基于中药超分子与肠道菌相互作用探讨大黄−黄连配伍和合的物质基础

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摘要: 基于中药超分子与肠道菌相互作用过程探讨大黄−黄连配伍和合的物质基础。采用扫描电子显微镜和动态光散射法表征大黄单煎液、黄连单煎液与配伍共煎液的形态学差异, 通过建立体外抑菌模型 (大肠杆菌 *E. coli*, 屎肠球菌 *E. faecium* 与枯草杆菌 *B. subtilis*) 初步评价大黄黄连配伍对肠道菌的损伤作用; 通过超高效液相色谱−串联质谱技术分析单煎与共煎的化学组分变化规律。大黄黄连共煎煮后呈浑浊状, 电镜下可见 300～400 nm 的类球形颗粒, 且较单煎液相比其汤剂相态更均一稳定; 并直接观察到二者配伍后形成的超分子和肠道菌的相互作用与单煎液相比明显不同, 超分子与肠道菌相互作用过程中维持类球形状态, 封闭着黄连或大黄中的药效成分, 可有效缓和对肠道菌的损伤, 为后续大黄−黄连配伍“和合”调控肠道菌群稳态研究提供参考。

关键词: 大黄; 黄连; 配伍; 超分子; 肠道菌

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Based on the interaction between supramolecules of traditional Chinese medicine and enterobacteria to explore the material basis of combination of *Rhei Radix et Rhizoma - Coptidis Rhizoma*

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Abstract: Based on the interaction between supramolecule of traditional Chinese medicine and enterobacteria, the material basis of *Rhei Radix et Rhizoma* and *Coptidis Rhizoma* was explored. Scanning electron microscopy (SEM) and dynamic light scattering (DLS) were used to characterize the morphological differences of *Rhubarb* single decoction, *Coptis* single decoction and *Rhubarb* and *Coptis* co-decoction. An *in vitro* antibacterial model (*E. coli*, *E. faecium* and *B. subtilis*) was established to evaluate the damage effect of the combination of *Rhei Radix et Rhizoma* and *Coptidis Rhizoma* on enterobacteria. Ultra high performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) was used to analyze the changes of chemical components of single decoctions and co-decoctions. The co-decoction of *Rhei Radix et Rhizoma* and *Coptidis Rhizoma* was turbid after decocting. The spherical particles of 300−400 nm were observed under SEM, and the co-decoction was more uniform and stable than that of single decoction. The interaction between supramolecules formed after the combination of *Rhei Radix et Rhizoma* and *Coptidis Rhizoma* and enterobacteria was significantly different from that of single decoction. In the process of interaction between supramolecules and enterobacteria, the spherical state was maintained, and the

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medicinal ingredients in *Coptidis Rhizoma* or *Rhei Radix et Rhizoma* were blocked, which could effectively alleviate the damage to enterobacteria. This study provided a reference for subsequent studies on the regulation of intestinal flora homeostasis by the combination of *Rhei Radix et Rhizoma* and *Coptidis Rhizoma*.

Key words: *Rhei Radix et Rhizoma*; *Coptidis Rhizoma*; compatibility; supramolecule; enterobacteria

肠道菌群失调与腹泻的发生、发展密切相关[1-4], 调节肠道菌群是中药治疗腹泻的重要途径与靶点[5,6]。中药水煎液经口服进入人体后与肠道菌群发生相互作用[7-9], 不仅影响了药效成分的结构与药效的发挥[10], 同时也对肠道菌群稳态结构产生了调控作用[11,12]。苦寒类中药在临床中被广泛的应用[13], 其对肠道菌群存在双向调节作用[14,15], 临床上往往因“苦寒败胃”而出现腹泻、腹痛等症状[16-18]。苦寒中药通过炮制[19]或者配伍后往往更为安全有效, 能较为显著地改善单味药的“苦寒败胃”之性, 例如三黄泻心汤[20]、黄连–甘草[21]、黄连–吴茱萸[22]、大黄–甘草[23]等药对的相互配伍。既往研究表明, 药对经过配伍共煎煮后产生“减毒增效”的原因与成分之间发生相互作用密切相关[24], 不仅改变复方汤剂的药效成分含量, 而且在与肠道菌群相互作用过程中发生竞争性抑制等进而影响成分的吸收、代谢过程[25]。

大黄与黄连均味苦性寒, 二者配伍可以有效缓和各自的大苦大寒之性。课题组前期发现, 大黄–黄连共煎煮时药效成分相互作用的过程, 是一个自发进行的化学放热反应而不是物理沉降所驱动的聚集, 二者共煎液中存在天然小分子无载体超分子库, 由蒽醌/鞣质类成分与生物碱类成分通过氢键、静电作用等非共价键相互作用而形成[26,27]。超分子延缓以小檗碱为代表的有效成分溶解释放过程,与单味药相比, 大黄–黄连共煎后汤剂苦味降低, 明显缓和小鼠的腹泻症状[28]。但前期研究尚未揭示大黄–黄连共煎液超分子与大黄、黄连单煎液相比, 在影响肠道菌方面有何差异。因此, 本研究以肠道中广为存在的大肠杆菌、枯草杆菌、屎肠球菌为对象初步探讨大黄–黄连配伍共煎煮后对肠道菌的调控作用, 为后续大黄–黄连药对配伍和合“苦寒之性”与体内肠道菌群的研究提供参考。

材料与方法

材料 大肠杆菌 (*Escherichia coli*, *E.coli*, 北京中医药大学生命科学学院); 枯草杆菌二联活菌颗粒 (屎肠球菌, *Enterococcus faecium*, *E. faecium*; 枯草杆菌, *Bacillus subtilis*, *B.subtilis*, 北京韩美药品有限公司)。大黄、黄连均购于北京同仁堂 (批号20201125、200301001), 分别为蓼科植物掌叶大黄 *Rheum palmatum* L. 的干燥根和根茎, 毛茛科植物黄连 *Coptis chinensis* Franch. 的干燥根茎。按照大黄–黄连1∶1称取药材装入隔渣袋, 加入8倍量水, 浸泡20 min, 大火煮沸后保持微沸15 min回流煎煮, 滤除药渣, 得到大黄黄连共煎液 (Rhubarb and Coptis co-decoction, RC), 同理可制得大黄单煎液 (Rhubarb single decoction, R)、黄连单煎液 (Coptis single decoction, C), 各水煎液样品直接经冷冻干燥后得到冻干粉末。

动态光散射法表征与浊度表征 使用去离子水将两单煎液冻干粉复溶至1 mg·mL^{-1}, 共煎液中含等量黄连 (2 mg·mL^{-1})。转移至比色皿中, 使用激光笔照射观察丁达尔效应。以去离子水为空白溶剂, 在浊度仪上测定样品的浊度值; 在马尔文粒度仪的Size模式下测定样品的平均粒径和多分散性指数 (polydispersity index, PDI), 在zeta模式下测定样品的电位 (zeta potential), 平行重复3次, 取平均值。

超高效液相色谱–串联质谱结构分析 (UPLC-MS/MS) 精密称取各单煎液冻干粉, 用色谱级甲醇稀释至1 mg·mL^{-1} (共煎液为2 mg·mL^{-1}), 超声1 h充分溶解, 0.22 μm微孔滤膜过滤后待测。TC-C18柱 (4.6 mm × 250 mm, 5 μm); 流动相为 (A) 0.1%甲酸水溶液和 (B) 乙腈, 梯度洗脱0～30 min, 4%～98% B; 流速0.3 mL·min^{-1}; 进样量5 μL。在正 (ESI$^+$) 和负 (ESI$^-$) 电喷雾电离模式下收集信息。毛细管温度和辅助气体加热器的温度均设置为350 ℃。扫描模式为Full MS, 分辨率为10 000, 扫描范围 *m/z* 150～1 500。

