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## 槲皮素对宫颈炎模型大鼠治疗效果的初步实验研究\*

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**摘要 目的:**探讨槲皮素对宫颈炎模型大鼠的治疗效果。**方法:**采用阴道注入苯酚胶浆制备大鼠宫颈炎模型,观察不同剂量(10  $\mu\text{mol}\cdot\text{L}^{-1}$ 、100  $\mu\text{mol}\cdot\text{L}^{-1}$ )槲皮素对大鼠白细胞总数、中性粒细胞数和比例影响,并对实验大鼠宫颈进行病理组织学和超微结构观察(观察细胞膜、线粒体及细胞核结构变化)。**结果:**槲皮素处理组白细胞总数下降,中性粒细胞计数和百分比均明显降低,与宫颈炎模型组比较差异有统计学意义( $P<0.05$ )。病理组织学观察结果显示槲皮素处理组炎症细胞数量较宫颈炎模型组减少,电镜观察结果也表明槲皮素处理组宫颈细胞超微结构损害程度依次减轻,胞核内染色质固缩情况减轻,逐渐可看到连续的核膜结构。**结论:**槲皮素能明显减轻宫颈组织充血水肿,对宫颈炎模型大鼠有一定的治疗作用。

**关键词:**槲皮素;宫颈炎;大鼠

**中图分类号:**R-33;R711.74 **文献标识码:**A **文章编号:**1673-6273(2018)02-222-04

## Assessment of the Therapeutic Effect of Quercetin on the Rat Model of Cervicitis\*

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**ABSTRACT Objective:** To investigate the therapeutic effect of quercetin on rats with cervicitis. **Methods:** Cervicitis model rats were prepared by vaginal injection of phenol mucilage, the effect of different doses of quercetin(10  $\mu\text{mol}\cdot\text{L}^{-1}$ , 100  $\mu\text{mol}\cdot\text{L}^{-1}$ ) on the number of white blood cells, number and proportion of neutrophil were observed, the rats cervical histopathological and ultrastructural findings(the changes of cell membrane, mitochondria and nuclear structure) were also detected. **Results:** White blood cells in quercetin group were decreased, counts and percentage of neutrophil cells were significantly reduced, which had statistical significance compared with cervicitis model group ( $P<0.05$ ); Histopathological observation results showed that the number of inflammatory cells in quercetin-treated group was lower than that in cervicitis model group, Electron microscopic observation also showed that the ultrastructural damage of cervical cells in quercetin-treated group was reduced, the chromatin condensation in the nucleus was alleviated, and the continuous nuclear membrane structure could be seen gradually. **Conclusion:** Quercetin can obviously reduce the total number of white blood cells, reduce the cell count and percentage of neutrophil cells, reduce the cervical tissue hyperemia and edema, which have a good therapeutic effect on cervicitis rats.

**Key words:** Quercetin; Cervicitis; Rat

**Chinese Library Classification(CLC):** R-33; R711.74 **Document code:** A

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### 前言

槲皮素是一种黄酮类化合物,具有多种生物活性,其中包括抗炎、扩张冠状动脉、清除自由基、下调由活性氧介导的下游信号通路<sup>[1,2]</sup>。关于槲皮素的抗炎作用,已有大量的文献报道。研究显示槲皮素及其主要代谢产物能够抑制炎症反应,对机体多种细胞发挥保护作用<sup>[3]</sup>。Javadi F 通过临床双盲、随机对照试验研究发现槲皮素能够抑制女性体内炎症因子的表达,改善妇女类风湿关节炎的临床症状<sup>[4]</sup>;Kayani WK 的研究证实黄酮类药物除了具有较强的抗氧化作用,还可以改善女性宫颈炎症状,并发挥止痛的作用<sup>[5]</sup>。但目前尚未有研究证实槲皮素对慢性宫

颈炎的疗效。因此,本研究建立了宫颈炎大鼠模型,通过宫颈局部给槲皮素治疗获得满意疗效,现报道如下。

### 1 材料与方法

#### 1.1 实验动物

3月龄雌性未孕 Wistar 大鼠 60只,体质量 200~250 g,由哈尔滨医科大学附属第一医院动物实验中心提供。

#### 1.2 造模及分组

随机分为3组,每组20只:正常组、模型组、治疗组。正常对照组:正常饲养,不做任何处理。宫颈炎模型组:乙醚麻醉大鼠后,将其仰卧固定。相对无菌环境下,手持1 mL注射器轻轻

