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评述

化学生物学专刊

氮杂糖应用于溶酶体蓄积症治疗的研究

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摘要 鞘糖脂代谢异常是一类罕见的遗传性疾病, 这类疾病种类繁多, 通常具有神经病变症状. 其中溶酶体蓄积症是这类疾病中较为典型的一类, 由溶酶体内参与鞘糖脂降解的酶或蛋白因子活性缺失导致代谢底物或者一些糖缀合物蓄积溶酶体内而引起. 目前这类疾病的主要治疗策略是酶替代疗法(ERT), 即为病人补充缺失的酶, 但这种策略固有的缺陷如重组酶无法通过血脑屏障等限制了其运用. 针对这种情况, 底物减少疗法(SRT)这种旨在减少鞘糖脂合成来匹配溶酶体降解能力的新型治疗策略被提了出来. *N*-烷基化氮杂糖因为能够抑制鞘糖脂合成过程中的关键酶—葡萄糖神经酰胺转移酶(CGT)被认为可以用于 SRT. 各种氮杂糖被设计合成以改善其抑制活性、选择性、生物利用度、生物安全性等特性. 其中 NB-DNJ 通过了临床试验, 已成功用于临床治疗. 氮杂糖的另一个特性是作为分子伴侣能够辅助突变酶正确折叠并稳定其构象使酶恢复活性, 这使得药理分子伴侣疗法(PCT)成为治疗溶酶体蓄积症的新型治疗策略. 氮杂糖具有小分子药物口服利用度高、中枢神经系统渗透性强、生物及药理特性明显等优点, 其用于治疗溶酶体蓄积症的临床试验不断增加, 在治疗溶酶体蓄积症方面明显的应用前景光明.

关键词溶酶体蓄积症
氮杂糖
底物减少疗法
药理分子伴侣疗法

1 引言

鞘糖脂是真核细胞细胞膜的重要组成成分, 在内质网和高尔基体内合成分泌至细胞膜^[1], 通过内吞作用进入细胞, 然后在溶酶体内降解^[2]. 当溶酶体内与鞘糖脂降解相关的酶及蛋白因子功能缺失时, 鞘糖脂代谢物即在溶酶体内贮积, 继而严重影响组织器官的正常功能, 导致溶酶体蓄积症(lysosomal storage disorders, LSDs). 溶酶体蓄积症的临床表现复杂多变, 常呈进行性加重, 常常伴有严重的神经症状, 严重影响患者的生活质量乃至生命安全.

虽然每一种 LSDs 均较少见, 但作为一组疾病而言, 其患病率在活产婴儿中达 1/7700, 美国每年约有

500~800 名 LSDs 患儿出生, 给社会造成极大的负担^[3]. 迄今我国尚无 LSDs 确切患病率的统计学资料, 但儿科诊断 LSDs 日益增多.

人们仍然不断尝试各种方法来治疗这类疾病并取得了很大的进步. 其中氮杂糖类化合物是一类在治疗溶酶体蓄积症中特别引起关注的小分子物质, 近年来有关探索氮杂糖类化合物在治疗各种溶酶体蓄积症中应用的研究报道很多. 本文将主要介绍有关溶酶体蓄积症的发病机制以及近年来氮杂糖用于溶酶体蓄积症治疗的研究进展.

2 溶酶体蓄积症

丝氨酸与十六棕榈酰辅酶 A 经内质网胞质表面^[4]

丝氨酸软脂酰转移酶(serine palmitoyltransferase, SPT)催化形成神经酰胺^[5],再以神经酰胺为底物经不同的酶催化途径形成不同的鞘糖脂.神经酰胺进入高尔基体经鞘磷脂合酶(sphingomyelinsynthase, SMS)催化形成鞘磷脂,主要分布在细胞膜和血浆脂蛋白中^[6];进入内质网经半乳糖基转移酶催化形成半乳糖神经酰胺,主要分布在神经系统,是髓鞘的主要组成部分^[7];经高尔基体胞质表面葡萄糖神经酰胺转移酶(ceramide galactosyltransferase, CGT)催化形成葡萄糖神经酰胺^[8].葡萄糖神经酰胺在高尔基体内转化成乳糖神经酰胺,乳糖神经酰胺经过不同酶催化过程最终形成三种鞘糖脂:红细胞系列糖鞘脂(globo-series)、神经节苷脂系列鞘糖脂(ganglio-series)以及乳糖系列鞘糖脂(lacto(neo)-series)^[9](图 1).

鞘糖脂通过内吞作用进入细胞后,在溶酶体内多种水解酶及蛋白因子的共同作用下降解,降解产物进入胞质循环利用^[10].一旦参与这个过程的水解酶或蛋白因子功能缺失,将导致鞘糖脂降解受阻,代谢底物蓄积在溶酶体内,超过一定界限时就会引起溶酶体蓄积症^[11].在某些溶酶体蓄积症中除了代谢底物蓄积外,也会伴有鞘糖脂等的累积^[12].目前已报道了 40 多种鞘糖脂病,它们大都由某种溶酶体酶或者重要的辅助因子缺失导致^[1].图 2 列出鞘糖脂降解途径中发生的一些常见鞘糖脂病.

