药物肠道渗透性预测模型研究进展

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摘要: 药物的肠道渗透性是口服药物发挥药效的决定性因素之一, 如何准确高效地评估化合物的渗透性已成为新药研发时的一大挑战。本文简介了几种常用的肠道渗透性预测模型的原理、优缺点和最新进展, 重点介绍准确度和效率较高的尤斯灌流和平行人工膜渗透模型, 并对未来渗透性模型的发展趋势进行展望, 以期为先导化合物的渗透性评价提供借鉴。

关键词: 肠道渗透性; 在体单向肠灌流; 外翻肠囊; 尤斯灌流; Caco-2; 平行人工渗透膜

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Advances in models for predicting drug intestinal permeability

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Abstract: Intestinal permeability is one of key factors determing absorption of oral drug products. It is a big challenge to assess permeability of compounds with high accuracy and high efficacy during research and development process. In this review, the principles, strengths, weaknesses and advances of common intestinal permeability models are summarized, with focus on Ussing chamber and parallel artificial membrane permeability assay. In addition, future trends of permeability models are briefly discussed. This review may provide a reference to accessing permeability of lead compounds.

Key words: intestinal permeability; single-pass intestinal perfusion *in vivo*; everted intestinal sac; Ussing chamber; Caco-2; parallel artificial membrane permeability assay

口服给药是最常见也是最方便的一种给药方式, 口服药物主要在肠道被吸收, 因此药物的肠道吸收特性是口服药物发挥药效的决定性因素之一。在新药研发过程中, 组合化学和高通量筛选手段的出现使得候选药物的数量大大增加的同时也存在大量吸收不良的化合物。若能在研发早期了解化合物的肠道吸收特性, 即可提高研发效率及节约成本。药物的肠道吸收特性由药物的理化性质、剂型和胃肠道生理病理状态等多种因素决定[1]。其中, 药物的肠道渗透性决定了药物被人体吸收的速率和程度, 最终影响药物的生物利用度。在生物药剂学分类系统 (biopharmaceutical classification system, BCS) 中, Amidon 等[2]将药物的肠道渗透性与溶解度作为药物分类的依据,

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现被广泛用于指导新药开发。

药物的肠道渗透性是指其跨肠壁细胞进入血液循环的能力，通常以单位时间或单位面积进入的药量衡量。药物的肠道渗透机制以被动跨膜渗透及主动转运两种方式为主[3]。高渗透性有利于药物跨细胞膜，到达作用靶点，从而发挥其药理学作用；而低渗透性药物则难以跨膜转运、发挥药效。若能在研发早期准确高效地评估化合物的渗透性将有助于避免后续临床前及临床研究时带来的挑战。目前各国法规中均被认可的用于预测药物肠道渗透性的模型有：动物体内或在体肠灌流模型、切除的动物肠道组织模型和单层培养上皮细胞模型[4−7]。本文将介绍体内外及非生理药物渗透性预测模型的原理与进展，以期为新药研发提供一些研究思路。

1 在体肠灌流模型

在体模型中肠灌流模型是较被认可的模型之一，这类模型的优点在于保留了肠道神经、内分泌输入和转运蛋白的完整性，血液及淋巴液的供应，从而保证了模型整体的生物活性，较接近机体内的状态，比体外及离体模型能更准确预测口服药物在体内的吸收情况。另外，利用该类模型还可同时测定肠道代谢物。不足之处在于需要的动物数量较大，成本高，不同动物组别之间存在一定差异[8]。