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插入大鼠阴道约 1 cm 处,缓慢注入 0.1 mL 25%苯酚胶浆,每 2 d 注射 1 次,连续 3 次<sup>[5]</sup>。槲皮素处理组(槲皮素浓度参阅文献 6,7):先制备宫颈炎模型,观察部分大鼠阴道口有白色分泌物流出,阴道充血、红肿,选择造模成功的 18 只大鼠按体重随机分为 2 组(槲皮素低、高浓度组):分组依据是根据槲皮素处理浓度不同,用 1 mL 注射器吸取浓度分别为  $10 \mu\text{mol}\cdot\text{L}^{-1}$ 、 $100 \mu\text{mol}\cdot\text{L}^{-1}$  的槲皮素注入大鼠阴道约 1 cm 处,注入剂量为 0.1 mL/200 g,2 次/日,连续 3 日,半个月后处死。正常对照组及宫颈炎模型组给以等量基质。

### 1.3 观察指标

**1.3.1 白细胞分类计数** 眼球取血 1 mL,EDTA 二钾盐抗凝,全自动血细胞计数分析测定血常规,重点比较白细胞分类的变化。

**1.3.2 宫颈组织学检查** 切取大鼠阴道至子宫分角处组织,10%甲醛固定,石蜡包埋,包埋好的蜡块固定于切片机上,切成薄片,苏木精-伊红染色 5 分钟,纯酒精脱水,再经二甲苯使切片透明,盖上盖玻片封固后光镜下观察病理组织学变化(放大倍数 $\times 200$ )。

**1.3.3 透射电子显微镜观察组织超微结构的变化** 切取大鼠

阴道至子宫分角处组织,2.5%戊二醛溶液固定子宫组织 24 小时,控制温度为  $4^{\circ}\text{C}$ ,PBS 漂洗,1%锇酸后固定,PBS 洗涤细胞 2 次,乙醇饱和醋酸铀染液块染,乙醇-丙酮梯度脱水,环氧树脂 618 包埋剂包埋,切片机切片后用柠檬酸铅、醋酸铀各染色 5 min,透射电镜观察细胞膜,线粒体及细胞核改变(放大倍数 $\times 10000$ )。

### 1.4 统计学分析

采用 SPSS11.5 对实验数据进行分析,实验结果以均值 $\pm$ 标准差(Mean $\pm$  SEM)表示,两样本之间的比较采用 t 检验,多样本之间的比较采用单因素方差分析, $P<0.05$  为差异具有统计学意义。

## 2 结果

### 2.1 各组大鼠白细胞分类计数的比较

宫颈炎模型组白细胞总数明显、中性粒细胞总数和百分比均显著高于阴性对照组,两组比较差异有统计学意义( $P<0.05$ ),槲皮素处理组白细胞总数、中性粒细胞总数和百分比均较宫颈炎模型组明显降低,差异有统计学意义( $P<0.05$ ),提示槲皮素能减轻炎症反应。

表 1 各组白细胞分类计数的比较( $\bar{x}\pm s$ )

Table 1 Comparison of the number and classification of white blood cells between different groups( $\bar{x}\pm s$ )

| Groups                           | Number | White blood cells<br>( $10^9/\text{L}$ ) | Neutrophil cells<br>( $10^9/\text{L}$ ) | percentage of neutrophil(%) |
|----------------------------------|--------|------------------------------------------|-----------------------------------------|-----------------------------|
| Negative control group           | 20     | $7.47\pm 3.1220$                         | $2.80\pm 0.40$                          | $36.99\pm 4.03$             |
| Cervicitis model group           | 20     | $16.63\pm 2.86^a$                        | $11.27\pm 1.86^a$                       | $71.88\pm 4.42^a$           |
| Low concentrations of quercetin  | 9      | $10.44\pm 3.26^b$                        | $5.65\pm 1.05^b$                        | $53.59\pm 5.89^b$           |
| High concentrations of quercetin | 9      | $8.88\pm 2.08^b$                         | $4.84\pm 1.01^b$                        | $52.53\pm 3.97^b$           |

