CT

2步法

80/95分钟完成实验流程

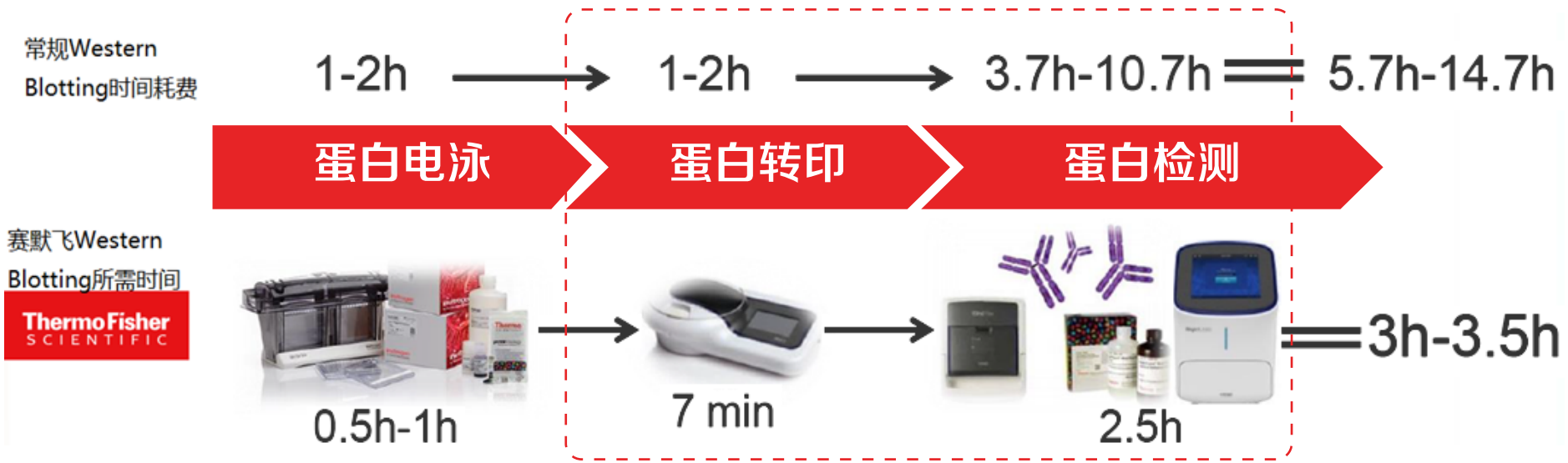
粗裂解物高达45% RT-qPCR体积

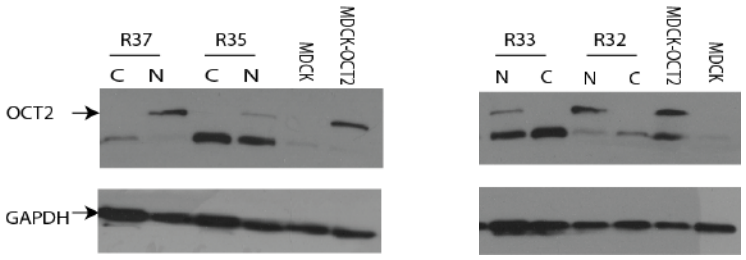
TaqMan™（A35378）和SYBR™ Green（A35381）



细胞裂解+终止液	逆转录	qPCR：TaqMan 或SYBR Green分析
7 min	35 min	40/50 min

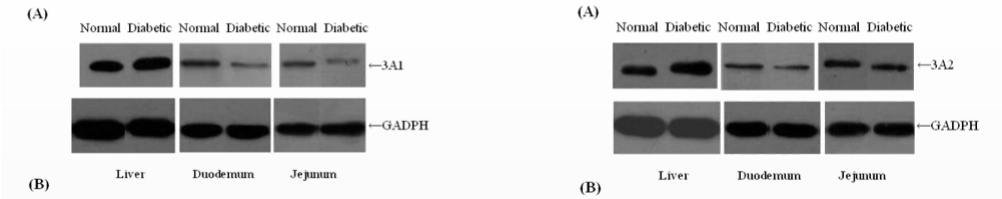
蛋白免疫印迹 Western blotting





引自余露山的PPT

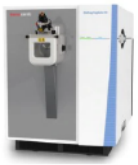
3.Western blot 蛋白印迹验证



引自刘李的PPT

药物、代谢物、底物等的分析

利用色谱–质谱技术实现定性/定量分析

样品前处理		采用多通路技术和 TurboFlow™ 技术的 Transcend™ II 系统		SOLAμ™ SPE 孔板
小分子药物生物分析		TSQ Quantis™三重四极杆质谱仪		Vanquish™ Flex HPLC
大分子药物生物分析		Orbitrap Exploris™ 240 高分辨质谱仪		TSQ Altis™ 三重四极杆质谱仪
代谢产物鉴定		Orbitrap ID-X™ Tribrid™ 质谱仪		TSQ™ 9000 三重四极杆 GC-MS/MS

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