阳和汤对兔膝关节炎木瓜蛋白酶模型 IL-1、TNF- α 的影响

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摘要 **目的:** 观察阳和汤对兔膝关节炎模型中白细胞介素-1(IL-1)、肿瘤坏死因子- α (TNF- α)的影响, 探讨阳和汤防治骨关节炎的作用机制。**方法:** 选取4~6月龄健康新西兰兔32只, 雌雄各半, 体质量(2.25 $\pm$ 0.25)kg; 采用随机数字表法分为对照组、模型组、阳和汤组、筋骨痛消丸组, 每组8只。适应性饲养2周后, 除对照组外, 其余3组均采用关节腔内注入木瓜蛋白酶复制膝关节炎兔模型: 轻度弯曲兔膝关节, 经关节正中或两侧进针, 将1.6%木瓜蛋白酶生理盐水溶液0.5 mL通过髌上韧带注入膝关节腔, 每隔3天注射1次, 连续3次。对照组与模型组予0.9%生理盐水灌胃; 阳和汤组予6 g/(kg·d)阳和汤灌胃; 筋骨痛消丸组予6 g/(kg·d)筋骨痛消丸灌胃。以上干预均1次/d, 每周7次, 共干预5周。停药1周后, 处死全部实验动物, 取兔股骨内髌关节软骨, 采用肉眼、光镜下进一步观察苏木素-伊红染色后的关节软骨组织形态学改变情况; 采用改良Mankin's评分标准评价软骨退变程度; 采用酶联免疫吸附实验(ELISA)检测血清和关节液中IL-1、TNF- α 含量。**结果:** 与对照组比较, 模型组兔的软骨表面凹凸不平、局部缺损, 软骨形态结构紊乱, 软骨细胞数量减少, 潮线紊乱消失, Mankin's评分增高, 血清和关节液中IL-1、TNF- α 含量增高, 差异具有统计学意义($P<0.05$)。与模型组比较, 阳和汤组、筋骨痛消丸组兔的软骨缺损减轻, 软骨形态结构较整齐, 软骨细胞数量增多, 潮线清晰, Mankin's评分降低, 血清和关节液中IL-1、TNF- α 含量降低, 差异具有统计学意义($P<0.05$)。与筋骨痛消丸组比较, 阳和汤组兔的软骨形态结构未见明显区别, 血清和关节液中IL-1、TNF- α 含量无明显区别, 差异无统计学意义($P>0.05$)。**结论:** 阳和汤对骨关节炎的软骨损伤具有修复作用, 能有效延缓关节软骨退变, 其作用机制可能与抑制IL-1、TNF- α 等炎症因子表达有关。

关键词 膝关节炎; 木瓜蛋白酶模型; 阳和汤; 白细胞介素-1; 肿瘤坏死因子- α ; 作用机制

骨关节炎(Osteoarthritis, OA)是一种以关节疼痛、僵硬、功能受限甚至关节畸形、残废为主要表现的骨科常见病, 目前中国已有超过1亿OA患者^[1]。药物是治疗骨关节炎的主要手段之一, 临床多采用非甾体抗炎药(nonsteroidal antiinflammatory drugs, NSAIDs)等为主的西药进行治疗, 虽可暂时缓解关节疼痛、改善功能, 但无法修复受损的关节软骨、阻

止OA进一步发展, 长期服用此类药物还会出现胃溃疡、胃出血、肝肾损害等不良反应。中医药可以修复损伤的软骨组织和细胞, 从而保护关节软骨, 防治骨关节炎具有疗效可靠、价格低廉、不良反应少的特色与优势^[2]。阳和汤源自清代王洪绪《外科证治全生集》, 原为治疗外科阴疽证的经典方剂, 现在广泛应用于内科杂症, 特别是痹证, 为攻补兼备、刚柔相

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济之治痹良方。有研究表明,阳和汤治疗膝骨关节炎的临床疗效明显,可有效缓解膝关节疼痛,改善膝关节功能^[3-6]。但是,有关阳和汤防治骨关节炎具体作用机制的研究还较少,也不够深入,值得进一步探讨。因此,本研究通过建立兔膝骨关节炎木瓜蛋白酶模型并予阳和汤灌胃,以探讨阳和汤防治骨关节炎的作用及其作用机制。

