

· 实验研究 ·

H-1152 对兔眼眼压及小梁组织超微结构的影响

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The effect of H-1152 on IOP and trabecular meshwork structure in rabbit

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Abstract Objective Glaucoma is an optic neuropathy caused by elevated intraocular pressure (IOP). To low IOP is still the major therapeutic approach to this disease. Our study was designed to investigate the effects of Rho-kinase inhibitor, H-1152, on IOP and ultrastructure of trabecular meshwork in rabbits. **Methods** 50 μ L of 10 mmol/L H-1152 was topically administered in the right eyes of 6 New Zealand white rabbits and the equal amount of physiology balance solution (PBS) was used at the same way in the left eyes as control. IOP was measured before and after the topical administration of H-1152 using pneumatometer. The ocular inflammatory manifestation was examined by the slit-lamp for the evaluation of adverse effects of H-1152. The morphologic change of rabbits trabecular meshwork was examined under the light and transmission electron microscopy. **Results** The IOP was significantly declined from 1 hour through 6 hours after application of 10 mmol/L (50 μ L) H-1152, showing a statistically significant difference in comparison with control group ($P < 0.05$). The maximum decrease scope of IOP was 50.6% at 2 hours after the topical administration of H-1152. Compared with the control groups, IOP reduction was the most dominant in the duration of 1 hour and 3 hours after H-1152 administration. No visible structural alteration was seen in trabecular meshwork tissue under the light microscope. However, the shortening of cells and widening of the extracellular spaces among the beams of the trabecular meshwork were displayed under the transmission electron microscopy in H-1152 group. No obvious adverse effect on eye tissue during the application duration of H-1152. **Conclusion** H-1152 decrease the IOP in rabbit probably by deterioration of the actin cytoskeleton and cellular adhesions and the expansion of the intercellular spaces in the juxtaganular region. The result suggests that H-1152 has the potential effect of IOP control as an anti-glaucoma medication.

Key words Rho-kinase inhibitor; glaucoma; intraocular pressure; trabecular meshwork

摘要 目的 观察 H-1152 对兔眼眼压以及小梁组织超微结构的影响,并探讨其作用机制。 **方法** 观察兔眼点用 H-1152 前后眼压变化,并用透射电镜分析兔眼小梁组织超微结构的变化。 **结果** H-1152 能明显降低兔眼眼压。局部点用 10 mmol/L 的 H-1152(50 μ L)后 1~6 h 的眼压较对照组降低($P < 0.05$)。点药后 2 h 下降至最低点,较处理前降低 50.6%。与对照组相比,点药后 1~3 h 眼压下降最明显。电镜下可见小梁间间隙增大,小梁组织中细胞外基质(ECM)减少。 **结论** H-1152 可有效降低兔眼眼压,其作用机制可能是通过降解小梁通道的 ECM,改变小梁通道的构型,使房水排出阻力减少,眼压降低。H-1152 有望成为治疗青光眼的有效药物。

关键词 H-1152; 青光眼; 眼压; 小梁网

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青光眼是一种不可逆的致盲性眼病,其发病机制尚不完全清楚,一般认为高眼压是导致视野和视神经损伤的主要的危险因素之一^[1-3]。目前,降眼压治疗仍是治疗青光眼的主要方法。我们前期的研究证实了

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Rho 激酶抑制剂 H-1152 对大鼠有较好的降眼压作用^[4]。值得注意的是,大鼠小梁网由被覆小梁上皮细胞的小梁柱形成网状,其间充满细胞外基质(extracellular matrix, ECM)。而兔眼房角没有真正的小梁网,仅有类似功能的房水丛^[5],其小梁组织的发育也不够完善。因此有必要研究 H-1152 对兔眼眼压以及兔小梁组织超微结构的影响,从而更深入地了解此药物通过小梁网途径调节眼压的机制。

1 材料与方法

1.1 材料

1.1.1 实验动物及分组 健康成年雄性新西兰大白兔8只(四川大学华西医学中心动物实验中心提供),兔龄4~5个月;体重2~3kg。经裂隙灯及直接检眼镜检查排除眼前节及眼底异常改变。其中6只用于眼压测量,另2只分别进行光镜和电镜标本的制备。

1.1.2 主要试剂及仪器 H-1152(德国SiChem GmbH公司);Pneumatometer眼压计(美国Digilab公司);光学显微镜(日本Olympus公司);透射电镜(H-600-IV型,日本Hitachi公司)。