溶酶体蓄积症的症状受蓄积的代谢物种类、蓄积部位及疾病严重程度的影响,因此溶酶体蓄积症的临床表现充满了个体差异性.但根据突变酶残留活性的大小仍可将突变酶基因携带者大致分为两类^[13]:

突变酶残留活性较低或者丧失的病人通常发病较早,症状一般在婴幼儿或者少年时期出现^[14];而突变酶残留活性稍高的个体,通常发病较晚或者不会有任何的临床症状^[15].

代谢物蓄积部位对溶酶体蓄积症的临床症状也有较大影响.溶酶体蓄积症中最常见的高雪氏病(Gaucher disease),是一种先天性脂质代谢障碍疾病,属于常染色体疾病^[16],在正常人群中的发病率为 1:40000~1:60000,在犹太人种的发病率高达 1:500~1:1000^[17].在高雪氏病中葡萄糖神经酰胺主要蓄积在巨噬细胞中,通常会表现出脾肿大、贫血、血小板减少、骨坏死等症状,另外巨噬细胞的吞噬作用也会进一步加剧症状的严重程度^[18].在法布瑞氏症(Fabry disease)中,代谢物主要蓄积在心脏及肾脏中,导致病人早期死于心血管疾病或者肾衰竭^[19].特别是神经节苷脂沉积症、山霍夫氏(Sandhoff)病、Tay-Sachs 病等一些代谢物蓄积部位为神经组织的疾病,都会伴有严重的神经症状,导致病人夭折^[20].

溶酶体蓄积症的分子机制可能是溶酶体内蓄积的鞘糖脂代谢物的直接毒性作用破坏细胞内正常的信号传导机制,导致细胞死亡或者溶酶体破裂.另外,2002 年 Mizukami 等^[21]发现炎症级联反应可能也是溶酶体蓄积症诱因之一,这个推论随后在 Jeyakumar 等^[22]完成的神经小胶质细胞的试验中得到证实.2004 年 Jeyakumar 等^[23]又一次证实了这个推论,在实验中他们用非甾体类抗炎药处理山霍夫氏病小鼠,从而延缓了山霍夫氏病的发展进程.其次,在一些溶酶体蓄积症中,神经节苷脂 GM1 在细胞内蓄积,

