

# 欧前胡素调节HMGB1-RAGE-NF- $\kappa$ B信号通路 对高糖诱导的视网膜内皮细胞损伤的影响

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**摘要** 该研究探讨欧前胡素(IMP)调节高迁移率族蛋白B1(HMGB1)-晚期糖基化终产物受体(RAGE)-核转录因子 $\kappa$ B(NF- $\kappa$ B)信号通路对高糖诱导的视网膜血管内皮细胞损伤的影响。使用10~320  $\mu$ mol/L的IMP处理高糖诱导的视网膜血管内皮细胞hRECs, 筛选最佳药物浓度; 将视网膜血管内皮细胞hRECs细胞分为正常糖组(NG组, 5.5 mmol/L葡萄糖)、高糖组(HG组, 25 mmol/L葡萄糖)、低浓度欧前胡素组(IMP-L组, 25 mmol/L葡萄糖+20  $\mu$ mol/L IMP)、中浓度欧前胡素组(IMP-M组, 25 mmol/L葡萄糖+40  $\mu$ mol/L IMP)、高浓度欧前胡素组(IMP-H组, 25 mmol/L葡萄糖+80  $\mu$ mol/L IMP)、高浓度欧前胡素+重组HMGB1蛋白组(IMP-H+rHMGB1组, 25 mmol/L葡萄糖+80  $\mu$ mol/L IMP+200  $\mu$ g/L rHMGB1)。CCK-8试剂盒检测hRECs细胞活性; 流式细胞仪检测hRECs细胞凋亡的情况; 管腔形成实验检测细胞血管生成; 酶联免疫吸附法(ELISA)检测细胞IL-6、肿瘤坏死因子- $\alpha$ (TNF- $\alpha$ )、白细胞介素-1 $\beta$ (IL-1 $\beta$ )、MDA水平和SOD水平; Western blot检测HMGB1-RAGE-NF- $\kappa$ B信号通路相关蛋白表达情况。结果显示, IMP可以促进高糖诱导的hRECs增殖, 且呈浓度依赖性, 选择浓度为20、40、80  $\mu$ mol/L的IMP进行后续实验; 与NG组比较, HG组细胞存活率、SOD活性降低, 细胞凋亡率, 管腔形成数, IL-6、TNF- $\alpha$ 、IL-1 $\beta$ 、MDA水平及HMGB1、RAGE、NF- $\kappa$ B p65蛋白表达水平升高( $P<0.05$ ); 与HG组比较, IMP-L组、IMP-M组、IMP-H组细胞存活率、SOD活性升高, 细胞凋亡率, 管腔形成数, IL-6、TNF- $\alpha$ 、IL-1 $\beta$ 、MDA水平及HMGB1、RAGE、NF- $\kappa$ B p65蛋白表达水平降低, 且呈浓度依赖性( $P<0.05$ ); 与IMP-H组比较, IMP-H+rHMGB1组细胞存活率、SOD活性显著降低, 细胞凋亡率, 管腔形成数, IL-6、TNF- $\alpha$ 、IL-1 $\beta$ 、MDA水平及HMGB1、RAGE、NF- $\kappa$ B p65蛋白表达水平显著升高( $P<0.05$ )。结果表明, IMP可能通过抑制HMGB1-RAGE-NF- $\kappa$ B信号通路, 降低炎症反应和细胞凋亡水平, 进而减轻高糖诱导的视网膜血管内皮细胞损伤。

**关键词** 欧前胡素; 高迁移率族蛋白B1-晚期糖基化终产物受体-核转录因子 $\kappa$ B; 高糖; 视网膜内皮细胞; 损伤

## Effect of Imperatorin on High Glucose Induced Retinal Endothelial Cell Injury by Regulating the HMGB1-RAGE-NF- $\kappa$ B Signaling Pathway

