评述

内皮型一氧化氮合酶与糖尿病肾病的关系研究

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摘要 糖尿病肾病(diabetic nephropathy, DN)是糖尿病的主要重症并发症之一, 是导致晚期肾病的头号因素, 也是糖尿病高致死率的主要因素, 在全球造成了严重的经济和健康问题。内皮型一氧化氮合酶(endothelial nitric oxide synthase, eNOS)主要表达于血管内皮细胞, 可催化底物L-精氨酸生成一氧化氮(nitric oxide, NO), 发挥各种生物学效应。本文从eNOS结构及其活性、eNOS与肾脏发育、eNOS与DN的发生发展、eNOS与DN的治疗4大方面进行了系统阐述, 以期进一步明确eNOS的作用范围及探索DN防治新思路。

关键词 内皮型一氧化氮合酶, 糖尿病肾病, 一氧化氮

糖尿病肾病(diabetic nephropathy, DN)是由遗传与环境因素相互作用的严重代谢性疾病并发症^[1], 是主要的糖尿病(diabetes mellites, DM)微血管并发症之一, 是DM高致死率以及导致晚期肾病的主要因素^[2], 在全球透析疾病中占据了高达35%~40%的比率^[3]。Huang等人^[4]报道指出, DM患者中约有20%~30%的人患有严重的肾损伤, 其肾小球滤过率明显低于60 mL/min, 在全球造成了严重的健康问题。

一氧化氮(nitric oxide, NO)在1987年被正式揭示为一种血管舒张因子^[5,6], 具有舒张血管、调节血压血流、血管重塑及血管平滑肌增生、抑制多种缩血管因子、抗血小板黏附聚集、参与神经调节和内分泌调节等重要生理功能^[7,8], 由一氧化氮合酶(nitric oxide synthase, NOS)作用于底物L-精氨酸生成。NOS由神经

型NOS(neuronal NOS, nNOS)、诱导型NOS(inducible NOS, iNOS)和内皮型NOS(endothelial NOS, eNOS)三种类型组成, 分别又叫作NOS1, NOS2, NOS3^[9~11]。其中, nNOS表达于神经和骨骼肌中, 产生的NO可作为细胞信号分子; iNOS表达于免疫细胞中, 其产生的NO可作为细胞毒性自由基前体物质防止病菌的入侵; eNOS则在血管内皮细胞中表达, 通过产生NO发挥调节血管的功能^[12]。eNOS可通过产生NO改善氧化损伤的视网膜血管及肾血管等微血管功能^[13~15], 在DN中发挥重要的调节作用。然而, 目前关于eNOS与DN相关性的系统报道比较缺乏, 本文将从eNOS结构与活性、eNOS与肾脏发育、eNOS与DN的相互关系进行陈述, 以期进一步明确eNOS的作用范围及探索DN防治新思路。

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1 eNOS结构及活性

1.1 eNOS结构

eNOS基因位于7号(7q35~q36)染色体上, 有26个外显子, 核苷酸链长度为21 kb^[16], 在功能结构上属于同(源)二聚体。每个单体由N-终端加氧酶结构域和C-终端还原酶结构域经中央钙调蛋白(calmodulin, CaM)结合序列连接而成^[17,18]。其中, N-端含有与四氢生物蝶呤(tetrahydrobiopterin, BH4)、血红素和L-精氨酸结合的位点; C-端含有与黄素单核苷酸(flavin mononucleotide, FMN)、黄素腺嘌呤二核苷酸(flavin adenine dinucleotide, FAD)以及烟酰胺腺嘌呤二核苷酸(nicotinamide adenine dinucleotide, NADPH)结合的位点^[19]。