1.2 方法

1.2.1 H-1152降眼压效应及不良反应观察 实验动物右眼为H-1152组,左眼为对照组,微量加样器于右眼结膜囊内点用10mmol/L的H-1152 50μL,同法左眼点用等量的磷酸盐缓冲液(phosphate buffered saline,PBS),在点药后0(即刻)、0.5、1、2、3、4、5、6h用Pneumatometer眼压计测量眼压变化,同时在裂隙灯下观察是否有结膜充血、角膜水肿、角膜后沉着物(kerato-precipitates,KP)、房水闪辉等眼部不良反应。

1.2.2 兔眼小梁组织光镜标本和电镜标本的制备

另取2只健康白兔,同上法右眼点H-1152,左眼点等量PBS。根据眼压曲线,在点药后2h眼压下降至最低点(图1),此时用耳缘静脉空气栓塞法处死动物。迅速摘除眼球,剥离眼后段组织,保留眼前节。沿矢状面将标本切成包含完整角膜、虹膜、小梁、部分睫状体和巩膜的组织块。浸泡于4%多聚甲醛溶液中至少72h。常规石蜡包埋。连续切片,厚度为5~7μm,苏木精-伊红染色,光学显微镜观察。实验动物处理步骤同上,光镜下定位小梁,将切好的2mm×5mm的包含完整小梁结构的组织块浸泡于3%戊二醛溶液中72h。修剪组织块成2mm×1mm,继续用3%戊二醛溶液固定48h。常规电镜标本处理染色,透射电镜观察。

1.3 统计学方法

采用SPSS 13.0统计学软件进行统计学处理。测得的眼压以 $\bar{x} \pm s$ 表示,H-1152组及对照组用配对t检验对2组各时间点的眼压进行比较,H-1152组给药前后不同时间点的眼压变化采用两因素的方差分析,各时间点间的两两比较采用LSD-t检验进行统计学分析。 $P < 0.05$ 为差异有统计学意义。

2 结果

2.1 H-1152对兔眼眼压的影响及不良反应观察

H-1152组和对照组0h的眼压分别为(20.42±0.66)mmHg和(19.50±0.83)mmHg(1mmHg=0.133kPa)(n=6),2组之间的眼压比较差异无统计学意义($P=0.090$)。H-1152组在10mmol/L H-1152(50μL)作用后1~6h的眼压较处理前明显降低($P < 0.05$)(图1)。点药后2h下降至最低点,较处理前降低了50.6%。与对照组相比点药后1~3h眼压下降最明显($P < 0.05$)。点药后裂隙灯检查均未见结膜充血、角膜水肿、KP、房水闪辉现象的发生。

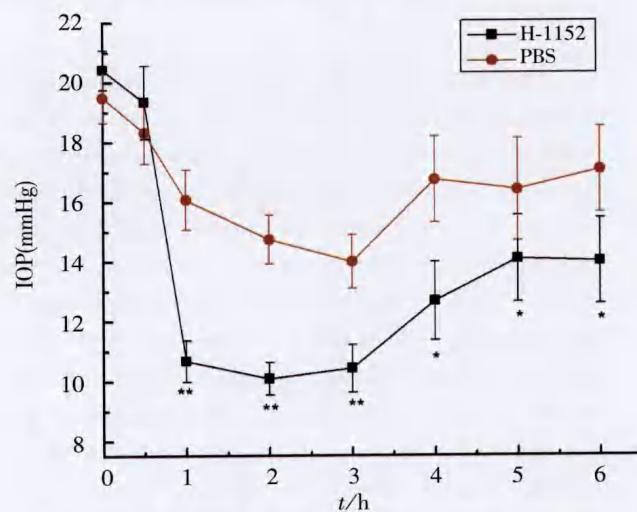


图1 H-1152对兔眼眼压的影响(n=6) 与给药即刻测量的眼压值比较,* $P < 0.05$;与给药前及对照眼比,** $P < 0.05$ (Two-way ANOVA,LSD-t test)

Fig. 1 Effect of H-1152 on IOP of rabbits (n=6) $F_{group}=9.642$, $P=0.017$, $F_{time}=5.483$, $P=0.020$; * $P < 0.05$ vs respective 0 h value, ** $P < 0.05$ vs respective control group (Two-way ANOVA, LSD-t test)