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**Abstract** This study investigated the effect of IMP (imperatorin) on high glucose induced retinal endothelial cell injury by regulating the HMGB1 (high-mobility group protein box 1)-RAGE (receptor for advanced glycation end products)-NF- $\kappa$ B (nuclear factor kappa-B) signaling pathway. High glucose induced hRECs in retinal endothelial cells were treated with IMP at a concentration of 10-320  $\mu$ mol/L to screen for the optimal drug concentration. hRECs (human retinal endothelial cells) were separated into normal glucose group (NG group, 5.5 mmol/L glucose), high glucose group (HG group, 25 mmol/L glucose), low concentration imperatorin group (IMP-L group, 25 mmol/L glucose+20  $\mu$ mol/L IMP), medium concentration imperatorin group (IMP-M group, 25 mmol/L glucose+40  $\mu$ mol/L IMP), high concentration imperatorin group (IMP-H group, 25 mmol/L glucose+80  $\mu$ mol/L IMP), and high concentration imperatorin+recombinant HMGB1 protein group (IMP-H+rHMGB1 group, 25 mmol/L glucose+80  $\mu$ mol/L IMP+200  $\mu$ g/L rHMGB1). CCK-8 assay kit was applied to detect the activity of hRECs cells. Flow cytometry was applied to detect the apoptosis of hRECs cells. The cell angiogenesis was detected by the lumen formation test. ELISA (enzyme linked immunosorbent assay) was applied to detect levels of IL-6, TNF- $\alpha$  (tumor necrosis factor- $\alpha$ ), IL-1 $\beta$  (interleukin-1 $\beta$ ), MDA, and SOD in cells. Western blot was applied to detect the expression of proteins related to the HMGB1-RAGE-NF- $\kappa$ B signaling pathway. The results showed that IMP was able to promote the proliferation of high glucose induced hRECs in a concentration dependent manner, subsequently, IMP concentrations of 20, 40, and 80  $\mu$ mol/L were selected for subsequent experiments. Compared with the NG group, the cell survival rate and SOD activity in the HG group were decreased; the apoptosis rate, the number of lumen formation, the levels of IL-6, TNF- $\alpha$ , IL-1 $\beta$ , MDA, and the expression of HMGB1, RAGE, NF- $\kappa$ B p65 proteins were increased ( $P<0.05$ ). Compared with the HG group, the cell survival rate and SOD activity were increased in the IMP-L group, IMP-M group, and IMP-H group; the apoptosis rate, the number of lumen formation, the levels of IL-6, TNF- $\alpha$ , IL-1 $\beta$ , MDA, and the expression of HMGB1, RAGE, NF- $\kappa$ B p65 proteins were decreased and in a concentration dependent manner ( $P<0.05$ ). Compared with the IMP-H group, the cell survival rate and SOD activity in the IMP-H+rHMGB1 group were significantly reduced; the apoptosis rate, the number of lumen formation, the levels of IL-6, TNF- $\alpha$ , IL-1 $\beta$ , MDA, and the expression of HMGB1, RAGE, NF- $\kappa$ B p65 proteins were significantly increased ( $P<0.05$ ). The results suggest that IMP may reduce inflammatory response and cell apoptosis by inhibiting the HMGB1-RAGE-NF- $\kappa$ B signaling pathway, thereby alleviating high glucose induced injury to retinal endothelial cells.

**Keywords** imperatorin; high-mobility group protein box 1-receptor for advanced glycation end products-nuclear factor kappa-B; high glucose; retinal endothelial cells; injury

随着人们生活水平的提高以及饮食习惯的改变, 糖尿病(diabetic mellitus, DM)的发病率不断增加, 而糖尿病视网膜病变(diabetic retinopathy, DR)是DM在临床上常见的并发症, 对成年人的视力造成严重影响, 并缺乏比较有效的治疗手段<sup>[1-2]</sup>。已有相关研究发现, DR发生的一个原因是人视网膜血管内皮细胞(human retinal endothelial cells, hRECs)功能障碍, 持续性的高糖刺激会一定程度增加活性氧水平, 诱导氧化应激以及炎症反应的发生, 并对血管屏障造成一定的损伤, 最终导致hRECs细胞的凋亡以及损伤<sup>[3]</sup>。同时, 视网膜新生血管已被确定为DR患者严重视力恶化的关键危险因素<sup>[4]</sup>。因此, 控

制高糖诱导的新生血管形成、探究减轻hRECs细胞损伤的机制, 对于临床治疗DR具有重要意义。欧前胡素(imperatorin, IMP)是从中草药中分离提纯得到的一种活性成分, 是具有多种生物活性的呋喃香豆素, 具有抗菌、抗炎、平喘及抗过敏等作用<sup>[5-6]</sup>。MA等<sup>[7]</sup>研究发现, IMP对炎症疾病具有一定的治疗以及防御的功能。既往研究显示, IMP能够抑制DM大鼠的高血糖<sup>[8]</sup>。故本研究推测IMP可能对DR进展有影响。高迁移率族蛋白1(high-mobility group protein box 1, HMGB1)/晚期糖基化终产物受体(receptor for advanced glycation end products, RAGE)/核转录因子 $\kappa$ B(nuclear factor kappa-B, NF- $\kappa$ B)信号通路是

参与调节炎症反应以及细胞应激的重要通路<sup>[9]</sup>。研究发现,在糖尿病的慢性炎症中, HMGB1-RAGE信号通路发挥重要作用<sup>[10]</sup>。近期研究表明, HMGB1-RAGE信号通路在DM和DR大鼠中被抑制<sup>[11-12]</sup>。故本研究推测IMP可能通过调节HMGB1-RAGE-NF- $\kappa$ B信号通路影响DR发展。因此,本研究主要探讨IMP调节HMGB1-RAGE-NF- $\kappa$ B信号通路对高糖诱导的视网膜内皮细胞损伤的影响。

