·基础研究·

# 金银花对变异链球菌UA159体外作用的实验研究

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【摘要】　目的　探讨金银花对变异链球菌UA159的体外抑制作用。方法　通过液体二倍稀释法测定金银花对变异链球菌UA159的最低抑菌浓度(minimium inhibitory concentration, MIC)；以溶剂DMSO溶解金银花粉末,配制不同浓度药液,加入菌液,同时设置溶剂对照组与菌液对照组。通过抑菌实验测定金银花对变异链球菌UA159生长及产酸作用,绘制生长曲线和产酸曲线；计算黏附率及黏附抑制率；采用结晶紫定量法测定金银花对变异链球菌UA159生物膜形成量的影响；利用正置显微镜、扫描电子显微镜(scanning electron microscope, SEM)观察生物膜形成情况及细菌数量变化。结果　金银花对变异链球菌UA159的MIC值为12.5 mg/mL；抑菌实验显示金银花对变异链球菌UA159生长、产酸、黏附的抑制作用较对照组强,差异均具有统计学意义($P < 0.05$),且随着药液浓度增强,抑制作用增加。结晶紫定量法结果显示药液组生物膜形成量与对照组相比下降,差异有统计学意义($P < 0.05$),同时正置显微镜下显示生物膜形成量明显下降。扫描电镜下,药液组0、6、12 h加入金银花细菌数量均明显下降。结论　金银花能够抑制变异链球菌UA159的生长、产酸,同时也能抑制其黏附及生物膜的形成。

【关键词】　金银花；　天然药物；　变异链球菌UA159；　龋病；　细菌生长；　最低抑菌浓度；细菌产酸；　黏附率；　黏附抑制率；　生物膜

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***In vitro* study of the effects of honeysuckle on *Streptococcus mutans* UA159**　　ZENG Huaqian[1,2], MAO Ling[1,2], JIN Yaheng[2], LI Shiting[1], XU Ai[1].　1. The Affiliated Stomatological Hospital of Southwest Medical University, Luzhou 646000, China;　2. School of Stomatology, Southwest Medical University, Luzhou 646000, China

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【Abstract】　**Objective**　To investigate the inhibitory effect of honeysuckle on *Streptococcus mutans* UA159 *in vitro*. **Methods**　We used a double-dilution method to measure the minimum inhibitory concentration (MIC) of honeysuckle against *Streptococcus mutans* UA159. Lonicerae lonicerae powder was dissolved in the solvent DMSO, different concentrations of liquid medicine were prepared, and bacterial liquid was added. The solution control group and bacterial liquid control group were set at the same time. The growth and acid production of UA159 were determined using antibacterial experiments. A growth curve and acid production curves were drawn, and the adhesion rate and adhesion inhibition rate were calculated. The effect of honeysuckle on the formation of *Streptococcus mutans* UA159 was determined by crystal violet quantification, and a microscope and a scanning electron microscope were used to observe biofilm formation and structural changes. **Results**　The MIC of honeysuckle against *Streptococcus mutans* UA159 was 12.5 mg/mL. The bacteriostatic experiments showed a difference in the growth, acid production and adhesion of UA159 after honeysuckle treatment ($P < 0.05$) compared with the controls, and the inhibitory effect increased as the drug liquid concentration increased. Crystal violet quantification showed a significant difference in biofilm formation between the pharmaceutical

liquid group and the control group ($P < 0.05$). Meanwhile, the forward microscope showed a significant decrease in biofilm formation. Under SEM, the number of bacteria decreased significantly at 0, 6 and 12 h after honeysuckle addition. **Conclusion**　Honeysuckle inhibits the growth and acid production of UA159 and inhibits adhesion and the formation of biofilms.

