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博 士 学 位 论 文

MK2 抑制剂对角膜碱烧伤的抗炎和抗新生 血管作用机制研究

**MK2 inhibitor selectively reduces alkali burn-induced
inflammation and neovascularization in cornea**

陈艳丰

指导教师姓名: 刘祖国教授

专业名称: 生理学

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陈艳丰

指导教师

刘祖国
教授

厦门大学

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摘要

眼化学烧伤是一类常见的眼外伤疾病，特别是在发展中国家。与酸性烧伤相比，碱性烧伤更为常见。碱性物质可快速穿透眼前房，导致慢性炎症和角膜浑浊，从而导致严重且持久的视觉缺损。尽管角膜移植手术是一项有效的治疗方法，然而其成功与否取决于角膜的炎症和新生血管是否得到控制。尽管目前有许多用于治疗角膜炎症和新生血管的药物，然而由于其有一定的副作用或仅仅通过缓解症状而非从根本途径进行治疗，因此亟须从发病机制入手寻找新的治疗办法。

持久的角膜炎症与新生血管，不仅会导致永久的视觉缺失，还会使行角膜手术的时机推迟。碱烧伤常伴随着大量炎症细胞浸润和炎症因子表达上调，促新生血管因子和抗新生血管因子的不平衡是导致新生血管生成的重要原因。目前碱烧伤抗炎和抗新生血管治疗方法包括激素、非甾体类抗炎药、柠檬酸盐、氩激光、光动力疗法等。然而以上治疗方法效果有限，特别是对于较大的炎性新生血管。此外，还有一些新兴的治疗方法，例如将相关炎症因子和促新生血管因子作为治疗靶点，但由于疗效有限且有一定副作用，因此其临床应用得到一定限制。

丝裂原活化蛋白激酶激酶 2 (Mitogen-activated protein kinase-activated protein kinase 2, MK2) 是 p38 丝裂原活化蛋白激酶 (Mitogen-activated protein kinase, MAPK) 的下游，可直接被 p38 MAPK 活化，其在术后肠粘连、胰腺炎、动脉粥样硬化、风湿性关节炎、肿瘤等疾病中发挥着重要的作用。由于 MK2 特异性增强炎症因子、趋化因子和细胞黏附因子 mRNA 翻译效率并提高其稳定性，因此 MK2 是许多炎症性疾病的治疗靶点。目前有关 MK2 在角膜炎症和新生血管形成中的作用还未有文献报道。

因此，本实验通过建立大鼠碱烧伤模型，研究 MK2 抑制剂对碱烧伤的抗炎和抗新生血管作用。我们在治疗的第 1、4、7 天通过裂隙灯观察 MK2 抑制剂对大鼠角膜炎症、新生血管、上皮修复的影响情况，采用免疫组织化学方法观察各组炎症细胞浸润，采用实时荧光定量-聚合酶链式反应 (Real-time polymerase chain reaction, Real-time PCR) 和蛋白印迹 (Western blot) 法检测各组单核细胞趋化蛋白(monocyte chemotactic protein, MCP)-1、巨噬细胞炎症蛋白(macrophage inflammatory protein, MIP)-1 α 、细胞间粘附分子(intercellular adhesion molecule,

ICAM)-1、血管细胞粘附分子(vascular cell adhesion molecule, VCAM)-1、白细胞介素(interleukin, IL)-6、IL-1 β 和血管内皮细胞生长因子(vascular endothelium growth factor, VEGF) 等促炎和促新生血管因子和抗新生血管因子色素上皮细胞衍生因子(pigment epithelium derived factor, PEDF) 的表达情况。通过细胞增殖实验和划痕实验检测 MK2 抑制剂对人角膜上皮细胞(human corneal epithelial cells, HCECs) 增殖和迁移的影响。此外, 采用 Draize 实验和免疫组织化学方法评估 MK2 抑制剂对正常角膜的毒副作用。

我们发现, MK2 抑制剂可抑制碱烧伤导致的 MK2 活化, 且具有抗炎和抗新生血管作用。一方面, MK2 抑制剂可减少角膜炎症指数、ED1 阳性巨噬细胞和多形核白细胞(polymorphonuclear, PMN) 阳性中性粒细胞浸润。另一方面 MK2 抑制剂可下调 IL-6、IL-1 β 、MIP-1 α 、ICAM-1 和 VCAM-1 等细胞因子表达。此外, MK2 抑制剂还可下调促新生血管因子和上调抗新生血管因子 PEDF 的表达。同时, 我们还发现 MK2 抑制剂不影响角膜上皮的增殖和迁移, 且对正常角膜无毒副作用。