## 1 材料与方法

### 1.1 实验细胞

视网膜内皮细胞hRECs(货号:XY-XB-1310)从上海恒雅生物科技有限公司购买。

### 1.2 主要试剂与仪器

DMEM培养基(货号:EVU301)购自北京孚博科技有限公司;IMP(货号:WKQ-0000413)购自四川省维克奇生物科技有限公司;重组HMGB1(recombinant HMGB1, rHMGB1)蛋白(货号:10326-H07E)购自北京义翘神州科技股份有限公司;细胞计数试剂盒8(cell counting kit-8, CCK-8)(货号:96992-100TESTS-F)购自上海益启生物科技有限公司;Annexin V-FITC/PI细胞凋亡检测试剂盒(货号:C00007-100)购自广州威佳科技有限公司;BCA试剂盒(货号:701780-480)购自艾美捷科技有限公司;IL-6酶联试剂盒(enzyme linked immunosorbent assay, ELISA)(货号:ZK-H2101)、肿瘤坏死因子- $\alpha$ (tumor necrosis factor- $\alpha$ , TNF- $\alpha$ ) ELISA试剂盒(货号:ZK-R3838)购自深圳子科生物科技有限公司;白细胞介素-1 $\beta$ (interleukin-1 $\beta$ , IL-1 $\beta$ ) ELISA试剂盒(货号:KJEIA0001D)购自北京康佳宏原生物科技有限公司;HMGB1、RAGE、NF- $\kappa$ B p65、GAPDH抗体(货号:ab92310、ab228861、ab32536、ab128915)购自英国Abcam公司;CO<sub>2</sub>细胞培养箱(型号:CellXpert)购自德国Eppendorf公司;凝胶成像仪(型号:ZF-208)购自上海金鹏分析仪器有限公司;显微镜(型号:DMi1)购自美国Cytok Biosciences公司等。

### 1.3 细胞培养与分组

1.3.1 细胞培养 将冻存的hRECs细胞取出来,放在37℃的培养箱中进行融化,将融化后的细胞4℃、3 000 r/min离心5 min,将下层的细胞收集起来,加入到DMEM培养基(含10%胎牛血清)中,放入37℃、5% CO<sub>2</sub>的细胞培养箱中进行常规传代培养,当细胞增

长到对数期时,进行细胞收集用于后续的实验。

1.3.2 药物浓度筛选 收集各组hRECs细胞并进行消化,并将其接种在96孔板(1×10<sup>5</sup>个/孔)中,培养24 h,加入25 mmol/L<sup>[13]</sup>葡萄糖培养细胞,在选定的孔中加入不同浓度的IMP(0、10、20、40、80、160  $\mu$ mol/L)培养基培养,以25 mmol/L葡萄糖和0  $\mu$ mol/L IMP处理的细胞作为高糖组(HG组),另以5.5 mmol/L葡萄糖培养的细胞作为正常糖组(NG组)。24 h后,向每个孔中加入10  $\mu$ L CCK-8溶液,37℃下孵育4 h,对各个孔450 nm处的吸光度(D)值进行检测,并计算细胞存活率(%)。

1.3.3 细胞分组 共将细胞分为6组。①NG组:加入5.5 mmol/L葡萄糖培养细胞;②HG组:加入25 mmol/L葡萄糖培养细胞;③低浓度欧前胡素组(IMP-L组):25 mmol/L葡萄糖+20  $\mu$ mol/L IMP共同培养细胞;④中浓度欧前胡素组(IMP-M组):25 mmol/L葡萄糖+40  $\mu$ mol/L IMP共同培养细胞;⑤高浓度欧前胡素组(IMP-H组):25 mmol/L葡萄糖+80  $\mu$ mol/L IMP共同培养细胞;⑥高浓度欧前胡素+重组HMGB1蛋白组(IMP-H+rHMGB1组):25 mmol/L葡萄糖+80  $\mu$ mol/L IMP+200  $\mu$ g/L rHMGB1共同培养细胞。待收集培养48 h的各组细胞进行后续研究。

1.3.4 流式细胞仪检测hRECs细胞凋亡情况 各组hRECs细胞经过48 h的培养后,用胰蛋白酶37℃消化3 min,收集细胞悬液,用预冷的PBS对其进行洗涤,3 000 r/min、4℃离心5 min去上清,在细胞沉淀中加入结合缓冲液对细胞浓度进行调整。再分别加入Annexin V-FITC和PI,室温避光反应15 min,借助流式细胞仪对细胞凋亡率进行检测。

1.3.5 管腔形成实验检测细胞血管生成情况 细胞(2×10<sup>5</sup>个/孔)接种到涂有Matrigel的96孔板上。按照1.3.2的方法培养48 h。然后,用倒置显微镜进行成像。利用ImageJ软件计数管腔形成数。

1.3.6 ELISA检测IL-6、TNF- $\alpha$ 、IL-1 $\beta$ 、MDA和SOD水平 收集各组hRECs细胞,4℃、3 000 r/min离心10 min,离心后收集上清,将其置于-80℃冰箱进行保存,并采用ELISA方法检测450 nm处的吸光度(D)值,并计算上清液中IL-6、TNF- $\alpha$ 、IL-1 $\beta$ 、MDA和SOD水平,其实验操作步骤完全按照试剂盒的说明书进行。