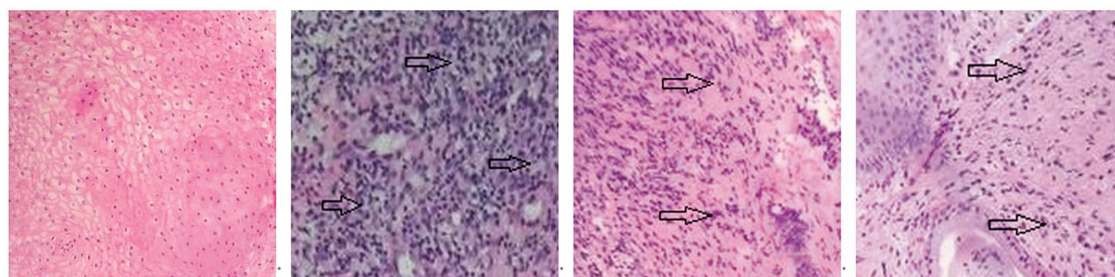
注:与阴性对照组比较  $^a P<0.01$ ; 与宫颈炎模型组比较  $^b P<0.01$ 。

Note: Compare with the negative control group  $^a P<0.01$ ; Compare with the cervicitis model group  $^b P<0.01$ .

### 2.2 各组大鼠宫颈组织形态观察

阴性对照组病理切片结果见宫颈上皮完整,结构正常,上皮细胞排列整齐(图 1A);宫颈炎模型组细胞间充满分泌物,大量炎细胞浸润,广泛出现细胞变性、坏死及细胞脱落现象(图

1B);治疗组随着槲皮素作用浓度的增加,炎症细胞数量及浸润范围减小,炎症细胞浸润明显减轻(图 1C、D,箭头所示为炎症细胞)。



A 阴性对照组 B 宫颈炎模型组 C 槲皮素低浓度组 D 槲皮素高浓度组

A:negative control group; B: cervicitis model group; C: low concentrations of quercetin; D: high concentrations of quercetin;

图 1 HE 染色法检测宫颈细胞形态( $200\times$ )

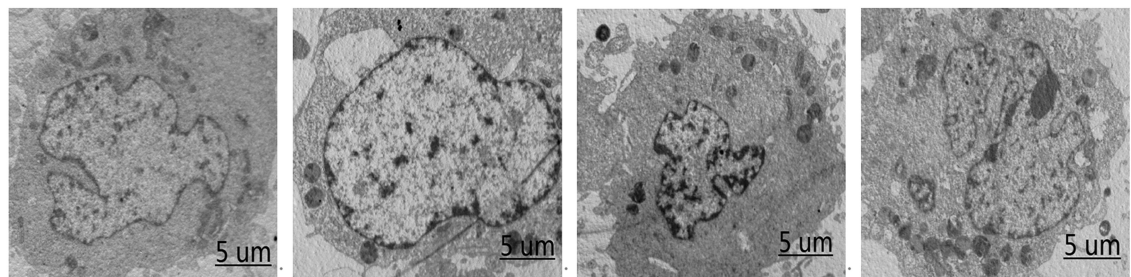
Fig.1 Characteristic of cervic cells detected by HE staining( $200\times$ )

电镜检测结果显示:阴性对照组宫颈细胞胞膜完整连续,核仁形态规则,呈圆形或椭圆形(图 2A);宫颈炎模型组细胞胞

膜不清晰,胞质内线粒体空泡变性,细胞核明显出现变形而不规则,核内染色质不均匀,核周边出现了异染色质的边移,(图

2B);  $10 \mu\text{mol}\cdot\text{L}^{-1}$ 、 $100 \mu\text{mol}\cdot\text{L}^{-1}$  的槲皮素处理组宫颈细胞超微结构损害程度依次减轻,胞核内染色质固缩情况减轻,逐渐可

看到连续的核膜结构(图 2C、2D)。



A 阴性对照组 B 宫颈炎模型组 C 槲皮素低浓度组 D 槲皮素高浓度组

A: negative control group; B: cervicitis model group; C: low concentrations of quercetin; D: high concentrations of quercetin

图 2 电镜观察宫颈细胞膜及细胞器改变(10000×)

