黄芪蜜炙对RAW264.7细胞能量代谢及极化的影响

李鸿昌[1], 裴 科[1*], 解旺洋[1], 孟祥龙[1], 于子涵[1], 李玟玲[1], 蔡 皓[2*]

(1. 山西中医药大学中药与食品工程学院, 中药炮制山西省重点实验室, 山西 晋中 030619; 2. 南京中医药大学药学院, 江苏 南京 210023)

摘要: 本研究以RAW264.7细胞为研究对象, 观察黄芪蜜炙对其能量代谢及极化的影响, 探讨蜜炙增强黄芪补中益气功效的科学内涵。将生品黄芪及蜜炙黄芪水煎液灌胃大鼠制备含药血清, 采用液质联用技术分析黄芪蜜炙前后含药血清中成分的变化。通过CCK-8实验检测细胞存活率, 筛选含药血清浓度。通过中性红吞噬实验、ATP检测试剂盒和NO检测试剂盒评价生品黄芪及蜜炙黄芪对细胞吞噬率、ATP能量代谢和NO分泌量的差异。通过ELISA试剂盒检测生品黄芪及蜜炙黄芪对RAW264.7细胞TNF-α分泌量的影响。通过流式细胞术评价生品黄芪及蜜炙黄芪对RAW264.7细胞极化的影响。采用液质联用技术筛选细胞裂解液和培养液中与糖酵解相关的差异代谢物。本实验获得山西中医药大学实验动物伦理委员会批准 (批准号: AWE202407352)。结果显示, 黄芪蜜炙后的水煎液灌胃大鼠后制备的含药血清中甜菜碱、氨基酸和芒柄花苷等成分含量升高。CCK-8实验结果表明, 生品黄芪和蜜炙黄芪各浓度含药血清对RAW264.7细胞均无细胞毒作用。含药血清给药细胞后, 吞噬指数和ATP的产量均有不同程度的提高, 正常状态下RAW264.7细胞的NO分泌量提高, 炎症状态下RAW264.7细胞的NO分泌量减少, 且蜜炙黄芪的效果优于生品黄芪。ELISA试剂盒的检测结果表明, 生品黄芪及蜜炙黄芪的含药血清均能呈浓度依赖性地促进TNF-α的分泌, 且蜜炙黄芪的促进效果强于生品黄芪。流式细胞术的检测结果表明, 生品黄芪及蜜炙黄芪的含药血清均能促进RAW264.7细胞向M1型极化, 并抑制向M2型极化, 蜜炙黄芪的效果优于生品黄芪。与生品黄芪相比, 蜜炙黄芪的含药血清细胞裂解液和培养液中与糖酵解相关的代谢物总体呈上升趋势, 表明蜜炙黄芪促进糖酵解的效果优于生品黄芪。综上所述, 蜜炙黄芪能促进RAW264.7细胞的极化及能量代谢, 并参与正向的免疫调节, 这与其补中益气功效的增强具有相关性。

关键词: 生品和蜜炙黄芪; 能量代谢; 极化; 补中益气

中图分类号: R917 **文献标识码**: A **文章编号**: 0513-4870(2025)02-0459-12

Effects of honey-processed *Astragalus* on energy metabolism and polarization of RAW264.7 cells

LI Hong-chang[1], PEI Ke[1*], XIE Wang-yang[1], MENG Xiang-long[1], YU Zi-han[1], LI Wen-ling[1], CAI Hao[2*]

(*1. School of Chinese Medicine and Food Engineering, Shanxi Key Laboratory for Chinese Medicine Processing, Shanxi University of Chinese Medicine, Jinzhong 030619, China; 2. School of Pharmacy, Nanjing University of Chinese Medicine, Nanjing 210023, China*)

Abstract: In this study, RAW264.7 cells were employed to investigate the effects of honey-processed *Astragalus* on their energy metabolism and polarization, and explore the scientific connotation of the enhanced efficacy of honey-processed *Astragalus* on invigorating spleen-stomach and replenishing Qi. The medicated sera

收稿日期: 2024-08-14; 修回日期: 2024-10-30.

基金项目: 国家自然科学基金面上项目 (82074022); 山西省科技创新人才团队项目(202304051001044); 国家自然科学基金联合基金重点项目(U21A20410); 分子中医药学国际联合研究中心开放课题基金资助项目 (2022JD-KF-01); 山西省中医药管理局项目 (2023ZYYA033); 山西中医药大学科研项目 (2022PY-TH-13).

*通讯作者 E-mail: peike_pk@sxtcm.edu.cn; haocai@njucm.edu.cn

DOI: 10.16438/j.0513-4870.2024-0785

were prepared by intragastric administration of rats with water extracts of crude and honey-processed *Astragalus*, and the composition changes of medicated sera of crude and honey-processed *Astragalus* were analyzed by using LC-MS technology. The cell survival rates were detected and the concentrations of medicated sera were screened through CCK-8 assay. The differences of cell phagocytic rates, ATP energy metabolism, and NO secretion between crude and honey-processed *Astragalus* were evaluated by using neutral red phagocytosis assay, ATP detection kit, and NO detection kit. The effects of crude and honey-processed *Astragalus* on TNF-*α* secretion of RAW264.7 cells were detected by employing ELISA kit. The effects of crude and honey-processed *Astragalus* on polarization of RAW264.7 cells were evaluated by utilizing flow cytometry. The differential metabolites related to glycolysis in cell lysates and culture media were screened by using LC-MS technology. The experiment was approved by the experimental animal ethics committee from Shanxi University of Chinese Medicine (No. AWE202407352). The results showed that the contents of betaine, amino acids, and ononin in the prepared medicated sera of rats treated by intragastric administration with water extracts of honey-processed *Astragalus* increased compared to those in crude one. The results of CCK-8 experiment showed that there were no cytotoxic effects on RAW264.7 cells in the medicated sera of crude and honey-processd *Astragalus* at different concentration. The phagocytic index and ATP yield both increased to varying degrees after administration of the medicated sera to cells. The secretion of NO in normal and inflammatory cells increased and decreased respectively, and the effect of honey-processed *Astragalus* was better than that of crude one. The results of ELISA kit showed that the medicated sera of both crude and honey-processed *Astragalus* could promote the secretion of TNF-*α* in a concentration-dependent manner, and the promoting effect of honey-processed *Astragalus* was stronger than that of crude one. The results of flow cytometry showed that the medicated sera of both crude and honey-processed *Astragalus* could promote M1-type polarization and inhibit M2-type polarization of RAW264.7 cells, and the effect of honey-processed *Astragalus* was better than that of crude one. Compared to crude *Astragalus*, the metabolites related to glycolysis in the cell lysates and culture media of the medicated sera of honey-processed *Astragalus* were generally on the rise, indicating that the effect on promoting glycolysis of honey-processed *Astragalus* was better than that of crude one. In summary, honey-processed *Astragalus* can promote the polarization and energy metabolism of RAW264.7 cells, and participate in positive immune regulation, which is correlated with its enhanced effect of invigorating spleen-stomach and replenishing Qi.

Key words: crude and honey-processed *Astragalus*; energy metabolism; polarization; invigorating spleen-stomach and replenishing Qi

黄芪为豆科植物蒙古黄芪或膜荚黄芪 *Astragalus membranaceus* (Fisch.) Bge. 的干燥根, 具有补气升阳、固表止汗和利水消肿的功效[1], 是历代医家所推崇的补气圣药, 其蜜炙后补中益气作用增强。传统中医理论认为, 黄芪的补中益气功效与增强免疫有关,《内经》曰“正气存内, 邪不可干”, 即当机体之气充足时, 病邪就不易侵袭机体, 含有现代免疫学“自稳”和“防御”两方面的功能[2]。现代研究表明, 气虚病症往往伴随免疫系统的紊乱, 故本文从免疫学的角度出发探讨蜜炙黄芪增强补中益气的机制。

巨噬细胞是遍及机体血液、淋巴和组织中的一种重要的免疫效应细胞[3], 具有抗原呈递、吞噬和分泌细胞因子等功能, 在机体内参与清除病原体、抗感染、免疫应答和免疫调节等过程[4], 是固有免疫系统的关键仲裁者[5]。巨噬细胞在参与免疫调节过程中会极化为不同的类型, 常见类型有 M1 型和 M2 型。M1 型巨噬细胞又名经典活化型巨噬细胞, 其在功能上可呈递抗原并促进免疫反应, 吞噬细菌与病毒, 然而过量的免疫应答亦会造成组织损害而对机体产生消极影响[6]。M2 型巨噬细胞又名替代活化型巨噬细胞, 具有抗炎作用, 可大量分泌 CD206 和 IL-10 等抗炎细胞因子, 同时 CD206[7]和 IL-10[8]也具有一定的免疫抑制性。