体外抑菌活性表征 将大黄单煎液、黄连单煎液冻干样品用无菌水分散至20 mg·mL^{-1}, 用培养基稀释成终浓度为0.5、0.25、0.125 mg·mL^{-1}的药物混悬液 (共煎液含等量黄连, 共煎液药物混悬液终浓度为1、0.5、0.25 mg·mL^{-1}), 每孔的最终体积为500 μL, 最后向每孔分别加入50 μL大肠杆菌混悬液 (2×10^3 CFU·mL^{-1})、枯草杆菌二联活菌混悬液 (含屎肠球菌1.35×10^4 CFU·mL^{-1}与枯草杆菌1.5×10^3 CFU·mL^{-1}), 在37℃恒温生化培养箱中培养12 h后, 收集上述48孔板中菌混悬液, 稀释10^{5}倍后使用涂布棒将细菌混悬液接种至营养琼脂板上。在37 ℃恒温生化培养箱中继续培养12 h后, 观察营养琼脂上的菌落情况。

体外抑菌活性形态学表征 向12孔板中各加入1 mL营养肉汤, 分别加入100 μL大肠杆菌混悬液

(2×10^3 CFU·mL^{-1})、枯草杆菌二联活菌混悬液 (含屎肠球菌 1.35×10^4 CFU·mL^{-1} 与枯草杆菌 1.5×10^3 CFU·mL^{-1}) 于 37 ℃恒温生化培养箱中培养 12 h。按照上述样品溶液配制方法, 用培养基将药物稀释成终浓度为 0.5 mg·mL^{-1} 的混悬液 (共煎液浓度为 1 mg·mL^{-1}), 继续在 37 ℃恒温生化培养箱中培养 12 h。弃去培养基, 加入 LIVE/DEAD BacLight™ 试剂, 于激光共聚焦显微镜观察菌体生长情况。自“继续在 37 ℃恒温生化培养箱中培养 12 h”后, 3 000 r·min^{-1} 离心 5 min 收集菌体, 加入 2.5% 戊二醛于 4 ℃固定 4 h。弃去戊二醛溶液, 分别在 30%、50%、70%、80%、90%、100% 乙醇中脱水, 每次 10 min。加入去离子水分散菌体, 转移至硅片上于扫描电子显微镜下观察。

统计方法　采用 SPSS 20.0 软件对结果进行 t 检验, 测量值以平均 ± 标准差 ($\bar{x} \pm s$) 表示, $P < 0.05$ 具有统计学意义。

结果

1　大黄黄连水煎液形态学表征

大黄−黄连共煎煮后汤剂呈黄褐色, 与单煎液相比, 有着明显差异且呈浑浊态 (图 1A), 经浊度仪测定, 大黄单煎液与黄连单煎液浊度值分别为 (38.87 ± 2.61) NTU、(54.32 ± 3.02) NTU, 二者配伍共煎煮后汤剂浊度值变大 (276.53 ± 0.58) NTU, 与单煎液相比具有极强的显著性差异 ($P < 0.001$, 与大黄单煎液相比; $P < 0.01$, 与黄连单煎液相比)。3 种汤剂均有明显的丁达尔效应 (图 1B), 结合浊度测定提示 3 种汤剂并不是真溶液体系, 在共煎液的分散体系中分散粒子的尺度大于单煎液。扫描电子显微镜结果显示 (图 1C), 大黄单煎液在镜下无明显颗粒或纤维聚集; 黄连单煎液中可见许多大小不一的簇状物, 分散较为均一; 共煎液中可见粒径约为 300 nm 的类球形颗粒且分散较为均匀, 提示大黄黄连在配伍共煎煮的过程中发生分子相互作用, 形成了超分子体系。动态光散射表征结果与扫描电子显微镜具有一致性 (图 1D、E), 其结果提示大黄−黄连经过共同煎煮后, 汤剂粒径变大 (365.3 ± 14.8) nm, 且分散程度较黄连单煎液更均匀稳定, 表明共煎液的浑浊相态稳定性好 (PDI = 0.236, ζ = −27 mV)。

2　大黄−黄连水煎液体外抑菌活性表征

以大肠杆菌、枯草杆菌二联活菌为对象, 通过平板涂布法、活死菌荧光染色法及扫描电子显微镜以观察大黄−黄连配伍共煎煮后对肠道菌的调控规律。

在大肠杆菌菌株测定中发现, 在给药梯度 0.5 mg·mL^{-1} 下, 大黄单煎液对大肠杆菌的抑制作用较弱, 黄连单煎液在此浓度下大肠杆菌存活率小于 5%, 平板上可见依

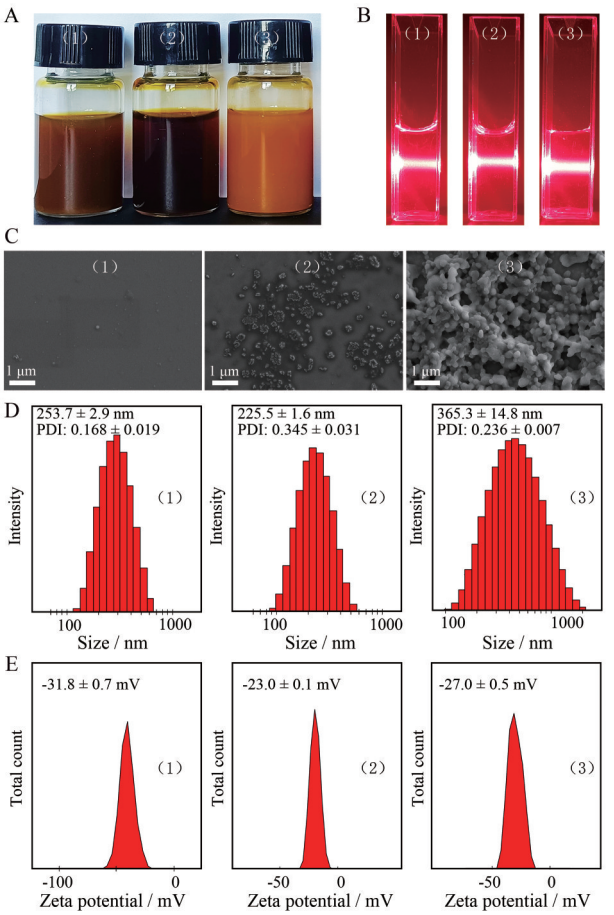

Figure 1　Morphological characterization. A: Liquid form; B: Tyndall effect characterization; C: Scanning electron microscope characterization; D: Particle size characterization; E: Potential characterization. (1) *Rheum palmatum* L.; (2) *Coptis chinensis* Franch.; (3) Co-decoction. PDI: polydispersity index.

稀的菌落, 而大黄−黄连共煎液对大肠杆菌的损伤作用减轻, 其平板菌落数显著多于黄连单煎液 (图 2A)。活死菌荧光染色结果 (图 3A) 可观察到大黄单煎液与对照组显示一致的大量绿色荧光 (活菌), 而黄连单煎液组中大量细菌被染成红色 (死菌), 提示菌体发生破碎致使染料进入。大黄−黄连共煎液中同样存在一定数量的死菌, 但相对于黄连单煎液, 其破坏性明显降低。通过扫描电子显微镜进一步观察药物作用下菌体的微观形貌特征, 如图 4A 所示, 对照组与大黄单煎组大肠杆菌菌体结构完整、边缘清晰, 呈现出规则的杆状。黄连单煎液与大肠杆菌共孵育 12 h 后, 可观察到在整个视野下大肠杆菌基本都存在不同程度的损伤, 菌体表面出现严重的皱缩、凹陷与菌体的破碎。大黄−黄连经过配伍共煎煮后, 共煎液对大肠杆菌的杀伤作用降低, 对菌体的破坏性有显著的改善作用, 菌体略显皱缩与凹陷但仍维持较为规则的杆状, 菌体结构完整性高。

