

doi:10.3971/j.issn.1000-8578.2014.06.010

• 基础研究 •

参归丸对顺铂化疗H22荷瘤小鼠的减毒增效作用及其机制



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Attenuated and Synergistic Effects of Angelica Sinensis and Radix Sophorae Flavescentis Decoction on H22 Tumor-bearing Mice Treated with Cisplatin and Its Mechanism
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Abstract:Objective To investigate the inhibitory effect of Angelica sinensis and Radix Sophorae Flavescentis Decoction (ARD) on H22 transplanted tumor and serum HIF1α and LDH in mice. **Methods** H22 hepatocellular transplantation tumor models were established to be randomly divided into the control, cisplatin , ARD and ARD plus cisplatin groups. General state of KM mice was observed to calculate effective bodyweight changes. Tumor inhibition rate, *q* value, as well as indexes of tumor, liver, spleen, kidney and thymus were calculated. Blood routine was applied to determine white blood cell count. Serum HIF1α and LDH were determined by ELISA and biochemistry method separately. **Results** The general states of ARD plus cisplatin group and ARD group were better than those of cisplatin group and control group. The tumor weights of treatment groups were lighter than that of control group(*P*<0.05). The tumor inhibiting rate of ARD plus cisplatin group was higher than those of ARD group and cisplatin group. ARD plus cisplatin had Synergistic effect. Increase of bodyweight minus tumor weight, and indexes of spleen and thymus in ARD group were higher than those in cisplatin group(*P*<0.05). Serum level of HIF-1α in control group was higher than those of ARD group and ARD plus cisplatin group(*P*<0.05). LDH activity in cisplatin group was higher than those in other treatment groups. (*P*<0.05). **Conclusion** ARD can inhibit the growth of transplanted tumor in mice, provide positive effect and reduce the side effect of cisplatin chemotherapy, which might have the relation with reducing HIF-1α concentration and LDH activity to a certain degree.

Key words: Angelica sinensis and Radix Sophorae Flavescentis pill; H22 hepatocarcinoma cell; Tumor inhibition rates; Hypoxia inducible factor (HIF1α); Lactate dehydrogenase(LDH)

摘 要: 目的 探讨参归丸的抑瘤作用及对顺铂化疗H22荷瘤小鼠的增效减毒作用。**方法** 建立小鼠H22肝癌移植瘤模型, 随机分为模型组、顺铂组、参归丸组、参归丸加顺铂联合组, 另设正常对照组。观察各组小鼠一般状况, 计算有效体重变化; 检测各给药组的抑瘤率、*q*值、肿瘤指数、肝脏、脾脏、胸腺、肾脏的器官指数。血常规检测白细胞计数, ELISA检测血清低氧诱导因子(HIF1α)的浓度, 血生化检测乳酸脱氢酶(LDH)活力。**结果** 参归丸组和联合组的一般状况优于模型组和顺铂组; 各治疗组的瘤体质量皆小于模型组的瘤体质量(*P*<0.05), 联合组的抑瘤率高于单用参归丸或顺铂组的抑瘤率, 联合用药疗效具有相加效应; 参归丸组有效体重增加、脾脏和胸腺指数高于顺铂组(*P*<0.05); 参归丸组和联合组血清HIF1α浓度低于模型组(*P*<0.05); 顺铂组血清LDH活性高于其他治疗

组(*P*<0.05)。**结论** 参归丸具有抑瘤作用和对顺铂化疗的增效减毒作用, 其机制可能在一定程度上与降低小鼠血清中HIF1α浓度和LDH活性有关。
关键词: 参归丸; H22细胞株; 抑瘤率; 低氧诱导因子; 乳酸脱氢酶
中图分类号: R730.53; R285
文献标识码: A

收稿日期: 2013-06-30; 修回日期: 2013-11-07
基金项目: 甘肃省高校基本科研业务费基金资助项目(2009-192-3); 国家科技支撑计划课题(2011BAI05B02)
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0 引言

恶性肿瘤在中医学中多属积的范畴，按发病阶段分为气滞血阻、瘀血内结、正虚瘀结3个证型，分别予以理气通络、活血消积；祛瘀软坚、调理脾胃；补益气血、活血化瘀。参归丸首见于《古今医鉴》卷九，由苦参、当归2:1的比例组成，有清热凉血之功。苦参性寒味苦，具有清热燥湿、杀虫、利尿功效，据《神农本草经》记载，苦参主心腹结气、症瘕积聚、溺有余沥、逐水、除痈肿等。当归性温味辛微苦，功效为补血活血、调经止痛、润燥滑肠。两药合用符合中医治疗肿瘤之扶正祛邪、攻补兼施的基本法则。研究证明多种苦参活性成分具有显著体内抗肿瘤活性^[1-3]，当归的活性成分具有免疫调节和促进造血的作用^[4-5]。本实验研究了参归丸对小鼠移植瘤的抑瘤作用及对顺铂化疗的增效减毒作用。