1.1 在体单向肠灌流模型 动物在体单向肠灌流 (single-pass intestinal perfusion, SPIP) (图 1a) 的主要步骤：量取麻醉动物 (通常是大鼠) 一定长度的肠段，两端插管，将药液用恒速泵灌流肠腔，每隔一定时间收集出口管中灌流液，测定药物浓度。目前该模型主要被应用于研究中药多成分环境下药物的渗透机制和药物在不同肠段的肠吸收特性[9−11]。Dong 等[12]参照 FDA 标准建立了 P-糖蛋白 (P-gp) 的 SPIP 模型，提出该模型较 Caco-2 和外翻肠囊法能得到更准确的药物渗透性分类。Jiang 等[13]采用该法测定了中药芫花中芫花素的肠道渗透性，发现芫花素在不同肠段的吸收存在差异，最佳吸收位点为十二指肠；且它的渗透性与浓度无关，说明存在被动扩散机制；若与丙磺舒 (多药耐药蛋白抑制剂) 一起灌流，发现渗透性增加，说明还同时存在多药耐药蛋白介导的外排机制。Sun 等[14]测定了刺黄柏总生物碱的肠道渗透性，发现总生物碱的渗透性较各单体显著增加，说明多成分之间存在相互作用；且与维拉帕米 (P-gp 抑制剂) 或硫胺素 (有机阳离子转运体抑制剂) 同时灌流后，渗透性增加，说明总生物碱可能是 P-gp 和有机阳离子转运体的底物。

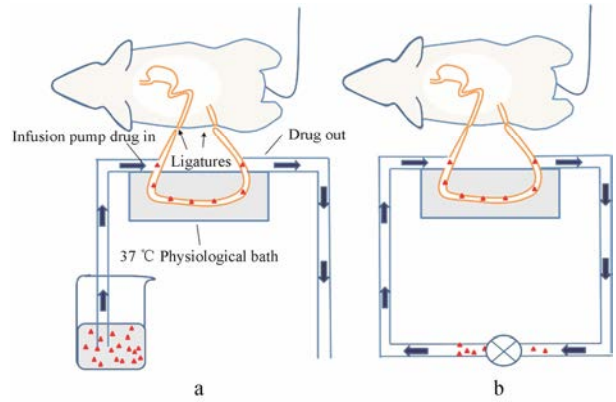

Figure 1 Schematic illustration of rat intestinal perfusion. a: Single-pass intestinal perfusion (SPIP) model; b: Doluisio's closed loop model

1.2 Doluisio 闭环肠灌流模型 Doluisio 闭环模型 (Doluisio's closed loop model) (图 1b) 是由 SPIP 衍生而来，需要借助三通阀来完成[15]。相对于 SPIP，闭环模型的优势在于由于灌流液在肠段中被持续循环，可明显减少灌流液的用量 (< 5 mL)，同时也节省药量 (适用于新药研发早期)[16]，适用于测定阿替洛尔等低渗透性药物[17]。缺点在于因为灌流液的浓度时刻在变化，需要频繁取样，另外由于灌流液浓度变化又不太明显，要求分析方法需要有较高的灵敏度[18]。Xu 等[19]采用该法确定了非诺贝特纳米悬液的跨膜转运方式为被动扩散，在测定肠道渗透性时用酚红来校正灌流液体积变化，避免对药物测定的影响。Lozoya-Agullo 等[20]应用该模型发现将溴酚蓝及考马斯亮蓝与阿替洛尔形成离子对后的阿替洛尔的结肠渗透性显著增加，且未破坏紧密连接或细胞膜，为测定低渗透性药物提供了新思路。

近年来学者认为以上两种肠灌流模型结果较为一致。Lozoya-Agullo 等[21]取大鼠小肠各段以 SPIP 和闭环模型测定了 12 种不同渗透性的模型药物，发现这两个模型的测定结果基本一致，尤其是空肠上段 ($R^2 = 0.95$)。取结肠段测定了 14 种不同结构类型的药物的渗透性，测定结果也基本一致 ($R^2 = 0.81$)[22, 23]。Ruiz-Picazo 等[17]认为闭环模型测得的各肠段的渗透性与人体数值相当。

2 离体肠囊模型

当麻醉对药物检测有影响时，可采用离体模型代替在体模型。此类模型也可用于研究药物在不同肠段的代谢情况及药物相互作用[24]。缺点在于离体条件下酶活力降低，会影响部分药物的渗透性数值，同时因肠黏膜细胞活力限制，整个实验需快速完成，因此取样点也有限[25]。该类模型多用于初步定性研究。