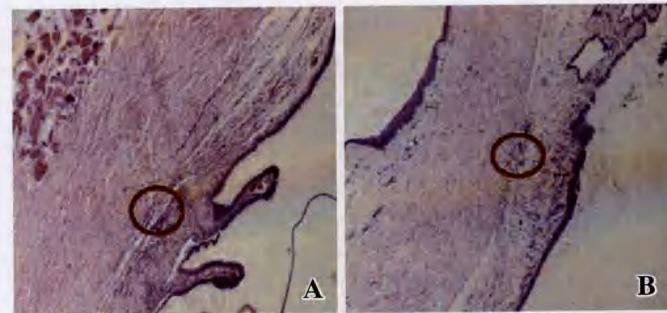


图2 光镜下兔眼小梁组织结构(圆圈标记处)(HE $\times 40$) A:对照组 B:H-1152组

Fig. 2 Structure of trabecular meshwork of rabbit eyes under the light microscope (circles)(HE $\times 40$) There is no visible structural alteration in trabecular meshwork tissue between the two groups A:control group B:H-1152 group

2.2 兔眼小梁组织学改变

光镜下兔眼房角组织结构在H-1152组与对照组未见明显区别(图2);兔的Schlemm管发育不完全,光

镜下难以准确识别。电镜下 H-1152 组较对照组小梁细胞趋于变短,细胞与细胞之间、细胞与 ECM 之间连接松弛,甚至消失,细胞外空隙增多、变大(图 3)。在 H-1152 组的小梁网中,未见明显的细胞损失或细胞碎片的积累,未显示 H-1152 存在明显细胞毒性。

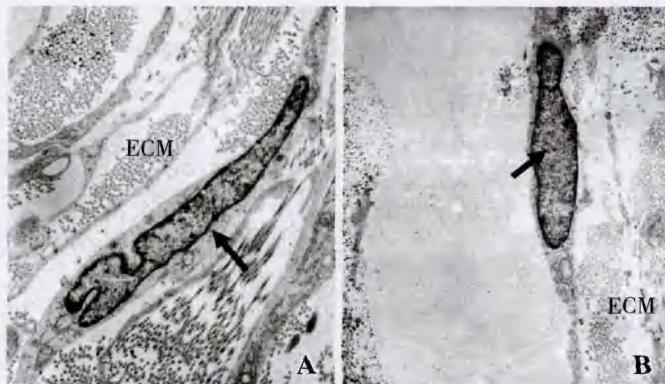


图 3 电镜下兔眼小梁细胞形态(箭头)($\times 8000$) A:对照组
B:H-1152 组

Fig. 3 Morphology of trabecular meshwork cells of rabbit eyes under the transmission electron microscope. The trabecular meshwork cells are short, and the intercellular junction between cells or cell and ECM is loose or absent in H-1152 group (arrow) ($\times 8000$) A: control group
B:H-1152 group

3 讨论

在房水引流方向,眼压一般通过小梁网途径及葡萄膜巩膜途径 2 条不同的途径进行调节^[6],后者约占总流出物的 10%^[7]。其中尤以小梁网途径更为重要,此途径通过改变小梁通道中肌动蛋白细胞骨架的分布及相关的细胞连接,促进房水的排出^[8]。经典抗青光眼药物,如 β 受体阻滞剂^[9],碳酸酐酶抑制剂^[9-10], α 受体激动剂^[9,11-12],前列腺素衍生物^[13-15],拟胆碱作用药物^[16]和非特异性肾上腺素受体激动剂等都广泛地应用于青光眼的治疗中。这些药物的作用机制多数是通过抑制房水生成和(或)促进房水外流,从而达成降眼压的目的。有研究表明,Rho 激酶与青光眼的发病有关^[16-17],一些药物可通过作用于小梁的肌动蛋白细胞骨架来调节眼压。已有众多的研究发现,在使用 Rho 激酶抑制剂 HA-1077 [1-(5-isoquinolinesulfonyl)-homopiperazine]、Y-27632 [(+)-R-trans-4-(1-aminoethyl)-N-(4-pyridyl)cyclohexanecarboxamide dihydrochloride] 和肌球蛋白轻链激酶抑制剂后,成功在兔眼^[16]、离体猪眼^[17-18]、牛眼^[19]和猴眼^[20]中提高房水外流的速率,显著降低眼压。

近来新合成的 H-1152 { (S)-(+) -2-methyl-1-

[(4-methyl-5-isoquinoline)sulfonyl]-homopiperazine} 是异喹琳(Isoquinoline)的衍生物,结构上比 HA-1077 多 2 个甲基(图 5),空间构型更利于与 Rho 激酶上的 ATP 结合位点互相作用,是比 HA-1077 具有更强选择性和亲和力的 Rho 激酶抑制剂^[21]。