1 实验材料

1.1 实验动物

选择健康新西兰兔 32 只,4~6 月龄,体质量 (2.25 ± 0.25) kg,雌雄各半,由福建中医药大学实验动物中心代购并饲养[许可证号:SCXK(闽)2012-0001]。按照随机数字表法分为对照组、模型组、阳和汤组、筋骨痛消丸组,每组 8 只。动物处理参照中华人民共和国科学技术部颁布的《关于善待实验动物的指导性意见》,并经福建中医药大学动物伦理委员会审核批准。

1.2 主要仪器、药品及试剂

生物组织包埋机(湖北省宏业医用仪器有限公司,BM-VII);石蜡切片机(美国 Thermo 公司,HM315);低温高速离心机(美国 BECKMAN 公司,64R);酶联免疫检测仪(美国 Biotek 公司,Elx808);光学显微镜(日本 Olympus 公司,BX40);酶标仪(美国 Biotek 公司,Elx808)。

阳和汤由福建中医药大学国医堂提供,采用清代王洪绪《外科证治全生集》原方(熟地黄 30 g,鹿角胶 9 g,麻黄 2 g,白芥子 6 g,肉桂 3 g,姜炭 2 g,生甘草 3 g),煎煮后制成浓度 1 g/mL 的溶液,55 mL/剂;筋骨痛消丸(河南省洛正制药厂,生产批号:Z10970117),由丹参、桂枝、香附、地黄、白芍、牛膝、秦艽等药物组成,以蒸馏水配制成浓度 1 g/mL 的溶液,36 mL/盒;木瓜蛋白酶(美国 Sigma 公司,生产批号:107147);兔白细胞介素-1(interleukin-1,IL-1)酶联免疫检测试剂盒(上海西唐生物科技有限公司,生产批号:F20190);兔肿瘤坏死因子- α (tumor necrosis factor- α ,TNF- α)酶联免疫检测试剂盒(上海西唐生物科技有限公司,生产批号:F2041);戊巴比妥钠(国药集团化学试剂有限公司,生产批号:WS20060401);0.9%NaCl 注射液(北京天坛生物制品股份有限公司);4%多聚甲醛、EDTA 脱钙液(上海国药集团)。

2 实验方法

2.1 动物模型制备

新西兰兔适应性饲养 2 周后,除对照组外,其余各组膝关节腔注入木瓜蛋白酶造模。选兔双膝为手术部位,耳缘静脉麻醉(戊巴比妥钠,30 mg/kg)后仰卧固定,常规备皮、消毒、铺巾。轻度弯曲兔膝关节,从关节的正中间或者关节的两侧进针,手下出现落空感则表示针头进入了关节腔,随后可往关节腔内注入 0.5 mL 1.6 %木瓜蛋白酶生理盐水溶液,每隔 3 d 注射 1 次,共注射 3 次。每日定时分 2 次于空旷场地驱赶,共强迫兔活动 30 min,连续驱赶 4 周。

2.2 干预方法

造模成功 4 周后,参照“人和动物体表面积折算的等效剂量比率表”进行换算。筋骨痛消丸具有活血行气、温阳通经、消肿止痛的作用,阳和汤与筋骨痛消丸均可有效缓解膝骨关节炎患者局部疼痛肿胀,疏利关节,临床效果显著。因此本研究选择筋骨痛消丸作为阳性对照。阳和汤组按 6 g/(kg·d)的量给予阳和汤灌胃,筋骨痛消丸组按 6 g/(kg·d)的量给予筋骨痛消丸灌胃,以上 2 组均给药 1 次/d,连续用药 5 周。对照组、模型组常规饲养,无其他特殊处理。停药 1 周后,经腹腔麻醉后取材,耳缘静脉空气栓塞处死全部动物。

2.3 取材

2.3.1 取材前准备 取材前 12 h 动物禁食不禁水,麻醉后取材。

2.3.2 血清采集 采用腹主动脉采血法。打开兔腹腔,分离腹主动脉,一次性采血针取血 5 mL,待血液静置分层后,低温高速离心机(4 ℃,3 000 r/min)离心 20 min,取血清,-80 ℃冰箱保存。