1.3.7 Western blot检测细胞中HMGB1-RAGE-NF- $\kappa$ B信号通路蛋白表达情况 对在不同条件下培养

的各组hRECs细胞进行收集,加入一定量的蛋白裂解液反应30 min,提取各组细胞总蛋白,并利用BCA试剂盒对蛋白总量进行测定。分别配置不同浓度的分离胶和浓缩胶进行SDS-PAGE凝胶电泳,将蛋白凝胶转至PVDF膜上,在冰上反应1 h,再用TBST溶液清洗,加入5%脱脂奶粉,室温封闭1 h,封闭后经TBST溶液清洗,分别加入一抗HMGB1、RAGE、NF- $\kappa$ B p65和GAPDH(均1:1 000稀释)4 °C过夜,加入TBST溶液洗涤;加入二抗(1:5 000稀释),室温孵育2 h,加入TBST溶液洗涤。用ECL试剂显影,用定影液定影后晾干,利用凝胶成像仪观察各个蛋白的表达情况,并以GAPDH为内参。最后用ImageJ软件进行定量。

#### 1.4 统计学分析

对实验数据的处理采用SPSS 25.0统计软件进行。计量资料用( $\bar{x}\pm s$ )表示,各组(如IMP-L组、IMP-M组、IMP-H组等)间比较采用 $F$ 检验,组间两两比较采用LSD- $t$ 检验。 $P<0.05$ 为差异有统计学意义。

## 2 结果

### 2.1 IMP对高糖诱导的hRECs细胞增殖的影响

与NG组比较,HG组的细胞存活率降低( $P<0.05$ );与HG比较,20~160  $\mu\text{mol/L}$ 的IMP可以显著促进hRECs细胞的增殖,选择浓度为20、40、80  $\mu\text{mol/L}$ 的IMP进行后续实验(表1)。

### 2.2 IMP对hRECs细胞凋亡的影响

与NG组比较,HG组的晚期凋亡率、早期凋亡率及总凋亡率增加( $P<0.05$ );与HG组比较,IMP-L组、IMP-M组、IMP-H组晚期凋亡率、早期凋亡率

及总凋亡率均降低( $P<0.05$ );与IMP-H组比较,IMP-H+rHMGB1组的晚期凋亡率、早期凋亡率及总凋亡率显著增加( $P<0.05$ )(图1和表2)。这表明IMP能够抑制HG诱导的hRECs细胞凋亡。

### 2.3 IMP对hRECs细胞血管生成的影响

与NG组比较,HG组的管腔形成数增加( $P<0.05$ );与HG组比较,IMP-L组、IMP-M组、IMP-H组管腔形成数均降低( $P<0.05$ );与IMP-H组比较,IMP-H+rHMGB1组的管腔形成数显著增加( $P<0.05$ )(图2和表3)。这表明IMP能够抑制HG诱导的hRECs细胞新生血管生成。

### 2.4 IMP对高糖诱导的hRECs细胞中IL-6、TNF- $\alpha$ 、IL-1 $\beta$ 的影响

与NG组比较,HG组的IL-6、TNF- $\alpha$ 、IL-1 $\beta$ 表达水平升高( $P<0.05$ );与HG组比较,IMP-L组、IMP-M组、IMP-H组IL-6、TNF- $\alpha$ 、IL-1 $\beta$ 表达水平降低( $P<0.05$ );与IMP-H组比较,IMP-H+rHMGB1组的IL-6、TNF- $\alpha$ 、IL-1 $\beta$ 表达水平显著增加( $P<0.05$ )(表4)。这表明IMP能够抑制HG诱导的hRECs细胞中炎症因子的表达。

### 2.5 IMP对高糖诱导的hRECs细胞氧化应激的影响

与NG组比较,HG组的SOD活性降低、MDA水平升高( $P<0.05$ );与HG组比较,IMP-L组、IMP-M组、IMP-H组SOD活性升高、MDA水平降低( $P<0.05$ );与IMP-H组比较,IMP-H+rHMGB1组的SOD活性降低、MDA水平升高( $P<0.05$ )(表5)。这表明IMP能够抑制HG诱导的hRECs细胞氧化应激。

### 2.6 IMP对hRECs细胞中HMGB1-RAGE-NF- $\kappa$ B信号通路的影响

与NG组比较,HG组的HMGB1、RAGE、NF- $\kappa$ B

表1 hRECs细胞存活率比较

Table 1 Comparison of hRECs cell survival rate

| 组别<br>Groups              | 24 h的细胞存活率/%<br>Cell survival rate of 24 h /% |
|---------------------------|-----------------------------------------------|
| NG                        | 100.00 $\pm$ 2.68                             |
| HG                        | 60.35 $\pm$ 3.95*                             |
| 10 $\mu\text{mol/L}$ IMP  | 58.32 $\pm$ 3.86                              |
| 20 $\mu\text{mol/L}$ IMP  | 72.32 $\pm$ 4.16 <sup>#</sup>                 |
| 40 $\mu\text{mol/L}$ IMP  | 80.31 $\pm$ 5.72 <sup>#</sup>                 |
| 80 $\mu\text{mol/L}$ IMP  | 93.46 $\pm$ 4.61 <sup>#</sup>                 |
| 160 $\mu\text{mol/L}$ IMP | 95.64 $\pm$ 4.60 <sup>#</sup>                 |

\* $P<0.05$ ,与NG组比较;<sup>#</sup> $P<0.05$ ,与HG组比较。

\* $P<0.05$  compared with NG group; <sup>#</sup> $P<0.05$  compared with HG group.