【**Key words**】　honeysuckle;　natural medicine;　*Streptococcus mutans* UA159;　caries;　growth of bacteria;　minimum inhibitory concentration;　acid-producing bacteria;　adhesion rate;　adhesion inhibition rate;　biofilm

龋病是一种常见的口腔疾病,不仅影响口腔健康还会影响咀嚼、消化、语言等功能,甚至导致错𬌗畸形,影响美观[1]。最近的研究发现龋病与肥胖症有关[2]。龋病的加重不仅会影响患者的生活质量,还会影响心理健康,甚至与抑郁症有关[3-4]。现代药理研究发现金银花是一种具有抗炎[5-6]、抗菌、抗肿瘤[7]、抗病毒[8]、抗氧化[9-10]、保肝[11]、免疫调节等生物活性的药物。临床上,金银花广泛应用于风热感冒、呼吸道感染、痈肿疔疮、炎症等。有研究发现金银花对葡萄球菌、溶血性链球菌、伤寒杆菌、肺炎球菌等有一定的抑制作用[12]。金银花制剂现广泛应用于口腔溃疡、牙龈炎、牙周病、牙髓根尖周病,并取得良好疗效,但对龋病的防治方面仍有所欠缺。变异链球菌为龋病的主要致龋菌,它的致龋性主要在于其具有产酸性、耐酸性及黏附力,有研究表明变异链球菌对牙面的黏附是口腔牙菌斑向致龋性菌斑转化的标志,也是其致龋的重要物质基础[13]。故本研究初步探讨金银花对变异链球菌的抑菌效果,为龋病防治的临床应用提供依据。

## 1　材料和方法

### 1.1　实验菌株

变异链球菌国际标准株UA159（简称UA159）由四川大学华西口腔医学院国家重点实验室提供。

### 1.2　主要试剂与仪器

脑心浸液培养基（brian heart infusion, BHI）、磷酸盐缓冲液（phosphate-buffered saline, PBS）、2.5%戊二醛、二甲基亚砜（北京索莱宝科技有限公司,中国）；金银花（海门,中国）；结晶紫及标准革兰染色液（上海碧云天生物技术有限公司,中国）；一次性无菌接种环、EP管、离心管（Bilogix Group,美国）；离心机（安徽中科中佳,中国）；正置显微镜（Olympus,日本）；扫描电子显微镜（FEI,美国）；全波长酶标仪（Thermo,美国）；YQX-Ⅱ厌氧培养箱（跃进,上海）；高压蒸汽灭菌锅（Panasonic,日本）。

### 1.3　菌株的复苏、培养和鉴定

取冻存变异链球菌UA159菌株,复苏,划线接种于BHI琼脂平板,37 ℃恒温厌氧箱（80% $N_2$、10% $H_2$、10%$CO_2$）培养24 h；BHI液体培养基增菌至对数期；离心,洗涤,重复3次,稀释重悬,调节菌液$OD_{600}$=1.0后再稀释100倍作为标准菌液。

### 1.4　金银花药液MIC值的测定

取500 mg金银花粉末,加入装有10 mL DMSO溶液的离心管,吹打混匀,最后配置成质量浓度为50 mg/mL的金银花原液。实验组7支离心管编号1~7,1号加入金银花原液1 mL,余试管依次按二倍梯度稀释法用1 mL BHI液体培养基依次按照二倍梯度稀释至药液浓度为25、12.5、6.25、3.125、1.5 625、0.78 125、0.390 625 mg/mL,最后各管均加入1 mL标准菌液。药液对照组加入1 mL金银花原液及1 mL培养基；溶剂对照组加入含DMSO溶剂的1 mL培养基和1 mL菌液；菌液对照组加入1 mL菌液和1 mL培养基。厌氧箱培养24 h,观察培养基情况,以浑浊度为指标检查有无细菌生长,结合酶标仪检测0、24 h后各试管内的$OD_{600}$值,0 h的$OD_{600}$值为初始值,计算$\Delta OD_{600}$值,确定MIC值。

### 1.5　UA159菌株生长曲线的测定

实验组分别稀释金银花原液至MIC及以下等比（1/2,1/4,1/8）3个浓度,取1 mL分别加入试管后再加入1 mL标准菌液,菌液对照组则加入等量培养基及菌液。检测培养0、4、8、12、24 h时各管中液体的$OD_{600}$值,绘制生长曲线图。