免疫细胞活化需要大量的能量和代谢中间物来满足生物合成的需求, 从而完成增殖、分化以及效应功能的执行, 以达到增强免疫的目的[9]。缺乏足够的能量供应会直接影响免疫细胞的功能, 提示能量代谢可能在免疫相关疾病治疗中扮演重要角色。因此, 推测蜜炙黄芪补中益气功效的增强可能与促进体内巨噬细胞的极化, 提高巨噬细胞和其他免疫细胞的能量代谢水平以及增强免疫功能有关。

材料与方法

细胞与动物 RAW264.7 细胞购自武汉普诺赛生命科技有限公司 (货号: CL-0190); SPF 级雄性 SD 大鼠

由斯贝福 (北京) 生物技术有限公司提供 [合格证号: 110324241100211213, 许可证号: SCXK (北京) 2019-0010]。本实验研究全过程严格遵守动物实验的各项伦理规定, 并获得山西中医药大学实验动物伦理委员会批准 (批准号: AWE202407352)。

药物与试剂 黄芪 (山西维康堂中药饮片有限公司, 批号: 221101); DMEM 高糖培养基 (Gibco, 货号: C11995500BT); 胎牛血清 (CellMax, 货号: SA211.02); 磷酸盐缓冲液 (美仑生物技术有限公司, 货号: MA0015); 脂多糖 (北京索莱宝科技有限公司, 货号: L8880); 中性红染色液 (上海碧云天生物技术有限公司, 货号: C0125); CCK-8 细胞增殖及细胞毒性检测试剂盒、TNF-α 试剂盒 (博士德生物工程有限公司, 货号: 18J13B60、EK0527); NO 检测试剂盒、增强型 ATP 检测试剂盒、BCA 蛋白浓度测定试剂盒 (上海碧云天生物技术有限公司, 货号: S0021S、S0027、P0010S); APC anti-mouse CD86 antibody、APC anti-mouse CD206 antibody、purified anti-mouse CD16/32 Antibody、胞内因子固定破膜剂 (武汉伊莱瑞特有限公司, 货号: E-AB-F0994E、E-AB-F1135E、E-AB-F0997A、E-CK-A109); 甲醇 (LC-MS 级)、乙腈 (LC-MS 级), 赛默飞世尔科技有限公司, 批号: LOT224617、LOT223164; 甲酸 (UPLC 级, 天津市光复精细化工研究所, 批号: F0167)。

仪器 HF90 型 CO_2 细胞培养箱和 Neofuge 23R 台式高速冷冻离心机 (上海力申科学仪器有限公司); Gen 5 型多功能酶标仪 (美国伯腾仪器有限公司); HC-3018 高速离心机 (安徽中科中佳科学仪器有限公司); BDS400 倒置生物显微镜 (重庆奥特光学仪器有限公司); NovoCyte2060R 流式细胞仪 (安捷伦生物有限公司); UltiMate 3000 型超高效液相色谱仪和 Q-Exactive 型四级杆−静电场轨道阱高分辨质谱仪 (美国 Thermo Fisher Scientific 公司)。

含药血清制备 SPF 级雄性 SD 大鼠 30 只, 体质量 (180 ± 20) g, 适应性喂养 7 天后, 随机分为 3 组, 分别灌胃生理盐水、生品黄芪及蜜炙黄芪水煎液, 每组 10 只。

生品黄芪及蜜炙黄芪水煎液的制备方法: 生品黄芪及蜜炙黄芪分别加水煎煮两次, 合并滤液并减压浓缩至浓度为 1 g·mL^{-1}, 4 ℃保存备用。生品黄芪及蜜炙黄芪水煎液按 2020 版《中华人民共和国药典》规定的成人日服最高剂量的 4 倍量进行灌胃给药[10], 每天 2 次, 连续给药 3 天。末次给药前禁食 12 h, 给药 40 min 后麻醉, 腹主动脉采血, 3 500 r·min^{-1} 离心 10 min, 吸取血清, 同组血清合并, 经 56 ℃, 30 min 灭活, −80 ℃保存备用[11]。

生品黄芪及蜜炙黄芪的高、中、低浓度含药血清通过空白血清与含药血清按比例分别进行稀释制得: 10% 含药血清 (空白血清−含药血清−培养基 0∶10%∶90%), 5% 含药血清 (空白血清−含药血清−培养基 5%∶5%∶90%), 2.5% 含药血清 (空白血清−含药血清−培养基 7.5%∶2.5%∶90%), 各组培养液中的血清比例均为 10%, 配制好后均 0.22 μm 滤膜过滤除菌[12]。

含药血清成分分析

色谱条件 Waters ACQUITY UPLC BEH C18 色谱柱 (2.1 mm × 100 mm, 1.7 μm); 柱温: 40 ℃; 流动相: 0.1% 甲酸水溶液 (A)−乙腈 (B); 流速: 0.3 mL·min^{-1}; 进样量: 5 μL; 梯度洗脱程序: 0～1 min, 5%～20% B; 1～5 min, 20%～50% B; 5～10 min, 50%～75% B; 10～14 min, 75%～100% B; 14～15 min, 100%～5% B; 15～16 min, 5% B。

质谱条件 采用电喷雾离子源 (ESI), 正、负离子同时采集。正离子模式下喷雾电压为 3.2 kV, 鞘气流速为 40 arb, 辅助气流速为 5 arb, 辅助气加热温度为 350 ℃; 负离子模式下喷雾电压为 2.5 kV; 鞘气流速为 38 arb; 辅助气流速为 10 arb; 辅助气加热温度 300 ℃; 毛细管温度为 320 ℃; 高压环形离子导入装置电压 (S-Lens RF Level) 为 50。检测模式采用全扫描/数据依赖二级扫描 (full MS/dd-MS^2), 扫描范围为 m/z 100～1 000, 一级质量分辨率为 70 000 FWHM, 二级质量分辨率为 17 500 FWHM, 碰撞能量为 30 eV。

CCK-8 细胞增殖实验 取对数生长期细胞, 将细胞悬液稀释至浓度为每毫升 1×10^5 个, 接种于 96 孔板, 置 37 ℃、5% CO_2 细胞培养箱中孵育 24 h。吸弃上清, 每孔加入 100 μL 含药血清继续培养 24 h。随后每孔加入 CCK-8 溶液 10 μL, 置 37 ℃、5% CO_2 细胞培养箱中孵育 40 min, 在酶标仪 450 nm 处测定吸光度。细胞存活率 (%) = $(A_2-A_0) / (A_1-A_0) \times 100\%$, 其中 A_0 是空白组的吸光度值, A_1 是对照组的吸光度值, A_2 是实验组的吸光度值。

中性红吞噬实验 参考文献[13], 并适当调整, 将 RAW264.7 (每毫升 4×10^5 个) 细胞接种于 96 孔板, 置 37 ℃、5% CO_2 细胞培养箱中孵育 24 h。吸弃上清, 用不同浓度的生品黄芪及蜜炙黄芪的含药血清和脂多糖 (LPS) 溶液 (1 μg·mL^{-1}) 处理细胞 24 h。吸弃上清, 加入 0.1% 中性红溶液置 37 ℃、5% CO_2 细胞培养箱中孵育 2 h。PBS 洗涤后加入细胞裂解缓冲液 (1% 冰醋酸∶乙醇 = 1∶1, 每孔 100 μL) 在室温下孵育 1 h, 在酶标仪 540 nm 处测定吸光度。吞噬指数采用以下方程式计算: 吞噬细胞指数 (PI) = A_1/A_0, 其中 A_1 是样品的吸光