1 材料和方法

1.1 材料

1.1.1 动物 SPF级昆明小鼠100只，雌雄各半，体重20~22 g，由甘肃中医学院SPF级动物实验中心提供，动物合格证号：SCXK(甘) 2011-0001，SPF级动物实验设施使用证号：SYXK(甘)2011-0001。

1.1.2 细胞株 H22肝癌瘤株由本实验室传代培养。

1.1.3 药物与试剂 RPMI1640(美国Invitro公司)，顺铂注射液，每只10 mg，齐鲁制药有限公司（批号：201002CF）；HIF1 α 试剂盒，上海源叶生物科技有限公司（批号：20121001A）；LDH试剂盒，南京建成生物工程研究所（批号：20121011）。参归丸为自制汤剂（0.45 g/ml）。

1.1.4 仪器 CO₂培养箱（NAPCO法国），SW-CJ-IF超净台（中国苏州净化设备厂），电子天平（LD100001，龙腾电子有限公司），Abacus Junior vet 5动物血清分析仪（兰桥医疗），酶标仪（680型，美国BD-RAD公司），UV1101紫外-可见分光光度仪（上海天美科学有限公司）。

1.2 方法

1.2.1 参归丸汤剂的制备 将当归饮片15 g，苦参饮片30 g放在陶土药锅中，加500 ml去离子水浸泡30 min，煎药1 h，将药液倒入烧杯中，再加500 ml去离子水浸泡30 min，煎药1 h，将两次煎出液混合后在水浴锅中水浴加热浓缩到100 ml，高压灭菌。药物浓度按生药量计算为0.45 g/ml。

1.2.2 动物模型制备 在无菌条件下，在SPF级实

验室内取100只KM小鼠，雌雄各半，实验室条件下适应环境4 d后，按随机数字表法分出雌雄各8只，设为正常对照组。取传代7天的腹水型H22小鼠腹水，用0.9%氯化钠溶液调至细胞浓度为2×10⁶个/毫升，将84只小鼠以75%乙醇消毒，按常规方法皮下注射配制好的H22肝癌细胞悬液0.2 ml，接种于昆明小鼠右前腋皮下，细胞计数约为4×10⁵个，以出现皮丘为皮下接种部位正确。5~7天内观察小鼠接种部位的肿瘤生长情况，接种部位触及到结节为接种成功^[6]。

1.2.3 模型动物分组及处理 第8天剔除接种不成功的小鼠，随机挑选接种成功的小鼠，雌雄各32只随机分为模型对照组、顺铂组、参归丸组和参归丸加顺铂组，每组16只。分组后称重，模型对照组灌胃和腹腔注射0.9%氯化钠溶液0.4 ml/20 g，0.2 ml/20 g；顺铂组灌胃0.9%氯化钠溶液0.4 ml/20 g，以每天2.0 mg/kg腹腔注射顺铂0.2 ml/20 g；参归丸组以每天9 g/kg灌胃0.4 ml/20 g参归丸药液，腹腔注射0.9%氯化钠溶液0.4 ml/20 g；参归丸加顺铂组组以每天9 g/kg灌胃参归丸药液，腹腔注射2.0 mg/kg顺铂0.2 ml；连续给药14天。

1.2.4 指标检测 (1)小鼠一般状态观察及计算。观察小鼠生存状态，记录小鼠的神态、发育、营养、活动、饮食、二便等，反映小鼠的一般情况。记录开始给药第1天和处死时的体质量，计算治疗前后的体质量变化。体质量变化=处死时的体质量-分组给药时的体质量。有效体质量变化=处死时的体质量-肿瘤质量-分组给药时的体质量。(2)计算抑瘤率、*q*值、肿瘤指数、器官指数。剥离瘤体组织，摘取肝、脾、肾、胸腺后分别称质量。计算抑瘤率、肿瘤、肝脏、肾脏、脾脏、胸腺等的指数。抑瘤率(%)=(模型对照组平均瘤体质量-实验组平均瘤体质量)/模型对照组平均瘤体质量。*q*值计算：两药联合应用时可能表现为拮抗、相加或协同。拮抗是指联合用药的效应低于强效应药物的效应。相加是联合用药的效应为两药的效应和。协同是指联合用药的效应大于两药各自效应的和，可以通过计算*q*值来具体确定两药联合应用时相互间的作用。 $q = E_{AB} / (E_A + E_B - E_A \times E_B)$ ^[7-8]。式中E_A为A药（参归丸）抑瘤率，E_B为B药（顺铂）抑瘤率，E_{AB}为联合用药组两药合用的抑瘤率， $q = 0.85 \sim 1.15$ 为两药作用相加， $q > 1.15$ 为两药作用增强(或协同)， $q < 0.85$ 为两药拮抗^[7-8]。器官指数=器官质量(mg)/体质量(g)。(3)血常规检测。在1.5 ml离心管中加入0.9%氯化钠溶液80 μ l，滴加