### 1.6　测定金银花药液对UA159产酸影响

实验组用BHI液体培养基（含1%蔗糖）按

照1.5方法稀释金银花原液后分别取4.5 mL加入试管,再加入标准菌液0.5 mL,菌液对照组加入等量培养基及菌液。每组设6个平行组,在培养的0、4、8、12、24、48 h时各组取出一支,离心,测pH值。0 h时的pH值作为初始pH值,计算每个时间段每组的ΔpH值,绘制产酸变化曲线。

### 1.7 测定金银花药液对UA159黏附影响

实验组按1.6方法稀释药液后取2.5 mL加入试管,菌液对照组则加入等量培养基,溶剂对照组加入等量含DMSO的培养基,再加入2.5 mL标准菌液,试管倾斜30°,24 h后取出,将管内液体倒入第二批相对应管中,原试管加入5 mL PBS摇晃震荡,再将液体吸取至第三批对应的试管中,PBS溶液再次震荡。将第2、3批试管离心(3 000 r/min,15 min),吸弃上清液,5 mL PBS溶液混匀。测各管$OD_{600}$值。三批试管的数据分别记为X、Y、Z值,黏附率=X/(X+Y+Z)×100%;黏附抑制率=(菌液对照组黏附率-实验组黏附率)/菌液对照组黏附率×100%。

### 1.8 金银花药液对UA159生物膜的影响

1.8.1 金银花药液对UA159生物膜形成量的影响 取96孔板,编号1~5组,每组设3个复孔,每孔加入100 μL标准菌液,在培养的0、6、12 h时分别在1~3组加入100 uL 20 mg/ mL的金银花药液,使最终药液浓度为10 mg/ mL后继续培养至24 h,4、5组分别为溶剂对照组及菌液对照组。24 h后吸弃孔内液体,PBS漂洗,室温干燥,甲醛固定15 min;按照使用说明对标本进行1%结晶紫染色液染色,再次漂洗干燥,加入200 μL的33%冰乙酸溶液溶解,酶标仪测定每孔$OD_{570}$值,评估生物膜的形成量。

1.8.2 变异链球菌生物膜结晶紫染色定性分析 取2个6孔板,编号1~5组,每组设2个复孔。每孔放入22 mm × 22 mm灭菌盖玻片一张,加入1 mL标准菌液。按1.8.1方法在培养的0、6、12 h于1、2、3组加入20 mg/mL的金银花药液,4组为溶剂对照组,5组为菌液对照组,1~5组均培养24 h后取出。吸干,PBS漂洗,吸出。5 mL甲醇固定15 min,洗净,风干。1%结晶紫染色5 min,PBS冲洗,滤纸吸干,正置显微镜镜下观察,拍照记录。

1.8.3 SEM观察金银花药液对UA159细菌数量的影响 15片氧化锆片打磨抛光,装入瓶内,用20 mL无水乙醇及UP超纯水进行超声震荡,高压蒸汽灭菌15 min,烘干。取1个24孔板,分5组,每组3个

复孔,放入氧化锆片,重复1.8.2方法加入菌液及药液,厌氧培养24 h后取出,吸干液体,充分漂洗;加2.5%戊二醛溶液2 mL,加盖4 ℃固定4 h后吸净液体,将氧化锆片转入一新的24孔板内,反复漂洗;随后依次50%、70%、80%、90%酒精梯度脱水各15 min,无水乙醇脱水2次后干燥。各组选取2枚,$CO_2$临界点干燥粘台,喷金,扫描电镜镜检,拍照。

### 1.9 统计学分析

采用SPSS Statistics 22.0对数据进行统计学分析。计量资料符合正态分布,数据采用$\bar{x} \pm s$表示,采取单因素方差分析比较,Leven检验方差齐性,方差齐采用LSD法分析,方差不齐采用Dunnett's法分析,$P < 0.05$为差异有统计学意义。

## 2 结 果

### 2.1 金银花药液MIC值的测定

观察1、2号试管表现为清亮的淡黄色液体并且不伴沉淀生长,3 ~ 7号、菌液及溶剂对照组均表现为不同程度的浑浊或沉淀。$\Delta OD_{600}$值结果见表1,与菌液组相比,1 ~ 4号及药液对照组差异有统计学意义($P < 0.05$),而5 ~ 7号、溶剂对照组差异无统计学意义($P > 0.05$),根据结果结合肉眼观察试管内无浑浊或沉淀生长的最低药物浓度即为金银花的MIC,最终测得金银花药液对UA159的MIC值为12.5 mg/mL。