度，A_0是空白对照的吸光度。

NO检测 将标准品稀释成不同浓度，按试剂盒说明书检测吸光度并制备标准曲线。收集RAW264.7细胞，调整细胞悬液浓度至每毫升4×10^5个后接种于96孔板，置37 ℃、5% CO_2细胞培养箱中孵育24 h。吸弃上清，分别加入不同浓度的生品黄芪及蜜炙黄芪的含药血清和1 μg·mL^{-1} LPS溶液，继续置37 ℃、5% CO_2细胞培养箱中孵育18 h。吸取上清液50 μL，加入Griess reagent I和Griess reagent II各50 μL，在酶标仪540 nm处测定吸光度。根据标准曲线计算样品中一氧化氮 (NO) 的浓度。采用1 μg·mL^{-1} LPS溶液诱导RAW264.7细胞24 h。吸弃上清，加入不同浓度的生品黄芪及蜜炙黄芪的含药血清继续置37 ℃、5% CO_2细胞培养箱中孵育18 h，检测给药前后NO的浓度变化，其余实验步骤同上。

ATP检测 按试剂盒说明书配制相关试剂，将标准品稀释成不同浓度并测定发光值，根据所得数据绘制标准曲线。将RAW264.7细胞以每毫升4×10^5个接种于12孔板，置37 ℃、5% CO_2细胞培养箱中孵育24 h。吸弃细胞培养液，PBS洗涤后加入不同浓度的生品黄芪及蜜炙黄芪的含药血清继续置37 ℃、5% CO_2细胞培养箱中孵育18 h。吸弃培养液，每孔加入100 μL细胞裂解缓冲液，反复吹打或晃动培养板以充分裂解细胞。裂解后于4 ℃、12 000×*g*离心5 min，取上清，用于后续的测定。加入ATP检测工作液反应3～5 min，随后每孔加入20 μL细胞裂解上清液，使用多功能酶标仪检测样品的发光值，根据标准曲线计算样品中ATP的浓度。使用BCA蛋白浓度测定试剂盒检测样品中蛋白的浓度，将ATP浓度换算成nmol·mg^{-1}蛋白的形式。

TNF-*α*检测 将标准品依次配制成说明书所述浓度，混匀，制备标准曲线。收集RAW264.7对数生长期细胞，以每毫升4×10^5个铺于96孔板，置37 ℃、5% CO_2细胞培养箱中孵育24 h。吸弃上清，加入空白血清、1 μg·mL^{-1} LPS溶液、生品黄芪及蜜炙黄芪的不同浓度含药血清继续置37 ℃、5% CO_2细胞培养箱中孵育18 h。吸取上清液离心，在酶标仪450 nm处测定吸光度，根据标准曲线计算浓度。

CD86和CD206检测

细胞培养及给药 将RAW264.7细胞以每毫升1.5×10^6个接种于6孔板，置37 ℃、5% CO_2细胞培养箱中孵育24 h。吸弃培养液，加入空白血清、1 μg·mL^{-1} LPS溶液、生品黄芪及蜜炙黄芪的不同浓度含药血清继续置37 ℃、5% CO_2细胞培养箱中孵育18 h。

CD86抗体孵育 吸弃培养液，用PBS漂洗一次。用含1% BSA的PBS将细胞吹打下来，300×*g*离心5 min，弃上清，调整细胞浓度至每毫升1×10^7个备用。在100 μL细胞悬液中加入2 μL Fc段封闭剂，轻柔吹打充分混匀后室温下孵育10 min。封闭结束后，在每100 μL细胞悬液中加入5 μL荧光标记的CD86抗体，混匀后置4 ℃避光孵育30 min。

CD206抗体孵育 Fc段封闭结束后，加入2 mL含1% BSA的PBS重悬细胞，300×*g*离心细胞悬液5 min，弃上清，加入200 μL含1% BSA的PBS重悬细胞。每管加入200 μL Fixation Buffer，轻柔混匀，室温避光孵育30～60 min。加入1 mL 1×permeabilization working solution，600×*g*离心5 min，弃上清。加入100 μL 1×permeabilization working solution，重悬细胞。对应的单染管和全染管加入相应的抗体5 μL，混匀。室温避光孵育30 min。每管加入2 mL Cell Staining Buffer 600×*g*离心5 min，弃上清，加入600 μL Cell Staining Buffer重悬细胞。调整仪器参数，上机检测。

RAW264.7细胞糖酵解差异代谢物分析

样品制备 将相同数量的RAW264.7细胞 (每毫升4×10^5个) 于6孔板中培养，每组设置6个复孔，培养24 h后，吸弃上清，PBS洗涤后加入含药血清继续培养18 h，收集细胞外培养基于液氮中速冻并储存在−80 ℃条件下，用于细胞外代谢组学分析。PBS洗涤后将细胞吹打下来，于4 ℃、1 200 r·min^{-1}离心10 min，收集细胞沉淀于液氮中速冻，储存在−80 ℃下，用于细胞内代谢组学分析。

细胞外代谢物提取 参考文献[14]并做适当修改，将细胞外液于4 ℃融化，在400 μL细胞外液中加入1.2 mL甲醇，涡旋30 s，冰浴中静置5 min，于4 ℃、13 000 r·min^{-1}离心10 min，收集上清并冻干。

细胞内代谢物提取 将细胞于4 ℃融化，反复冻融5次后，加入75%甲醇溶液2 mL，置冰上超声破碎15 min，于4 ℃、13 000 r·min^{-1}离心10 min，收集上清并冻干[15]。在冻干后的细胞上清液和细胞裂解物中加入400 μL 1%甲酸水溶液复溶，于4 ℃、13 000 r·min^{-1}离心10 min，上清置液相小瓶中。另通过混合各自的等分试样作为QC样本，空白为提取溶剂。

色谱条件 Waters ACQUITY UPLC HSS T3色谱柱 (100 mm × 2.1 mm, 1.8 μm)；柱温：40 ℃；流动相：2%甲酸水 (A)−乙腈 (B)；流速：0.3 mL·min^{-1}；进样量：3 μL；梯度洗脱程序：0～2 min, 1% B; 2～10 min, 1%～20% B; 10～15 min, 20%～100% B; 15～16 min, 100% B; 16～17 min, 100%～1% B; 17～18 min, 1% B。

质谱条件 采用电喷雾离子源 (ESI)，正、负离子同时采集。正离子模式下喷雾电压为3.2 kV，鞘气流

速为40 arb, 辅助气流速为5 arb, 辅助气加热温度为350 ℃; 负离子模式下喷雾电压为2.5 kV, 鞘气流速为38 arb, 辅助气流速10 arb, 辅助气加热温度300 ℃。毛细管温度为320 ℃, 高压环形离子导入装置电压 (S-Lens RF Level) 为50。检测模式采用全扫描/数据依赖二级扫描 (Full MS/dd-MS 2), 扫描范围为 *m/z* 80～1 000, 一级质量分辨率为70 000 FWHM, 二级质量分辨率为17 500 FWHM, 碰撞能量为15、20、30 eV。

数据处理 采用SPSS 20.0对数据进行单因素方差分析 (one-way analysis of variance, one-way ANOVA), 柱状图使用GraphPad Prism 9绘制。数据以均数 ± 标准差 ($\bar{x} \pm s$) 表示, 以 $P < 0.05$ 为差异具有统计学意义。

结果

1 含药血清成分分析

采用LC-MS对生品黄芪及蜜炙黄芪的含药血清成分进行分析, 见表1[16-24]和图1。结果显示, 与生品黄芪的含药血清相比, 蜜炙黄芪的含药血清中葡萄糖、岩藻糖、赖氨酸、酪氨酸、脯氨酸、亮氨酸、色氨酸、甜菜碱、芒柄花苷、黄芪甲苷、毛蕊异黄酮葡萄糖苷、毛蕊异黄酮的含量均有增加, 而芒柄花素和精氨酸的含量均有降低。

2 生品黄芪及蜜炙黄芪的含药血清对RAW264.7细胞活性及吞噬能力的影响

与空白血清相比, 生品黄芪及蜜炙黄芪的各浓度含药血清均可促进细胞增殖且呈浓度依赖性增长, 除蜜炙黄芪的2.5%含药血清外, 其余均具有显著性。蜜炙黄芪的5%和10%含药血清的细胞存活率分别为 (174.75 ± 30.51) %和 (181.50 ± 16.66) %, 显著高于生品黄芪的各浓度含药血清, 所选含药血清的浓度对RAW264.7细胞均无细胞毒作用, 因此, 这些浓度将被选择进行后续的研究 (图2A)。

采用中性红吞噬法评估生品黄芪及蜜炙黄芪的含药血清对RAW264.7细胞吞噬能力的影响。结果显示, 与空白血清相比, 生品黄芪及蜜炙黄芪的各浓度含药血清均可显著提高RAW264.7细胞吞噬指数。蜜炙黄芪的10%含药血清的细胞吞噬能力显著高于生品黄芪的各浓度含药血清, 其吞噬率为 (179.33 ± 11.85) % (图2B)。