1.2 eNOS活性

在人类eNOS序列中, 当NADPH结合于eNOS还原酶结构域后, 其衍生的电子传递到该结构域中的FAD中, 随后至FMN, 最后传递至加氧酶结构域中的亚铁血红素中, 亚铁血红素铁在BH4的辅助下结合O₂并催化L-精氨酸逐步合成NO^[12,20]。该电子传递过程发生于eNOS两个单体之间, 故eNOS二聚体成为了电子传递偶联的结构前提。

eNOS功能活性的调节可从维持二聚体结构或调节电子从还原酶区域传递至加氧酶区域的整个偶联过程两条途径得以实现^[12]。其中, 辅酶BH4、Ca²⁺/CaM以及磷酸化修饰对eNOS活性起关键的调节作用。

(1) eNOS与BH4。BH4是eNOS的重要辅助因子, 其结合发生在亚铁血红素与二聚体的交界面上^[21], 可以使eNOS的稳定性提高, 并使之与底物L-精氨酸的结合力增强^[22]。此外, BH4还可调节亚铁血红素辅基的氧化还原反应^[12]。墨蝶呤脱氢酶是一种稳定的BH4前体物质, 补充墨蝶呤脱氢酶会使视网膜中的BH4含量增多, 并且可以增强内皮中eNOS的修复能力以及抵御氧化损伤的能力^[23]。

辅助因子BH4的生物活性是导致eNOS失偶联的主要因素之一。离体研究发现, 当视网膜血管中的eNOS辅助因子BH4被过氧化物消耗过多时, 会导致eNOS解偶联并催化产生大量O²⁻而非NO^[14,24]。而当补充含有BH4的体外组织匀浆后, eNOS的功能又恢复正常并催化产生NO^[14]。同时, 促红细胞生成素也能增强BH4的生物活性, 避免由于eNOS失偶联诱发的氧化应

激损伤的出现^[25,26]。作为超氧阴离子与NO相互作用后的产物之一, 活性氧(reactive oxygen species, ROS)与过氧硝酸盐可诱导BH4降解及eNOS失偶联, 进而减少内皮型NO的产生, 最终影响血管舒张和内皮细胞增殖^[27]。

(2) eNOS与Ca²⁺/CaM(图1)。在没有任何修饰的情况下, eNOS二聚体是没有活性的, 这是由于还原酶结构域中存在两种可调节N端和C端之间偶联过程的自动抑制区域^[28,29]。抑制区可与两个结构域之间的可变链接区相互作用, 进而阻止中央区与CaM结合。由于CaM只有与Ca²⁺形成复合物后才能与eNOS结合, 因此eNOS的激活需依赖于细胞内的Ca²⁺浓度^[12]。Siragusa和Fleming^[20]进一步指出, eNOS的活性可依细胞内Ca²⁺浓度及CaM与Ca²⁺的结合状态而不同。细胞内Ca²⁺水平提高后, Ca²⁺与CaM结合形成复合物, 复合物与eNOS中央结构域结合, 使eNOS发生构象变化并激活, 使电子从还原结构域传递至氧化结构域, 进而催化NO的生成^[30,31]。内皮细胞凹陷小窝内的三种小窝蛋白与Ca²⁺/CaM依赖性的eNOS交互作用后可调节改变eNOS的功能活性^[32]。小窝蛋白-1可直接与eNOS中的CaM结合区域连接, 进而使eNOS激活受阻, 影响NO生成^[33]。Feron等人^[34]研究发现, 当eNOS与小窝蛋白-1结合后

图1 Ca²⁺/CaM与eNOS活性(网络版彩图)
Figure 1 Ca²⁺/CaM and the activity of eNOS (color online)