表1 UA159在不同金银花药液浓度下的$\Delta OD_{600}$值

Table 1 $\Delta OD_{600}$ value of UA159 in the presence of different concentrations of honeysuckle

$\bar{x} \pm s$

| Honeysuckle concentration/(mg/mL) | $\Delta OD_{600}$ | $F$ | $P$ |
|---|---|---|---|
| 25(No.1) | 0.036 ± 0.006* | | < 0.001 |
| 12.5(No.2) | 0.084 ± 0.003* | | < 0.001 |
| 6.25(No.3) | 0.194 ± 0.002* | | < 0.001 |
| 3.125(No.4) | 0.285 ± 0.001* | | < 0.001 |
| 1.5625(No.5) | 0.397 ± 0.003 | 5 672.286 | 0.386 |
| 0.78125(No.6) | 0.398 ± 0.004 | | 0.561 |
| 0.390625(No.7) | 0.397 ± 0.006 | | 0.386 |
| 25(Honeysuckle control) | 0.025 ± 0.004* | | < 0.001 |
| 0(Solvent control) | 0.404 ± 0.002 | | 0.205 |
| 0(Bacteria control) | 0.400 ± 0.003 | | |

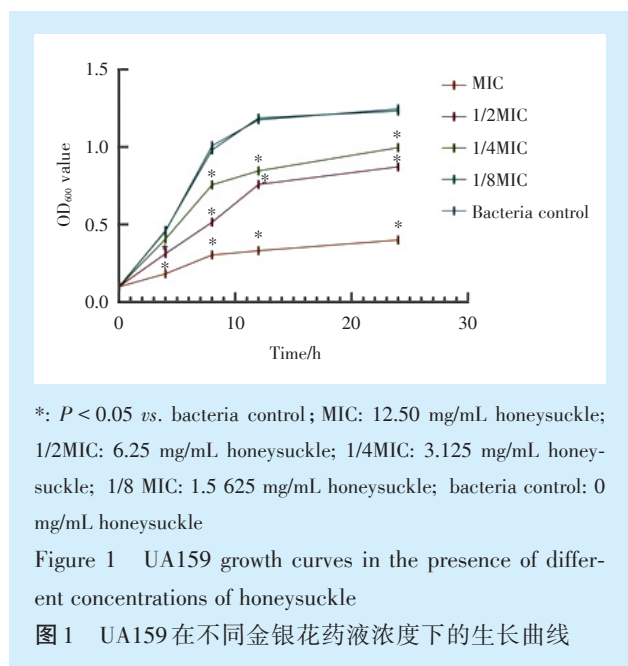*: $P < 0.05$ *vs.* bacteria control group

### 2.2 UA159生长曲线的测定

24 h内不同浓度金银花溶液对UA159生长影响情况见表2、图1。结果显示:UA159生长速度随着药液浓度的增加而逐渐变缓。菌液组在前8 h

表 2 UA159 在不同时间点不同金银花药液浓度下的 $OD_{600}$ 值

Table The $OD_{600}$ value of UA159 in the presence of different concentrations of honeysuckle and at various time points $\bar{x} \pm s$

| Time/h | Groups | | | | | $F$ | $P$ | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | MIC | 1/2MIC | 1/4MIC | 1/8 MIC | Bacteria control | | $P_1$ | $P_2$ | $P_3$ | $P_4$ |
| 4 | 0.183 ± 0.004 | 0.314 ± 0.001 | 0.408 ± 0.003 | 0.462 ± 0.002 | 0.458 ± 0.001 | 6 729.677 | < 0.001 | < 0.001 | < 0.001 | 0.077 |
| 8 | 0.304 ± 0.003 | 0.515 ± 0.008 | 0.757 ± 0.002 | 0.996 ± 0.004 | 1.002 ± 0.001 | 14 841.016 | < 0.001 | < 0.001 | < 0.001 | 0.121 |
| 12 | 0.332 ± 0.005 | 0.759 ± 0.003 | 0.847 ± 0.003 | 1.180 ± 0.004 | 1.175 ± 0.002 | 13 632.478 | < 0.001 | < 0.001 | < 0.001 | 0.266 |
| 24 | 0.401 ± 0.002 | 0.873 ± 0.003 | 0.997 ± 0.004 | 1.239 ± 0.003 | 1.243 ± 0.001 | 46 048.769 | < 0.001 | < 0.001 | < 0.001 | 0.110 |