3 生品黄芪及蜜炙黄芪的含药血清对RAW264.7细胞NO分泌的影响

采用Griess法检测生品黄芪及蜜炙黄芪的含药血

Table 1 Differential components in medicated sera of crude and honey-processed *Astragalus*

No.	Name	t_R/min	Formula	Adduct	*m/z*		Error/10^{-6}	Fragment ion (*m/z*)	Change
					Expected	Observed			
1	Lysine[16]	0.79	$C_6H_{14}N_2O_2$	$[M+H]^+$	147.112 8	147.112 8	0	84.081 5, 130.086 4, 56.050 6	Up
2	Tyrosine[16]	1.31	$C_9H_{11}NO_3$	$[M+H]^+$	182.081 2	182.081 1	−0.55	165.054 7, 136.075 8	Up
3	Proline[17]	0.90	$C_5H_9NO_2$	$[M+H]^+$	116.070 6	116.070 8	1.72	70.066 0	Up
4	Leucine[18]	1.54	$C_6H_{13}NO_2$	$[M+H]^+$	132.101 9	132.101 7	−1.51	132.076 9	Up
5	Tryptophan[19]	3.05	$C_{11}H_{12}N_2O_2$	$[M+H]^-$	205.097 2	205.097 0	−0.98	203.081 7, 142.064 9, 116.049 0	Up
6	Betaine[20]	0.87	$C_5H_{11}NO_2$	$[M+H]^+$	118.086 3	118.086 4	0.85	58.066 1	Up
7	Calycosin-7-glucoside[21]	2.07	$C_{22}H_{22}O_{10}$	$[M+H]^+$	447.128 6	447.128 4	−0.45	229.162 9	Up
8	Formononetin[22]	6.82	$C_{16}H_{12}O_4$	$[M-H]^-$	267.066 2	267.066 3	0.37	252.042 4, 223.039 7	Down
9	Calycosin[22,23]	4.31	$C_{16}H_{12}O_5$	$[M-H]^-$	283.061 2	283.061 4	0.71	283.264 2, 239.200 9	Up
10	Ononin[24]	5.32	$C_{22}H_{22}O_9$	$[M+COOH]^-$	475.124 6	475.124 5	−0.21	269.045 5, 241.050 8	Up
11	Fucose	1.63	$C_6H_{12}O_5$	$[M+H]^+$	165.075 8	165.075 6	−1.21	123.044 3, 119.049 5, 95.049 7	Up
12	Astragaloside	5.38	$C_{41}H_{68}O_{14}$	$[M+COOH]^-$	829.459 1	829.460 5	1.69	–	Up
13	Glucose	0.85	$C_6H_{12}O_6$	$[M-H]^-$	179.056 1	179.055 4	−3.91	–	Up
14	Arginine	0.80	$C_6H_{14}N_4O_2$	$[M+H]^+$	175.119 0	175.118 6	−2.28	–	Down

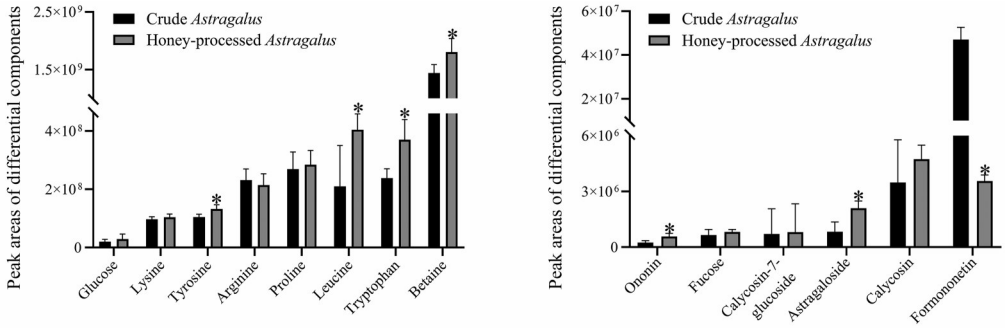

Figure 1 Peak areas of differential components in medicated sera of crude and honey-processed *Astragalus* ($n = 5$, $\bar{x} \pm s$). $^*P < 0.05$ *vs* crude *Astragalus* group

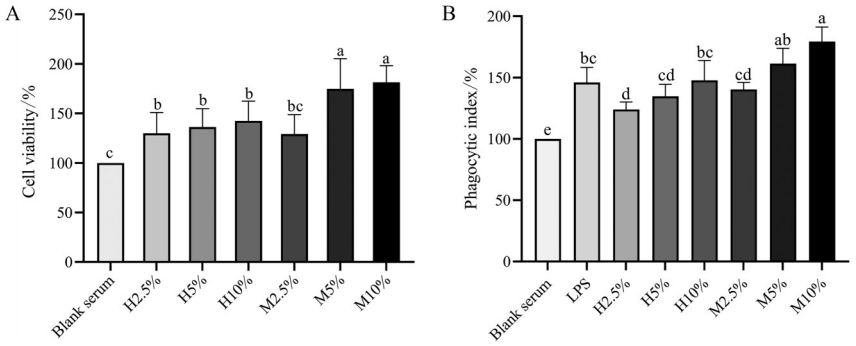

Figure 2 Effects on activity and phagocytosis of RAW264.7 cells in medicated sera of crude and honey-processed *Astragalus*. A: Differences of cell activity between medicated sera of crude and honey-processed *Astragalus* (n = 3, $\bar{x} \pm s$); B: Differences of cell phagocytosis between medicated sera of crude and honey-processed *Astragalus* (n = 3, $\bar{x} \pm s$). a−e: Absence of same letter between any two groups indicates significance, $P < 0.05$. H represents crude *Astragalus* and M represents honey-processed *Astragalus*

清对RAW264.7细胞分泌NO的影响。结果显示, 生品黄芪的5%和10%以及蜜炙黄芪的各浓度含药血清的NO分泌量均高于空白血清, 其中生品黄芪的10%以及蜜炙黄芪的各浓度含药血清的NO分泌量与空白血清相比均具有显著性。蜜炙黄芪的各浓度含药血清的NO分泌量显著高于生品黄芪的各浓度含药血清, 蜜炙黄芪的10%含药血清的NO分泌量最高, 达到4.55 ± 0.16 μmol·L^{-1} (图3A)。

此外, 使用LPS将RAW264.7细胞诱导为炎性细胞 (M1型), 随后分别使用空白血清、生品黄芪及蜜炙黄芪的不同浓度含药血清进行干预。结果显示, 与未经干预的LPS相比, 生品黄芪及蜜炙黄芪的各浓度含药血清均可显著抑制炎性细胞NO的分泌, 抑制作用呈浓度依赖性增强且蜜炙黄芪的效果优于生品黄芪 (图3B)。此结果证明了生品黄芪及蜜炙黄芪对RAW264.7细胞具有抗炎和免疫的双向调节作用。

4 生品黄芪及蜜炙黄芪的含药血清对RAW264.7细胞TNF-α分泌及能量代谢的影响

巨噬细胞激活后会分泌TNF-α协助参与免疫调节, 在宿主防御中发挥着重要的作用。采用TNF-α ELISA试剂盒检测生品黄芪及蜜炙黄芪的含药血清对TNF-α分泌的影响。结果显示, 与空白血清相比, 生品黄芪的各浓度以及蜜炙黄芪的5%和10%含药血清均可促进RAW264.7细胞分泌TNF-α且呈浓度依赖性增长, 其中生品黄芪的10%以及蜜炙黄芪的5%和10%含药血清具有显著性。与生品黄芪的含药血清相比, 蜜炙黄芪的5%和10%含药血清能显著促进TNF-α的分泌, 提示蜜炙黄芪促进免疫的功效优于生品黄芪 (图4A)。

ATP是能量的“通用货币”, 能直接反映生物体能量代谢水平的高低。采用增强型ATP检测试剂盒检测生品黄芪及蜜炙黄芪的含药血清对能量代谢的影响。结果显示, 生品黄芪的10%以及蜜炙黄芪的各浓度含药血清的ATP产量均显著高于空白血清, 蜜炙黄芪的各浓度含药血清的ATP产量均显著高于生品黄芪的各浓度含药血清的ATP产量, 且ATP产量呈浓度依赖性增强, 蜜炙黄芪的10%含药血清的ATP产量最高, 达到3.65 ± 0.08 nmol·mg^{-1}。蜜炙黄芪促进能量代谢的