H-1152 是高效、特异的 Rho 激酶抑制剂,其抑制常数是 1.6 nmol/L,而 HA-1077 和 Y-27632 的抑制常数分别为 0.33 mol/L 和 0.44 mol/L^[22]。

细胞骨架不仅在维持细胞形态、承受外力、保持细胞内部结构的有序性方面发挥重要作用,而且还参与许多重要的生命活动。来自 Rho 家族小型鸟苷三磷酸酶的 Rho A 蛋白在肌动蛋白细胞骨架的调节中起关键作用^[23]。Rho A 蛋白通过传送细胞外信号至下游效应器,如 Rho 激酶,发挥作用。Rho 激酶是一类丝氨酸-苏氨酸蛋白激酶,主要是通过抑制肌球蛋白轻链的磷酸化来提高肌球蛋白 II 的活性^[24]。已有研究表明,使用 Rho 激酶抑制剂 HA-1077、Y-27632 可通过抑制肌动蛋白轻链的磷酸化来改变小梁细胞的形态,从而改善房水外流^[16-17]。本研究结果表明,H-1152 可以显著降低兔眼眼压,点药后 1~3 h,眼压降低幅度较大。点药眼的对侧眼眼压也有一定程度的下降,推测可能是通过结膜吸收或其他途径进入全身血液循环系统,从而作用于对侧眼。对小梁网的形态学研究表明,H-1152 可能是通过调节小梁细胞之间的收缩性,从而舒张小梁网,扩大引流表面,促进房水外流。

研究表明,HA-1077、Y-27632 和 H-1152 等 Rho 激酶抑制剂均可通过调节房水外流阻力来降低眼压。尽管这些可能的“抗青光眼药物”降低房水阻力的作用机制尚未完全清楚,明确这些药物在小梁网和房水动力学的作用机制,对开发以小梁组织为作用位点的新型药物十分重要。兔眼与大鼠眼结构不同,其小梁网具有更多的网状组织^[5]。但本研究发现,在局部使用了 H-1152 之后,这 2 种动物的眼压及小梁超微结构的改变十分类似。这也说明,与网状结构一样,肌动蛋白细胞骨架和细胞连接的结构,对眼部房水阻力的形成同样有作用。因此,这些包括兔在内的动物模型,均适用于筛选以肌动蛋白为靶点的降眼压药物。此外,有研究显示 Rho 激酶抑制剂除了能够有效地降眼压外,还能提高眼部血流量,维持神经节细胞的存活及轴突

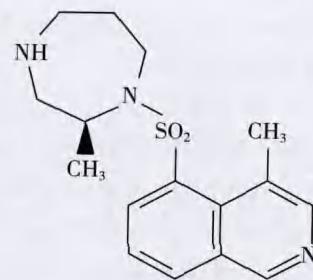


图 5 H-1152 的化学结构
Fig. 5 The chemical structure of H-1152

的再生等^[22,25],提示Rho激酶抑制剂有一定治疗青光眼的应用前景。

综上所述,H-1152对具有网状小梁的兔眼也具有很好的降眼压作用,可能是通过干扰肌动蛋白细胞骨架及细胞连接的结构,与在鼠眼中的降眼压作用及机制类似。综合本研究与其他相关研究结果^[16-22,26],说明Rho激酶抑制剂具有开发为新型的抗青光眼药物的潜力。