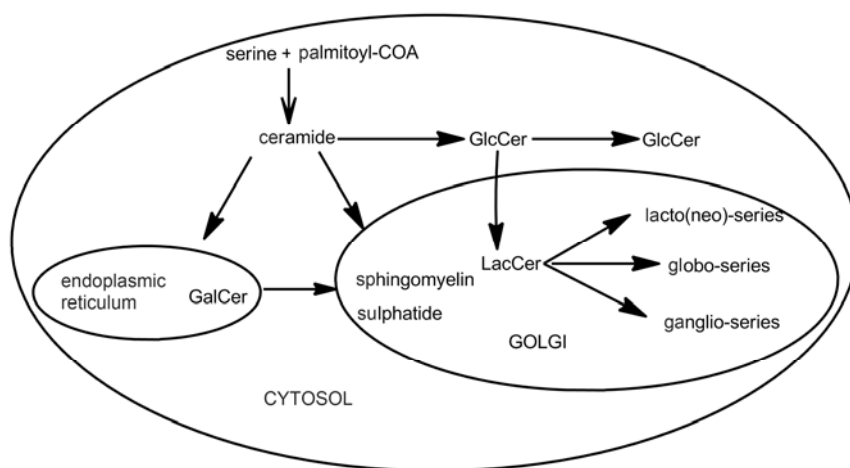


图 1 鞘糖脂的生物合成

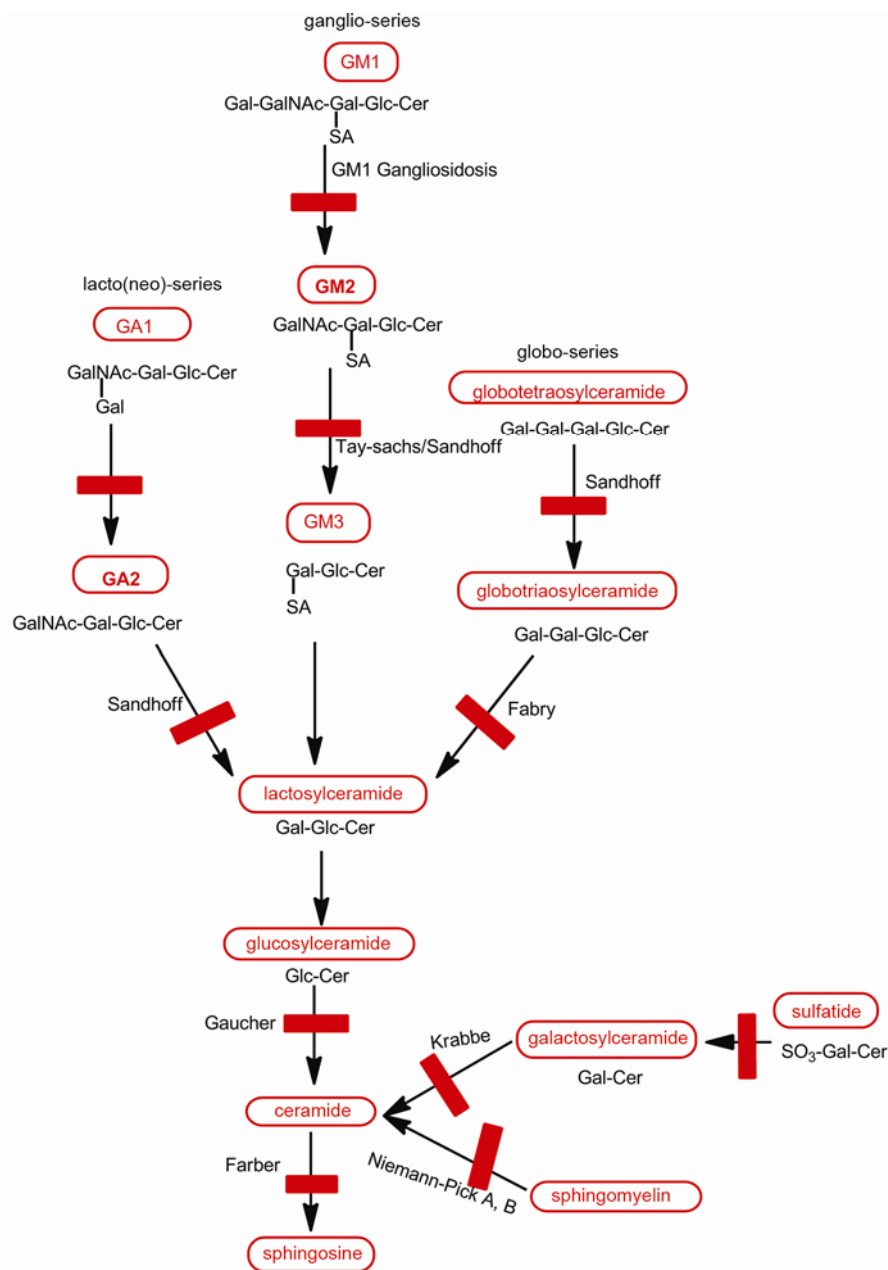


图2 鞘糖脂降解途径中发生的一些鞘糖脂病。Cer: 神经酰胺; GalNAc: 乙酰氨基半乳糖; Gal: 半乳糖; Glc: 葡萄糖; SA: 唾液酸

会消耗内质网内储存的钙离子, 导致蛋白合成受阻, 内质网内非折叠蛋白数量增多, 进而激活细胞内非折叠蛋白应答通路, 造成神经元细胞破坏^[11]。

3 溶酶体蓄积症的治疗策略

过去 20 年内, 有关溶酶体蓄积症治疗的研究取

得了激动人心的进展。如图 3 所示, 目前的治疗策略主要有 3 种: 酶替代疗法(enzyme replacement therapy, ERT)、底物减少疗法(substrate reduction therapy, SRT)和药理分子伴侣疗法(pharmacological chaperone therapy, PCT), 其中酶替代疗法和底物减少疗法已经被用于临床治疗。此外, 骨髓移植、基因及干细胞疗法等治疗策略也在研究中。

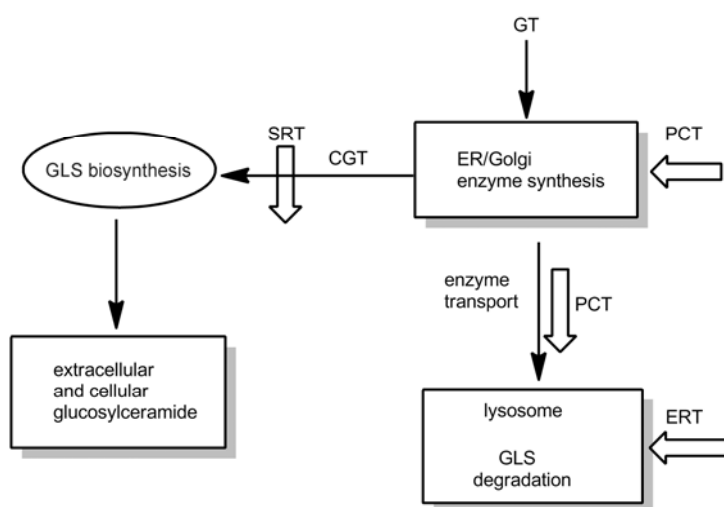


图3 溶酶体蓄积症治疗策略

3.1 以酶为基础的治疗

为病人补充缺失的酶以使鞘糖脂降解、从而减轻鞘糖脂病的各种症状已经被证明是一个有效的办法。1992年,重组 β -葡萄糖脑苷脂酶已经被批准用于高雪氏病的治疗,定期静脉注射这种重组酶能够有效缓解I型高雪氏病的各种症状^[24]。注射重组半乳糖苷酶治疗Fabry病也已在进行临床I/II期试验,已有的数据表明其可以缓解鞘糖脂代谢物在溶酶体内的蓄积^[25]。该疗法也被批准用于治疗I、VI型黏多糖症以及Pompe病等其他溶酶体蓄积症^[26]。然而酶替代疗法也有其局限性,重组酶无法透过血脑屏障,因而对神经系统症状无效^[27];另外,其价格比较昂贵。

通过骨髓移植来使病人体内产生能够合成正常酶的细胞,也是一种补充缺陷酶的方案。骨髓移植可以用于治疗溶酶体蓄积症首先在I型粘多糖病人身上得到证实^[28],它能够有效缓解各种临床症状;此外临床研究发现,骨髓移植疗法也能够改善II型和VI型粘多糖病、Krabbe病及其他一些溶酶体蓄积症的临床症状^[29]。