其酶的活性会减弱, 而当两者解离后eNOS功能增强。

(3) eNOS磷酸化(图2)。蛋白质磷酸化修饰对eNOS活性调控起着非常重要的作用^[35]。目前已知的对eNOS功能起关键作用的磷酸化位点包括Y81, S615, S633, S1177, S114, T495以及Y657, T497, Ser635等, 其中Y81, S114, Y657磷酸位点位于eNOS加氧酶结构域, T495位于中央钙调蛋白结合区域, 而S615, S633, S1177位于氧化还原酶区域^[12]。位点磷酸化修饰通过多种多样的激酶途径影响eNOS活性, 且不同的磷酸化位点对eNOS活性的作用不同。其中, S615和S1177磷酸化可通过干扰还原酶结构域中的自动抑制区的活动而S633磷酸化可通过调节eNOS与CaM的依赖关系, 以增强eNOS活性, 促进NO生成。以上三个位点主要通过蛋白激酶B(protein kinase B, PKB)、蛋白激酶A(protein kinase A, PKA)、腺苷酸活化蛋白激酶(adenosine monophosphate activated protein kinase, AMPK)、蛋白激酶G(protein kinase G, PKG)、CaM等被磷酸化。而S114, Y657和T495三个位点主要通过PKC、AMPK、丙酮酸激酶2(pyruvate kinase 2, PYK2)实现磷酸化, 且该三个位点的磷酸化已经被证实可抑制eNOS活性影响NO生成^[12]。Ser635是eNOS另一个非常重要的磷酸化位点, 可增强eNOS活性, 但在促使其磷酸化的激酶方面却存在争议。此外, 由PKC激活的Thr497位点磷酸化会使eNOS活性降低^[28,36]。

除了以上三大因素可影响eNOS活性外, ROS、血管紧张素II、非对称二甲基精氨酸、糖基化终末产物(advanced glycation end products, AGEs)、维生素D以及肿瘤坏死因子- α (tumor necrosis factor- α , TNF- α)等都参与了eNOS的调节^[37]。

2 eNOS与肾脏发育

eNOS分子主要表达在肾小球毛细血管、入球和出球小动脉、肾内小动脉等血管的内皮细胞中^[38]。eNOS释放基础量的NO可调节正常的血管张力及血栓阻力, 维持内皮的稳态^[39], 进而调节肾小球滤过系数和肾髓质血流。

eNOS在胚胎期小鼠肾脏的发育阶段具有重要作用。包翠芬等人^[40]通过免疫组化及免疫印迹法发现, eNOS在14天胎龄小鼠肾脏开始呈阳性表达, 随后含量逐渐增多, 并于出生时含量最高; 同时, eNOS在胚胎期

图2 eNOS磷酸化与eNOS活性(网络版彩图)

Figure 2 Phosphorylation of eNOS and its activity (color online)

肾脏的表达部位最早开始于生肾区并在此区逐渐减少, 随后表达于近端小管及远端小管并在此区逐渐增多, 出生时表达至最高峰^[40]。此外, Han等人^[39]对胎龄为14、16、18、20天的胚胎鼠肾脏及出生后1、3、7、14及21天的新生幼鼠肾脏进行免疫细胞化学和免疫印迹分析发现, eNOS在调节未成熟肾脏血流动力学方面扮演着关键角色。

胚胎小鼠低肾血流量、低肾小球滤过率是促使eNOS表达的主要因素, 而细胞自身生长发育与增殖、母体环境及肾脏血流动力学也与之有关; 围产期缺氧情况下, NO是维持肾血流量, 保护肾免受损伤的关键^[40]。此外, 胚胎及新生动物eNOS表达受母体雌激素影响^[41]。在培养的内皮细胞中应激刺激、氧张力、转化生长因子 β 1(transforming growth factor β 1, TGF β 1)、PKC、TNF- α 、溶血磷脂胆碱和细胞增殖程度, 均可通过调节eNOS基因转录及其mRNA的稳定性, 影响eNOS表达水平^[42]。

3 eNOS与DN

3.1 DN

DN与遗传因素、高糖引发的代谢紊乱、氧化应激、AGEs、炎症反应以及eNOS异常等有关。肾小球

内皮及附着的多糖蛋白复合物受损会改变肾小球滤过膜的通透性, 并导致DN发生^[1]。DN早期典型的病理表现包括肾小球肥大、肾小球基底膜增厚、肾小球系膜扩张增宽; 而后期则主要表现为肾小球系膜溶解、肾小球微动脉瘤或结节样变(Kimmelstiel-Wilson nodule)^[43,44]。此外, 在DN发生发展的各阶段蛋白尿呈进行性增多, 其中DN最早的症状是微量蛋白尿(>30 mg/d)伴有肾小球高滤过, 随着病情发展为大量蛋白尿(>300 mg/d)并出现肾小球滤过减少, 最终出现终末期肾病^[45]。