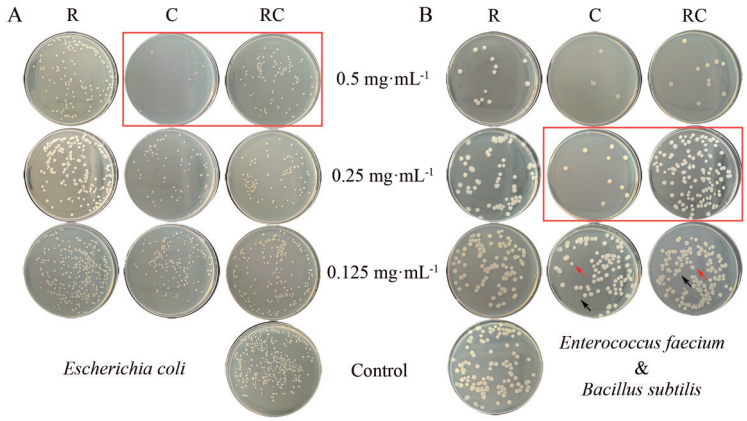

Figure 2 Characterization of antibacterial activity *in vitro*. A: Plate coating of *E.coli*; B: Plate coating of *E. faecium* (black arrow) and *B. subtilis* (red arrow). R: Rhubarb single decoction; C: Coptis single decoction; RC: Rhubarb and Coptis co-decoction

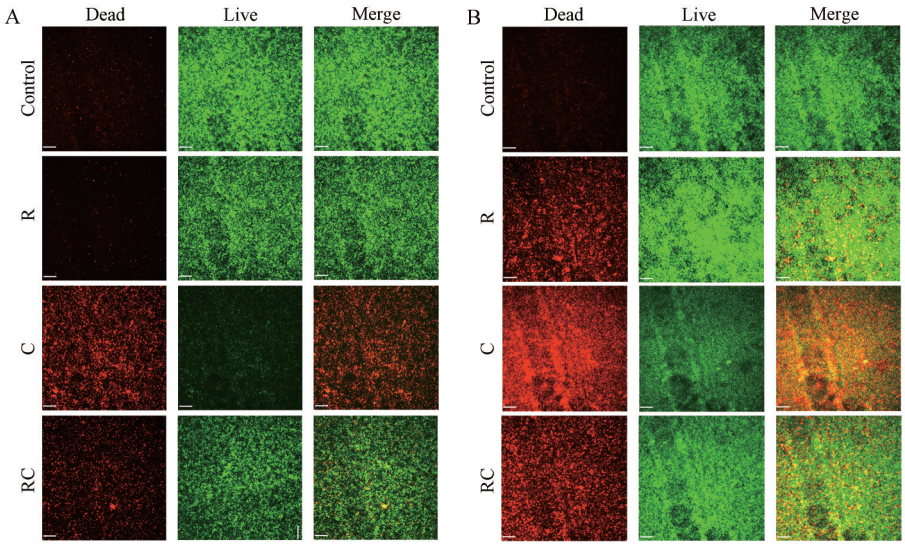

Figure 3 Staining characterization of live and dead bacteria. A: Staining characterization of *E. coli*; B: Staining characterization of *E. faecium* and *B. subtilis*. Scale = 60 μm

枯草杆菌二联活菌为枯草杆菌与屎肠球菌的混合体系, 二者的初始浓度与生长周期具有差异性。对照组与大黄单煎液的平板接种图均鲜见屎肠球菌, 黄连单煎液与共煎液在给药梯度为0.125 mg·mL^{-1}时可见零星的屎肠球菌, 且浓度为0.25 mg·mL^{-1}时, 共煎液可以较好的缓和黄连单煎液对菌的损伤, 菌落生长状态趋近于对照组 (图2B)。枯草杆菌二联菌的活死菌染色与平板涂布具有一致性, 其结果显示给药组对枯草二联菌均有不同程度抑制作用, 其中红色荧光强度 (死菌数量) 黄连单煎液 > 大黄黄连共煎液 > 大黄单煎液 (图3B)。扫描电子显微镜表征显示 (图4B), 对照组视野中仅可见零星的几个屎肠球菌, 绝大多数为枯草杆菌, 结合平板涂布图提示枯草杆菌与屎肠球菌在不经过药物干预下共培养12 h, 枯草杆菌占优势; 大黄单煎液对枯草杆菌与屎肠球菌的影响都较小, 电镜视野下与对照组基本保持一致。经黄连单煎液与共煎液干预后, 镜下均可观察到比例相当的枯草杆菌与屎肠球菌, 推测黄连的加入对枯草杆菌抑制作用强于屎肠球菌, 使得屎肠球菌与枯草杆菌在竞争氧气或养分的过程中能有一定优势。黄连单煎液对菌体的破坏性更强, 枯草杆菌与屎肠球菌均出现了明显的凹陷与皱缩, 而大黄与黄连经过共同煎煮后药性更为缓和, 对菌体的完整性保存度更高, 视野中小部分菌体略显褶皱, 大部分菌体仍维持规则的杆状与椭球状。

体外抑菌实验显示, 大黄–黄连共煎体系与菌作用起效的形式与单煎液存在明显差异, 大黄–黄连经过配伍共煎煮后, 含等量黄连组分的共煎液的药性缓和, 大肠杆菌、枯草杆菌、屎肠球菌的损伤得到了明显的改善。

3 大黄–黄连水煎液的超高效液相色谱–串联质谱 (UPLC-MS/MS) 成分分析

为了探究大黄黄连配伍共煎煮后, 形成的相对浑

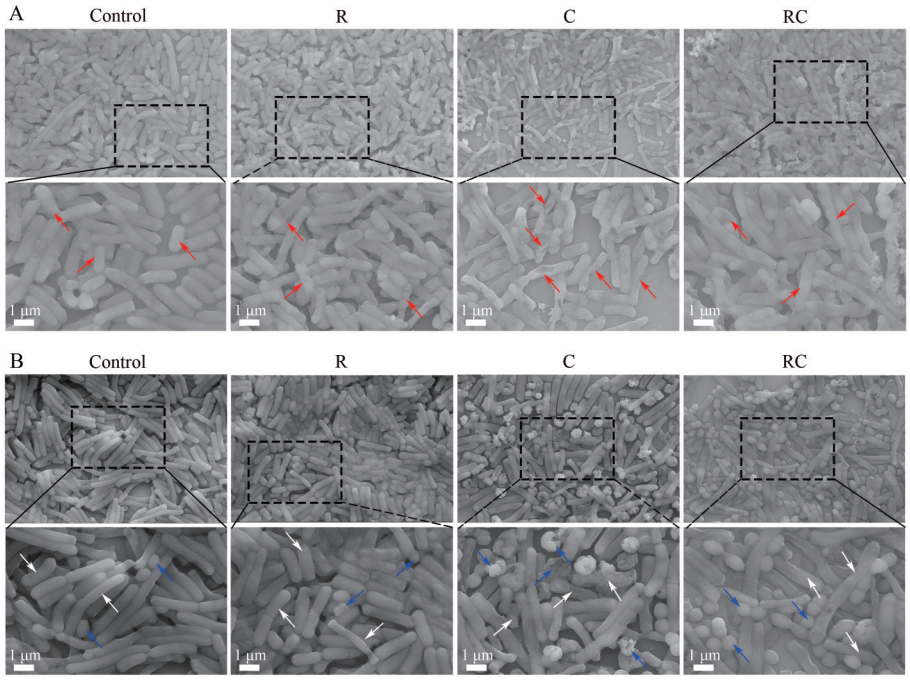

Figure 4　Morphological characterization of bacteria. A: SEM characterization of *E. coli* (red arrow); B: SEM characterization of *E. faecium* (blue arrow) and *B. subtilis* (white arrow). SEM: scanning electron microscopy