基因疗法目前尚处在实验室阶段,主要针对带有神经症状的溶酶体蓄积症。2004年,Ohashi小组^[30]将腺病毒重组的 β -半乳糖脑苷脂酶基因注射到小鼠的脑腔内,结果显示可以缓解Krabbe病的神经系统症状,使脑部细胞内 β -半乳糖脑苷脂酶含量明显上升,逆转了相关代谢物的积累过程。他们又将从人胎

儿分离出的细胞经过遗传工程处理为可以过表达 β -葡萄糖醛酸酶的细胞,并注入小鼠脑腔内,观察发现 β -葡萄糖醛酸酶被大量合成,广泛地清除了溶酶体内积蓄的酶底物。类似的试验在其他动物模型上也已经开展,并且取得了令人满意的结果^[31~34]。

3.2 氮杂糖在治疗溶酶体蓄积症中的应用

氮杂糖是糖环中氧原子被氮原子所取代的一类类糖化合物(糖模拟物),因其结构与糖类相似,因而可以被糖苷酶等识别^[35]。氮杂糖的糖苷酶抑制活性被广泛认知。脱氧野尻霉素(DNJ)系列化合物能够强烈地抑制 α -葡萄糖苷酶介导的N-聚糖蛋白糖基化。例如,NB-DNJ可以使HIV的N-聚糖gp120蛋白^[36]构象发生改变,从而阻断病毒的感染过程^[37]。由于这个化合物对 α -葡萄糖苷酶I的选择性较低,副作用较大,在完成二期临床试验后被迫停止^[38]。但是这个试验为后来NB-DNJ作为SRT药物的研究提供了至关重要的药物安全性信息。经过最近十几年的研究,将氮杂糖用于溶酶体蓄积症治疗的研究取得了很大的进展。

3.2.1 底物减少疗法

部分鞘糖脂病相关的突变酶仍残留有部分活性,只是不足以降解泵入溶酶体内的待降解底物,从而导致底物在溶酶体内蓄积,也就是疾病的病理取决于残留酶的活性与待降解底物的比例^[39]。基于这个前提,如果减少底物的合成以匹配溶酶体的底物降

解能力, 那么就可以缓解溶酶体蓄积症的各种临床症状. 由于一大部分鞘糖脂是在高尔基体表面葡萄糖神经酰胺转移酶(CGT)作用下将葡萄糖基连接到神经酰胺形成葡萄糖神经酰胺再经过多步酶催化反应得到, 那么抑制 CGT 就可以减少细胞内鞘糖脂的合成, 进而减少鞘糖脂代谢底物在溶酶内的蓄积, 使溶酶体蓄积症的各种症状得到缓解^[40]. 研究发现 *N*-烷基野尻霉素及 *N*-烷基半乳糖型野尻霉素能够强烈抑制葡萄糖神经酰胺转移酶(CGT), 从而抑制许多鞘糖脂的合成^[41]. 这个发现展示了氮杂糖在治疗溶酶

体蓄积症中的应用价值.

Butters 等^[42]发现具有葡萄糖及半乳糖立体化学, 同时氮原子上烷基侧链长度大于 3 的氮杂糖衍生物都能较好地抑制 CGT 的活性, 能够抑制鞘糖脂的合成. 表 1 列出了可能用于 SRT 的部分氮杂糖化合物; 其中 α -葡萄糖苷酶 I 抑制活性及 CGT 抑制活性主要通过体外酶实验得到^[42], 而细胞毒性试验则在 MDBK 细胞上进行评价^[43].

碳链的长短对氮杂糖的 CGT 抑制活性及细胞毒性影响很大, 其抑制活性在一定范围内随着碳链的

表 1 部分氮杂糖的酶抑制活性及细胞毒性结果

结构式	缩写名称	α -葡萄糖苷酶 I (IC ₅₀)	CGT (IC ₅₀)	细胞增殖 (CC ₅₀)
	DNJ	1.44 $\mu\text{mol/L}$	2 mmol/L 无抑制	>5 mmol/L
	DGJ	2 mmol/L 无抑制	2 mmol/L 无抑制	>10 mmol/L
	NB-DNJ	0.57 $\mu\text{mol/L}$	20.4 $\mu\text{mol/L}$	>10 mmol/L
	NB-DGJ	2.13 $\mu\text{mol/L}$	30 $\mu\text{mol/L}$	>10 mmol/L
	NN-DNJ	1.33 $\mu\text{mol/L}$	1.6 $\mu\text{mol/L}$	237 $\mu\text{mol/L}$
	NN-DGJ	13%抑制 (500 $\mu\text{mol/L}$)	10.6 $\mu\text{mol/L}$	237 $\mu\text{mol/L}$
	<i>N</i> -7-oxadecyl-DNJ	0.29 $\mu\text{mol/L}$	3.2 $\mu\text{mol/L}$	>5 mmol/L
	<i>N</i> -7-oxadecyl-DGJ	10%抑制 (500 $\mu\text{mol/L}$)	Nd	>5 mmol/L