2.3.3 关节液采集 兔髌骨内缘行纵行切口,由皮肤向下逐层分离至关节囊,反复屈伸关节将关节液挤压至髌上囊内侧,轻轻划开关节囊,注入 1.5 mL 生理盐水反复抽吸后将关节液尽量抽出,低温高速离心机(4 ℃,15 000 r/min)离心 15 min,取上清液,-80 ℃冰箱保存。

2.3.4 软骨组织采集 切开兔膝关节囊,分离软组织后快速切取股骨内髁,粗修后于 4%多聚甲醛中固定 24 h,10% EDTA 脱钙完全后修切,常规石蜡包埋后切片。

2.4 指标及检测方法

2.4.1 软骨组织形态学观察 取上述的软骨组织石蜡切片,常规脱蜡后,分别进行苏木素-伊红染色;

苏木素染液 20 min,10%盐酸酒精分色 3 s,自来水返蓝 20 min,0.5%伊红染液 5 s。染色完成后均采用 95%乙醇、无水乙醇脱水,二甲苯透明,光学树脂固

封。光镜下对组织结构进行观察及图像采集。
2.4.2 软骨损伤程度评价 采用 Mankin's 评分标准^[7-8]评价软骨的损伤程度。见表 1。

表 1 Mankin's 评分标准
Table 1 Mankin's score

软骨结构		软骨细胞		蛋白多糖染色		潮线完整性	
项目	评分	项目	评分	项目	评分	项目	评分
光整如常	0	数量如常	0	正常	0	完整	0
表面细小裂隙	1	弥漫性增多	1	染色轻度减退	1	多重潮线	1
裂隙达移行层	2	大量簇集细胞	2	染色中度减退	2	血管侵入潮线	2
裂隙达放射层	3	数量明显减少	3	染色重度减退	3		
裂隙达钙化层	4			染色完全消失	4		
软骨层脱落	5						

2.4.3 血清及关节液中白细胞介素-1、肿瘤坏死因子- α 含量检测 运用酶联免疫吸附法(enzyme-linked immunosorbent assay,ELISA)检测血清及关节液中 IL-1、TNF- α 含量。方法及步骤如下:按照 ELISA 试剂盒的操作步骤依次操作,以酶标仪(波长 450 nm)读取 A 值,绘制标准曲线并计算各样品的 IL-1、TNF- α 浓度。

2.5 统计学方法

用 SPSS 20.0 软件进行数据统计,计量资料以($\bar{x}\pm s$)表示,数据符合正态分布采用单因素方差分析,若方差齐,使用 LSD-*t* 检验比较两两之间差异,若方差不齐,则采用 Games-Howell 比较两两之间差异;数据不符合正态分布,采用多样本秩和检验进行两两比较。*P*<0.05 为差异有统计学意义。

3 结果

3.1 4组实验情况

实验期间各给药组新西兰兔精神状态好,排便、体温和皮毛等未见异常,其体质量、进食量与对照组基本一致。实验过程动物无死亡。

3.2 4组软骨组织形态学比较

3.2.1 4组软骨组织肉眼观察 ① 对照组:股骨内侧髌关节软骨色泽鲜红,表面光滑,无软骨破坏和骨赘形成。② 模型组:股骨内侧髌软骨无光泽,局部凹凸不平,可见软骨缺损及骨赘形成。③ 阳和汤组:股骨内侧髌软骨表面欠光滑,可见轻微软骨缺损和骨赘形成,其程度明显较模型组轻。④ 筋骨痛消丸组:股骨内侧髌软骨表面欠光泽,缺损和骨赘较模型组轻。见图 1。

注:A 为对照组,B 为模型组,C 为阳和汤组,D 为筋骨痛消丸组。
Note: A is control group, B is model group, C is Yanghe decoction group, D is Jingu Tongxiao pill group.