参考文献

- Quigley HA, Broman AT. The number of people with glaucoma worldwide in 2010 and 2020 [J]. *Br J Ophthalmol*, 2006, 90 (3): 262-267
- Kaufman PL, Gabelt BT, Cynader M. Introductory comments on neuroprotection [J]. *Surv Ophthalmol*, 1999, 43 (1): 89-90
- Weinreb RN, Khaw PT. Primary open-angle glaucoma [J]. *Lancet*, 2004, 363 (9422): 1711-1720
- Yu M, Chen X, Wang N, et al. H-1152 effects on intraocular pressure and trabecular meshwork morphology of rat eyes [J]. *J Ocul Pharmacol Ther*, 2008, 24 (4): 373-379
- Chen CC, Yeh LK, Liu CY, et al. Morphological differences between the trabecular meshworks of zebrafish and mammals [J]. *Curr Eye Res*, 2008, 33 (1): 59-72
- Kaufman PL. Pressure-dependent outflow. //Hart WM, ed. *Adler's Physiology of the Eye* [M]: Clinical Application [M]. 9th ed. St Louis: Mo: Mosby-Yearbook Inc., 1992: 307-335
- Weinreb RN. Uveoscleral outflow: the other outflow pathway [J]. *J Glaucoma*, 2000, 9 (5): 343-345
- Johnson M. What controls aqueous humour outflow resistance [J]? *Exp Eye Res*, 2006, 82 (4): 545-557
- Alward WL. Medical management of glaucoma [J]. *New Engl J Med*, 1998, 339 (18): 1298-1307
- Schuman JS. Antiglaucoma medications: a review of safety and tolerability issues related to their use [J]. *Clin Therapeut*, 2000, 22 (2): 167-208
- LeBlanc RP. Twelve-month results of an ongoing randomized trial comparing brimonidine tartrate 0.2% and timolol 0.5% given twice daily in patients with glaucoma or ocular hypertension. Brimonidine Study Group 2 [J]. *Ophthalmology*, 1998, 105 (10): 1960-1967
- Katz LJ. Brimonidine tartrate 0.2% twice daily vs timolol 0.5% twice daily: 1-year results in glaucoma patients. Brimonidine Study Group [J]. *Am J Ophthalmol*, 1999, 127 (1): 20-26
- Camras CB. Comparison of latanoprost and timolol in patients with ocular hypertension and glaucoma: a six-month masked, multicenter trial in the United States. The United States Latanoprost Study Group [J]. *Ophthalmology*, 1996, 103 (1): 138-147
- Brandt JD, van Denburgh AM, Chen K, et al. Comparison of once- or twice-daily bimatoprost with twice-daily timolol in patients with elevated IOP: a 3-month clinical trial [J]. *Ophthalmology*, 2001, 108 (6): 1023-1032
- Nordmann JP, Mertz B, Yannoulis NC, et al. A double-masked randomized comparison of the efficacy and safety of unoprostone with timolol and betaxolol in patients with primary open-angle glaucoma including pseudoexfoliation glaucoma or ocular hypertension. 6 month data [J]. *Am J Ophthalmol*, 2002, 133 (1): 1-10
- Honjo M, Inatani M, Kido N, et al. Effects of protein kinase inhibitor, HA1077, on intraocular pressure and outflow facility in rabbit eyes [J]. *Arch Ophthalmol*, 2001, 119 (8): 1171-1178
- Rao PV, Deng PF, Kumar J, et al. Modulation of aqueous humor outflow facility by the Rho kinase-specific inhibitor Y-27632 [J]. *Invest Ophthalmol Vis Sci*, 2001, 42 (5): 1029-1037
- Rao PV, Deng PF, Sasaki Y, et al. Regulation of myosin light chain phosphorylation in the trabecular meshwork: role in aqueous humour outflow facility [J]. *Exp Eye Res*, 2005, 80 (2): 197-206
- Rosenthal R, Choritz L, Schlott S, et al. Effects of ML-7 and Y-27632 on carbachol- and endothelin-1-induced contraction of bovine trabecular meshwork [J]. *Exp Eye Res*, 2005, 80 (6): 837-845
- Tian B, Kaufman PL. Effects of the Rho kinase inhibitor Y-27632 and the phosphatase inhibitor calyculin A on outflow facility in monkeys [J]. *Exp Eye Res*, 2005, 80 (2): 215-225
- Sasaki Y, Suzuki M, Hidaka H. The novel and specific Rho-kinase inhibitor (S)-(+)-2-methyl-1-[(4-methyl-5-isoquinolinesulfonyl)homopiperazine as a probing molecule for Rho-kinase-involved pathway [J]. *Pharmacol Therapeut*, 2002, 93 (2-3): 225-232
- Bertrand J, Winton MJ, Rodriguez-Hernandez N, et al. Application of Rho antagonist to neuronal cell bodies promotes neurite growth in compartmented cultures and regeneration of retinal ganglion cell axons in the optic nerve of adult rats [J]. *J Neurosci*, 2005, 25 (5): 1113-1121
- Hall A. Rho GTPases and the actin cytoskeleton [J]. *Science*, 1998, 279 (5350): 509-514
- Wettschureck N, Offermanns S. Rho/Rho-kinase mediated signaling in physiology and pathophysiology [J]. *J Mol Med*, 2002, 80 (10): 629-638
- Bertrand J, di Polo A, McKerracher L. Enhanced survival and regeneration of axotomized retinal neurons by repeated delivery of cell-permeable C3-like Rho antagonists [J]. *Neurobiol Dis*, 2007, 25 (1): 65-72
- Rao VP, Epstein DL. Rho GTPase/Rho kinase inhibition as a novel target for the treatment of glaucoma [J]. *Bio Drugs*, 2007, 21 (3): 167-177

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