增长而增大,但是当碳链增长至 18 个碳原子时,继续增长碳链已经不能改善其 CGT 抑制活性^[44]. 随后的研究发现,细胞实验的结果与酶实验结果存在一定的误差,在酶水平上 NN-DNJ、NN-DGJ 对 CGT 的抑制活性要优于 NB-DNJ 和 NB-DGJ,而细胞水平的评价结果恰恰相反. 其可能原因如图 4 所示^[45]. 在葡萄糖基加到神经酰胺的过程中,神经酰胺必须全部或部分地从磷脂双分子层中提取出来. CGT 是一个跨膜蛋白,其与神经酰胺有两个结合位点,其中结合位点 I 靠近酶的活性中心,可以将神经酰胺从磷脂双分子层中提取出来(图 4(a)). NB-DNJ 与神经酰胺竞争性地结合这个位点,阻断了神经酰胺从磷脂双分子层中提取的过程,进而抑制鞘糖脂的合成,(图 4(b)). NN-DNJ 则由于本身碳链较长,致使它与结合位点的结合需要经历一个从磷脂双分子层中提取的过程,(图 4(c)). 结合位点 II 则主要分布在磷脂双分子层中,它与神经酰胺结合,辅助其从磷脂双分子层中提取. *N*-烷基长链的氮杂糖抑制剂除了可以与神经酰胺竞争结合位点 I 之外,同时与神经酰胺竞争这个位点,阻断神经酰胺从磷脂双分子层中的提取,进而抑制鞘糖脂的合成. 因而长链氮杂糖(大于 5 个碳)可以同时与 CGT 的两个位点结合,因此在酶水平上更好地抑制 CGT,但由于在细胞水平上需要经历一个从磷脂双分子层中提取的过程,所以它会在细胞水平的实验中表现出明显的活性差异.

随着 *N*-烷基链的增长,氮杂糖的细胞毒性也随之增加. 这是因为随着碳链的增加,氮杂糖的疏水作用随之增加,更容易镶嵌在细胞膜上形成孔洞,造成细胞膜溶解^[46].

在这些化合物中, NB-DNJ(Miglustat)已经作为一个口服治疗溶酶体蓄积症的药物在临床上运用^[47],被用来治疗 I 和 III 型高雪氏病、GM1 和 GM3 型神经节苷脂病、Niemann-Pick 病等溶酶体蓄积症. 但是 NB-DNJ 缺乏选择性,同时对 α -葡萄糖苷酶也有较强的抑制作用,所以其副作用较为严重,因而只被推荐用于酶替代疗法不适用的病人^[48].

值得注意的是, NB-DGJ 在高雪氏病的组织培养模型^[49]、体内耐受性模型^[50]及山霍夫氏病的动物模型^[51]上的实验中显示了与 NB-DNJ 几乎相等的活性,它同样可以减少脑组织中的鞘糖脂的浓度^[51],延长实验动物的寿命^[52]. 但是与 NB-DNJ 相比, NB-DGJ 在这些试验中没有出现诸如肠胃不适、体重降低、内

质网糖苷酶抑制等副作用,表现了比 NB-DNJ 更好的选择性及耐受性.