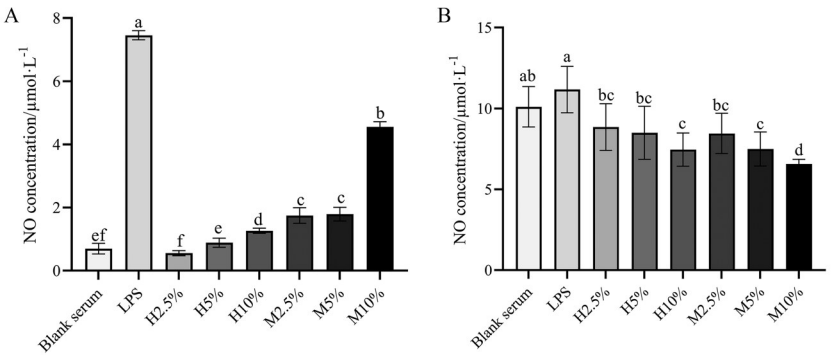

Figure 3 Effects on NO secretion of normal and inflammatory RAW264.7 cells in medicated sera of crude and honey-processed *Astragalus*. A: NO secretion of normal cells after intragastric administration (n = 3, $\bar{x} \pm s$); B: NO secretion of inflammatory cells after intragastric administration (n = 3, $\bar{x} \pm s$). a−f: Absence of same letter between any two groups indicates significance, $P < 0.05$

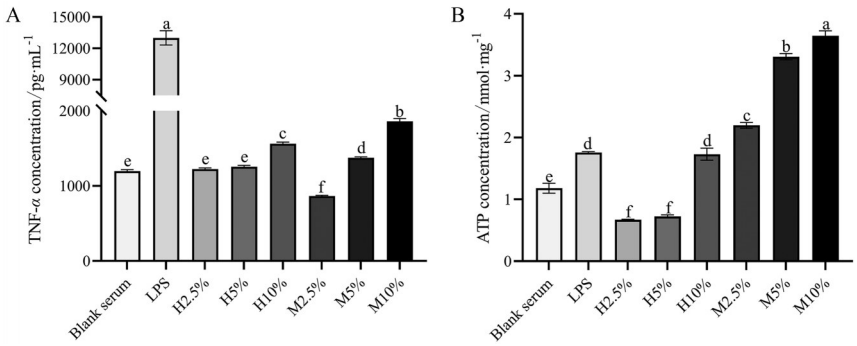

Figure 4 Effects on TNF-α secretion and energy metabolism of RAW264.7 cells in medicated sera of crude and honey-processed *Astragalus*. A: Difference of TNF-α secretion between medicated sera of crude and honey-processed *Astragalus* (n = 3, $\bar{x} \pm s$); B: Difference of ATP yield between medicated sera of crude and honey-processed *Astragalus* (n = 3, $\bar{x} \pm s$). a−f: Absence of same letter between any two groups indicates significance, $P < 0.05$

效果优于生品黄芪 (图4B)。

5 生品黄芪及蜜炙黄芪的含药血清对RAW264.7细胞极化的影响

巨噬细胞在免疫过程中会极化为M1型和M2型巨噬细胞, 二者的标志因子分别为CD86和CD206。通过流式细胞术检测生品黄芪及蜜炙黄芪的含药血清对RAW264.7细胞极化的影响。结果显示, 图5A中空白血清的CD86表达比例为5.88%, 生品黄芪的各浓度含药血清的CD86表达比例分别为5.77%、9.14%和9.18%, 呈浓度依赖性增长。蜜炙黄芪的各浓度含药血清的CD86表达比例分别为8.01%、9.05%和10.32%, 同样呈浓度依赖性增长, 略低于脂多糖的12.77%, 且蜜炙黄芪的各浓度含药血清均高于生品黄芪的各浓度含药血清。图5B中空白血清的CD206表达比例为14.75%, 生品黄芪的各浓度含药血清的CD206表达比例分别为7.89%、6.64%和3.66%, 呈浓度依赖性降低。蜜炙黄芪的各浓度含药血清的CD206表达比例分别为5.32%、5.00%和1.16%, 同样呈浓度依赖性降低。蜜炙黄芪的各浓度含药血清均低于空白血清和生品黄芪的各浓度含药血清。

在给予生品黄芪及蜜炙黄芪的含药血清后, M1型和M2型巨噬细胞的标志因子均有表达, 但M2型的比例低于M1型。与空白血清相比, 生品黄芪及蜜炙黄芪的含药血清的M1型比例呈上升趋势, M2型比例呈下降趋势, 说明在免疫过程中会促进巨噬细胞向M1型极化, 并有减弱巨噬细胞向M2型极化的趋势, 其中蜜炙黄芪的效果更好。

6 生品黄芪及蜜炙黄芪的含药血清对糖酵解的影响

糖酵解是能量代谢的一种方式, 巨噬细胞激活后主要通过糖酵解产生能量参与免疫调节[25]。采用LC-MS技术对细胞培养液及裂解液进行代谢组学分析, 筛选与糖酵解相关的差异代谢物。

本部分实验结果见表2[26,27]和图6。图6A为RAW264.7细胞内的糖酵解代谢产物分析结果。与空白血清及生品黄芪的含药血清相比, 蜜炙黄芪的含药血清细胞裂解液中葡萄糖-1-磷酸、葡萄糖、3-磷酸甘油酸、丙酮酸的含量均显著上升 ($P < 0.05$); 3-磷酸甘油醛、乳酸、1,3-二磷酸甘油酸的含量均高于空白血清及生品黄芪的含药血清, 但无显著性差异。图6B为RAW264.7细胞外的糖酵解代谢产物分析结果。蜜炙黄芪的10%含药血清培养液中乳酸的含量显著高于空白血清及生品黄芪的2.5%和5%含药血清, 略高于生品黄芪的10%含药血清; 葡萄糖的含量显著高于空白血清和生品黄芪的各浓度含药血清; 1,3-二磷酸甘油酸的含量显著高于空白血清及生品黄芪的各浓度含药血清。蜜炙黄芪的含药血清培养液中葡萄糖-1-磷酸、3-磷酸甘油酸、丙酮酸的含量均高于生品黄芪的含药血清, 但无显著性差异。3-磷酸甘油醛、果糖1,6-二磷酸的含量均低于生品黄芪的含药血清, 但无显著性差异。糖酵解是巨噬细胞在参与免疫调节时主要的能量代谢方式, 研究结果显示, 黄芪经蜜炙后, 其细胞内、外液中糖酵解代谢产物总体呈上升趋势, 说明其能促进RAW264.7细胞的糖酵解, 从而产生更多能量参与免疫调节。

讨论

传统中医中的气虚型疾病常伴随着免疫系统紊乱 (包括免疫细胞、免疫组织以及免疫器官的失调), 细胞因子水平表达失常, 组织结构以及器官正常形态变异等[28], 这类似于现代医学的免疫学疾病。黄芪在中医临床上常用来治疗气虚病症, 因此可以从中医的气虚理论出发, 通过与现代免疫学思维的结合来阐释蜜