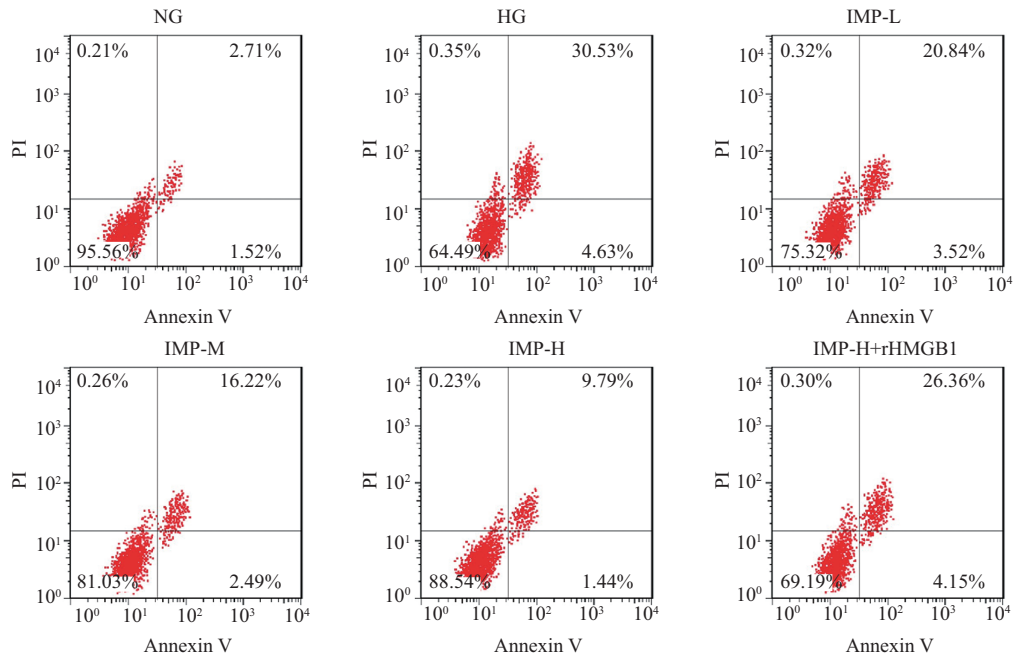


图1 流式细胞术检测细胞凋亡

Fig.1 Apoptosis was detected by flow cytometry

表2 IMP对hRECs细胞凋亡的影响

Table 2 Effects of IMP on apoptosis of hRECs

| 组别<br>Groups | 晚期凋亡率/%<br>Late apoptosis rate /% | 早期凋亡率/%<br>Early apoptosis rate /% | 总凋亡率/%<br>Total apoptosis rate /% |
|--------------|-----------------------------------|------------------------------------|-----------------------------------|
| NG           | 2.71±0.69                         | 1.52±0.40                          | 4.23±1.09                         |
| HG           | 30.53±2.89*                       | 4.63±0.83*                         | 35.16±2.35*                       |
| IMP-L        | 20.84±1.52 <sup>#</sup>           | 3.52±0.69 <sup>#</sup>             | 24.36±2.26 <sup>#</sup>           |
| IMP-M        | 16.22±1.03 <sup>#&amp;</sup>      | 2.49±0.51 <sup>#&amp;</sup>        | 18.71±1.17 <sup>#&amp;</sup>      |
| IMP-H        | 9.79±1.27 <sup>#&amp;▼</sup>      | 1.44±0.32 <sup>#&amp;▼</sup>       | 11.23±1.13 <sup>#&amp;▼</sup>     |
| IMP-H+rHMGB1 | 26.36±1.79 <sup>@</sup>           | 4.15±0.87 <sup>@</sup>             | 30.51±3.32 <sup>@</sup>           |

\* $P<0.05$ , 与NG组比较; <sup>#</sup> $P<0.05$ , 与HG组比较; & $P<0.05$ , 与IMP-L组比较; ▼ $P<0.05$ , 与IMP-M组比较; @ $P<0.05$ , 与IMP-H组比较。

\* $P<0.05$  compared with NG group; <sup>#</sup> $P<0.05$  compared with HG group; & $P<0.05$  compared with IMP-L group; ▼ $P<0.05$  compared with IMP-M group;

@ $P<0.05$  compared with IMP-H group.

