·论　著·

人参皂苷Rg3与5-氟尿嘧啶联用对结肠癌小鼠肿瘤的血管生成与肿瘤生长抑制效果的实验研究

赵娅菽[1]，　邓丽聪[2]，　曹　玥[3]，　马步云[4]，　李　月[1]，　徐靖怡[1]，　李　红[1]，　黄　英[1△]

1. 四川大学华西基础医学与法医学院 病理生理学教研室（成都 610041）；2. 四川资阳市第一人民医院 肿瘤中心（资阳 641300）；

3. 成都中医药大学附属医院 病理科（成都 610075）；4. 四川大学华西医院 超声科（成都 610041）

【摘要】 **目的**　评价人参皂苷Rg3和5-氟尿嘧啶（5-fluorouracil, 5-FU）联用对结肠癌小鼠肿瘤的血管生成与肿瘤生长的抑制作用。**方法**　建立CT26结肠癌荷瘤小鼠模型，建模成功后将40只荷瘤小鼠随机分为对照组、人参皂苷Rg3组、5-FU组及Rg3与5-FU联合组。5-FU组按20 mg/kg剂量腹腔注射，0.2 mL/只，每天1次，连续注射10 d；Rg3组，按20 mg/kg剂量灌胃，0.2 mL/只，每天1次，连续灌胃21 d；Rg3+5-FU联合组用药剂量和方式同5-FU组和Rg3组；对照组腹腔注射生理盐水0.2 mL/d，连续注射10 d。通过免疫组化检测血管内皮细胞生长因子（VEGF）和CD31的表达，检测肿瘤组织微血管密度（MVD），彩色多普勒血流成像（CDFI）检测血流信号及肿瘤坏死情况；观测小鼠的生活质量、存活率、肿瘤体积、肿瘤质量及抑瘤率。**结果**　治疗21 d后，与对照组相比，各治疗组肿瘤体积、肿瘤质量均明显减少，联合组减少最为明显。Rg3组、5-FU组及联合组抑瘤率分别为29.96%、68.78%及73.42%，单用Rg3对肿瘤生长具有一定的抑制作用，但5-FU单用或与Rg3联用对肿瘤的抑制作用更强，联合组抑瘤率虽然高于5-FU组水平，但差异无统计学意义（$P>0.05$）。彩色多普勒检测显示，Rg3组和联合组小鼠肿瘤内部可见明显的多局部、较大的肿瘤坏死区域，而5-FU组和对照组仅有较小的肿瘤坏死区域，联合组的肿瘤坏死率为（55.63±3.12）%，高于其他各组水平（$P<0.05$）。CDFI检测小鼠肿瘤内血流情况显示，联合组肿瘤内血流信号多为0-Ⅰ级，对照组血流信号最丰富，多为Ⅱ-Ⅲ级，Rg3组和5-FU组肿瘤内血流信号丰富程度介于对照组和联合组之间。与对照组相比，Rg3组、5-FU组和联合组肿瘤组织MVD和VEGF表达水平均降低，其中联合组降低最为显著（$P<0.05$）。HE染色结果提示，对照组小鼠肿瘤的坏死情况明显，血管较多；Rg3组、5-FU组血管较少，肿瘤内出现空隙状坏死；联合组的肿瘤组织血管最少，可见条索状坏死。自治疗开始后18 d小鼠开始出现死亡，对照组小鼠在42 d全部死亡，此时Rg3组、5-FU组和联合组分别还有3只、5只、7只小鼠存活，存活率分别为30%、50%、70%。治疗开始后60 d时，各组小鼠全部死亡。**结论**　人参皂苷Rg3与5-FU联用不仅能够显著抑制结肠癌小鼠肿瘤的血管生成，并且显著抑制了肿瘤生长，提高了荷瘤小鼠的生活质量、延长了生存期。