3.2 eNOS低表达与DN

eNOS耗损会加速糖尿病鼠并发DN, 尤其是中晚期DN^[46]。研究人员在电子显微镜下发现, eNOS敲除的糖尿病鼠出现了以系膜间胶原蛋白IV沉积、系膜细胞及细胞外基质增多为主要表现的系膜扩张, 致密层增厚, 内皮下腔隙增大, 肾小球毛细血管内皮细胞形态异常、胞核增大、纤维沉积, 肾小球基底膜变宽, 最终造成肾小球肥大及高血压、蛋白尿、肾小球系膜基质扩张以及Kimmelstiel-Wilson结节^[47], 且其在肾脏结构改变和功能损伤方面没有性别差异^[48]。此外, 低灌注则会导致肾小球缺血, 近球细胞肾素释放进而促使高血压的形成, 促进DN形成^[49]。

DN本质上是一种糖尿病微血管并发症, 其发生发展与内皮功能紊乱密切相关^[1]。微血管内皮功能受到血管内皮生长因子(vascular endothelial growth factor, VEGF)、内皮素(endothelin, ET)、血管生成素及NO等的共同调节^[50]。VEGF是一种关键的血管细胞生成因子, 可刺激内皮细胞增殖和游移, 而其作用的发挥离不开NO的正常生成^[50]。VEGF-A可通过磷肌醇-3-激酶/PKB使还原酶结构域中S615位点磷酸化, 进而激活eNOS以催化生成NO, NO激活鸟苷酸环化酶以发挥舒张血管的作用, 而NO又可负反馈调节VEGF诱导的内皮细胞增殖, 即VEGF-A到NO下游信号之间的偶联在维持肾小球内皮完整性方面发挥重要作用^[51]。Nakagawa等人^[47]发现, 在DN动物实验中, eNOS敲除鼠比对照鼠表现出更为明显的肾脏肥大及蛋白尿增多、系膜扩张, VEGF不再起肾脏保护作用, 反而加剧其病情进展。大量研究指出, DM患者体内NO生物活性大大降低^[52,53], 且伴有eNOS表达下调及活性降低^[54]。而Tufro和Veron^[55]发现, 表现为低NO生物活性的糖尿病患者

往往伴有高水平的VEGF-A。Nakagawa等人称这一现象为“VEGF-NO失偶联”, 并在机制上将VEGF与NO的存在情况与晚期肾病联系起来^[47], 并指出高血糖可能是引起VEGF-NO失偶联的主要原因^[56], 而胰岛素可很好地阻断eNOS敲除DM鼠中VEGF的过表达^[47]。研究报道进一步指出, eNOS基因敲除小鼠可并发DN, 具体表现为结节性肾小球硬化、肾小球滤过率降低、高血压以及VEGF mRNA在肾脏的过度表达^[47]。而补充L-精氨酸和墨蝶呤后, 糖尿病鼠蛋白尿和肾小球基底膜变厚情况得到改善, 并伴有eNOS二聚体结构逆转和磷酸化, 这一现象表明, 提高eNOS活性可以延缓DN的进程^[57]。