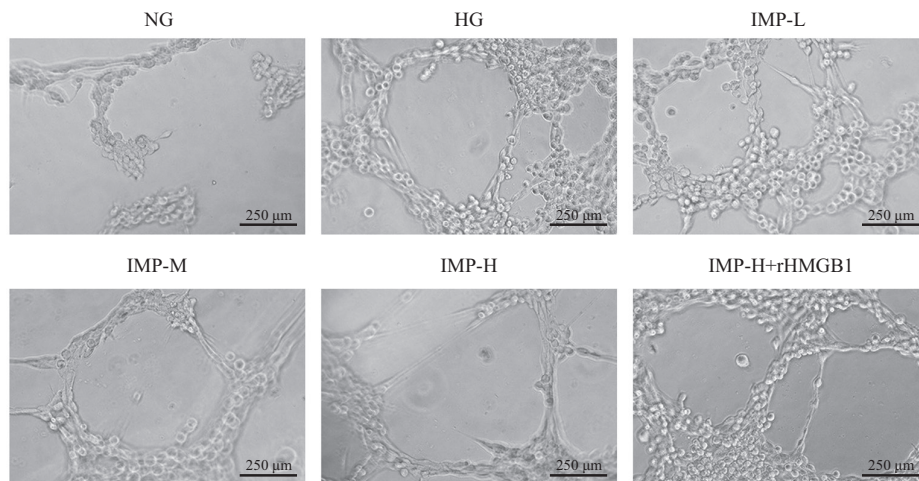


图2 管腔形成实验检测细胞血管生成

Fig.2 Cell angiogenesis was detected by lumen formation assay

表3 IMP对hRECs细胞血管生成的影响  
Table 3 Effects of IMP on angiogenesis of hRECs

| 组别<br>Groups | 管腔形成数<br>Number of lumen formation |
|--------------|------------------------------------|
| NG           | 35.23±3.12                         |
| HG           | 124.31±10.41*                      |
| IMP-L        | 101.92±9.23 <sup>#</sup>           |
| IMP-M        | 79.14±8.06 <sup>#&amp;</sup>       |
| IMP-H        | 54.63±5.24 <sup>#&amp;▼</sup>      |
| IMP-H+rHMGB1 | 115.46±9.67 <sup>@</sup>           |

\* $P<0.05$ , 与NG组比较; <sup>#</sup> $P<0.05$ , 与HG组比较; <sup>&</sup> $P<0.05$ , 与IMP-L组比较; <sup>▼</sup> $P<0.05$ , 与IMP-M组比较; <sup>@</sup> $P<0.05$ , 与IMP-H组比较。  
\* $P<0.05$  compared with NG group; <sup>#</sup> $P<0.05$  compared with HG group; <sup>&</sup> $P<0.05$  compared with IMP-L group; <sup>▼</sup> $P<0.05$  compared with IMP-M group;  
<sup>@</sup> $P<0.05$  compared with IMP-H group.

表4 IMP对高糖诱导的hRECs细胞IL-6、TNF-α、IL-1β的影响  
Table 4 Effects of IMP on IL-6, TNF-α, IL-1β in high-glucose induced hRECs

| 组别<br>Groups | IL-6 /ng·L <sup>-1</sup>      | TNF-α /ng·L <sup>-1</sup>    | IL-1β /pg·mL <sup>-1</sup>    |
|--------------|-------------------------------|------------------------------|-------------------------------|
| NG           | 7.92±0.82                     | 8.99±0.92                    | 23.95±2.61                    |
| HG           | 30.06±2.31*                   | 35.05±3.25*                  | 166.37±17.25*                 |
| IMP-L        | 20.75±2.81 <sup>#</sup>       | 20.24±2.14 <sup>#</sup>      | 124.37±13.16 <sup>#</sup>     |
| IMP-M        | 15.92±1.63 <sup>#&amp;</sup>  | 13.08±1.49 <sup>#&amp;</sup> | 70.42±8.24 <sup>#&amp;</sup>  |
| IMP-H        | 11.05±1.27 <sup>#&amp;▼</sup> | 7.15±1.81 <sup>#&amp;▼</sup> | 41.35±4.36 <sup>#&amp;▼</sup> |
| IMP-H+rHMGB1 | 20.40±2.16 <sup>@</sup>       | 17.64±1.32 <sup>@</sup>      | 113.29±12.07 <sup>@</sup>     |

\* $P<0.05$ , 与NG组比较; <sup>#</sup> $P<0.05$ , 与HG组比较; <sup>&</sup> $P<0.05$ , 与IMP-L组比较; <sup>▼</sup> $P<0.05$ , 与IMP-M组比较; <sup>@</sup> $P<0.05$ , 与IMP-H组比较。  
\* $P<0.05$  compared with NG group; <sup>#</sup> $P<0.05$  compared with HG group; <sup>&</sup> $P<0.05$  compared with IMP-L group; <sup>▼</sup> $P<0.05$  compared with IMP-M group;  
<sup>@</sup> $P<0.05$  compared with IMP-H group.