$P_1$: MIC group *vs*. bacteria control group; $P_2$: 1/2 MIC group *vs*. bacteria control group; $P_3$: 1/4 MIC group *vs*. bacteria control group; $P_4$: 1/8 MIC group *vs*. bacteria control group; MIC: 12.50 mg/mL honeysuckle; 1/2 MIC: 6.25 mg/mL honeysuckle; 1/4 MIC: 3.125 mg/mL honeysuckle; 1/8 MIC: 1.5 625 mg/mL honeysuckle; bacteria control: 0 mg/mL honeysuckle



*: $P < 0.05$ *vs*. bacteria control; MIC: 12.50 mg/mL honeysuckle; 1/2MIC: 6.25 mg/mL honeysuckle; 1/4MIC: 3.125 mg/mL honey-suckle; 1/8 MIC: 1.5 625 mg/mL honeysuckle; bacteria control: 0 mg/mL honeysuckle

Figure 1 UA159 growth curves in the presence of different concentrations of honeysuckle

图 1 UA159 在不同金银花药液浓度下的生长曲线

内增长迅速,后逐渐变缓,12 h 后趋于稳定;1/8MIC 组与菌液组相比差异无统计学意义($P > 0.05$),1/4MIC、1/2MIC、MIC 与菌液组相比每个时间点的差异均具有统计学意义($P < 0.05$)。不同药物浓度组组间比较,差异均具有统计学意义($P < 0.05$)。

### 2.3 测定金银花药液对 UA159 产酸影响

各组 pH 值随着时间的推移均呈下降趋势,8 h 前速度更快,随后减缓,结果见图 2,表 3。1/8MIC 与菌液组差异无统计学意义($P > 0.05$)。MIC、1/2MIC、1/4MIC 组与菌液组相比抑制作用明显,差



*: $P < 0.05$ *vs*. bacteria control; MIC: 12.50 mg/mL honeysuckle; 1/2MIC: 6.25 mg/mL honeysuckle; 1/4MIC: 3.125 mg/mL honey-suckle; 1/8 MIC: 1.5 625 mg/mL honeysuckle; bacteria control: 0 mg/mL honeysuckle

Figure 2 Acid production curves of UA159 in the presence of different concentrations of honeysuckle
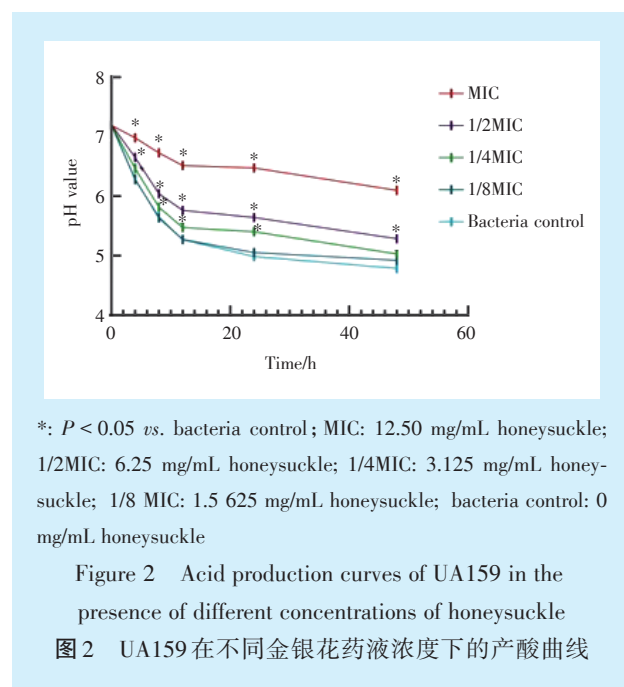
图 2 UA159 在不同金银花药液浓度下的产酸曲线