浊汤剂 (超分子相态) 对肠道菌损伤减轻背后的化学物质基础差异, 通过超高效液相色谱−串联质谱分析大黄单煎液、黄连单煎液和共煎液在正负离子模式下的化学成分异同 (图 5)。在大黄黄连共煎液中共鉴定出 85 种化合物, 其中生物碱类成分 29 种, 蒽醌类成分 21 种, 有机酸类成分 15 种, 鞣质类成分 7 种, 其他类成分 13 种 (表 1)。共煎液中来源于大黄、黄连的成分分别为 52 种、51 种, 其中 18 种成分为大黄单煎液与黄连单煎液所共有, 主要为有机酸类化合物。

化合物 **15** 被鉴定为表儿茶素 ($C_{15}H_{14}O_6$), 负离子模式下可在 *m/z* 289.072 0 观测到其准分子离子峰 $[M-H]^-$。通过失去 H_2O, CO_2, C_2H_2O, C_3H_6O, $C_6H_6O_2$ 等分子后得到 6 个碎片离子 *m/z* 271.060 8 $[M-H-H_2O]^-$, 245.081 7 $[M-H-CO_2]^-$, 227.070 4 $[M-H-CO_2-H_2O]^-$, 203.070 8 $[M-H-CO_2-C_2H_2O]^-$, 187.039 2 $[M-H-CO_2-C_3H_6O]^-$, 179.034 1 $[M-H-C_6H_6O_2]^-$。儿茶素通过 C 环

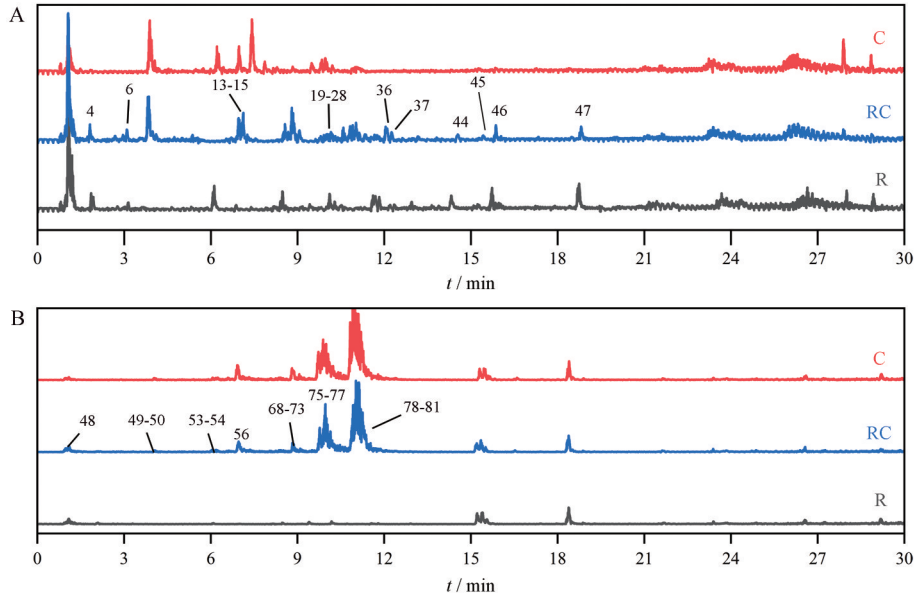

Figure 5　Ultra performance liquid chromatography-tandem mass spectrometry characterization (total ion flow diagram). A: Negative ion mode; B: Positive ion mode

的1,4键、1,3键和1,2键发生断裂可观测到*m/z* 165.018 4 $[M-H-^{1,2}A^-]^-$, 137.023 3 $[M-H-^{1,3}A^-]^-$, 125.023 1 $[M-H-^{1,4}A-]^-$等碎片离子。

化合物**46**与**47**分别被鉴定为大黄酸、大黄素, 属于蒽醌类化合物, 二者都是大黄的特征成分, 在负离子模式下更容易产生如CO和CO_2等中性分子。大黄酸(rhein)的分子式为$C_{15}H_8O_6$, *m/z* 283.024 8为其准分子离子峰, 可观测到大黄酸的特征离子碎片为*m/z* 239.034 8 $[M-H-CO_2]^-$, 211.039 7 $[M-H-CO_2-CO]^-$, 183.044 4 $[M-H-CO_2-CO-CO]^-$和155.049 0 $[M-H-CO_2-CO-CO-CO]^-$。大黄素(emodin)的准分子离子峰为*m/z* 283.024 8, 去质子化分子通过失去CO或CO_2中性基团, 可得到碎片离子*m/z* 241.050 5 $[M-H-CO]^-$, 225.055 2 $[M-H-CO_2]^-$和181.064 6 $[M-H-CO_2-CO_2]^-$。蒽醌类化合物是大黄发挥抗菌、抗氧化等功效的药效成分之一[29], 蒽醌及其衍生物的抗菌活性与取代基的极性呈正相关, 极性越强抗菌效果越强[30,31], 例如大黄酸(3-COOH)、大黄素(3-OH)、芦荟大黄素(3-CH_2OH)的极性取代基有助于抗菌活性提高, 而大黄素甲醚(-OCH_3)、大黄酚(-CH_3)的供电子基团引入使得抗菌活性降低[32]。

生物碱类成分主要源于黄连, 均在正离子模式(ESI^+)下响应, 准分子离子峰通常表现为$[M]^+$或$[M+H]^+$, 易于脱去CH_3、CO、CO_2、CH_2、CH_4等中性碎片。化合物75和80分别被鉴定为表小檗碱和小檗碱, 二者为同分异构体, 具有相似的裂解规律。*m/z* 336.122 9 $[M]^+$为小檗碱的准分子离子峰, 分别失去一分子CH_3和CH_4得到两个碎片离子*m/z* 321.099 6 $[M-CH_3]^+$和320.091 8 $[M-CH_4]^+$, 再连续各失去一分子CH_2和CO可得到碎片离子*m/z* 306.076 2 $[M-CH_4-CH_2]^+$和292.096 7 $[M-CH_4-CO]^+$。黄连中的生物碱类成分如小檗碱、黄连碱、巴马汀等, A环上的亚甲二氧基与C环季铵结构为其抗菌活性所必备[33], C8、C9、C13的结构变化同样也会引起抗菌活性差异[34,35]。小檗碱及其他异喹啉生物碱因多环平面结构和C环的季铵氮驱动与细菌细胞膜相互作用, 促进生物靶点结合, 从而产生较强的抗菌活性[36,37], N7位季铵化后其衍生物抗菌活性弱于小檗碱[38]。

UPLC-MS/MS结果表明共煎液中的化学成分源于大黄单煎液和黄连单煎液, 并未产生新化合物, 说明超分子体系的形成是两药对间成分相互作用的产物。课题组前期研究发现大黄黄连超分子部位中发现诸多小分子自组装体, 例如大黄素-黄连碱纳米颗粒、大黄酸-黄连碱纳米纤维、大黄酸-小檗碱纳米颗粒[38]等, 其结合位点为3-COOH/3-OH与7-N^+, 它们组装机制具有相似性, 都是大黄酸3-COOH、大黄素3-OH与小檗碱/黄连碱环上的N^+通过静电作用相互靠近, 依靠π-π作用与氢键作用, 分子相互堆叠形成自组装体, 组装的过程引起了电子云密度的改变, 蒽醌类组分H-2、H-4、H-5、H-6、H-7, 生物碱类组分H-8、H-11、H-12、H-13化学位移向高场方向发生波动[26]。