2.1 外翻肠囊模型 外翻肠囊模型 (everted intestinal sac) 最早于 1954 年提出[26]。其基本步骤为: 取一定长度的肠段一端结扎, 并翻转肠段使肠黏膜层在外、浆膜层在内, 缓冲液冲洗后结扎另一端, 形成肠囊 (图 2), 向肠囊内注入空白缓冲液 (受体侧), 然后将肠囊置于含药缓冲液 (供体侧), 整个装置通入碳合气 (95% O_2/5% CO_2) 置于 37 ℃恒温水浴中, 定时从肠囊内取样检测, 实验结束后测量肠囊内表面积[27]。该法的主要优点在于由于肠囊内浆膜层体积小, 黏膜层药物经肠段转运后可在浆膜侧浓集, 易于检测药物, 尤其是低渗透性药物; 缺点在于测定的转运数值为肠腔侧膜和基底侧膜的转运总和; 残留的黏膜肌肉层可能会导致药物黏附, 使得测定结果偏低[28]; 肠囊内液体停滞不动, 这与机体小肠的蠕动状态的生理条件不同有关。

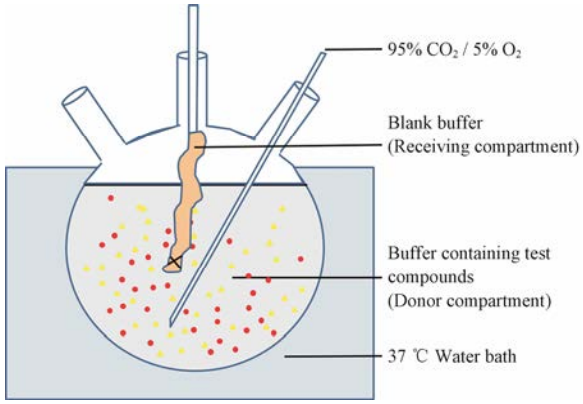

Figure 2 Schematic illustration of everted intestinal sac

Liu 等[29]首先采用该法初步考察黄连水煎液在不同肠段内的吸收情况, 结果检测到 7 个生物碱成分能入肠吸收, 进一步用 SPIP 法确认及定量分析。对于葛根芩连汤中小檗碱的 BCS 属性的探究, 他们也先采用外翻肠囊法定性看出葛根芩连汤中小檗碱可透过肠壁吸收, 再采用 SPIP 确认, 发现小檗碱随生药浓度增加吸收速率增大, 表明存在被动扩散[30]。Zhu 等[31]采用大鼠外翻肠囊模型考察了黄芩苷在不同肠段的表观渗透系数 (apparent permeability coefficients, P_{app}), 以及白芷对黄芩苷不同肠段吸收的影响, 确定白芷提取物能促进黄芩水提液中黄芩苷的小肠吸收, 尤其是十二指肠的吸收。

2.2 未外翻肠囊模型 未外翻肠囊模型 (non-everted intestinal sac, NEIS) 最早由 Kaul 等[32]于 1981 年提出, 该模型是用未经外翻的肠囊, 探究药物自浆膜侧至黏膜侧的转运。优点在于因不必外翻肠囊, 避免了对肠组织的破坏, 较外翻肠囊法利于保持活性, 因此 Ruan 等[33]建议以此法替代外翻肠囊法。Genty 等[34]发现, 当肠囊被外翻后经主动转运的药物的渗透性数值会增加, 而经被动扩散转运的药物渗透性在肠囊外翻前后不变, 于是建议用 NEIS 测定主动转运药物的渗透性。Wada 等[35]采用该法解释了 P-gp 底物罗丹明 123 口服给药后的药时曲线双峰现象是由 P-gp 在各肠段的活性差异导致。Ruan 等[33]和 Palle 等[36]还采用该法考察天然产物的肠道代谢情况。Gamal 等[37]采用该法筛选出能提高氨磺必利渗透性的液体纳米自组装乳化药物传递系统。