eNOS减少或缺乏除了与VRGF相互作用参与DN的发生发展外, 还可能通过炎症通路、肾素-血管紧张素-醛固酮系统等发挥作用。eNOS及NO减少可导致VEGF过量表达, 进而引发机体出现炎症状态、巨噬细胞浸润和内皮细胞异常增殖, 促发DN^[58]。与此同时, eNOS缺乏同时伴随高脂饮食还可通过刺激小鼠NF- κ B通路, 加剧炎症反应, 上调组织因子进而促发血液凝固, 最终加剧DN的发展^[59]。此外, 实验证实eNOS(-/-) db/db小鼠会迅速出现DN症状, 而当给与小鼠血管紧张素转换酶抑制剂, 即肾素-血管紧张素系统抑制剂后, DN小鼠的蛋白尿、肾小球硬化等症状明显改善^[60]。另一实验给eNOS基因敲除的DN小鼠补充醛固酮受体阻断剂, 明显改善了其DN症状, 且效果优于单纯的肾素-血管紧张素系统抑制剂^[61]。随后, Tesch等人^[62]通过动物实验报道称, 给eNOS(-/-)所致的DN小鼠联合补充肾素-血管紧张素系统抑制剂和趋化因子受体2(chemokine (C-C motif) receptor 2, CCR2)抑制剂可明显改善巨噬细胞聚集所致炎症损伤、肾小球硬化等DN症状。由以上三个实验可推论出, eNOS可能通过与功能亢进的肾素-血管紧张素-醛固酮系统相互作用, 参与DN的发生发展。此外, 糖尿病高血糖环境可诱导p-AMPK水平降低、AMPK活性减弱, 进而下调eNOS的表达, 导致细胞外基质在肾脏的聚积, 进而引发DN^[63]。

此外, DN的发生发展与足突细胞凋亡有关^[1]。足突细胞是肾小球滤过膜的重要组成部分, 而足突细胞减少是肾小球早期形态改变的表现之一, 参与DN的发生发展过程^[1,64]。高糖环境下, ROS释放增多, 激活聚腺苷二磷酸-核糖聚合酶作用于足突细胞使其凋亡脱落, 而足突细胞减少后无法被其他物质替代, 裸露出来的

部分基底膜只能由存活的足突细胞通过改变自身大小及形状来覆盖^[65]。研究指出, eNOS不足会诱导足突细胞减少, 而足突细胞减少会导致VEGF缺乏, 从而导致毛细血管塌陷^[66]。

3.3 eNOS过表达与DN

以上大量动物实验证实, eNOS缺乏的DM鼠容易迅速发展成为中晚期DN, 然而, 单纯使eNOS在肾脏过表达却未必能改善DN。糖尿病肾小球内皮过表达伴随高水平的过氧化物会导致eNOS失偶联, 二聚体减少使得eNOS成为过氧化物的主要来源, 参与DN的发生。高糖环境下, 大量的ROS会导致BH4氧化并使eNOS失偶联, 而eNOS失偶联又会进一步加剧细胞内过氧化物的产生并使NO生物活性降低^[14], 形成恶性循环(图3)。因此, 在eNOS过表达的情况下, 要使其发挥积极的调节作用需要一些辅助因子, 比如提高内皮细胞内BH4水平或促红细胞生成素并减少过氧化物生成。

此外, 有文献指出, eNOS在早期DN患者体内水平升高, 而随着病情发展eNOS及其产生的NO降低, 并最终导致晚期DN的发生^[46], 即eNOS在DN的不同发展期其表达和影响不一样。穆长征等人^[67]通过动物实验进一步指出, DM鼠第2周开始出现NO含量及eNOS表达明显增高, 并伴有肾小球滤过率增高。eNOS表达增多、NO生成及活性提高, 可使肾入球小动脉、肾小球毛细血管等舒张, 肾小球滤过率大大增高, 从而参与DN早期病变的发生。

3.4 eNOS基因多态性与DN

eNOS基因多态性与终末期肾病有关。对于包括DN在内的慢性疾病而言, 基因图谱是主要的不可变危险因素^[68]。基因变异可减少eNOS产生进而使NO生成减少, 损害血管内皮功能及血管舒缩调节功能, 引起肾功能恶化, 是肾病包括DN病情进展的主要因素之一^[69]。一项荟萃分析指出, 在与DN相关的16个基因中鉴定发现24种基因变异体^[70]。其中rs891512, rs1799983, rs2070744和rs869109213基因多态性可作为DM的基因标志物^[71], 而基因G894T错义突变(rs1799983)、第4内含子的一个27 bp副本以及启动子的T786C单核苷酸多态性(rs2070744)这三种基因多态性与DN易感性密切相关^[72]。其中, G894T(rs1799983)是与2型DN有关的最常见的基因多态性表现^[73], 其可