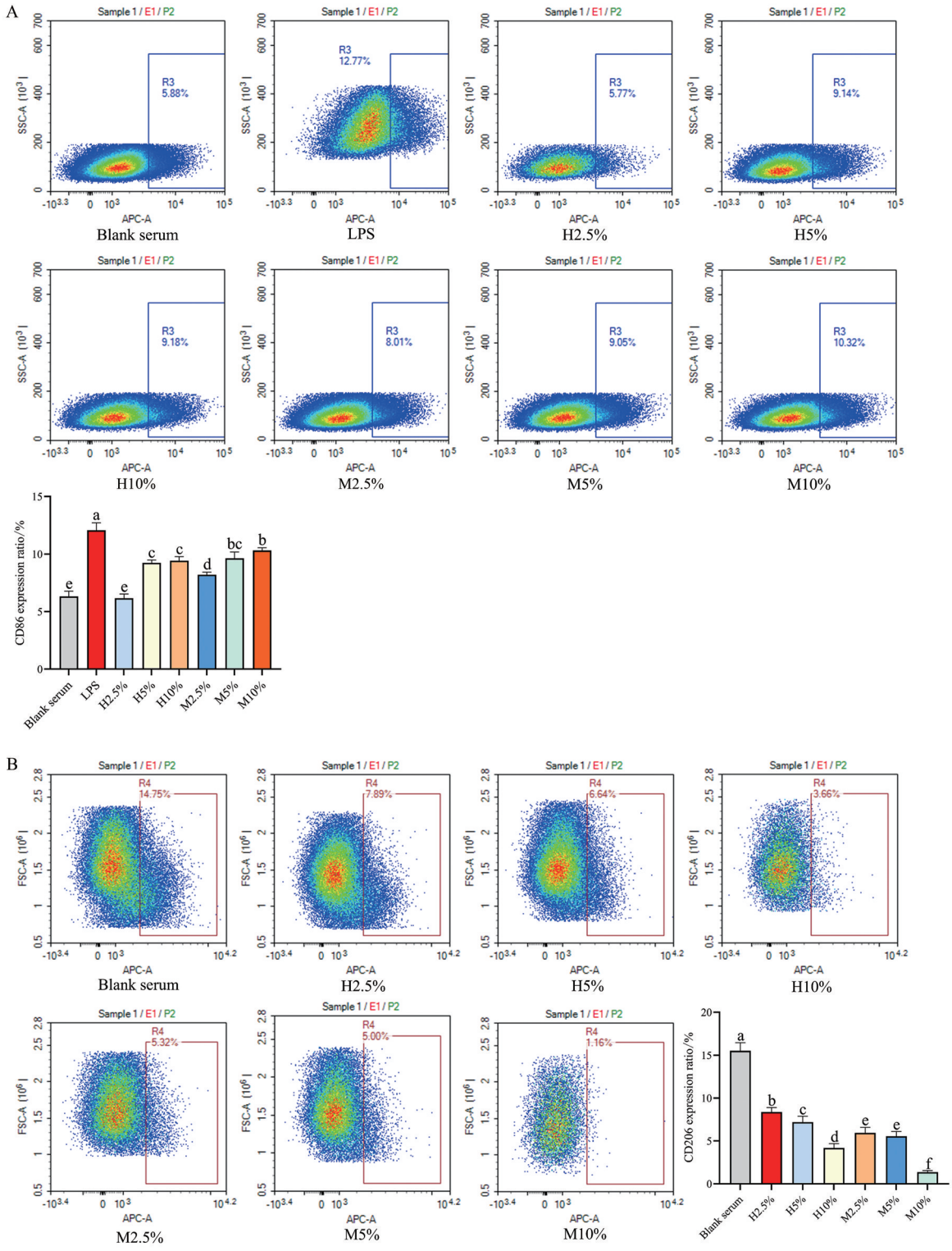

Figure 5 Effects on expression of M1 and M2 marker factors (CD86 and CD206) of RAW264.7 cells in medicated sera of crude and honey-processed *Astragalus*. A: Effects on CD86 expression of RAW264.7 cells in medicated sera of crude and honey-processed *Astragalus* (n = 3, $\bar{x} \pm s$); B: Effect on CD206 expression of RAW264.7 cells in medicated sera of crude and honey-processed *Astragalus* (n = 3, $\bar{x} \pm s$). a−f: Absence of same letter between any two groups indicates significance, $P < 0.05$

炙黄芪增强补中益气的科学内涵。

巨噬细胞在免疫系统中扮演着极其重要的角色，它可通过吞噬病原体、提呈抗原或分泌免疫活性物质的方式在机体的抗肿瘤、抗感染以及免疫调节等方面发挥作用[29]。激活后的巨噬细胞通过分泌免疫活性物质如NO和TNF-α等参与免疫调节。NO是中枢神经

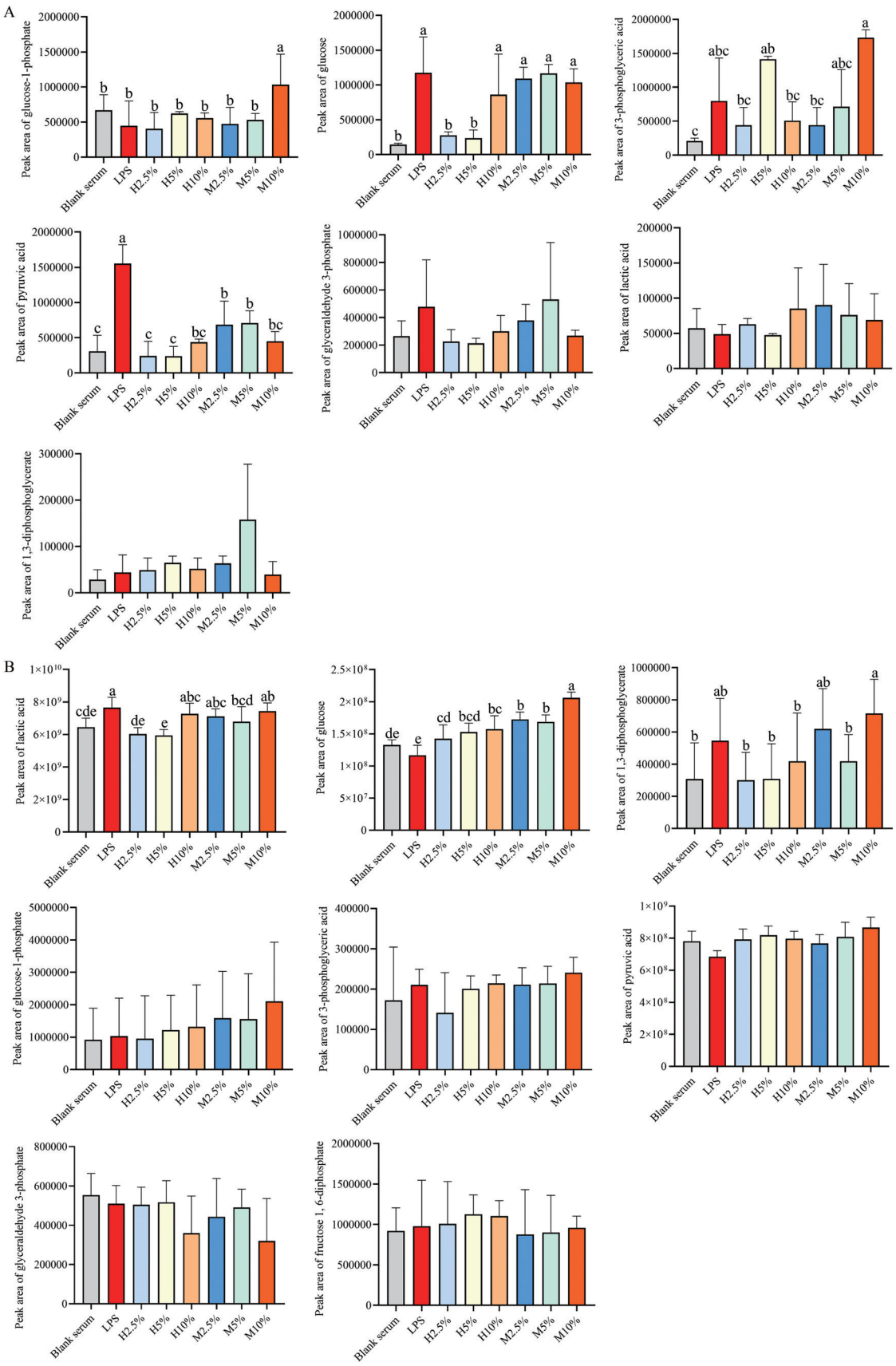

Figure 6 Effects on glycolysis of RAW264.7 cells in medicated sera of crude and honey-processed *Astragalus*. A: Peak areas of glycolytic metabolites in cell lysate (n = 6, $\bar{x} \pm s$); B: Peak areas of glycolytic metabolites in cell culture medium (n = 6, $\bar{x} \pm s$). a−e: Absence of same letter between any two groups indicates significance, $P < 0.05$

Table 2 Differential metabolites associated with glycolysis in cell lysate (A) and cell culture medium (B)