4 大黄-黄连水煎液超分子与肠道菌相互作用

大黄-黄连水煎液的体外抑菌电镜表征中, 样品前处理不经乙醇脱水或减少脱水次数, 电镜视野下可观察到大量共煎液中的类球形颗粒。大黄-黄连药效分子互作形成的超分子与肠道菌相互作用的过程中, 依然维持类球形颗粒, 大肠杆菌、枯草杆菌与屎肠球菌的表面可见大量颗粒附着(图6)。结构的完整性说明分子内部的排列仍未受到破坏, 大黄-黄连相互作用反应强烈且结合紧密($K_a = 4.885\times10^3$、$K_d = 1.048\times10^{-4}$)[26], 使得相互作用形成的超分子与菌体作用过程仍维持其类球形结构, 表现为微观层面各自组装体的组装形式未发生改变, 仍封闭着大黄-黄连各自药效成分的药效基团(如生物碱7N^+、蒽醌3-COOH/3-OH), 使得药效基团不能有效与肠道菌相互识别结合而发挥作用, 同时课题组前期研究表明, 与单味药相比药效成分缓慢地从超分子体系中游离[28], 因此对大黄-黄连配伍共煎后对肠道菌起保护作用。

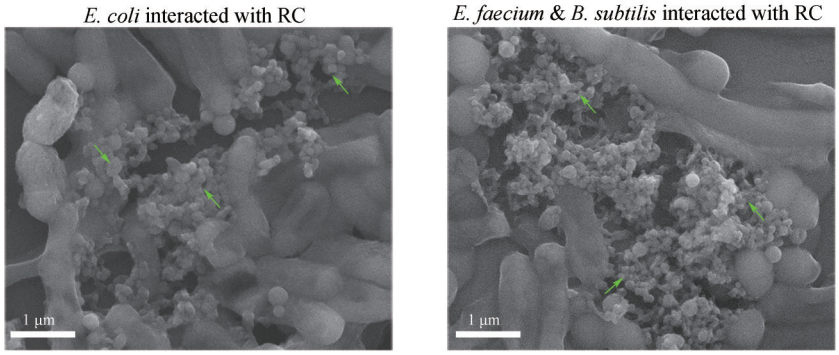

Figure 6 Interaction of RC (green arrow) with *E. coli*, *E. faecium* and *B. subtilis*

Table 1 Chemical composition analysis of UPLC-MS/MS in single and co-decoction of *Rheum palmatum* L. and *Coptis chinensis* Franch. *Confirmed by standard substances

NO.	t_R /min	Identification	Formula	Precursor ion	Theoretical (*m*/*z*)	Experimental (*m*/*z*)	Error ($\times10^{-6}$)	Fragment ion (*m*/*z*)	Source
1	1.05	Glucose	$C_6H_{12}O_6$	$[M-H]^-$	179.055 0	179.054 4	−3.35	161.043 7 $[M-H-H_2O]^-$	R, C, RC
2	1.06	Sucrose	$C_{12}H_{22}O_{11}$	$[M-H]^-$	341.107 8	341.107 3	−1.47	179.054 3 $[M-H-C_6H_{10}O_5]^-$, 161.043 8 $[M-H-C_6H_{10}O_5-H_2O]^-$ 113.022 7 $[M-H-C_6H_{10}O_5-H_2O-CH_4O_2]^-$	R, C, RC
3	1.07	Quinic acid	$C_7H_{12}O_6$	$[M-H]^-$	191.055 0	191.054 5	−2.62	173.044 1 $[M-H-H_2O]^-$, 127.038 4 $[M-H-H_2O-CH_2O_2]^-$ 93.032 8 $[M-H-H_2O-CH_2O_2-H_2O_2]^-$	R, C, RC
4	1.81	Citric Acid	$C_6H_8O_7$	$[M-H]^-$	191.018 6	191.018 4	−1.05	85.027 7 $[M-H-H_2O-CO_2-CO_2]^-$	R, C, RC
5	2.95	Galloylglucose	$C_{13}H_{16}O_{10}$	$[M-H]^-$	331.065 9	331.066 2	0.91	169.012 7 $[M-H-C_6H_{10}O_5]^-$, 125.022 8 $[M-H-C_6H_{10}O_5-CO_2]^-$	R, RC
6*	3.07	Gallic acid	$C_7H_6O_5$	$[M-H]^-$	169.013 1	169.012 8	−1.78	125.022 9 $[M-H-CO_2]^-$	R, C, RC
7	3.97	Pantothenic acid	$C_9H_{17}NO_5$	$[M-H]^-$	218.102 2	218.102 1	−0.46	–	R, C, RC
8	4.05	Vanillic acid	$C_8H_8O_4$	$[M-H]^-$	167.033 8	167.033 6	−1.20	123.043 8 $[M-H-CO_2]^-$	R, RC
9	4.13	3-Hydroxy-4-methoxybenzoic acid 3-*O*-*β*-*D*-glucopyranoside	$C_{14}H_{18}O_9$	$[M-H]^-$	329.086 7	329.087 2	1.52	167.033 4 $[M-H-C_6H_{10}O_5]^-$	R, C, RC
10	4.58	Gentisic acid	$C_7H_6O_4$	$[M-H]^-$	153.018 2	153.018 0	−1.31	109.028 1 $[M-H-CO_2]^-$	R, C, RC
11	5.35	Catechin-5-*O*-glucoside	$C_{21}H_{24}O_{11}$	$[M-H]^-$	451.123 4	451.123 6	0.44	289.070 8 $[M-H-C_6H_{10}O_5]^-$, 245.080 8 $[M-H-C_6H_{10}O_5-CO_2]^-$	R, RC
12	5.50	Procyanidin B	$C_{30}H_{26}O_{12}$	$[M-H]^-$	577.134 0	577.134 2	0.35	289.070 8 $[M-H-C_{15}H_{12}O_6]^-$, 245.080 5 $[M-H-C_{15}H_{12}O_6-CO_2]^-$	R, RC
13	6.79	1,6-Digalloyl glucopyranose	$C_{20}H_{20}O_{14}$	$[M-H]^-$	483.076 9	483.077 2	0.62	169.012 8 $[M-H-C_{13}H_{14}O_9]^-$, 125.022 7 $[M-H-C_{13}H_{14}O_9-CO_2]^-$	R, RC
14	6.88	Methyl gallate	$C_8H_8O_5$	$[M-H]^-$	183.028 7	183.028 7	0.00	124.014 7 $[M-H-C_2H_3O_2]^-$	R, C, RC
15*	6.96	Epicatechin	$C_{15}H_{14}O_6$	$[M-H]^-$	289.070 6	289.070 7	0.35	271.060 3 $[M-H-H_2O]^-$, 245.080 7 $[M-H-CO_2]^-$ 227.070 2 $[M-H-CO_2-H_2O]^-$, 203.070 0 $[M-H-CO_2-C_2H_2O]^-$ 187.038 7 $[M-H-CO_2-C_3H_6O]^-$, 165.019 1 $[M-H-^{1,2}A^-]^-$ 179.033 2 $[M-H-C_6H_6O_2]^-$, 137.022 8 $[M-H-^{1,3}A^-]^-$ 125.022 8 $[M-H-^{1,4}A^-]^-$	R, RC
16	7.09	3-*O*-Feruloylquinic acid	$C_{17}H_{20}O_9$	$[M-H]^-$	367.102 3	367.102 0	−0.82	193.049 2 $[M-H-C_7H_{10}O_5]^-$, 191.054 6 $[M-H-C_{10}H_8O_3]^-$ 173.043 8 $[M-H-C_{10}H_8O_3-H_2O]^-$ 149.059 2 $[M-H-C_7H_{10}O_5-CO_2]^-$ 134.035 7 $[M-H-C_7H_{10}O_5-CO_2-CH_3]^-$	R, C, RC
17	8.53	Procyanidin B 3″-*O*-gallate	$C_{37}H_{30}O_{16}$	$[M-H]^-$	729.145 0	729.143 5	−2.06	577.132 9 $[M-H-C_7H_4O_4]^-$, 289.070 6 $[M-H-C_{22}H_{16}O_{10}]^-$	R, RC
18	8.81	4-*O*-Feruloylquinic acid	$C_{17}H_{20}O_9$	$[M-H]^-$	367.102 3	367.101 8	−1.36	193.049 2 $[M-H-C_7H_{10}O_5]^-$, 191.054 7 $[M-H-C_{10}H_8O_3]^-$ 173.044 1 $[M-H-C_{10}H_8O_3-H_2O]^-$ 149.059 1 $[M-H-C_7H_{10}O_5-CO_2]^-$ 134.034 1 $[M-H-C_7H_{10}O_5-CO_2-CH_3]^-$	R, C, RC
19	9.77	Sennoside C	$C_{42}H_{40}O_{19}$	$[M-H]^-$	847.208 0	847.207 1	−1.06	685.154 1 $[M-H-C_6H_{10}O_5]^-$	R, RC
20*	9.80	Sennoside A	$C_{42}H_{38}O_{20}$	$[M-H]^-$	861.187 2	861.186 7	−0.58	699.134 0 $[M-H-C_6H_{10}O_5]^-$, 386.100 3 $[M-H-C_{22}H_{19}O_{12}]^-$	R, RC
21	9.83	Hydroxycinnamic acid	$C_9H_8O_3$	$[M-H]^-$	163.038 9	163.038 8	−0.61	119.048 5 $[M-H-CO_2]^-$	R, RC
22	9.89	Aloeemodin glucoside	$C_{21}H_{20}O_{11}$	$[M-H]^-$	431.097 2	431.096 9	−0.70	269.044 5 $[M-H-C_6H_{10}O_5]^-$	R, RC
23	10.00	Rhein glucoside	$C_{21}H_{18}O_{11}$	$[M-H]^-$	445.076 5	445.076 3	−0.45	283.023 6 $[M-H-C_6H_{10}O_5]^-$, 239.033 9 $[M-H-C_6H_{10}O_5-CO_2]^-$	R, RC