以上结果表明, MK2 抑制剂对大鼠碱烧伤角膜具有抗炎和抗新生血管作用, 因此其有望成为临床上治疗角膜碱烧伤的新药。

关键词: MK2; 角膜碱烧伤; 炎症

Abstract

Ocular chemical burns are a common trauma encountered worldwide particularly in the developing countries. They are more frequently a cause of injury than exposure to injurious acids. Caustic agents can readily penetrate into the anterior ocular surface and produce chronic inflammation and opacification resulting in severe and permanent visual impairment. Even though keratoplasty is a viable therapeutic option, the success of this procedure depends on first resolving inflammation and neovascularization (NV) with drugs. Currently, many of the drugs used for this purpose are somewhat problematic since they can have side effects and only provide symptomatic relief rather than target the mechanisms underlying inflammation and NV. This limitation has prompted numerous studies to delineate mechanisms underlying the pathogenesis of chronic inflammation and NV.

Unrelenting and dysregulated corneal inflammation and NV, are common sequels of an alkali burn that can lead to persistent visual impairment and delay performing penetrating keratoplasty. Alkali burns induce chemokine driven immune cell corneal infiltration accompanied by rises in proinflammatory cytokines levels. In addition, the tenuous balance between pro-angiogenic and anti-angiogenic factors can be disrupted leading to corneal NV. Thus, suppression of these maladaptive injury-induced responses is essential for reducing losses in corneal transparency and hastening wound healing. Various medical and surgical options such as steroids, nonsteroidal inflammatory agents, citrate, argon laser photocoagulation, and photodynamic therapy are used to treat corneal inflammation and inflammatory NV induced by an alkali burn⁴; however, sometimes, these therapies are ineffective, especially for large inflammatory NV. The novel approaches under investigation to improve treatment of chemical burns include manipulating the inflammatory and angiogenesis-related factors by means of monoclonal antibodies, receptor modification, aptamers, and inhibitors of candidate inflammatory and/or angiogenesis pathways. Even though

some of these options look promising, each one of them can have side effects that limit their usefulness in restoring corneal transparency and optical properties needed for normal vision.

Mitogen-activated protein kinase-activated protein kinase-2 (MAPKAPK2 or MK2) is an intracellular serine/threonine kinase substrate downstream from p38 mitogen-activated protein kinase (MAPK) and its activation by p38 is implicated in many inflammatory diseases including postoperative ileus, pancreatitis, atherosclerosis rheumatoid arthritis and cancer. Accordingly, it is an established drug target for treating many inflammatory diseases since its activation selectively induces the translation and increases stability of proinflammatory cytokine, chemokine and cell adhesion factor mRNA. There are no reports describing a role for MK2 in mediating corneal inflammation and inflammatory NV.

We determined if a MK2 inhibitor, MK2i, improves cornea wound healing by inhibiting inflammation and neovascularization (NV) caused by burning rat corneas with alkali. The effects of MK2i on inflammation, NV and epithelial damage were assessed under a slit lamp microscope 1, 4 and 7 days after injury. Immune cell infiltration was evaluated with immunohistochemistry. Real-time PCR and Western blot analysis were performed to assess pro-angiogenic and proinflammatory cytokine expression levels including monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-1 α (MIP-1 α), intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), IL-6, IL-1 β and VEGF as well as anti-angiogenic cytokine PEDF levels. Cell proliferation assay and scratch wound assays were conducted to measure cell proliferation and migration in human corneal epithelial cells (HCECs). Draize test and immunohistochemistry were performed to determine if MK2i altered ocular surface health.

MK2i inhibited alkali-induced MK2 activation as well as rises in inflammation and NV based on: a) blunting rises in inflammatory index, inflammatory cell infiltration, ED1+ macrophage and PMN+ neutrophil infiltration; b) suppressing IL-6 and IL-1 β gene expression along with those of MIP-1 α , ICAM-1 and VCAM-1; c) reducing angiogenic gene expression levels and NV whereas anti-angiogenic PEDF

levels increased. In contrast, MK2i did not affect HCEC proliferation and migration and had no detectable side effects on ocular surface integrity.

We show here that MK2i selectively inhibited alkali-induced corneal inflammation and NV. MK2i may be a viable option for clinical management of corneal alkali burns.

Keywords: mitogen-activated protein kinase-activated protein kinase 2 (MK2); cornea alkali burn; inflammation

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