Fig.2 Cervical cell membrane and cell organelles changes detected by electron microscopy(10000×)

### 3 讨论

研究显示我国 90% 以上的经产妇都患有宫颈炎,近 50% 的育龄妇女都患有不同程度的宫颈炎<sup>[4-7]</sup>。慢性宫颈炎因其病程通常比较长,容易迁延,反复发作,对女性的身体健康会产生较大的伤害,严重影响女性生活质量。临床表现包括白带增多,白带呈现黄白色或红色,有时还会出现腥臭味,并可出现宫颈糜烂<sup>[8]</sup>。引起宫颈炎的主要原因有经期不卫生,不洁净的性生活、机体抵抗力低下、流产、分娩等<sup>[9]</sup>。

宫颈炎的药物治疗方法很多,分为局部用药和全身用药,全身用药适应于全身症状明显且病情较重者,而局部用药是治疗慢性宫颈炎常见的方法之一,适用于病灶表浅且糜烂面积相对较小的病例<sup>[9-11]</sup>。主要药物治疗方法包括局部阴道灌洗及局部上药和中药治疗<sup>[12,13]</sup>。其中,中药治疗的方式较为常见,也是疗效较好的治疗方式之一,不会使患者产生耐药性,治疗的效果显著。截至目前,国内对宫颈炎的诊断和治疗尚无统一的共识,但是局部的药物治疗仍然是改善病情的重要方法<sup>[14,15]</sup>。

宫颈炎模型的建立是研究该病的重要手段,对于疾病的预防、诊断、治疗具有指导意义。本研究选取体质量 200~250 g 的雌性未孕 Wistar 大鼠,易于饲养,体形适中,在探讨宫颈炎病理转归的同时也可作为筛选和验证治疗宫颈炎的药物提供可靠的研究手段。结果显示造模成功的 Wistar 大鼠宫颈阴道部呈淡红色细颗粒状外观,显微镜下看到宫颈管单层柱状上皮菲薄,其下间质透出橙红色,这些表现均与临床宫颈炎症状一致。

槲皮素具有广泛的生理和药理活性,在我国资源丰富,是一个很经济的黄酮类药物<sup>[16-19]</sup>。有研究显示槲皮素能够抑制人体的内皮细胞氧化应激与炎症的发生,食用含有槲皮素的植物原料能够促进人类健康,其作用机制可能与槲皮素代谢物抑制内皮氧化应激与炎症反应有关。生理剂量的槲皮素能有效抑制单细胞趋化蛋白-1(MCP-1)、细胞间黏附因子-1(ICAM-1)和血管细胞黏附因子-1(VCAM-1)的基因表达,有效减少 ROS 的过量生成并逆转线粒体膜电位下降,从而发挥抑制内皮氧化应激的功能<sup>[20]</sup>。国内外有不少关于槲皮素抗炎疗效的文章,但是尚未见其应用于宫颈炎中,本实验首次将该药物作用于宫颈炎

大鼠模型中,希望为临床治疗提供新的药物选择。有研究显示中药外用治疗慢性宫颈炎治疗方法多样、不良反应小且疗效较好,通过喷敷法、中药灌洗法、栓剂上药法、散剂蘸涂法等均可有效发挥治疗作用<sup>[13]</sup>。因为槲皮素具有较好的抗炎、抗氧化作用,而且天然低毒,所以我们将其应用于宫颈炎的治疗。

本实验结果显示宫颈炎模型大鼠白细胞数目较正常对照大鼠明显上升,给予槲皮素治疗后白细胞总数下降,中性粒细胞总数和百分比均明显降低。此外,造模成功的宫颈炎大鼠排出物中有大量灰白色脓液,阴道水肿,宫颈充血,槲皮素可改善宫颈组织形态学损伤,减轻阴道及宫颈的充血水肿状态。病理组织学结果及超微结构观察也从不同角度证实了槲皮素对宫颈炎大鼠宫颈组织的保护作用。这些研究结果均证实了槲皮素对大鼠宫颈炎有较好治疗效果,但对其作用的具体机制,还需要进一步探讨。

综上所述,槲皮素对宫颈炎模型大鼠有一定改善作用,能明显减轻宫颈组织充血水肿,降低炎症细胞浸润,其确切机制有待进一步研究。

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