图 1 肉眼观察关节软骨结构

Figure 1 Observation of articular cartilage structure with naked eyes

3.2.2 4组软骨组织光镜观察 ① 对照组:软骨表面光滑、完整,软骨组织的 4 层结构(表浅层、移行层、辐射层、钙化层)分层清晰,软骨细胞成柱状排列,分布均匀,潮线清晰完整。② 模型组:软骨表面

不光滑,出现多个裂隙及缺损,软骨结构分层不清,且细胞数量减少、分布不均匀,可见细胞簇集,潮线紊乱消失。③ 阳和汤组:软骨表面较光滑,软骨细胞比模型组排列整齐,软骨细胞增多,可见少量软骨

细胞簇集现象,潮线较模型组清晰、完整。④ 筋骨痛消丸组:软骨表面光滑,细胞排列尚整齐,可见软骨

细胞簇集现象,潮线清晰、完整程度较模型组好。

注:A 为对照组,B 为模型组,C 为阳和汤组,D 为筋骨痛消丸组。

Note: A is control group, B is model group, C is Yanghe decoction group, D is Jingu Tongxiao pill group.

图 2 光镜观察关节软骨病理显微结构(石蜡切片-HE 染色)($\times 100$)

Figure 2 Observation of pathological microstructure of articular cartilage by light microscopy (Paraffin-HE staining)($\times 100$)

3.3 4 组关节软骨形态学改变 Mankin's 评分比较

见表 2。

3.4 4 组血清及关节液中 IL-1、TNF- α 比较

见表 3。

4 讨 论

4.1 阳和汤能有效修复骨关节炎的软骨损伤

骨关节炎以关节疼痛、肿胀、活动受限甚至关节畸形为临床症状,以关节软骨退变和继发性骨质增生为病理特点。木瓜蛋白酶关节内注射动物模型与人类骨关节炎类似^[9],呈现出早期骨关节炎的病理改变。本研究运用木瓜蛋白酶关节内注射法建立兔骨关节炎模型,采用苏木素-伊红染色方法在光镜

表 2 4 组关节软骨形态学改变 Mankin's 评分比较($\bar{x} \pm s$)

Table 2 Comparison of Mankin's score in morphological change of articular cartilage in four groups ($\bar{x} \pm s$)

组 别	n	Mankin's 评分
对照组	8	0.47 $\pm$ 0.03
模型组	8	8.41 $\pm$ 0.07 ¹⁾
筋骨痛消丸组	8	5.38 $\pm$ 0.06 ²⁾
阳和汤组	8	5.43 $\pm$ 0.05 ²⁾

注:与对照组比较,1) $P < 0.05$;与模型组比较,2) $P < 0.05$ 。

Note: Compared with the control group, 1) $P < 0.05$; Compared with the model group, 2) $P < 0.05$.

表 3 4 组血清及关节液中 IL-1、TNF- α 比较($\bar{x} \pm s$)

Table 3 Comparison of IL-1 and TNF- α in serum and articular fluid in four groups($\bar{x} \pm s$)

组 别	n	血 清		关 节 液	
		IL-1	TNF- α	IL-1	TNF- α
对照组	8	55.06 $\pm$ 1.02	71.22 $\pm$ 0.68	5.86 $\pm$ 0.34	3.93 $\pm$ 0.17
模型组	8	86.27 $\pm$ 1.27 ¹⁾	92.39 $\pm$ 0.61 ¹⁾	31.71 $\pm$ 1.01 ¹⁾	49.07 $\pm$ 1.34 ¹⁾
筋骨痛消丸组	8	67.30 $\pm$ 1.48 ²⁾	66.29 $\pm$ 0.71 ²⁾	19.03 $\pm$ 0.27 ²⁾	31.10 $\pm$ 0.44 ²⁾
阳和汤组	8	64.67 $\pm$ 1.07 ²⁾	69.26 $\pm$ 0.39 ²⁾	19.59 $\pm$ 0.59 ²⁾	30.35 $\pm$ 0.60 ²⁾

注:与对照组比较,1) $P < 0.05$;与模型组比较,2) $P < 0.05$ 。

Note: Compared with the control group, 1) $P < 0.05$; Compared with the model group, 2) $P < 0.05$.