表5 IMP对高糖诱导的hRECs细胞氧化应激的影响  
Table 5 Effects of IMP on oxidative stress of hRECs induced by high glucose

| 组别<br>Groups | SOD /U·L <sup>-1</sup>          | MDA /nmol·mL <sup>-1</sup>      |
|--------------|---------------------------------|---------------------------------|
| NG           | 213.26±22.15                    | 107.26±11.27                    |
| HG           | 67.15±7.14*                     | 507.23±51.02*                   |
| IMP-L        | 86.23±8.36 <sup>#</sup>         | 416.27±42.37 <sup>#</sup>       |
| IMP-M        | 136.27±14.27 <sup>#&amp;</sup>  | 315.26±32.14 <sup>#&amp;</sup>  |
| IMP-H        | 198.72±20.15 <sup>#&amp;▼</sup> | 154.22±16.33 <sup>#&amp;▼</sup> |
| IMP-H+rHMGB1 | 76.59±8.69 <sup>@</sup>         | 462.31±47.15 <sup>@</sup>       |

\* $P<0.05$ , 与NG组比较; <sup>#</sup> $P<0.05$ , 与HG组比较; <sup>&</sup> $P<0.05$ , 与IMP-L组比较; <sup>▼</sup> $P<0.05$ , 与IMP-M组比较; <sup>@</sup> $P<0.05$ , 与IMP-H组比较。  
\* $P<0.05$  compared with NG group; <sup>#</sup> $P<0.05$  compared with HG group; <sup>&</sup> $P<0.05$  compared with IMP-L group; <sup>▼</sup> $P<0.05$  compared with IMP-M group;  
<sup>@</sup> $P<0.05$  compared with IMP-H group.

p65蛋白水平升高( $P<0.05$ ); 与HG组比较, IMP-L组、IMP-M组、IMP-H组HMGB1、RAGE、NF-κB p65蛋白水平均降低( $P<0.05$ ); 与IMP-H组比较, IMP-H+rHMGB1组的HMGB1、RAGE、NF-κB p65蛋白水平显著升高( $P<0.05$ )(图3和表6)。这表明IMP能够抑制HG诱导的hRECs细胞HMGB1-RAGE-NF-κB信号通路激活。

3 讨论

DR是DM的主要并发症之一, 据相关研究发现, DR的发生受到氧化应激等多种机制的调控<sup>[14]</sup>。已有研究发现, 在整个DR病理的进展中, 内皮细胞损伤发挥着关键性的作用<sup>[15]</sup>。据相关研究发现, 内皮细胞长期处于高糖状态下, 内皮细胞的活性会受到一定的抑制, 并且这会诱导内皮损伤的加剧<sup>[16]</sup>。

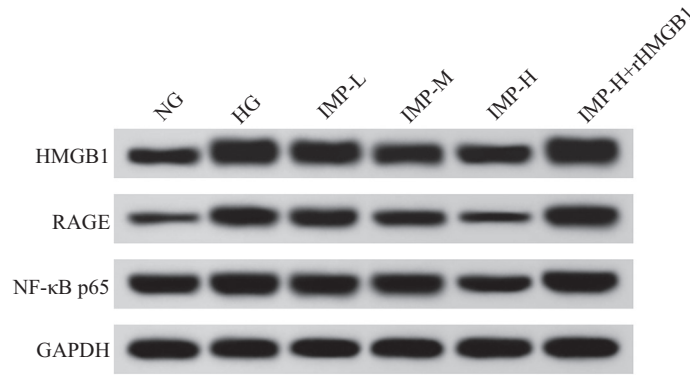


图3 Western blot检测hRECs细胞中HMGB1、RAGE、NF-κB p65蛋白表达

Fig.3 Western blot analysis of HMGB1, RAGE, NF-κB p65 protein expression in hRECs

表6 各组hRECs细胞中HMGB1-RAGE-NF-κB信号通路相关蛋白水平

Table 6 Levels of protein associated with HMGB1-RAGE-NF-κB signaling pathway in each group of hRECs

| 组别<br>Groups | HMGB1/GAPDH                  | RAGE/GAPDH                   | NF-κB p65/GAPDH              |
|--------------|------------------------------|------------------------------|------------------------------|
| NG           | 0.57±0.06                    | 0.21±0.03                    | 0.72±0.08                    |
| HG           | 1.65±0.17*                   | 0.83±0.09*                   | 1.32±0.14*                   |
| IMP-L        | 1.36±0.14 <sup>#</sup>       | 0.62±0.07 <sup>#</sup>       | 1.11±0.12 <sup>#</sup>       |
| IMP-M        | 1.01±0.11 <sup>#&amp;</sup>  | 0.44±0.05 <sup>#&amp;</sup>  | 0.83±0.09 <sup>#&amp;</sup>  |
| IMP-H        | 0.66±0.07 <sup>#&amp;▼</sup> | 0.24±0.03 <sup>#&amp;▼</sup> | 0.53±0.06 <sup>#&amp;▼</sup> |
| IMP-H+HMGB1  | 1.53±0.16 <sup>@</sup>       | 0.75±0.08 <sup>@</sup>       | 1.16±0.12 <sup>@</sup>       |