20 μl新鲜血液，以动物血清分析仪检测血常规。
(4)HIF-1α和LDH检测。按照ELISA试剂盒说明书检测血清HIF-1α，按照血生化试剂盒说明书检测血清LDH。

1.3 统计学方法

应用SPSS17.0统计软件进行单因素方差分析。样本实验数据以平均值±标准差 ($\bar{x} \pm s$) 表示。以 $P < 0.05$ 为差异有统计学意义。

2 结果

2.1 荷瘤小鼠的一般状况

接种成功后，瘤块体积逐渐增大，外观呈球形、半球形或山脉状。初期能活动，后来大多逐渐变硬。分组前荷瘤小鼠身形匀称（瘤体隆起处除外），动作敏捷，反应灵活，皮毛光滑，进食进水正常；分组7天后，随着实验时间的继续延长，顺铂组小鼠最先出现精神萎靡，饮食活动减少，聚集一团，皮毛成揪失去光泽，下腹部不洁净，尿液变黄等现象，参归丸加顺铂组中上述现象的出现比顺铂组较晚。模型组中上述现象的出现又晚一些，参归丸组中上述现象的出现最晚。从饮食、活动及毛发色泽来看，参归丸组小鼠生存质量和生存状态均明显高于顺铂组。一般状况比较表明顺铂整体状况最差。

2.2 各组小鼠体质量和有效体质量给药前后的变化结果见表1。

2.3 各组瘤体质量、抑瘤率的测定及q值计算

结果见表 2。 $q = E_{AB} / [E_A + (1 - E_A)E_B] = 0.709 \div [0.436 + (1 - 0.436) \times 0.600] \approx 0.92$ 说明联合用药有相加作用。

2.4 荷瘤小鼠各器官（肝脏、脾脏、肾脏、胸腺）指数比较

结果见表 3。

2.5 荷瘤小鼠白细胞计数比较

结果见表4。

2.6 荷瘤小鼠血清HIF1α 浓度和 LDH活性比较

结果见表5。

3 讨论

本研究中小鼠移植瘤于注射瘤细胞悬液7日后形成，即中医上的癌毒湿邪瘀久化热，炼液为痰，酿生痰浊，积而为病，治当清热燥湿。参归丸由当归、苦参以1:2比例组成，具有清热燥湿的功效，以参归丸治疗皮下移植瘤方药对证，而且现代研究认为苦参中的苦参碱^[1]、氧化苦参碱^[2]、槐果碱^[9]、槐定碱^[10]等多种碱类物质对多种肿瘤细胞

具有G₀/G₁期周期阻滞、增殖抑制、分化诱导、凋亡诱导和抗恶病质活性，苦参黄酮类物质抗肿瘤活性更强^[3]，为本实验取得较好疗效提供了研究基础。本研究模型对照组小鼠肿瘤均值5.5 g > 1 g，参归丸组肿瘤抑制率43.6% > 30%，参归丸组小鼠体质量增加，参归丸联合顺铂组小鼠体质量下降不超过15%，实验中各组小鼠未死亡，符合原卫生部药政管理局制定的治疗恶性肿瘤的中药药效评价标准^[11]。

实验中参归丸组及参归丸联合顺铂给药组能增加顺铂化疗荷瘤小鼠的有效体质量；改善荷瘤小鼠的生存状态；对顺铂化疗所致中性粒细胞和单核细胞减低有升高作用；参归丸能够拮抗顺铂化疗引起的脾脏指数和胸腺指数降低，有升高脾脏指数和胸腺指数的作用，对小鼠重要免疫器官具有一定的保护作用；q值为0.92, 说明参归丸和顺铂联合应用存在相加作用。单体研究证实参归丸中的当归多糖有改善机体免疫，对化疗引起的骨髓抑制有促进造血作用^[4-5]。当归多糖能够提高放疗小鼠的胸腺和脾脏指数，外周血红细胞计数和白细胞计数，保护骨髓免于放疗所致的骨髓微核的形成，促进骨髓造血^[12]。研究结果未显示肾脏指数的明显改变，可能与肾脏不参与机体免疫调节有关。