Continued

NO.	t_R /min	Identification	Formula	Precursor ion	Theoretical (*m*/*z*)	Experimental (*m*/*z*)	Error ($\times10^{-6}$)	Fragment ion (*m*/*z*)	Source
24	10.09	Epi/catechin gallate	$C_{22}H_{18}O_{10}$	$[M-H]^-$	441.081 6	441.081 3	−0.68	331.045 1 $[M-H-C_6H_6O_2]^-$, 289.070 5 $[M-H-C_7H_4O_4]^-$ 271.060 4 $[M-H-C_7H_6O_5]^-$, 169.012 7 $[M-H-C_{15}H_{12}O_5]^-$ 245.080 6 $[M-H-C_7H_4O_4-CO]^-$, 125.022 7 $[M-H-C_7H_6O_5-^{1,4}A^-]^-$ 179.033 6 $[M-H-C_7H_4O_4-C_6H_6O_2]^-$	R, RC
25	10.10	Sennoside D	$C_{42}H_{40}O_{19}$	$[M-H]^-$	847.208 0	847.206 8	−1.42	685.155 8 $[M-H-C_6H_{10}O_5]^-$	R, RC
26	10.11	Cinnamoyl glucose	$C_{15}H_{18}O_7$	$[M-H]^-$	309.096 8	309.097 1	0.97	147.043 4 $[M-H-C_6H_{10}O_5]^-$	R, RC
27*	10.22	Sennoside B	$C_{42}H_{38}O_{20}$	$[M-H]^-$	861.187 2	861.186 3	−1.05	699.134 0 $[M-H-C_6H_{10}O_5]^-$, 386.100 3 $[M-H-C_{22}H_{19}O_{12}]^-$	R, RC
28	10.27	Resveratrol-4′-*O*-(6″-galloyl) glucoside	$C_{27}H_{26}O_{12}$	$[M-H]^-$	541.134 0	541.133 7	−0.55	227.071 5 $[M-H-C_7H_4O_4-C_6H_{10}O_5]^-$, 169.012 8 $[M-H-C_{20}H_{20}O_7]^-$	R, RC
29	10.73	Rhein 1-*O*-(6′-*O*-acetyl)-glucoside	$C_{23}H_{20}O_{12}$	$[M-H]^-$	487.087 1	487.087 4	0.62	283.023 8 $[M-H-C_8H_{12}O_6]^-$, 239.033 7 $[M-H-C_8H_{12}O_6-CO_2]^-$	R, RC
30	10.83	Aloesin	$C_{19}H_{22}O_9$	$[M-H]^-$	393.118 0	393.117 6	−1.02	231.065 0 $[M-H-C_6H_{10}O_5]^-$	R, RC
31	10.90	Azelaic acid	$C_9H_{16}O_4$	$[M-H]^-$	187.096 4	187.096 0	−2.14	125.095 8 $[M-H-CO_2-H_2O]^-$	R, C, RC
32	11.01	2-Cinnamoyl-1-galloylglucose	$C_{22}H_{22}O_{11}$	$[M-H]^-$	461.107 8	461.107 1	−1.52	313.055 5 $[M-H-C_9H_8O_2]^-$, 169.012 7 $[M-H-C_{15}H_{16}O_6]^-$ 151.001 9 $[M-H-C_{15}H_{18}O_7]^-$, 147.043 5 $[M-H-C_{13}H_{14}O_9]^-$	R, C, RC
33	11.98	Torachrysone	$C_{14}H_{14}O_4$	$[M-H]^-$	245.080 8	245.080 6	−0.82	230.056 8 $[M-H-CH_3]^-$, 187.038 4 $[M-H-CH_3-CH_3-CO]^-$	R, RC
34	12.03	Torachrysone 8-*O*-glucoside	$C_{20}H_{24}O_9$	$[M-H]^-$	407.133 6	407.133 2	−0.98	245.080 8 $[M-H-C_6H_{10}O_5]^-$, 230.057 2 $[M-H-C_6H_{10}O_5-CH_3]^-$	R, RC
35	12.04	Chrysophan-1-*O*-glucoside	$C_{21}H_{20}O_9$	$[M-H]^-$	415.102 3	415.102 1	−0.48	253.049 5 $[M-H-C_6H_{10}O_5]^-$, 225.054 7 $[M-H-C_6H_{10}O_5-CO]^-$	R, RC
36	12.09	Emodin glucoside	$C_{21}H_{20}O_{11}$	$[M-H]^-$	431.097 2	431.096 9	−0.70	269.044 4 $[M-H-C_6H_{10}O_5]^-$	R, RC
37	12.23	Chrysophanol	$C_{15}H_{10}O_4$	$[M-H]^-$	253.049 5	253.049 7	0.79	225.054 5 $[M-H-CO]^-$	R, RC
38	12.78	Torachrysone 8-*O*-(6′-*O*-acetyl)-glucoside	$C_{22}H_{26}O_{10}$	$[M-H]^-$	449.144 2	449.144 6	0.89	245.080 8 $[M-H-C_8H_{12}O_6]^-$, 230.057 6 $[M-H-C_8H_{12}O_6-CH_3]^-$	R, RC
39	12.84	Emodin 8-*O*-(6′-*O*-acetyl)-glucoside	$C_{23}H_{22}O_{11}$	$[M-H]^-$	473.107 8	473.107 9	0.21	268.036 8 $[M-H-C_8H_{13}O_6]^-$	R, RC
40	13.11	Physcion glucoside	$C_{22}H_{22}O_{10}$	$[M-H]^-$	445.112 9	445.112 9	0.00	283.060 0 $[M-H-C_6H_{10}O_5]^-$, 268.037 2 $[M-H-C_6H_{10}O_5-CH_3]^-$ 240.042 0 $[M-H-C_6H_{10}O_5-CH_3-CO]^-$	R, RC
41	13.16	Physcion	$C_{16}H_{12}O_5$	$[M-H]^-$	283.060 0	283.060 5	1.77	268.036 5 $[M-H-CH_3]^-$, 240.040 6 $[M-H-CH_3-CO]^-$	R, RC
42	13.18	Chrysophanol 1-*O*-(6′-*O*-acetyl)-glucoside	$C_{23}H_{22}O_{10}$	$[M-H]^-$	457.112 9	457.113 4	1.09	253.049 7 $[M-H-C_8H_{12}O_6]^-$, 239.070 3 $[M-H-C_8H_{12}O_6-CH_2]^-$	R, RC
43	13.97	Physcion 8-*O*-(6′-*O*-acetyl) glucoside	$C_{24}H_{24}O_{11}$	$[M-H]^-$	487.123 4	487.123 7	0.62	283.060 6 $[M-H-C_8H_{12}O_6]^-$, 268.037 4 $[M-H-C_8H_{12}O_6-CH_3]^-$ 240.041 1 $[M-H-C_8H_{12}O_6-CH_3-CO]^-$	R, RC
44	14.54	3-Methyl-rhein	$C_{16}H_{10}O_6$	$[M-H]^-$	297.039 3	297.039 7	1.35	253.049 6 $[M-H-CO_2]^-$, 225.054 5 $[M-H-CO_2-CO]^-$	R, RC
45	15.40	Aloe-emodin	$C_{15}H_{10}O_5$	$[M-H]^-$	269.044 4	269.044 9	1.86	240.041 9 $[M-H-CO-H]^-$, 223.038 6 $[M-H-CO-H_2O]^-$ 183.043 8 $[M-H-CO-CO_2-CH_2]^-$	R, RC
46*	15.84	Rhein	$C_{15}H_8O_6$	$[M-H]^-$	283.023 7	283.0243 8	2.40	239.034 0 $[M-H-CO_2]^-$, 211.038 9 $[M-H-CO_2-CO]^-$ 183.043 7 $[M-H-CO_2-CO-CO]^-$	R, RC
47*	18.80	Emodin	$C_{15}H_{10}O_5$	$[M-H]^-$	269.044 4	269.044 5	0.37	241.049 4 $[M-H-CO]^-$, 225.054 5 $[M-H-CO_2]^-$ 181.064 2 $[M-H-CO_2-CO_2]^-$	R, RC
48	0.91	Arginine	$C_6H_{14}N_4O_2$	$[M+H]^+$	175.118 9	175.118 4	−2.86	116.070 3 $[M+H-CH_5N_3]^+$	R, C, RC