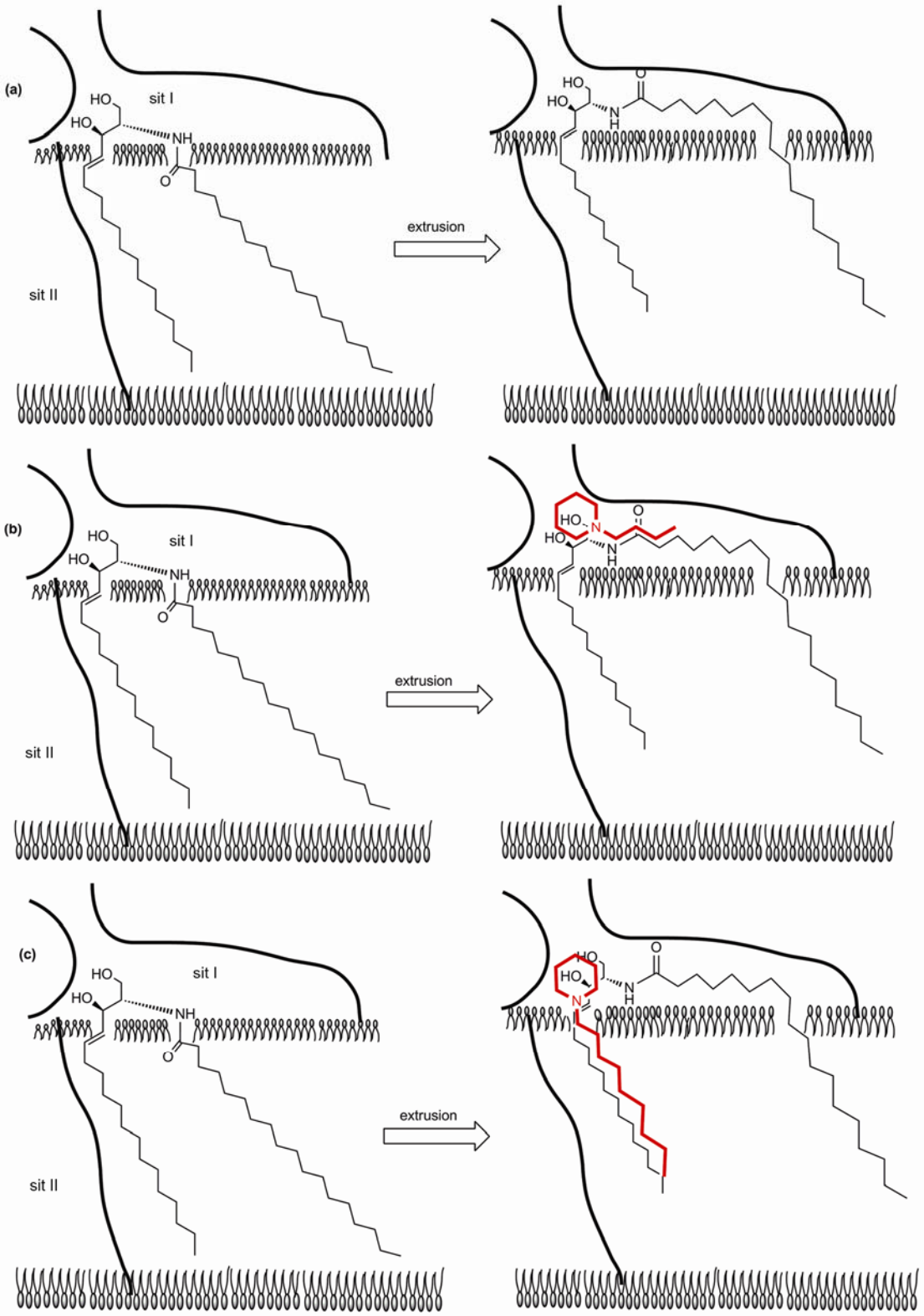
3.2.2 药理分子伴侣疗法

药理分子伴侣疗法(PCT)也被称为活性位点特异性分子伴侣疗法(active-site-specific chaperone therapy),是用小分子化合物作为口服药物治疗溶酶体蓄积症的一种新型治疗策略. 在溶酶体蓄积症中由于基因突变导致水解酶在内质网中不能正确折叠,从而被内质网质量控制机制降解^[53]. 但是这些突变酶可以与合适的小分子伴侣结合而帮助其部分恢复酶的活性,如果能够被转运到溶酶体中,它们依然能够发挥水解作用增加溶酶体的降解能力,缓解溶酶体蓄积症的临床症状. 研究发现,突变酶的竞争性抑制剂在亚抑制浓度范围内与突变酶结合,可以充当药理分子伴侣诱导并稳定突变酶形成合适构象,从而逃逸内质网质量控制机制,使突变酶能够被转运到溶酶体中,发挥酶的功能,缓解糖脂的蓄积,最后小分子与突变酶活性中心解离^[54]. 小分子抑制剂能够口服且可到达体内绝大部分组织包括中枢神经系统,因而药理分子伴侣疗法能够缓解神经系统症状,是一种有吸引力的治疗策略^[54].

小分子物质必须满足以下 3 个条件,才可以成为高效的药理分子伴侣: 1. 与目标蛋白活性位点有足够高的亲和力; 2. 足够的细胞通透性及恰当的亚细胞分布; 3. 能够从目标蛋白活性位点顺利解离.

Asano 等^[55]研究了氮杂糖对 α -半乳糖苷酶抑制能力及其对突变半乳糖苷酶活性增强能力之间的关系. 结果显示,对 α -半乳糖苷酶抑制能力越强,对突变半乳糖苷酶活性增强能力也越强. 很多氮杂糖抑制剂与酶的亲和力都非常高(10^{-6} ~ 10^{-9} mol/L),且其与酶活性位点之间的结合是可逆的,这为氮杂糖在 PCT 中的运用提供了依据.

2000 年, Butters 等^[56]发现,细胞器内的药物浓度是一个时间依赖性过程,在细胞内抑制内质网 α -葡萄糖苷酶所需要的 *N*-alkyl-DNJ 浓度比酶水平上实验所需要浓度要高出 100~1000 倍. *N*-烷基氮杂糖像一般的两亲分子一样通过触发的方式透过细胞膜进入细胞,持续时间通常少于 1 min^[57],但是进入内质网的难度较大,而且在内质网中的药物浓度难以维持. 这可能与氮杂糖进入内质网受限及具有多重耐药性机制的 P 蛋白有关. 在 2001 年, Morjani 等^[58]就



发现在多重耐药性细胞株中鞘糖脂的水平明显升高. 在鞘糖脂的分解再生过程中, P 蛋白是蛋白酶体中葡萄糖神经酰胺等一系列鞘糖脂代谢物的翻转酶^[59]. 由此可知, 葡萄糖神经酰胺能够被 P 蛋白识别, 那么作为其类似物 *N*-烷基氮杂糖也应该被 P 蛋白识别, 但是体外实验表明, *N*-烷基氮杂糖不能够和鞘糖脂竞争性结合 P 蛋白^[60]. 尽管看起来它们之间有明显的联系, 但是这种联系是复杂的, 具体的过程目前尚不清楚.

进入内质网的能力对于氮杂糖在 PCT 中的应用至关重要, 它决定了给药剂量及药物的实际疗效^[61]. 目前利用氮杂糖来增加突变酶的活性已经在实验中得到证实. 但是在这些实验中只有氮杂糖在胞质内浓度高达 5~500 $\mu\text{mol/L}$ 时, 在内质网中才能够达到有效浓度, 这种给药浓度显然不适合在人体上运用. 目前只有 NB-DNJ 在临床实验阶段取得了较好的效果, 每天口服 300 mg 可以在血浆中达到 6 $\mu\text{mol/L}$ 的浓度, 在细胞内达到 5 $\mu\text{mol/L}$ 的浓度, 同时在其有效浓度下也能够部分地抑制 CGT 活性, 因此其 PCT 临床研究取得了不错的结果^[62]. 当修正突变酶所需要的分子伴侣在内质网内达到的浓度高于 20 $\mu\text{mol/L}$ 时, 内质网就会主动地泵出腔内药物, 这样就会在一些药物富集的部位引起一系列的副反应. 在小鼠试验中 *N*-长链烷基的 DNJ 衍生物有更好的分子伴侣活性, 而且能够在肠胃内存留更长的时间^[63], 但同时也带来了生物膜破坏等一系列的毒副作用^[46].