恶性肿瘤的发生和转移与肿瘤细胞的耐受缺氧能力及代谢有关。HIF-1α是组织细胞对低氧的适应性反应，是独立于血氧浓度的敏感控制因子，能够激活细胞内代谢废物清除基因^[13]及VEGF的表达^[14]，促进肿瘤组织代谢产物的清除及血管生成。模型组HIF-1α过度表达，预后差^[15]。HIF-1α可以成为肿瘤治疗的潜在靶点，参归丸能够通过下调HIF-1α抑制肿瘤细胞内代谢产物的清除，促进肿瘤细胞死亡，其分子机制可能与苦参碱能够剂量依赖性地降低肺腺癌细胞HIF-1α mRNA的表达^[16]有关。LDH同工酶是诊断心肝肾损伤的重要指标，细胞膜损伤后释放到细胞外。队列研究表明顺铂导致肾脏毒性及LDH高^[17]。LDH升高，肿瘤患者预后差^[18]。参归丸降低顺铂化疗所致血清LDH活性升高可能与苦参碱能够保护细胞膜的完整性，减少LDH释放，降低血清LDH的活性有关^[19]。当归长于补血，濡养心肾等组织，减少顺铂伤津耗气所致心肾细胞损伤^[20]。精血同源，血能生精，发挥肾（中医认为肾为先天之本，包括了肾脏、性腺、肾上腺等的功能）藏精功能，使肾气健旺，恢复肾主骨生髓（骨髓和脑髓）的功能，通过

表1 各组小鼠体质量及有效体质量变化比较($\bar{x}\pm s$)

Table1 Changes of bodyweight and bodyweight minus tumor weight($\bar{x}\pm s$)

Groups	Sample number	Body-mass on the 1st day of treatment(g)	Body-mass on the execution day (g)	Change of body-mass(g)	Change of body-mass(g) minus tumor weight
Normal	16	31.8±1.2	40.0±2.8	8.1±2.4	8.1±2.4
Model	16	29.4±0.9	37.5±1.2	8.1±1.2	2.5±1.7 ^a
Cisplatin	16	29.8±1.3	22.5±1.2 ^{a,b}	-6.3±2.2 ^{a, b}	-8.5±2.3 ^{a,b}
ARD	16	29.6±1.1	34.9±1.3 ^c	5.3±1.6 ^c	2.2±1.5 ^{a,c}
ARD+cisplatin	16	29.7±0.9	26.5±1.3 ^{a,b,d}	-3.2±1.5 ^{a,b,c,d}	-4.8±1.7 ^{a,b,c,d}

Notes:^a: $P=0.000$, compared with normal group;^b: $P=0.000$,compared with model group;^c: $P=0.000$, compared with cisplatin group;^d: $P=0.000$, compared with angelica sinensis and radix sophorae flavescentis decoction (ARD) group

表2 各组瘤体质量($\bar{x}\pm s$)、肿瘤指数($\bar{x}\pm s$)及抑瘤率的比较

Table2 Tumor weights($\bar{x}\pm s$), tumor indexes($\bar{x}\pm s$) and tumor inhibition rates

Groups	Sample number	Tumor weights (g)	Tumor indexes(mg/g)	Tumor inhibition rates
Model	16	5.5±1.3	148.6±35.9	—
Cisplatin	16	2.2±0.6 ^b	93.0±24.4 ^b	60.0%
ARD	16	3.1±0.8 ^{b,c}	88.1±20.5 ^b	43.6%
ARD + cisplatin	16	1.6±0.3 ^{b,c,d}	59.7.0±11.8 ^{b, c,d}	70.9%

Notes:^b: $P=0.000$, compared with model group;^c: $P=0.000$, compared with cisplatin group;^d: $P=0.000$,compared with ARD group;—:not applicable

表3 肝癌 H22荷瘤小鼠肝脏指数、脾脏指数、肾脏指数、胸腺指数比较($\bar{x}\pm s$)

Table3 Indexes of liver, spleen, kidney, thymus in H22 tumor-bearing mice($\bar{x}\pm s$)