引起第7外显子出现错义置换进而调节eNOS的活性^[73]。案例对照实验表明, eNOS-rs1799983氧化应激相关多态性对马来西亚2型DN的发生有着重要的影响^[72]。eNOS基因第7外显子G894T多态性位点与血液NO水平和血管反应性下降有关^[74]。而关于eNOS第7外显子的G894T(Glu298Asp)变异与DN的关联性研究显示出一定的差异性^[75]。程洁等人^[75]提出eNOS基因区两个主要变异: 第7外显子上298位的Glu-Asp的错义、第4内含子上的a/b变异; 并进一步指出, 在汉族人群中, a等位基因可能通过影响eNOS基因表达使NO生成减少, 增加DN易感性。

4 eNOS与DN治疗

当前, 研究人员通过药物及基因工程技术在调节eNOS表达量并最终防治DN(尤其是中晚期DN)的发生发展方面进行了大量研究。Oe等人^[76]发现, 给eNOS基因敲除并伴有VEGF抑制剂诱导的DN小鼠补充蛋白激酶激活受体2可改善其肾小球内皮和足突细胞损伤。实验报道, 给eNOS基因敲除的DN鼠补充肾素-血管紧张素系统抑制剂后可明显减轻其蛋白尿、肾小球硬化、小管间质损伤等DN症状, 减缓DN的进一步发展^[60]。此外, 实验指出, 400 mg/(kg·d)川陈皮素的补充可明显提高大鼠肾脏组织中p-AMPK, AMPK和eNOS的表达, 提高机体的抗氧化能力, 进而改善DN^[77]。Piperazine ferulate(PF)可上调肾小球内皮细胞eNOS表达及活性, 降低血液肌酐及尿素氮水平, 保护肾脏并延缓DN的发生^[2]。维生素D可上调ET-1以及ET-B受体mRNA表达以及eNOS mRNA表达, 进而使eNOS在Swiss Webster鼠肾脏的表达增多^[78], 这一结果证实了ET与NO共同调节动脉血管张力, 维持血管稳态的重要作用。锻炼与eNOS在肾脏的表达之间也有联系。例如, Miyauchi等人^[79]研究发现, 参与锻炼的小鼠肾脏eNOS活性降低而iNOS活性却保持不变, 而Tastekin等人^[80]却指出, 锻炼和高蛋白饮食对eNOS在肾脏的表达几乎无影响, 但却可以上调iNOS在肾脏的表达。此外, 补充外源性H₂S连同三基因疗法(CBS/CSE/3MST)可使eNOS脱同型半胱氨酸并下调小窝蛋白-1, 最终提高eNOS功能活性改善肾损伤^[32]。Huang等人^[81]提出, 白藜芦醇可通过激活PKB/eNOS/NO通路以及Sirt1/ER通路而改善DM引发的血管病变。非诺贝特可通过氧

图 3 eNOS与DN发生发展(网络版彩图)
Figure 3 eNOS and the occurrence and development of DN (color online)

化酶体增殖激活受体 α (peroxisome proliferator-activated receptors α , PPAR α)及AMPK α 相关的信号通路,促进eNOS磷酸化,抑制氧化应激以发挥NO的生物活性,减少DM肾脏损伤标志物,平衡血管内皮依赖性的

收缩和舒张功能,改善DM小鼠入球小动脉内皮依赖性舒张反应(endothelium-dependent relaxation, EDR)及肾功能紊乱并降低血脂^[82].此外,L-精氨酸-NO途径对肾脏的作用引发了科研热潮,口服L-精氨酸延缓慢性肾功能衰竭已经入临床实践阶段.研究指出,将eNOS基因传递给肾脏占位鼠,使其eNOS表达上调,NO释放增多,阻止肾功能的进一步衰退^[83],也不失为一种防治措施(表1).