2.3 尤斯灌流模型 尤斯灌流模型 (Ussing chamber) 最早于 1949 年提出[38], 它可以研究药物在特定肠段的渗透。尤斯灌流模型的基本操作: 取目标离体肠段剪成合适的肠段, 如图 3 所示, 固定于样品夹 (a) 中, 安装于两个扩散池 (b) 之间。两个扩散池一侧加入含药缓冲液 (供体侧), 另一侧为空白缓冲液 (受体侧), 并从通气口 (c) 通入碳合气, 保持肠组织的活性。药物加入供体侧后, 在受体侧取样检测药物不同时间的累计量, 实验过程中通过水浴加热装置 (d) 保持缓冲液温度为 37 ℃[39]。在该装置内通气的意义不仅在于给扩散池内液体供氧并维持溶液 pH 值, 另外供气产生的气泡可促进液体在各池内循环流动, 并促进加入药物的混匀。一些学者认为肠段表面的浆膜层和一部分的肌肉层会阻碍药物和氧气扩散, 因此主张肠段表面需剥膜, 但 Sjögren 等[40]经实验证明剥不剥膜对渗透性测定无影响, 具体可参考 Kisser 等[41]撰写的最新技术指南。该法缺点之一在于需严格保证实验过程中细胞膜的完整性。若细胞膜完整性被破坏, 经跨细胞膜转运的药物就会通过细胞旁路途径发生“渗漏”。因此, Li 等[42]提出渗透比值的概念, 在该模型下, 以测得的荧光黄 (跨细胞旁路转运的经典药物) 的渗透性数值来校正药物的渗透性数值, 该比值与人体内数值有更高的一致性, 可更准确预测 BCS 分类。缺点之二是由于前置时间较长加上肠道活力的限制, 该方法通量低, 一套装置 1 天可能只能测定 1 个药物浓度[41]。即便这样, 但由于准确度高, 该法仍被广泛使用, 近年来对于该模型的改进集中在缓冲液配方。Wuyts 等[43]分别以空腹和进食状态下的人工胃液替换缓冲液, 可测定进食对于某些药物肠道渗透性的影响。Forner 等[44]提出将供体侧液体换成改良的人工胃液, 受体侧加入牛血清蛋白和消泡剂 B, 可用于测定低溶解度药物 (BCS II 和 IV 类) 的渗透性测定。Lozoya-Agullo 等[23]发现尤斯灌流和大鼠体内肠灌流测得的数值较为一致 (R^2 = 0.77), 但

以大鼠肠段测得的数值高于人体肠段，原因可能是该模型中大鼠肠段表现出更多细胞旁路渗透。因此，Miyake 等[45]建议若用转运指数代替 P_{app}，大鼠肠段得到的结果与人体较为一致，另外他们还对装置本身进行了微型化。

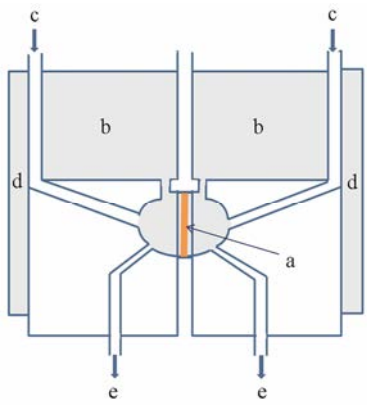

Figure 3　Schematic illustration of Ussing chamber.　a: Sample holder; b: Chamber; c: Vent; d: Heating unit; e: Waste

3　体外细胞模型

由于原代培养的肠上皮细胞不能形成有序排列的单层细胞，目前常培养永生化细胞来测定药物渗透性。最常用的即为人结肠癌细胞上皮细胞 (human colon carcinoma cell line, Caco-2)。但其有许多缺点，如缺少肠壁黏液层、缺少某些代谢酶、培养周期长和实验结果变异性大[46]。因此，近年来对于细胞模型的改进层出不穷 (表 1)[18, 47, 48]。总结可得出：经被动扩散转运的药物 (尤其是高渗透性)，细胞模型测得的渗透性结果与人体较一致，但主动转运药物的一致性较低，主要原因可能是这些细胞模型中转运体的表达量偏低。近年来，科学家热衷于开发 3D 细胞模型用于测定药物渗透性。Lee 等[49]利用预先涂有人工细胞膜的圆底 96 孔板建立了球体 3D Caco-2 细胞模型，测定 22 种模型药物的渗透性后发现该法较平行人工膜渗透模型 (parallel artificial membrane permeability assay, PAMPA) 与人体数值一致性更高。Haraguchi 等[50]应用组织工程细胞片技术 (cell sheet engineering)，构建了较胶原凝胶等支架构建的 3D 模型更为致密的细胞模型，用于测定药物渗透性。