下观察软骨组织,软骨形态学及 Mankin's 评分结果显示,模型组的软骨表面出现多个裂隙及缺损,软骨结构分层不清,且细胞数量减少、分布不均匀,可见细胞簇集,潮线紊乱消失,与对照组比较 Mankin's 评分明显升高。而经过阳和汤干预后,兔膝关节软骨形态及 Mankin's 评分均明显改善,这表明阳和汤

能有效修复骨关节炎的软骨损伤。

中医学认为,骨关节炎属于“骨痹”范畴,“本”乃肝肾亏虚、正气不足,“标”乃风、寒、湿邪、血虚寒凝^[10]。因此,骨关节炎的治疗应遵循“急则治痹痛,缓则补肝肾、益气血”的原则,并随风寒湿痰随证加减^[11]。阳和汤方中熟地黄温补养血,鹿角胶温阳、益

精血,二药合用,温阳补血;姜炭、肉桂药性辛热,共奏温阳散寒、温通血脉之功;白芥子辛温,可达皮里膜外,温化寒痰,通络散结;麻黄辛温达卫,宣通毛窍,开腠理,散寒凝;甘草解毒而调和诸药。可见,阳和汤堪为攻补兼备、刚柔相济之治痹良方。

4.2 阳和汤能有效调控 IL-1、TNF- α 等炎性因子

骨关节炎以关节软骨破坏和骨重建为特征,其发病机制尚不明确,研究发现涉及多因素、多机制、多环节。其中,有研究显示 IL-1、TNF- α 与骨关节炎关系密切^[12-13],这些炎性因子在骨关节炎患者中明显升高^[14-15],与骨关节炎的严重程度呈正相关^[16-17],是骨关节炎重要的致病因素,可多方位介导关节软骨退变,导致关节软骨破坏^[18-19]。

本研究运用 ELISA 法检测血清及关节液中 IL-1、TNF- α 含量,结果表明模型组血清及关节液中 IL-1、TNF- α 的表达与对照组比较明显升高,而阳和汤组血清及关节液中 IL-1、TNF- α 的表达较模型组比较明显降低,这提示阳和汤能够调控 IL-1、TNF- α 等炎性因子,促进骨关节炎患者损伤的软骨组织修复。

5 小 结

阳和汤可促进关节软骨修复,延缓关节软骨退变,其作用机制可能与抑制 IL-1、TNF- α 这些炎性因子的表达有关。

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Effects of Yanghe Decoction on IL-1 and TNF- α of Papain in Rabbit Knee Osteoarthritis

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ABSTRACT Objective: To observe the effect of Yanghe decoction on interleukin 1 (IL-1) and tumor necrosis factor- α (TNF- α) in rabbit knee osteoarthritis model, and to explore the mechanism of Yanghe decoction in the prevention and treatment of osteoarthritis. **Methods:** The 32 healthy New Zealand rabbits aged 4-6 months, half male and female, body weight (2.25 ± 0.25) kg, were randomly divided into the control group, the model group, the Yanghe decoction group and the Jingu Tongxiao pill group according to the method of random number table, with 8 cases in each group. After two weeks of adaptive feeding, in addition to the control group, the remaining three groups were injected with papain into the articular cavity to replicate the rabbit model of knee osteoarthritis. The rabbit knee joint was slightly bend, insert the needle through the middle or both sides of the joint, and 0.5 mL 1.6% Papain saline solution was injected into the knee joint cavity through the superior patellar ligament, all were injected every three days, three consecutive times. The control group and the model group were given 0.9% saline, the Yanghe decoction group was given 6 g/(kg·d) Yanghe decoction, the Jingu Tongxiao pill group was given 6 g/(kg·d) Jingu Tongxiao pills. All groups were intervened once a day, seven times a week, continuous intervention for five weeks. One week after drug withdrawal, all experimental animals were sacrificed, and the rabbit femoral medial condyle articular cartilage was taken. The morphological changes of articular cartilage stained with hematoxylin-eosin were further observed by naked eye and light microscope, and the degree of cartilage degeneration was evaluated by modified Mankin's score standard. The levels of interleukin-1 (IL-1) and tumor necrosis factor- α (TNF- α) in serum and synovial fluid were detected by enzyme-linked immunosorbent assay (ELISA). **Results:** Compared with the control group, the model group's cartilage surface was uneven and local defects, the morphology and structure of cartilage was disordered, the number of chondrocytes decreased, the tidal line disorder disappeared, the Mankin's score increased, and the contents of IL-1 and TNF- α in serum and synovial fluid increased, and the difference was statistically significant ($P < 0.05$). Compared with the model group, the cartilage defects in the Yanghe decoction group and the Jingu Tongxiao pill group were alleviated, the morphology and structure of cartilage was neat, the number of chondrocytes increased, the tidal line was clear, the Mankin's score decreased, and the levels of IL-1 and TNF- α in serum and synovial fluid decreased, and the difference was statistically significant ($P < 0.05$). Compared with the Jingu Tongxiao pill group, there was no significant difference in the morphology and structure of cartilage and the contents of IL-1 and TNF- α in serum and synovial fluid in Yanghe decoction group ($P > 0.05$). **Conclusion:** Yanghe decoction can repair the cartilage injury of osteoarthritis and effectively delay the degeneration of articular cartilage, and its mechanism may be related to the inhibition of the expression of inflammatory factors such as IL-1 and TNF- α .