表 3 UA159 在不同金银花药液浓度不同时间点下的 ΔpH 值

Table 3 The ΔpH value of UA159 at different time points and with various concentrations of honeysuckle $\bar{x} \pm s$

| Time/h | Groups | | | | | $F$ | $P$ | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | MIC | 1/2MIC | 1/4MIC | 1/8 MIC | Bacteria control | | $P_1$ | $P_2$ | $P_3$ | $P_4$ |
| 4 | 0.211 ± 0.002 | 0.544 ± 0.002 | 0.721 ± 0.002 | 0.911 ± 0.003 | 0.921 ± 0.002 | 52 483.080 | < 0.001 | < 0.001 | < 0.001 | 0.082 |
| 8 | 0.465 ± 0.004 | 1.161 ± 0.002 | 1.376 ± 0.003 | 1.556 ± 0.001 | 1.551 ± 0.005 | 55 928.373 | < 0.001 | < 0.001 | < 0.001 | 0.095 |
| 12 | 0.677 ± 0.005 | 1.442 ± 0.006 | 1.720 ± 0.005 | 1.928 ± 0.003 | 1.928 ± 0.007 | 28 329.583 | < 0.001 | < 0.001 | < 0.001 | 1.000 |
| 24 | 0.718 ± 0.003 | 1.562 ± 0.004 | 1.788 ± 0.004 | 2.201 ± 0.008 | 2.213 ± 0.001 | 3 756.354 | < 0.001 | < 0.001 | < 0.001 | 0.410 |
| 48 | 1.096 ± 0.002 | 1.918 ± 0.003 | 2.161 ± 0.006 | 2.285 ± 0.006 | 2.292 ± 0.001 | 43 781.215 | < 0.001 | < 0.001 | < 0.001 | 0.066 |

$P_1$: MIC group *vs*. bacteria control group; $P_2$: 1/2 MIC group *vs*. bacteria control group; $P_3$: 1/4 MIC group *vs*. bacteria control group; $P_4$: 1/8 MIC group *vs*. bacteria control group; MIC: 12.50 mg/mL honeysuckle; 1/2 MIC: 6.25 mg/mL honeysuckle; 1/4 MIC: 3.125 mg/mL honeysuckle; 1/8 MIC: 1.5 625 mg/mL honeysuckle; bacteria control: 0 mg/mL honeysuckle

异均具有统计学意义($P < 0.05$)。

### 2.4 测定金银花药液对UA159黏附影响

随着金银花药液浓度的增加，UA159的黏附率下降，黏附抑制率增加，结果如表4。溶剂组与菌液组间的黏附率与黏附抑制率差异无统计学意义（$P > 0.05$）；不同金银花药液组与菌液组间的黏附率与黏附抑制率差异有统计学意义（$P < 0.05$）。

表4 不同金银花药液浓度对UA159粘附的影响

Table 4 Effects of different concentrations of honeysuckle on the adhesion of UA159 $\bar{x} \pm s$

| Groups | Adhesion rate(%) | F | P | Adhesion inhibition rate(%) | F | P |
|---|---|---|---|---|---|---|
| MIC | 27.68 ± 0.45* | | < 0.001 | 32.28 ± 0.46* | | < 0.001 |
| 1/2MIC | 29.87 ± 0.38* | | < 0.001 | 27.49 ± 0.98* | | < 0.001 |
| 1/4MIC | 32.46 ± 0.32* | 1 235.534 | < 0.001 | 20.67 ± 0.69* | 1 033.858 | < 0.001 |
| 1/8MIC | 38.67 ± 0.37* | | < 0.001 | 14.89 ± 1.21* | | < 0.001 |
| Solvent control | 43.85 ± 0.34 | | 0.301 | 0.39 ± 0.30 | | 0.522 |
| Bacteria control | 44.16 ± 0.20 | | | 0 | | |