4　非生理模型

4.1　平行人工膜渗透模型　PAMPA 由 Kansy 等[51]于 1998 年提出，以人工磷脂作为生物膜来模拟药物跨膜的屏障，用于测定药物的被动跨膜渗透。随后因商品化的 96 孔滤板 PAMPA 具有高通量优势被用于新药早期研发[52]。商品化的 96 孔滤板 PAMPA 装置结构如图 4 所示，在上层板 (受体侧) 每孔底部的滤膜上预先涂有磷脂溶液，下层板为供体侧。操作流程为：首先将装置放在室温预平衡 30～60 min；然后在供体侧加入含药缓冲液，受体侧加入空白缓冲液；再将上层板置于下层板上方，确保磷脂膜能完全接触到供体液；盖好盖子，将整套装置置于一个密闭恒湿的容器，以避免溶液挥发[53]。在这个过程中，药物分子会从供体侧扩散，穿过磷脂膜，进入到受体侧。待扩散完毕分别吸取受体液和供体液测定药物浓度，计算得出 P_{app}。该模型的优点在于高通量、低成本。由于 95% 药物都是经被动跨膜转运，PAMPA 市场前景巨大。有人计算过，用商品化的 PAMPA 进行药物早期筛选，筛选一批药物需要 4 h，每周可测定 650 个化合物；而采用 Caco-2 细胞模型测定渗透性，待细胞长成紧密单层至少需要 21 天，且每周只能测定 50 个化合物。且用 Caco-2 测定要比 PAMPA 多花费 50～100 倍的实验经费[54, 55]。缺点在于它仅用于研究药物的被动渗透，对于通过主动转运的药物，不宜用 PAMPA 模拟，如用 PAMPA 预测阿米洛利和头孢氨苄等亲水性药物的渗透性，会出现假阳性结果[56]，这可能是因为这些药物是肠道转运体的底物。近年来，研究者在传统 PAMPA 基础上通过对参比药物、磷脂

Table 1　Comparison of different cell models [18, 47, 48]

Cell model	Advantage	Limitation
TC-7 (Caco-2 subclone)	Shorter culture time (2 weeks)	Tighter junction than Caco-2
Caco-2/HT-29-H (Caco-2/HT-29-MTX coculture)	Produces mucus	Looser tight junction than Caco-2; low transporter expression; longer culture time (24 days); difficult technique due to different seeding times of cells
IEC-18	Permeability for drugs with low passive transcellluar transport is close to *in vivo*	Absence of active transport
2/4/A1	Relatively fast method (only 3−4 days); permeability for drugs with low passive transcellluar transport is close to *in vivo*	Absence of active transport; fewer microvilli than Caco-2
MDCK	Relatively fast method (only 3−4 days); permeability for drugs with low passive transcellluar transport is higher than Caco-2 but still underpredicted	Low active transport; non-human, non intestinal origin

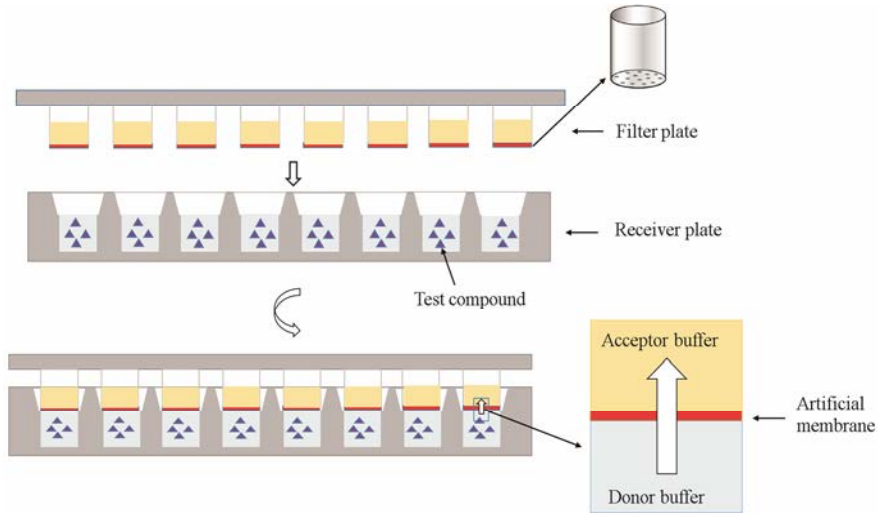

Figure 4 Schematic illustration of parallel artificial membrane permeability assay (PAMPA)