No.	Name	t_R/min	Formula	Adduct	*m/z*		Error/ 10^{-6}	Fragment ion (*m/z*)
					Expected	Observed		
1	Glucose-1-phosphat (A)	0.93	$C_6H_{13}O_9P$	$[M-H]^-$	259.022 4	259.022 6	0.77	259.022 6, 223.831 2, 221.834 3, 96.968 1
	Glucose-1-phosphat (B)	6.95	$C_6H_{13}O_9P$	$[M-H]^-$	259.022 4	259.022 5	0.39	223.107 9, 221.842 3, 128.033 9, 130.085 9
2	Glucose[26] (A)	0.87	$C_6H_{12}O_6$	$[M-H]^-$	179.056 1	179.056 4	1.67	179.055 1, 161.044 4, 101.022 9
	Glucose (B)	0.88	$C_6H_{12}O_6$	$[M-H]^-$	179.056 1	179.056 3	1.12	101.022 8
3	Pyruvic acid (A)	1.45	$C_3H_4O_3$	$[M-H]^-$	87.008 8	87.008 7	−1.15	87.007 2
	Pyruvic acid (B)	1.37	$C_3H_4O_3$	$[M-H]^-$	87.008 8	87.008 9	1.15	87.007 1
4	Glyceraldehyde 3-phosphate (A)	1.48	$C_3H_7O_6P$	$[M+H]^-$	171.005 3	171.005 6	1.75	148.977 2, 130.966 5, 89.071 6
	Glyceraldehyde 3-phosphate (B)	1.36	$C_3H_7O_6P$	$[M+H]^-$	171.005 3	171.005 4	0.58	148.977 1, 130.966 5, 89.071 5
5	Lactic acid[27] (A)	1.36	$C_3H_6O_3$	$[M-H]^-$	89.024 4	89.024 5	1.12	71.012 2, 89.022 8
	Lactic acid (B)	1.33	$C_3H_6O_3$	$[M-H]^-$	89.024 4	89.024 5	1.12	89.022 8
6	1,3-Diphosphoglycerate (A)	8.65	$C_3H_8O_{10}P_2$	$[M-H]^-$	264.952 0	264.952 7	2.64	–
	1,3-Diphosphoglycerate (B)	8.65	$C_3H_8O_{10}P_2$	$[M-H]^-$	264.952 0	264.952 7	2.64	–
7	Fructose 1,6-diphosphate (A)	8.27	$C_6H_{14}O_{12}P_2$	$[M-H]^-$	338.988 8	338.989 4	1.77	–
	Fructose 1,6-diphosphate (B)	8.27	$C_6H_{14}O_{12}P_2$	$[M-H]^-$	338.988 8	338.989 5	2.06	–
8	3-Phosphoglyceric acid (A)	17.39	$C_3H_7O_7P$	$[M-H]^-$	184.985 7	184.986 6	4.87	–
	3-Phosphoglyceric acid (B)	17.39	$C_3H_7O_7P$	$[M-H]^-$	184.985 7	184.986 4	3.87	–

系统中的信息传递介质, 巨噬细胞通过释放大量NO对病毒、细菌和真菌等微生物及肿瘤细胞产生细胞毒作用, 并通过诱导机体产生炎症反应来抵御外源物质的侵害[30]。同时, NO也是一把“双刃剑”, 其过量分泌也会对机体造成损伤。巨噬细胞在免疫过程中会极化为不同类型, M1型巨噬细胞能分泌大量促炎细胞因子如TNF-α等。当机体处于正常状态时, 适量分泌的TNF-α能上调组织修复和增强免疫机能[31]。而M2型巨噬细胞在特定条件下可产生大量的抗炎细胞因子, 如IL-10和CD206等, 除了具有抗炎作用外还有一定的免疫抑制活性。

免疫细胞的正常生命活动和效应功能的执行需要三磷酸腺苷 (ATP) 形式的能量, 缺乏足够的能量供应会直接影响免疫细胞的功能[32]。免疫细胞激活后的代谢格局与非活化的免疫细胞截然不同, 这与肿瘤细胞的生长类似, 即发生了“代谢重编程”现象, 同时免疫细胞的表型和功能又会受到代谢的调控。激活的免疫细胞利用类似于“Warburg”效应的方式进行代谢, 如M1型巨噬细胞、激活的中性粒细胞和树突状细胞等主要利用糖酵解产生的ATP来维持细胞的功能[33], 静息的免疫细胞, 如M2型巨噬细胞等, 一般利用三羧酸循环与氧化磷酸化偶联产生的ATP来维持细胞的功能[34]。

本研究证明了黄芪经蜜炙后, 葡萄糖、岩藻糖、赖氨酸、酪氨酸、脯氨酸、亮氨酸、色氨酸、甜菜碱、芒柄花苷、黄芪甲苷、毛蕊异黄酮、毛蕊异黄酮葡萄糖苷等成分含量升高; 其中葡萄糖有益于能量代谢, 是机体重要的能量来源和代谢的中间产物, 岩藻糖、氨基酸、甜菜碱、黄芪甲苷、芒柄花苷、毛蕊异黄酮、毛蕊异黄酮葡萄糖苷能够有效增强细胞免疫和体液免疫功能, 提高免疫细胞活性, 释放免疫活性物质, 进而增强机体的免疫功能[35-38], 毛蕊异黄酮除具有免疫功能外, 还能通过抑制NF-κB蛋白的活化达到抗炎作用[38-41]。黄芪及蜜炙黄芪的含药血清能通过提高巨噬细胞的增殖及吞噬能力, 增加TNF-α的分泌和促进巨噬细胞向M1型极化并减弱巨噬细胞向M2型极化的趋势, 进而参与免疫调节。在能量代谢方面, 生品黄芪和蜜炙黄芪均能促进巨噬细胞的糖酵解及ATP的产生, 其中蜜炙黄芪的功效优于生品黄芪。此外, 生品黄芪及蜜炙黄芪均能促进RAW264.7细胞正常状态下NO的产生, 也能抑制炎症状态下NO的分泌, 具有抗炎免疫双向调节作用。综上所述, 蜜炙黄芪能促进巨噬细胞RAW264.7极化及能量代谢, 参与正向的免疫调节。因此, 蜜炙黄芪补中益气功效的增强可能与促进体内巨噬细胞极化及能量代谢和增强免疫功能有关, 还可能与增强体内其他免疫细胞能量代谢及免疫功能有关。

作者贡献: 李鸿昌负责总体实验的开展、数据分析及文章撰写; 裴科负责实验的整体设计、指导及文章修改; 解旺洋、于子涵、李玟玲参与部分实验; 孟祥龙负责维护相关实验设备; 蔡皓负责文章的整体修改和校对。

利益冲突: 所有作者均声明不存在利益冲突。

References

[1] Chinese Pharmacopoeia Commission. Pharmacopoeia of the People's Republic of China (中华人民共和国药典) [S]. Vol I. Beijing: China Medical Science Press, 2020: 315-316.

[2] Li FT. Study on the Immune Regulation Mechanism of Ginseng Based on Intestinal Flora and Chromatography-Mass Spectrometry (基于肠道菌群和色谱质谱技术研究林下参免疫调节作用机制) [D]. Changchun: Changchun University of Chinese Medicine, 2022.

[3] Cuan B. Preparation of Cordycepin and Its Anti-inflammatory

and Immunorhodulatory Activities (北虫草菌素制备及其抗炎、免疫调节活性研究) [D]. Harbin: Northeast Forestry University, 2021.

[4] Yu YF, Zhang X, Sang R, et al. Effects of total flavonoids of *Astragalus* on secretion levels of cytokines and mediators in RAW264.7 cells under normal conditions [J]. Agric Sci J Yanbian Univ (延边大学农学学报), 2018, 40: 1-7.

[5] Keewan E, Naser SA. Notch-1 signaling modulates macrophage polarization and immune defense against *Mycobacterium avium paratuberculosis* infection in inflammatory diseases [J]. Microorganisms, 2020, 8: 1006.

[6] Shapouri-Moghaddam A, Mohammadian S, Vazini H, et al. Macrophage plasticity, polarization, and function in health and disease [J]. J Cell Physiol, 2018, 233: 6425-6440.

[7] Zhang XY, Zhong R, Cui HJ, et al. Inhibitory effects of modified Sijunzi Decoction on transplanted tumor of CT26 colorectal cancer in mice and influence on protein expressions of tumor-associated macrophages CD68 and CD206 [J]. Acad J Shanghai Univ Tradit Chin Med (上海中医药大学学报), 2019, 33: 61-65.

[8] Zhang MY. The Role and Mechanism of IL-10 in Macrophage-Mediated Tumor Chemotherapy Resistance (IL-10 在巨噬细胞介导肿瘤耐药中的作用及机制研究) [D]. Changchun: Jilin University, 2023.

[9] Ghesquière B, Wong BW, Kuchnio A, et, al. Metabolism of stromal and immune cells in health and disease [J]. Nature, 2014, 511: 167-176.