Groups	Sample number	Liver indexes (mg/g)	Spleen indexes (mg/g)	Kidney indexes (mg/g)	Thymus indexes (mg/g)
Normal	16	39.8±8.0	3.5±0.5	5.0±1.2	3.0±0.9
Model	16	69.2±7.2 ^a	10.3±3.4 ^a	4.8±1.0	2.8±0.6
Cisplatin	16	53.9±8.9 ^a	2.3±0.7 ^b	5.2±0.8	0.8±0.2 ^{a,b}
ARD	16	71.1±9.3 ^a	11.5±4.4 ^{a,c}	5.3±0.8	2.5±0.6 ^c
ARD + cisplatin	16	67.0±7.2 ^a	2.6±0.7 ^{b,d}	4.4±2.1	1.2±0.2 ^{a, b,d}

Notes:^a: $P=0.000$,compared with normal group;^b: $P=0.000$,compared with model group;^c: $P=0.004$ (liver index), $P=0.000$ (spleen index), $P=0.002$ (thymus index), compared with cisplatin group;^d: $P=0.000$ (spleen index), $P=0.002$ (thymus index),compared with ARD group

表4 肝癌 H22荷瘤小鼠白细胞计数比较($n=10, \bar{x}\pm s$)

Table4 White cell counts of H22 tumor-bearing mice($n=10, \bar{x}\pm s$)

Groups	White cell $1\times 10^9/L$	Lymphocyte $1\times 10^9/L$	Monocyte $1\times 10^9/L$	Neutrophile granulocyte $1\times 10^9/L$
Normal	3.74±1.52	2.32±0.93	0.13±0.13	1.30±0.27
Model	24.68±1.37 ^a	15.57±1.40 ^a	1.46±0.65 ^a	7.67±1.67 ^a
Cisplatin	6.16±1.65 ^{a,b}	4.97±1.60 ^{a,b}	0.06±0.04 ^{a, b}	1.11±0.80 ^b
ARD	14.59±5.04 ^{a,b,c}	7.56±4.73 ^{a, b,c}	0.61±0.62 ^{a, b, c}	9.63±13.11 ^{a,c}
ARD + cisplatin	9.78±3.64 ^{a,b,c,d}	4.01±1.47 ^{a, b,d}	0.28±0.33 ^{a, b, c,d}	5.39±2.30 ^{a,c,d}

Notes:by rank test;^a: $P=0.000$,compared with normal group;^b: $P=0.000$,compared with model group;^c: $P=0.000$,compared with cisplatin group;^d: $P=0.000$,compared with ARD group

表5 各组小鼠血清HIF-1 α 、LDH水平比较($\bar{x}\pm s$)

Table5 Serum levels of HIF-1 α and LDH activities($\bar{x}\pm s$)

Groups	Sample number	HIF-1 α (pg/ml)	LDH (u/L)
Normal	10	4.061±0.640	3999.35±295.55
Model	10	7.298±0.838 ^a	3631.56±262.03
Cisplatin	10	3.443±1.159 ^b	7456.70±506.58 ^{a,b}
ARD	10	3.24±0.70 ^b	4049.39±246.04 ^c
ARD + cisplatin	10	3.57±1.09 ^b	4378.56±962.22 ^c

Notes:^a: $P=0.000$,compared with normal group;^b: $P=0.000$,compared with model group;^c: $P=0.000$,compared with cisplatin group

耳窍反映出来，为减轻顺铂化疗引起的中医肾系的性腺抑制^[21]、肾毒性^[17,22]、精子及DNA（先天之本的物质基础）毒性^[23]、神经毒性^[24]、耳毒性^[25]、骨髓抑制^[26]提供了中医学理论依据。研究表明富含黄酮和生物碱的吊灯树果实提取物能够显著消除顺铂化疗所致睾丸毒性^[21]，顺铂等化疗药与抗肿瘤的 植物药物联合应用能够增强多药耐药肿瘤细胞对顺铂等化疗药物的敏感度^[27]。因此，研究具有抑瘤作用及对化疗药物具有增效减毒作用的中药及

其有效单体逐渐成为中医药研究的热点。参归丸能够降低HIF-1 α 的浓度水平，消弱肿瘤细胞耐受低氧的能力，促进肿瘤细胞死亡；参归丸能够降低LDH的活性，减少LDH对正常细胞的损伤，从而对顺铂化疗有减毒作用。苦参丸方药中含有多种促进肿瘤细胞凋亡及调节免疫监视的抗肿瘤成分，苦参碱注射液和当归多糖制剂已经应用于临床。苦参黄酮、当归内酯等抗肿瘤成分有待于进一步开发。

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[编辑：周永红；校对：杨 卉]