*: $P < 0.05$ *vs.* bacteria control group；MIC: 12.50 mg/mL honeysuckle; 1/2MIC: 6.25 mg/mL honeysuckle; 1/4MIC: 3.125 mg/mL honeysuckle; 1/8 MIC: 1.5 625 mg/mL honeysuckle; bacteria control: 0 mg/mL honeysuckle; solvent control: 0 mg/mL honeysuckle

### 2.5 金银花药液对UA159生物膜形成量的影响

与菌液组相比，0、6、12 h点加入金银花药液组的细菌生物膜形成量下降，差异有统计学意义（$P < 0.05$），而菌液组与溶剂组间差异无统计学意义（$P > 0.05$），见表5。

### 2.6 变异链球菌生物膜结晶紫染色定性分析

显微镜下观察UA159菌体呈紫红色，链状或短链状排列，0、6、12 h加入药液后生物膜形成量均明显下降（图3a~3c）。溶剂对照组（图3d）与菌液对照组（图3e）相比无明显差异。

### 2.7 SEM观察金银花药液对UA159细菌数量的影响

UA159为椭圆形，成对或呈短链状分布。在0、6、12 h三个时间点加入金银花药液后，细菌数量均明显下降，但趋势减缓（图4a~4c）。溶剂组与菌液组均可见菌体大量团聚，无明显差异（图4d、4e）。

表5 金银花药液对UA159生物膜形成量的影响

Table 5 The effect of honeysuckle on UA159 biofilm formation $\bar{x} \pm s$

| Groups | OD$_{570}$ | F | P |
|---|---|---|---|
| Honeysuckle concentration(0 h) | 0.154 ± 0.004* | | < 0.001 |
| Honeysuckle concentration(6 h) | 0.232 ± 0.006* | | < 0.001 |
| Honeysuckle concentration(12 h) | 0.291 ± 0.007* | 521.336 | < 0.001 |
| Solvent control | 0.338 ± 0.003 | | 0.547 |
| Bacteria control | 0.335 ± 0.008 | | |

*: $P < 0.05$ *vs.* bacteria control group

## 3 讨 论

变异链球菌被认为是非致病性口腔微生物群落向菌斑生物膜过渡过程中最相关的细菌[14]。变异链球菌具有耐酸性，致使局部牙体组织脱矿，如果不加控制，最终导致龋病产生[15]。目前临床上
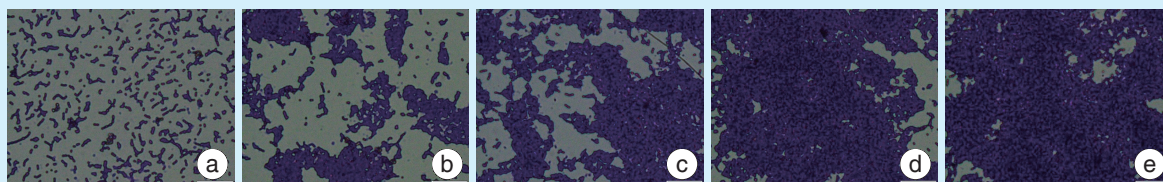


a: 10 mg/mL honeysuckle added at 0 h, the UA159 number decreased significantly; b: 10 mg/mL honeysuckle added at 6 h, the UA159 number decreased significantly; c: 10 mg/mL honeysuckle added at 12 h, the UA159 number decreased, but the trend decreased, and biofilm aggregation trend increased; d: solvent control: no significant difference compared with the bacteria control group; e: bacteria control group, UA159 grew well

Figure 3 The effect of honeysuckle on UA159 biofilm formation was observed under a microscope (×100)