【关键词】　人参皂苷Rg3　　5-氟尿嘧啶　　结肠癌　　肿瘤生长　　肿瘤血管生成

Inhibitory Effect of Ginsenoside Rg3 Combined With 5-Fluorouracil on Tumor Angiogenesis and Tumor Growth of Colon Cancer in Mice: An Experimental Study　*ZHAO Yashu*[1], *DENG Licong*[2], *CAO Yue*[3], *MA Buyun*[4], *LI Yue*[1], *XU Jingyi*[1], *LI Hong*[1], *HUANG Ying*[1△]. 1. Department of Pathophysiology, West China School of Basic Medical Sciences and Forensic Medicine, Sichuan University, Chengdu 610041, China; 2. Cancer Center, First People's Hospital of Ziyang, Ziyang 641300, China; 3. Department of Pathology, Hospital of Chengdu University of Traditional Chinese Medicine, Chengdu 610075, China; 4. Department of Ultrasound, West China Hospital, Sichuan University, Chengdu 610041, China*
△ Corresponding author, E-mail: huangying68@163.com

【**Abstract**】　**Objective**　To evaluate the inhibitory effect of ginsenoside Rg3 combined with 5-fluorouracil (5-FU) on tumor angiogenesis and tumor growth in colon cancer in mice. **Methods**　CT26 mouse model of colon cancer was established and the mice were randomly assigned to the control group, the ginsenoside Rg3 group, the 5-FU group, and the Rg3 combined with 5-FU group. The 5-FU group was injected intraperitoneally at the dose of 20 mg/kg, 0.2 mL/animal, and once a day for 10 days. Treatment for the Rg3 group was given at the dose of 20 mg/kg, 0.2 mL/animal, and once a day for 21 days via gastric gavage. The dose and the mode of treatment for the Rg3+5-FU combination group were the same as those for the 5-FU and the Rg3 group. The control group was intraperitoneally injected with 0.2 mL/d of normal saline for 10 days. The expression of vascular endothelial growth factor (VEGF) and CD31 and the microvascular density (MVD) of the tumor tissues were examined by immunohistochemistry. The blood flow signals and tumor necrosis were examined by color Doppler flow imaging (CDFI). The quality of life, survival rate, tumor volume, tumor mass, and tumor inhibition rate of the mice were monitored. **Results**　After 21 days of treatment, the tumor volume and the tumor mass of all treatment groups were significantly decreased compared with those the control group, with the combination treatment group exhibiting the most significant decrease. The tumor inhibition rates of the Rg3 group, the 5-FU group,

and the combination group were 29.96%, 68.78%, and 73.42%, respectively. Rg3 treatment alone had inhibitory effect on tumor growth to a certain degree, while 5-FU treatment alone or 5-FU combined with Rg3 had a stronger inhibitory effect on tumor growth. The tumor inhibition rate of the combination group was higher than that of the 5-FU group, but the difference was not statistically significant ($P>0.05$). Color Doppler ultrasound showed that there were multiple localized and large tumor necrotic areas that were obvious and observable in the Rg3 group and the combination group, and that there were only small tumor necrotic areas in the 5-FU group and the control group. The tumor necrosis rate of the combination group was (55.63 ± 3.12)%, which was significantly higher than those of the other groups ($P<0.05$). CDFI examination of the blood flow inside of the tumor of the mice showed that the blood flow signals in the combination group were mostly grade 0-Ⅰ, and that the blood flow signals in the control group were the most abundant, being mostly grade Ⅱ-Ⅲ. The abundance of the blood flow signals in the Rg3 and 5-FU groups were between those of the control group and the combination group. Compared with those of the control group, the expression levels of MVD and VEGF in the tumor tissues of the Rg3 group, the 5-FU group, and the combination group were significantly decreased, with the combination group showing the most significant decrease ($P<0.05$). HE staining results indicated that there was significant tumor necrosis in mice in the control group and that there were more blood vessels. In contrast, in the tumor of the Rg3 group and the 5-FU group, there were fewer blood vessels and necrotic gaps appeared within the tumors. In the combination group, the tumor tissues had the fewest blood vessels and rope-like necrosis was observed. The mice started dying on the 18th day after treatment started, and all the mice in the control group died on the 42nd day. By this time, there were 3, 5, and 7 mice still alive in the Rg3 group, the 5-FU group, and the combination group, respectively, presenting a survival rate of 30%, 50%, and 70%, respectively. All mice in all the groups died on day 60 after treatment started. **Conclusion**　Ginsenoside Rg3 combined with 5-FU can significantly inhibit tumor angiogenesis and tumor growth of colon cancer in mice and improve the survival and quality of life of tumor-bearing mice.