氮杂糖可以应用于药理分子伴侣疗法首先在由 α -半乳糖苷酶 A 缺陷引起的 Fabry 病中被发现. 研究发现突变 α -半乳糖苷酶 A 的活性能够在亚抑制浓度下, 被不同种类的 α -半乳糖苷酶 A 抑制剂所增强, 其中也包括 1-脱氧半乳糖野尻霉素(DGJ)^[64]. 这个发现促使 FDA 批准了数个分子伴侣疗法应用于 Fabry 病的临床试验. Fabry 病的一期临床试验已经成功完成,

而且在临床试验中也没有报道有不良事件发生(www.amicustherapeutics.com). 随后药理分子伴侣疗法又被证明适用于高雪氏病^[63]、Tay-Sachs 病^[65]、Sandhoff 病^[65]和 GM1-神经节苷脂病^[66].

以高雪氏病为例, 目前寻找用于治疗的药理分子伴侣的研究主要集中于筛选 β -葡萄糖脑苷脂酶的抑制剂. 表 2 中列举了部分活性较好的氮杂糖类化合物结构, 它们对 β -葡萄糖脑苷脂酶的抑制活性(IC₅₀)和目前所处的临床阶段的数据. 表 2 中所列举的氮杂糖都能够增加突变酶的活性. 但是大部分氮杂糖化合物在内质网中达到有效浓度时, 其在胞内基质中的浓度已经超过阈值, 同时抑制了其他酶的活性^[67], 由此带来了一系列的副作用, 因此发展新型分子伴侣非常必要.

本课题组^[72]在寻找新型药理分子伴侣方面做出了努力. 我们设计合成了一系列 *N*-烷基内酰胺型氮杂糖, 其对正常细胞 GC 酶抑制的 IC₅₀ 全部大于 100 $\mu\text{mol/L}$, 大部分都超过 0.5 mmol/L(抑制活性很弱); 其中有 14 个化合物对最常见的 N370S 突变型葡萄糖脑苷脂酶活性的增强作用超过 2 倍, 共有 7 个化合物超过 3 倍(能够使突变酶活性增强到两倍以上即具有成为药理分子伴侣的潜力), 其中活性最好的化合物(图 5, 化合物 1)对突变酶活性的增强作用更是达到了 6 倍, 大大超过了目前在临床上应用的 NB-DNJ(其对酶的激活作用约为 2.3 倍^[53])及处于临床试验阶段的化合物 IFG(对酶的激活作用约为 3 倍^[72]). 通过初步的构效关系研究以及计算机辅助的与 GC 酶晶体结构的分子对接和能量优化实验, 我们发现羰基在稳定突变酶构象中起到了重要的作用, 同时羰基的存在对减小化合物的毒性也可能具有重要的意义. 最近, 我们又首次合成了 *N*-烷基内酰胺七元环型氮杂糖, 活性评价结果显示其对突变酶活性同样有增强作用, 其中活性最好的化合物(图 5, 化合物 2)对突变

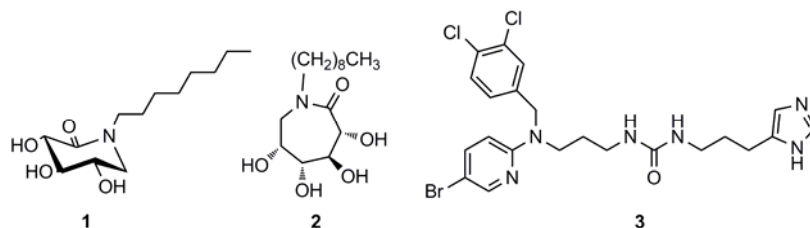
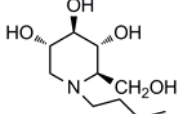
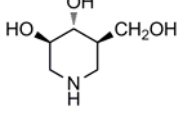
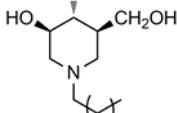
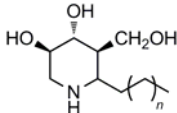
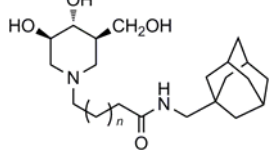
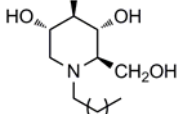
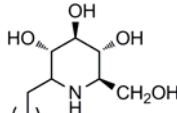
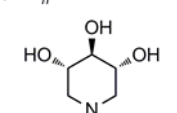
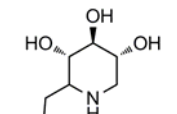
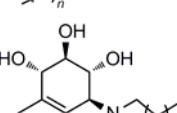