KEY WORDS knee osteoarthritis; papain model; Yanghe decoction; interleukin-1; tumor necrosis factor- α ; mechanism

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Effect of Intermittent High Intensity Aerobic Exercise on Exercise Endurance of Patients with Stroke Complicated with Coronary Heart Disease

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ABSTRACT Objective: To observe the effect of intermittent high intensity aerobic exercise on exercise endurance and activities of daily living in patients with stroke complicated with coronary heart disease. **Methods:** A total of 180 patients with stroke complicated with coronary heart disease, were treated in Shanghai Fourth Rehabilitation Hospital, from February 2015 to December 2017. The patients were randomly divided into the control group, the medium intensity group and the high intensity group according to the method of random number table, with 60 cases in each group. All the three groups were given routine drug treatment of stroke complicated with coronary heart disease. The control group was only given routine rehabilitation treatment, including stroke limb motor function exercise, cardiac rehabilitation training and physiotherapy, five times a week, treatment for 1 to 2 hours each time, continuous treatment for three months. On the basis of the control group, the patients in the medium intensity group were given the aerobic exercise training guided by the results of cardiopulmonary exercise test (CPET) evaluation. After 5 minutes of relaxation and warm-up, the patients used C580R riding bicycle for power treadmill training. The aerobic exercise intensity was 60% peak power (PP), 10 groups were trained each time, 3 minutes for each group, and 1 minute for rest in different groups, continuous training for 40 minutes, once a day, once every other day, three times a week, continuous treatment for three months. On the basis of the control group, the patients in the high intensity group were given the aerobic exercise training guided by the results of CPET evaluation, the training method was the same as the medium intensity group, but only the intensity of aerobic exercise was adjusted to 80% PP. The cardiopulmonary function was evaluated by maximum metabolic equivalents (METs) and left ventricular ejection fraction (LVEF), and the exercise endurance was evaluated by CPET peak power (PP), exercise duration (ED), peak oxygen uptake (peak VO_2) and anaerobic threshold (AT). The activities of daily living of the patients was evaluated by Barthel index. **Results:** There was no significant difference in the scores of PP, ED, peak VO_2 , AT, LVEF, METs and Barthel index in the three groups before treatment ($P > 0.05$). Compared with before treatment, the PP, ED, peak VO_2 , AT, LVEF, METs and Barthel index in the three groups increased significantly after treatment for three months, and the difference was statistically significant ($P < 0.05$). Compared with the control group, the PP, ED and peak VO_2 in the medium and high intensity groups were significantly higher after treatment for three months, the AT and LVEF in the high intensity group were significantly higher after treatment for three months, and the difference was statistically significant ($P < 0.05$), but there was no significant difference in AT and LVEF in the medium intensity group after treatment for three months ($P > 0.05$). Compared with the medium intensity group, the PP, ED, peak VO_2 , METs and LVEF in the high intensity group were significantly higher after treatment for three months, and the difference was statistically significant ($P < 0.05$). **Conclusion:** Intermittent high intensity aerobic exercise can effectively improve the cardiac function and exercise endurance of stroke patients with coronary heart disease, improve the activities of daily living, and improve the prognosis of patients, which is worthy of clinical application.

KEY WORDS stroke; coronary heart disease; aerobic exercise; high intensity; exercise endurance

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