[10] Cao LL, Pei K, Liu R, et al. Material basis and mechanism of "Shanghuo" response caused by warm nature of Astragali Radix based on "fraction deduction" [J]. Chin Tradit Herb Drugs (中草药), 2022, 53: 4350-4364.

[11] Huang J, Zhang J, Xu ZW. Effect of *Astragalus* polysaccharide on the proliferation of bone marrow mesenchymal stem cells and the expression of stem cell factor *in vitro* [J]. J Fudan Univ Med Sci (复旦学报 医学版), 2011, 38: 343-348.

[12] Li Q, Wu YZ, Guan LC, et al. Effect of different concentrations of Astragali Radix containing serum on CYP24a1, CYP27b1 mRNA and protein in osteogenic differentiation of aging bone marrow mesenchymal stem cells [J]. Chin J Exp Tradit Med Form (中国实验方剂学杂志), 2020, 26: 49-55.

[13] Li YG, Yu P, Fu WW, et al. Ginseng-Astragalus-oxymatrine injection ameliorates cyclophosphamide-induced immunosuppression in mice and enhances the immune activity of RAW264.7 cells [J]. J Ethnopharmacol, 2021, 279: 114387.

[14] Li SY, Li K, Qin XM, et al. Study on active constituents of *Astragalus* polysaccharides for injection to regulate metabolism of macrophages based on LC-MS metabolomics [J]. Chin Tradit Herb Drugs (中草药), 2020, 51: 1575-1585.

[15] Fan XH, Li K, Yang YD, et al. Screening for anti-inflammatory components of *Astragalus* polysaccharide and metabolomics research based on molecular weight distribution [J]. Acta Pharm Sin (药学学报), 2022, 57: 783-792.

[16] Zhao MY, Bi CH, Li MJ, et al. Rapid analysis of components in Longshengzhi capsules based on UPLC-Q-TOF/MS technology [J]. Mod Tradit Chin Med Mater Med World Sci Technol (世界科学技术−中医药现代化), 2024. http://kns.cnki.net/kcms/detail/11.5699.R.20241021.1149.002.html.

[17] Zhao Y, Weng YJ, Yang CL. Simultaneous determination of betaines and proline in plant tissue under salt stress by HPLC-ESI-MS [J]. J Instrum Anal (分析测试学报), 2004, 23: 83-86.

[18] Zhao TT, Pei K, Yu ZH, et al. Research on differences between saccharides ingredients and other chemical components of Astragali Radix and honey-processed Astragali Radix [J]. Chin Tradit Herb Drugs (中草药), 2023, 54: 1586-1596.

[19] Zhang JQ, Fu XM, Zhuo Y, et al. Identification of chemical components based on UPLC-Q-TOF-MS/MS and evaluation of anti-aging activity *in vitro* for Yangxue Qingnao Wan [J]. China J Chin Mater Med (中国中药杂志), 2024, 49: 4672-4686.

[20] Cheng SH, Lei XY, Kang W, et al. Analysis of chemical constituents of Zhuang Medicine Longzuan Tongbi Granules by LC-MS/MS [J]. Chin Pharm J (中国药学杂志), 2024, 59: 818-826.

[21] Li RR. Investigation of Chemo Markers of Hengshan Astragali Radix by LC-MS Metabolomics (基于液质联用技术的恒山黄芪中特征成分研究) [D]. Taiyuan: Shanxi University, 2021.

[22] Lv YH. Study on the Pharmacodynamic Material Basis and Network Pharmacology of Modified Danggui Buxue Decoction in Treating Leukopenia (加味当归补血汤治疗白细胞减少症的药效物质基础和网络药理学研究) [D]. Jinan: Shandong University of Traditional Chinese Medicine, 2023.

[23] Yang L, Li MY, Hu WQ, et al. Analyzed the absorbed components of Bufei Pills and its mechanism of action in treating respiratory disorders and recovery period of novel coronavirus infections through HPLC-Q-TOF-MS/MS integrated with network pharmacology [J]. China J Chin Mater Med (中国中药杂志), 2024. https://doi.org/10.19540/j.cnki.cjcmm.20240715.201.

[24] Gao WJ, Li SS, Xiang XM, et al. Analysis of material basis of famous classical formula baoyuantang based on UPLC-Q-TOF-MS/MS [J]. Chin J Exp Tradit Med Formulae (中国实验方剂学杂志), 2024, 30: 243-248.

[25] Yang XP, Weng XF, Tan Z, et al. Energy metabolism in immune cells [J]. Chin Bull Life Sci (生命科学), 2016, 28: 200-207.

[26] Shi LX. Immune Activity Screening and Preliminary Structure Study of Oligosaccharide Fragments Degraded by Trifluoroacetic Acid for Active *Astragalus* Polysaccharides APS-II (黄芪活性多糖 APS-II三氟乙酸降解寡糖片段的免疫活性筛选与结构初探) [D]. Taiyuan: Shanxi University, 2021.

[27] Zhang W. Study on the Origins and Generationpathways of Lactate and Effects of ^{13}C on Ionization and Fragmentation of ^{13}C-labled Compounds (乳酸的来源和生成途径研究及^{13}C对^{13}C标记化合物质谱行为的影响) [D]. Hangzhou: Zhejiang

University, 2017.

[28] Liu JQ. Study on the Immunoregulation and Metabonomics of Astragalus, Ginseng and Their Combination (补气药黄芪、人参及其配伍免疫调节和代谢组学研究) [D]. Beijing: China Academy of Chinese Medical Sciences, 2019.

[29] Xu ZC. Characterization of *Isaria cicadae* Miquel Polysaccharide JCH-1 and Its Immunomodulatory Mechanism on RAW264.7 Cells (蝉花多糖JCH-1理化性质及其对RAW264.7细胞免疫调节机制研究) [D]. Guangzhou: Guangzhou University of Chinese Medicine, 2019.

[30] Zhang Y. Physicochemical Properties, Antioxidant and Immunomodulatory Activities of Polysaccharides from the Stem, Leaf and Flower of *Dendrobium candidum* (铁皮石斛茎、叶、花多糖理化性质及抗氧化、免疫调节活性研究) [D]. Hangzhou: Zhejiang University, 2016.

[31] Yan SH. Anti-inflammatory Effect and Regulation on Associated Signal Transduction Pathway of Doramectin (多拉菌素的抗炎作用及其对相关信号转导通路的调控) [D]. Changchun: Jilin University, 2013.

[32] Zhang Y, Fu Y. Advances in energy metabolism and inflammatory immunity [J]. J Med Res (医学研究杂志), 2022, 51: 6-9.

[33] Jha AK, Huang SCC, Sergushichev A, et al. Network integration of parallel metabolic and transcriptional data reveals metabolic modules that regulate macrophage polarization [J]. Immunity, 2015, 42: 419-430.

[34] Pearce EL, Pearce EJ. Metabolic pathways in immune cell activation and quiescence [J]. Immunity, 2013, 38: 633-643.

[35] Wang XY, Gao Y, Tang JF, et al. Research progress of *Astragalus* and its drug pairs [J]. Chin Arch Tradit Chin Med (中华中医药学刊), 2024. http://kns. cnki. net/kcms/detail/21.1546. R.20240611.1535.002.html.

[36] Tong X. The pharmacology study of active constituents from *Astragalus* [J]. Lishizhen Med Mater Med Res (时珍国医国药), 2011, 22: 1246-1249.

[37] Li XP, Yu MX, Kuang TR, et al. Research progress on the antitumor effect of flavonoid derivatives [J]. Acta Pharm Sin (药学学报), 2021, 56: 913-923.

[38] Wu J, Tong FC. Chemical composition, pharmacological action and therapeutic application of *Astragalus* [J]. J Binzhou Med Univ (滨州医学院学报), 2024, 47: 68-75.

[39] Zhao TT, Pei K, Yu ZH, et al. Research progress in the influence of honey-processing on the chemical composition, pharmacological action and application of Astragali Radix [J]. Chin J Hosp Pharm (中国医院药学杂志), 2024, 44: 113-122.

[40] Li SF, Yang RX, Sun J, et al. Research progress of effective components of *Astragalus* on prevention and treatment of organ fibrosis [J]. Acta Pharm Sin (药学学报), 2023, 58: 2168-2179.

[41] Wang R, Wu MT, Wan MSN, et al. Research progress of calycosin [J]. Yunnan Chem Technol (云南化工), 2019, 46: 56-57.