图 5 一些新型的药理分子伴侣

表 2 文献报道的部分可能用于高雪氏病药理分子伴侣疗法的氮杂糖

结构式	名称或缩写	对 β -GC 的 IC_{50}	临床阶段
	(NB-DNJ), miglustat Zavesca1	270 $\mu\text{mol/L}$ ^[68]	pre-clinical GD
	isofagomine (IFG)	40 nmol/L ^[54]	phase 1 GD
	<i>N</i> -alkyl-IFG, $n = 2, 7$	$n = 2$, 44 $\mu\text{mol/L}$ ^[54] ; $n = 7$, >100 $\mu\text{mol/L}$ ^[69]	pre-clinical GD
	6- <i>C</i> -alkyl-IFG, $n = 2, 4, 5, 6, 7$	$n = 2$, 160 $\mu\text{mol/L}$ ^[69] ; $n = 7$, 0.6 $\mu\text{mol/L}$ ^[69]	pre-clinical GD
	adamantyl amide IFG, $n = 1, 2, 3$	$n = 1$, 18 $\mu\text{mol/L}$ ^[53] ; $n = 2$, 11 $\mu\text{mol/L}$ ^[53] ; $n = 3$, 94 $\mu\text{mol/L}$ ^[53]	pre-clinical GD
	<i>N</i> -nonyl-deoxynojirimycin (NN-DNJ), $n = 7$	0.66 $\mu\text{mol/L}$ ^[70]	pre-clinical GD
	α -1- <i>C</i> -alkyl-deoxynojirimycin, $n = 2, 7$	$n = 2$, 100 $\mu\text{mol/L}$ ^[70] ; $n = 7$, 0.27 $\mu\text{mol/L}$ ^[70]	pre-clinical GD
	1,5-dideoxy-iminoxylitol (DIX)	2.3 $\mu\text{mol/L}$ ^[54]	pre-clinical GD
	α -1- <i>C</i> -alkyl-DIX, $n = 2, 7$	$n = 7$, 2.2 $\mu\text{mol/L}$ ^[70]	pre-clinical GD
	<i>N</i> -alkyl- β -valienamine, $n = 6, 10, 12$	$n = 6$, 0.502 $\mu\text{mol/L}$ ^[71] ; $n = 10$, 0.085 $\mu\text{mol/L}$ ^[71] ; $n = 12$, 0.093 $\mu\text{mol/L}$ ^[71]	pre-clinical GD

酶活性的增强效果达到了 2.4 倍^[73]。

最近，有人用高通量筛选方法获得了非氮杂糖类新型药理分子伴侣。例如，图 5 中化合物 **3** 就是通过此途径得到的，它对正常葡萄糖脑苷脂酶没有抑制活性，但它同样能够增加突变葡萄糖脑苷脂酶的活性^[74]。

4 总结与展望

溶酶体蓄积症由溶酶体内参与鞘糖脂降解的酶或蛋白因子活性缺失导致代谢底物或者一些糖缀合物蓄积而引起。目前的治疗策略主要有酶替代疗法(ERT)、底物减少疗法(SRT)、药理分子伴侣疗法(PCT)等。*N*-烷基氮杂糖因为能够抑制葡萄糖神经酰胺转移酶(CGT)从而减少鞘糖脂合成, 匹配溶酶体降解能力。同时, 一些氮杂糖能够作为突变酶的分子伴侣辅

助其正确折叠并稳定其构象从而恢复酶的活性, 这是氮杂糖能够用于治疗溶酶体蓄积症的基础。各种类型的氮杂糖被合成并且评价其在治疗溶酶体蓄积症中的应用潜力, 其中 NB-DNJ 通过了临床试验, 成功地用于临床治疗各种溶酶体蓄积症。但是随着 NB-DNJ 的应用, 各种副作用的报道逐渐增多。寻找具有高选择性、高亲和力的能够用于 SRT、PCT 的新氮杂糖化合物应用于溶酶体蓄积症的治疗, 仍将会是研究的热点课题。

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Iminosugars for the treatment of lysosomal storage disorders

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Abstract: The metabolic disorders of glycosphingolipid (GSL) are a relatively rare group of inherited diseases that have diverse and often neurodegenerative symptoms. And the lysosomal storage disorder is typical one of these diseases. It is caused by lysosomal storage of GSL substrates or several other glycoconjugates, due to deficiency in the activity of enzymes or protein factors involved in the degradation of glycosphingolipids in the lysosome. Now the main treatment strategy for these diseases is enzyme replacement therapy (ERT) which uses direct infusion of the recombinant enzyme into patients. However, the inherent defects of this strategy, for example, the recombinant enzymes cannot pass the blood-brain barrier, limit its application. So the second strategy called substrate reduction therapy (SRT), which is involved in reducing the synthesis of glycosphingolipids to match the degradation activity of lysosome, is proposed. N-Alkylated iminosugars were thought to be used in SRT for its inhibition of the key enzyme, ceramide glucosyltransferase (CGT), in GSL biosynthesis. Many iminosugars were designed and synthesized for improving their inhibitory potency, bioavailability, enzyme selectivity, and biological safety. After a successful clinical evaluation, one compound, namely *N*-butyl-deoxynojirimycin (NB-DNJ), has been used in the clinical treatment. On the other hand, iminosugars as chemical chaperones, can assist enzyme folding and stabilize the conformation of mutant enzymes to rescue their activity. This feature makes the pharmacological chaperone therapy (PCT) as an alternative novel therapeutic strategy for the treatment of lysosomal storage disorders. Given the ability of small molecules to be orally available, to penetrate the central nervous system (CNS), and to have well-characterized pharmacological properties, the iminosugars are increasingly used in the treatment of lysosomal storage disorders, and would have a bright future.

Keywords: lysosomal storage disorders, iminosugars, substrate reduction therapy, pharmacological chaperone therapy