\* $P<0.05$ , 与NG组比较; <sup>#</sup> $P<0.05$ , 与HG组比较; <sup>&</sup> $P<0.05$ , 与IMP-L组比较; <sup>▼</sup> $P<0.05$ , 与IMP-M组比较; <sup>@</sup> $P<0.05$ , 与IMP-H组比较。

\* $P<0.05$  compared with NG group; <sup>#</sup> $P<0.05$  compared with HG group; <sup>&</sup> $P<0.05$  compared with IMP-L group; <sup>▼</sup> $P<0.05$  compared with IMP-M group;

<sup>@</sup> $P<0.05$  compared with IMP-H group.

DUAN等<sup>[17]</sup>研究发现,暴露于高糖环境下的内皮细胞,其增殖活性受到抑制,但其迁移能力增强。此外,视网膜血管生成是DR发病的重要机制。RECs对视网膜屏障起重要作用,可感知血糖变化,高糖刺激视网膜血管生成可加速糖尿病视网膜病变<sup>[18]</sup>。因此,通过探究减轻视网膜血管内皮细胞损伤的机制,可以为DR的治疗提供一种新的方向。本研究结果显示,与NG组比较, HG组细胞存活率降低,细胞凋亡率和管腔形成数升高,提示高糖环境会损伤视网膜血管内皮细胞。

IMP是从中草药中提取得到的,具有毒性低、活性强的优点。相关研究发现, IMP在糖尿病肾损伤以及相关炎症反应中发挥重要作用<sup>[19]</sup>。LIAO等<sup>[20]</sup>研究发现, IMP在血管性痴呆中发挥抗氧化的作用。近期研究显示,含IMP的生肌化瘀方能够促进糖尿病大鼠创面愈合<sup>[21]</sup>。据相关研究表明, IL-1 $\beta$ 、IL-6和TNF- $\alpha$ 的水平均与机体的炎症有关,它们的水平高低可以反映细胞或者组织的损伤程度<sup>[22-23]</sup>。SOD是清除氧自由基的内源性抗氧化剂,其水平是机体

氧化应激水平的标志物<sup>[24]</sup>。MDA是脂质过氧化产物,其水平高低可以反映细胞氧化应激水平<sup>[25]</sup>。本研究结果显示,与HG组比较, IMP-L组、IMP-M组、IMP-H组细胞存活率、SOD活性升高,细胞凋亡率,管腔形成数, IL-6、TNF- $\alpha$ 、IL-1 $\beta$ 、MDA表达水平降低,表明IMP可以促进视网膜血管内皮细胞增殖,抑制细胞凋亡、血管生成、炎症反应与氧化应激,且具有浓度依赖性。

HMGB1是一种分泌蛋白,可以对其信号通路的下游进行激活,从而促进炎症因子的释放<sup>[26]</sup>。研究发现, HMGB1可以和RAGE相结合,从而介导其信号通路下游蛋白的磷酸化,进一步诱导神经损伤的发生<sup>[27]</sup>。NF- $\kappa$ B是重要的炎症反应调控因子,在相关炎症(如骨关节炎)的发生发展过程中发挥重要作用<sup>[28]</sup>。已有相关研究发现,通过抑制NF- $\kappa$ B信号通路可以在一定程度上减少炎症因子的释放,进一步减缓炎症反应以及氧化过程<sup>[29]</sup>。研究发现, NF- $\kappa$ B p65是NF- $\kappa$ B的转录活性形式<sup>[30]</sup>。研究发现,抑制HMGB1-RAGE-NF- $\kappa$ B活化能够减轻DR大鼠视网膜炎症<sup>[31]</sup>,

且抑制NF- $\kappa$ B信号通路,可促进高糖诱导的hRECs细胞增殖<sup>[32]</sup>。本研究发现,IMP处理高糖诱导的hRECs细胞后,HMGB1、RAGE、NF- $\kappa$ B p65蛋白水平显著降低,提示IMP可能通过调控HMGB1-RAGE-NF- $\kappa$ B信号通路来发挥作用。为验证该通路的作用,本研究进一步用重组HMGB1蛋白和高浓度IMP共同处理高糖诱导的hRECs细胞,结果发现rHMGB1促进HMGB1、RAGE、NF- $\kappa$ B p65蛋白表达,并逆转高糖条件下IMP对hRECs细胞凋亡的抑制作用,提示IMP可能通过抑制HMGB1-RAGE-NF- $\kappa$ B信号通路,减轻高糖诱导的视网膜血管内皮细胞损伤。

综上所述,IMP可能通过抑制HMGB1-RAGE-NF- $\kappa$ B信号通路,降低炎症反应和氧化应激水平,进而减轻高糖诱导的视网膜血管内皮细胞损伤。这为新药的开发提供了一定的基础。但是本研究仅在细胞上进行了体外实验,后续会进一步开展体内实验进行验证。

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