图3 显微镜下观察金银花药液对UA159生物膜形成的影响 (×100)
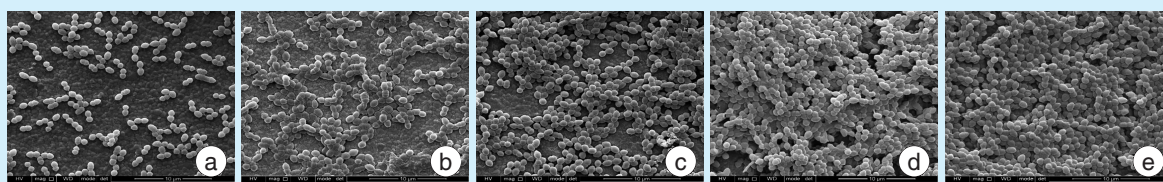
a: 10 mg/mL honeysuckle added at 0 h, UA159 grew in pairs; b: 10 mg/mL honeysuckle added at 6 h, the total number of UA159 decreased significantly; c: 10 mg/mL honeysuckle added at 12 h, the total number of UA159 decreased, but the trend weakened; d: in the control group, there were no significant differences compared with the bacteria control group; e: in the bacteria control group, UA159 grew well

Figure 4　The effect of honeysuckle on the total amount of UA159 bacteria was observed under a scanning electron microscope (×10 000)

图4　扫描电镜下观察金银花药液对 UA159 细菌数量的影响 (×10 000)

普遍使用的氟化物防龋效果是值得肯定的,但氟化物长期使用会带来口腔组织着色、改变味觉等问题,甚至氟中毒等情况的发生。金银花作为天然药物,价格低,产量大,应用领域广泛,安全性高。主要化学成分有挥发油、黄酮类化合物、有机酸、环烯醚萜类,其中的绿原酸类是有机酸的主要有效成分,具有广泛的抗氧化、抗菌作用[16]。现已有研究发现天然药物黄柏、肉桂、丁香的提取物对变异链球菌都有抑制作用[17-18],但目前国内外关于金银花与变异链球菌致龋性研究较少。

本实验采用光密度的值来表示待检样品中细菌的浓度,测得 MIC 为 12.5 mg/mL。在生长实验中,药液组细菌与标准株生长趋势基本一致,但 $OD_{600}$ 值均低于菌液组,说明金银花可能会影响细菌的增殖速度,但并不影响其生长的基本趋势。从产酸曲线上看,加入药液后 pH 值随着药物浓度的增加而升高,产酸的抑制作用增强,说明金银花能抑制变异链球菌的产酸能力。

细菌的黏附能力是牙菌斑生物膜形成的基础,变异链球菌具有多种高亲和力的表面黏附素,这导致其即使没有蔗糖也能定植[19]。它表面与黏附有关的结构有黏附蛋白、胞外多糖等。变异链球菌表面的表面蛋白 P1 目前被研究较多,10 个以上的蛋白 P1 与唾液 GP340 相互作用导致黏附作用的产生[20]。本研究发现随着金银花浓度增加,细菌黏附率呈下降趋势,且黏附抑制率上升,说明金银花能抑制其黏附。但目前抑制黏附的具体机制尚不清楚,有待进一步研究。

生物膜的特殊条件可以保护微生物免受宿主免疫细胞和药物治疗,并使微环境在低 pH 时稳定,导致牙釉质脱矿[21]。牙菌斑生物膜基质的主要成分有变异链球菌分泌的胞外多糖,其能促进细菌在牙面的定植,起到生物膜骨架的作用,稳定菌斑基本结构。本实验测量 $OD_{570}$ 值以胞外多糖的量来间接衡量被膜的生成量,结果显示:与菌液组相比,金银花药液培养 24 h 后的 $OD_{570}$ 值呈减少趋势。另外在体外建立变异链球菌生物膜模型,再利用光学显微镜初步观察生物膜形成后的样貌,同时利用 SEM 观察细菌数量变化,结果显示加入 10 mg/mL 金银花药液组在 3 个时段均降低了细菌的数目,同时减缓了生物膜的形成速度,初步证实金银花能抑制生物膜的形成。

综上所述,金银花可抑制变异链球菌的生长、产酸、黏附、生物膜形成。本次研究初步证实了金银花的抑菌作用,为龋病的防治提供了一定的实验依据。但目前关于金银花抗菌作用机制多停留在药理指标,化学成分与药理作用的关系仍未探索,且缺少对变异链球菌黏附相关蛋白、基因及动物模型的研究,因此,未来将从这些方面进一步深入研究。

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