比例、缓冲液、pH 值、浓度和渗透时间等因素进行优化[57−59]，以得到更为准确的模型。

4.2 计算机模型 尽管有 PAMPA 等高通量模型，在药物设计早期，仍需要更加快速方便的、能对先导化合物的渗透性进行正确预测的方法。计算机模型的出现大大提高了药物研发速度，可较早地淘汰渗透性差的药物，减少投资风险。ADMET Predictor、GastroPlus、Discovery Studio、Sybyl 和 Schrödinger 等商业化软件运用定量构效关系、自由能差法、分子动力学模拟等理论，被大型制药公司用于化合物结构的早期初筛[60]。建立新的计算机模型时，预测结果常用 PAMPA 来验证[61, 62]。Wang 等[63]用分子动力学方法模拟了阿替洛尔等 6 种 β 阻滞剂的转运过程，预测结果与 Caco-2 实验结果基本一致。Pade 等[64]建立的 MechPeff 模型可根据肠段生理特点和药物的理化参数，预测药物在啮齿类动物小肠的有效渗透性 (effective permeability, P_{eff})。

上述每个模型的优缺点汇总在表 2 中，以便于比较。

5 结语与展望

美国食品药品监督管理局 (FDA) 和欧盟药品管理局 (EMA) 法规推荐结合不同的模型测定药物渗透性，提高 BCS 分类判定的准确性。另外，针对运用同一种模型在不同实验室的测定结果存在变异性的现象[46]，作者建议在实验室中建立新的渗透性模型时，分 3 个步骤考察系统适应性[65]：① 对于模型中主要参数设定进行优化，以确保适应本实验室；② 测定模型药物的渗透性数值，与文献中数值比较，确保系统适应性达标；③ 在待测药物测定时，务必同时测定模型药物的渗透性以确保方法的重现性和可靠性。理想的渗透性预测模型必须具备构建简单、成本低、准确和高通量等特点。然而由于药物经胃肠道黏膜的转运是一个复杂的动态过程，仅采用一种模型预测体内渗透性有很大困难。目前在药物研发早期，制药企业常采用 PAMPA 和计算机模型，再辅以细胞模型作为初步验证。在未来，渗透性模型的发展将会走向两种趋势：一是更加简单高效的计算机模型，用于早期研发以高效地筛选出成药性良好的原料药；二

Table 2 Advantages and limitations of different permeability models

Model	Advantage	Limitation
In situ perfusion	Close to *in vivo*; blood flow and nerve retain	Need surgery and anesthesia; low throughout; unable to screen drugs
Ex vivo gut sac	Intestinal structure retains; permeability differs in intestinal segments; both human and animals tissues application; can be used to study transport mechanism	Limited tissue viability; membrane integrity might be volatile among experiments, thus leading to unreliable determination
In vitro cell models	Can be used to study transcellular and paracellular passive diffusion, active transport; automatic in some conditions	Intra-laboratory variations because of culture conditions; low transporters expression; lack of mucous layer
PAMPA	Simple and high throughout; automatic; support various pH range and high cosolvent concentration	No active transport; transport depends on lipid components and pH
Computer models	Rapid; low cost	Accuracy improvements need training; require verification by other models

是更加复杂的、接近体内的体外或在体模型，可准确地预测原料药的渗透性机制。计算机技术的发展日新月异，为非生理模型的探索提供了便利，但由于体内过程涉及参数多变，在体及离体肠灌流仍不失为可靠的后期检验模型。随着 3D 打印技术的发展，3D 人体器官、类器官微流控芯片模型或许将成为更加准确的药物渗透性预测模型。

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