5 总结

DN是由遗传与环境因素相互作用的疾病^[1],是糖尿病微血管并发症之一,是导致终末期肾病的头号原因^[2].DN的病理改变主要涉及肾小球内皮细胞、基底膜、足突细胞、肾小球系膜等.eNOS活性与其重要辅助因子BH4、不同位点的磷酸化、及Ca²⁺/CaM密切相关.eNOS参与了胚胎期肾脏的发育.生理量的eNOS可在辅助因子BH4帮助下催化精氨酸生成NO,NO可与VEGF、ET-1等共同维持血管内皮稳态;而糖尿病高糖环境下,eNOS可出现各种失偶联从而参与DN的发生发展.eNOS减少或生物活性降低可导致VEGF-NO失偶联,从而失去两者偶联状态下对血管内皮功能的维稳作用,也可通过肾素-血管紧张素-醛固酮系统、炎症通路等导致内皮细胞过度增殖、肾小球肥大、蛋白尿增多、肾小球硬化等,促进DN发生发展.eNOS基因多态性与DN易感性尤其是终末期肾病相关.在DN的防治方面,尤其是DN中晚期eNOS缺乏或过低时,

表 1 eNOS与DN治疗

Table 1 eNOS and the treatment of DN

干预因素	干预机制及效果
蛋白激酶激活受体2	改善eNOS缺乏状态下肾小球内皮和足突细胞损伤
肾素-血管紧张素抑制剂	抑制eNOS缺乏状态下功能亢进的肾素-血管紧张素系统活性,改善DN
川陈皮素	上调p-AMPK/AMPK/eNOS表达,提高抗氧化能力,改善DN
Opiprazine	上调NOS3表达及活性,保护肾脏及延缓DN发展
维生素D	内皮素1与内皮素B受体结合/eNOS信使RNA上调/NO增多,血管舒张
外源性H2S+三基因疗法(CBS/CSE/3MST)	eNOS脱同型半胱氨酸/下调小窝蛋白-1,提高eNOS活性改善肾损伤
白藜芦醇	VEGF-B, p-AMPK及eNOS磷酸化增强;激活PKB/eNOS/NO通路,改善糖尿病血管病变
非诺贝特	LKB1/AMPK/eNOS磷酸化/NO增多,抑制氧化应激,改善肾脏血管内皮功能
口服L-精氨酸	增加eNOS底物,NO增多,延缓DN
eNOS基因传递	eNOS表达上调,NO增多,延缓DN

可通过各种药物制剂上调肾小球内皮细胞eNOS表达及活性, 并适当补充BH4及L-精氨酸以防eNOS脱偶联, 必要时直接进行eNOS基因传递以防止DN的发生发展. 然而需要注意的是, eNOS在内皮细胞过表达反而可能会加剧DN的发展, 这其中机制很可能是其辅因子BH4不足导致eNOS失偶联及超氧化物水平升高所致.

因此, 虽然大量研究证实了eNOS与DN关联很大, 但要使其在临床使用中发挥积极作用还存在很多困难, 比如在DN的不同发展阶段不同程度地上调eNOS的表达可能会导致完全相反的结果. 要找到能够有效改善DN的eNOS表达量, 进而推广eNOS用于改善DN的治疗方案, 还有待进一步的研究, 以保证其安全有效.

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A review of endothelial nitric oxide synthase and diabetic nephropathy

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Diabetic nephropathy (DN), one of the main complications of diabetic mellitus (DM), is the major factor that causes advanced end-stage renal disease and also leads to the high mortality of DM. Endothelial nitric oxide synthase (eNOS), mainly expressed in vascular endothelial cells, catalyzes the formation of nitric oxide (NO) from substrate L-arginine, which is closely related to DN. In this article, the structure and activity of eNOS, the relationship between eNOS and kidney development, the relationship between eNOS and occurrence and development of DN, and the association between eNOS and the treatment of DN are reviewed.

endothelial nitric oxide synthase, diabetic nephropathy, nitric oxide

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