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NO.	t_R /min	Identification	Formula	Precursor ion	Theoretical (*m*/*z*)	Experimental (*m*/*z*)	Error ($\times10^{-6}$)	Fragment ion (*m*/*z*)	Source
49	4.06	Danshensu	$C_9H_{10}O_5$	$[M+H]^+$	199.060 1	199.059 5	−3.01	181.048 9 $[M+H-H_2O]^+$, 163.038 5 $[M+H-H_2O-H_2O]^+$	C, RC
50	4.07	Caffeic acid	$C_9H_8O_4$	$[M+H]^+$	181.049 5	181.048 9	−3.31	163.038 5 $[M+H-H_2O]^+$	C, RC
51	5.09	Tryptophan	$C_{11}H_{12}N_2O_2$	$[M+H]^+$	205.097 1	205.096 8	−1.46	161.107 0 $[M+H-CO_2]^+$	R, C, RC
52	5.41	Isoferulic acid	$C_{10}H_{10}O_4$	$[M+H]^+$	195.065 1	195.064 7	−2.05	177.054 0 $[M+H-H_2O]^+$	C, RC
53	6.03	Magnocurarine	$C_{19}H_{24}NO_3^+$	$[M]^+$	314.175 0	314.174 1	−2.86	269.116 5 $[M-C_2H_7N]^+$, 237.090 1 $[M-C_2H_7N-CH_3OH]^+$	C, RC
54	6.15	Dihydro-11-hydroxy-stepholidine-glucoside	$C_{25}H_{31}NO_{10}$	$[M+H]^+$	506.202 1	506.201 0	−2.17	344.147 7 $[M+H-C_6H_{10}O_5]^+$ 208.096 0 $[M+H-C_6H_{10}O_5-C_8H_8O_2]^+$ 190.085 4 $[M+H-C_6H_{10}O_5-C_8H_8O_2-H_2O]^+$	R, C, RC
55	6.79	9-*O*-Berberine glucoside	$C_{25}H_{26}NO_9^+$	$[M]^+$	484.160 2	484.159 5	−1.45	322.105 9 $[M-C_6H_{10}O_5]^+$, 308.091 1 $[M-C_6H_{10}O_5-CO]^+$	C, RC
56	6.95	Magnoflorine	$C_{20}H_{24}NO_4^+$	$[M]^+$	342.169 9	342.168 9	−2.92	297.110 8 $[M-C_2H_7N]^+$, 282.087 3$[M-C_2H_7N-CH_3]^+$ 265.084 7 $[M-C_2H_7N-CH_3OH]^+$ 237.089 8 $[M-C_2H_7N-CH_3OH-CO]^+$	C, RC
57	7.35	11-Hydroxy-stepholidine-glucoside	$C_{25}H_{29}NO_{10}$	$[M+H]^+$	504.186 4	504.185 0	−2.78	342.132 1 $[M+H-C_6H_{10}O_5]^+$ 188.069 8 $[M+H-C_6H_{10}O_5-C_8H_8O_2-H_2O]^+$	C, RC
58	7.55	Magnoflorine glucoside	$C_{26}H_{34}NO_9^+$	$[M]^+$	504.222 8	504.220 9	−3.77	342.168 7 $[M-C_6H_{10}O_5]^+$	C, RC
59	7.69	8,9-Di-demethyl-epiberberine	$C_{18}H_{14}NO_4^+$	$[M]^+$	308.091 7	308.091 2	−1.62	280.095 7 $[M-CO]^+$, 278.080 2 $[M-CH_2O]^+$ 265.072 2 $[M-C_2H_3O]^+$	C, RC
60	8.00	Dihydrojatrorrhizine	$C_{20}H_{22}NO_4^+$	$[M]^+$	340.154 3	340.153 6	−2.06	325.129 0 $[M-CH_3]^+$, 324.121 1 $[M-CH_4]^+$	C, RC
61	8.05	8-Oxocoptisine	$C_{19}H_{13}NO_5$	$[M+H]^+$	336.086 6	336.086 1	−1.49	318.074 9 $[M+H-H_2O]^+$, 308.091 1 $[M+H-CO]^+$ 294.075 9 $[M+H-CO-CH_2]^+$	C, RC
62	8.23	Menisperine	$C_{21}H_{26}NO_4$	$[M]^+$	356.185 6	356.185 0	−1.68	311.127 3 $[M-C_2H_7N]^+$, 251.107 5 $[M-C_2H_7N-CH_3OH-CO]^+$	C, RC
63	8.34	Demethyleneberberine glucoside	$C_{25}H_{28}NO_9$	$[M]^+$	486.175 8	486.174 9	−1.85	324.121 5 $[M-C_6H_{10}O_5]^+$, 308.089 7 $[M-CH_4]^+$	C, RC
64	8.35	Dihydropalmatine	$C_{21}H_{23}NO_4$	$[M+H]^+$	354.169 9	354.168 9	−2.82	338.139 1 $[M+H-CH_4]^+$, 324.121 0 $[M+H-CH_3-CH_3]^+$ 322.143 9 $[M+H-CH_4-CH_4]^+$	C, RC
65	8.51	Demethylenepiberberine	$C_{19}H_{18}NO_4^+$	$[M]^+$	324.123 0	324.122 5	−1.54	309.098 1 $[M-CH_3]^+$, 294.074 6 $[M-CH_3-CH_3]^+$ 266.080 2 $[M-CH_3-CH_3-CO]^+$	C, RC
66	8.56	Columbamine	$C_{20}H_{20}NO_4^+$	$[M]^+$	338.138 6	338.137 5	−3.25	322.106 0 $[M-CH_4]^+$, 294.111 1 $[M-CH_4-CO]^+$ 308.090 3 $[M-CH_4-CH_2]^+$, 306.111 0 $[M-CH_4-CH_4]^+$ 294.111 1 $[M-CH_{4-}-CO]^+$	C, RC
67	8.60	Noroxyhydrastinine	$C_{10}H_9NO_3$	$[M+H]^+$	192.065 5	192.065 6	0.52	149.059 3 $[M+H-CONH]^+$	C, RC
68*	8.71	Jatrorrhizine	$C_{20}H_{20}NO_4^+$	$[M]^+$	338.138 6	338.137 9	−2.07	322.105 7 $[M-CH_4]^+$, 294.111 1 $[M-CH_4-CO]^+$ 308.090 3 $[M-CH_4-CH_2]^+$, 306.111 0 $[M-CH_4-CH_4]^+$ 294.111 1 $[M-CH_{4-}-CO]^+$	C, RC
69*	8.78	Coptisine	$C_{19}H_{14}NO_4^+$	$[M]^+$	320.091 7	320.090 9	−2.50	292.095 5 $[M-CO]^+$	C, RC
70	8.85	Berberrubine	$C_{19}H_{16}NO_4^+$	$[M]^+$	322.107 3	322.106 5	−2.48	307.082 5 $[M-CH_3]^+$, 294.110 8 $[M-CO]^+$	C, RC

Continued

NO.	t_R /min	Identification	Formula	Precursor ion	Theoretical (*m*/*z*)	Experimental (*m*/*z*)	Error ($\times10^{-6}$)	Fragment ion (*m*/*z*)	Source
71	8.86	11-Hydroxyl-groenlandicine	$C_{19}H_{16}NO_5^+$	$[M]^+$	338.102 2	338.1017	−1.48	322.070 3 $[M-CH_4]^+$, 310.106 1 $[M-CO]^+$, 306.074 2 $[M-CH_4O]^+$	C, RC
72	8.88	Demethyleneberberine	$C_{19}H_{18}NO_4^+$	$[M]^+$	324.123 0	324.121 7	−4.01	266.079 7 $[M-CH_3-CH_3-CO]^+$	C, RC
73	9.11	Oxyepiberberine	$C_{20}H_{17}NO_5$	$[M+H]^+$	352.117 9	352.117 3	−1.70	336.085 2 $[M+H-CH_4]^+$, 322.070 0 $[M+H-CH_3-CH_3]^+$ 308.090 3 $[M+H-CH_3-CH_3-CH_2]^+$ 294.074 6 $[M+H-CH_3-CH_3-CO]^+$	C, RC
74	9.36	Ferulic acid	$C_{10}H_{10}O_4$	$[M+H]^+$	195.065 1	195.064 6	−2.56	177.054 1 $[M+H-H_2O]^+$	C, RC
75*	9.87	Epiberberine	$C_{20}H_{18}NO_4^+$	$[M]^+$	336.123 0	336.121 8	−3.57	320.090 3 $[M-CH_4]^+$, 306.073 9 $[M-CH_4-CH_2]^+$ 292.095 2 $[M-CH_4-CO]^+$	C, RC
76	10.12	Dehydrocorydaline	$C_{22}H_{24}NO_4^+$	$[M]^+$	366.169 9	366.168 8	−3.00	350.137 0 $[M-CH_4]^+$, 336.121 1 $[M-CH_3-CH_3]^+$ 334.141 6 $[M-CH_4-CH_4]^+$, 322.141 2 $[M-CH_3-CH_3-CH_2]^+$	C, RC
77	10.49	13-Methylepiberberine	$C_{21}H_{20}NO_4^+$	$[M]^+$	350.138 6	350.137 9	−2.00	334.107 5 $[M-CH_4]^+$, 320.090 6 $[M-CH_3-CH_3]^+$ 306.110 8 $[M-CH_4-CO]^+$	C, RC
78*	10.74	Worenine	$C_{20}H_{16}NO_4^+$	$[M]^+$	334.107 3	334.106 4	−2.69	319.083 6 $[M-CH_3]^+$, 306.110 7 $[M-CO]^+$	C, RC
79*	10.92	Palmatine	$C_{21}H_{22}NO_4^+$	$[M]^+$	352.154 3	352.153 2	−3.12	336.121 6 $[M-CH_4]^+$, 322.106 0 $[M-CH_4-CH_2]^+$ 308.126 8 $[M-CH_4-CH_2-CH_2]^+$	C, RC
80*	11.06	Berberine	$C_{20}H_{18}NO_4^+$	$[M]^+$	336.123 0	336.122 0	−2.98	320.090 5 $[M-CH_4]^+$, 306.074 8 $[M-CH_4-CH_2]^+$ 292.095 5 $[M-CH_4-CO]^+$	C, RC
81	11.79	13-Methylberberine	$C_{21}H_{20}NO_4^+$	$[M]^+$	350.138 6	350.138 1	−1.43	334.107 2 $[M-CH_4]^+$, 320.090 6 $[M-CH_3-CH_3]^+$ 306.111 1 $[M-CH_4-CO]^+$	C, RC
82	12.89	Acacetin	$C_{16}H_{12}O_5$	$[M+H]^+$	285.075 7	285.075 4	−1.05	270.051 1 $[M+H-CH_3]^+$, 242.056 5 $[M+H-CH_3-CO]^+$ 211.074 8 $[M-C_2H_2O_3]^+$	C, RC
83*	16.52	Oxyberberine	$C_{20}H_{17}NO_5$	$[M+H]^+$	352.117 9	352.117 4	−1.42	336.084 7 $[M+H-CH_3]^+$, 322.069 8 $[M+H-CH_3-CH_3]^+$ 308.090 5 $[M+H-CH_3-CH_3-CH_2]^+$ 294.074 9 $[M+H-CH_3-CH_3-CO]^+$	C, RC
84	21.44	Eleostearic acid	$C_{18}H_{30}O_2$	$[M+H]^+$	279.231 8	279.231 5	−1.07	261.221 2 $[M+H-H_2O]^+$	R, C, RC
85	25.35	Palmitic acid	$C_{16}H_{32}O_2$	$[M+H]^+$	257.247 5	257.246 6	−3.50	239.236 1 $[M+H-H_2O]^+$	R, C, RC

讨论

基于中药超分子化学理论, 本研究从大黄–黄连配伍共煎煮后与单煎液的相态差异出发, 通过动态光散射与扫描电子显微镜横向对比单煎液与共煎液的差异, 并建立体外抑菌模型进一步评价配伍共煎液对肠道菌的调控规律, 最后通过超高效液相–串联质谱分析汤剂浑浊背后的化学成分组成。实验结果显示大黄黄连共煎液眼观浑浊, 电镜下为300～400 nm的类球形颗粒, 大黄黄连二者配伍后可有效改善对肠道菌(大肠杆菌 *E. coli*, 屎肠球菌 *E. faecium* 与枯草杆菌 *B. subtilis*) 的损伤作用, 共煎液缓和肠道菌的损伤与大黄黄连成分之间形成超分子体系密切相关。本研究从肠道菌的角度出发, 初步探究大黄黄连配伍使用减轻小鼠腹泻作用、平和药性的原因, 为后续苦寒药对配伍的药性研究提供参考。

本研究初步考察了大黄、黄连单煎液及其共煎液对肠道菌的损伤程度, 并从电镜直接观察到超分子与肠道菌的相互作用过程, 从体外抑菌模型反映苦寒药对配伍与单味中药之间影响肠道菌生长存在明显差异, 这为后期采用正常动物和模型动物探讨苦寒中药配伍“驱邪不伤正”奠定基础; 其次, 大肠杆菌、枯草杆菌、屎肠球菌为肠道中的兼性菌, 后期对于肠道益生菌 (例如厌氧菌) 仍需选择更为针对的菌种进行研究, 以期充分理解中药配伍“和合”的科学内涵。

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利益冲突: 本文所有作者声明不存在利益冲突关系。

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