【**Key words**】　Ginsenoside Rg3　　5-fluorouracil　　Colon cancer　　Tumor growth　　Tumor angiogenesis

结肠癌是一种常见的消化道恶性肿瘤,其发病率及死亡率居全球癌谱前列且呈上升趋势[1-2]。2020年全球结肠癌发病率为11.4/10万,死亡率为5.4/10万;中国结肠癌发病率为13.1/10万,死亡率为6.8/10万[2]。目前,结肠癌的主要治疗方法包括手术、放化疗、免疫疗法等。5-氟尿嘧啶(5-fluorouracil, 5-FU)是治疗结肠癌的一线化疗药物,在晚期和转移性结肠癌的治疗中有着公认的疗效,可明显延长患者的生存期,但由于其能够引发严重的不良反应,因此,5-FU在临床上的应用受到了一定的限制[3]。抗血管生成是肿瘤治疗的研究热点之一,肿瘤的血管生成与结肠癌进展、转移有显著的相关性[4]。目前,已有药物被用于肿瘤的抗血管生成治疗,包括抗血管内皮生长因子(vascular endothelial growth factor, VEGF)/VEGFR抗体,如贝伐珠单抗、阿帕替尼等[5]。人参皂苷Rg3是一种从红参中提取出来的中药单体成分,已明确其具有显著的抑制肿瘤新生血管生成等作用,它能够靶向多条与肿瘤有关的信号通路,如MAP、JNK及NF-κB等。此外,人参皂苷Rg3还与细胞凋亡、免疫、逆转化疗药物的耐药性等有关,其在临床上已被证明可通过抑制肿瘤血管生成从而提高患者的生存期[6]。本实验检测了VEGF和CD31的表达、肿瘤组织微血管密度(MVD)以及血流信号,观测了小鼠的生活质量、存活率、肿瘤体积、肿瘤质量及抑瘤率和肿瘤坏死情况等指标。本实验旨在观察人参皂苷Rg3和5-FU联用对小鼠CT26结肠癌模型是否具有比单药更显著的抑制肿瘤血管生成和肿瘤生长的效果,并同时观察荷瘤小鼠的生存质量和生存期,以期为结肠癌的中西医结合治疗提供一定的实验依据,并进一步扩大人参皂苷Rg3的临床使用范围。

1　材料与方法

1.1　材料

CT26结肠癌细胞株来源于四川大学国家重点实验室;雄性BALB/c小鼠(6~8周龄,体质量18~22 g)购自成都达硕实验动物有限公司;彩色多普勒血流显像(color Doppler flow imaging, CDFI)超声仪(Philips IU22, Holland);5-FU购自天津金耀氨基酸有限公司,国药准字号为H12020959;人参皂苷Rg3提取于我国东北红参,纯度不低于95%,由中国亚泰药业公司提供,批号:R20100101;小鼠VEGF单克隆抗体购自美国Santa Cruz,稀释比例为1:200;小鼠CD31单克隆抗体和LSAB试剂盒购自日本Dako公司,CD31单克隆抗体稀释比例为1:100。

1.2　模型建立、分组及给药

将CT26结肠癌细胞悬液以0.1 mL/只(2×10^6 mL^{-1}细胞)接种于BALB/c小鼠右臀部皮下,接种7 d后,接种部位可扪及直径约2~3 mm大小的瘤块,即小鼠CT26结肠癌模型造模成功。取40只荷瘤小鼠随机分为4组(每组10只)进行实验室取材及检测:5-FU组按20 mg/kg剂量腹腔注射,

0.2 mL/只, 每天1次, 连续注射10 d后, 休息至第21天处死取材; Rg3组, 按20 mg/kg剂量灌胃, 0.2 mL/只, 每天1次, 连续灌胃21 d后处死取材; Rg3+5-FU联合组用药剂量和方式同5-FU组和Rg3组; 对照组腹腔注射生理盐水0.2 mL/d, 连续注射10 d, 休息至第21天处死取材。另取40只荷瘤小鼠随机分为4组(n=10), 分组及用药处理同上, 但不进行人为处死, 观察各组小鼠用药后毒副反应和生存情况。本实验按照我国《实验动物福利伦理审查指南(GB/T 35892-2018)》要求, 规范落实实验动物福利伦理。

1.3　免疫组织化学染色

肿瘤标本用体积分数10%中性福尔马林液固定, 石蜡包埋和切片。采用EnVision二步法进行免疫组化染色(一抗分别为抗小鼠VEGF单克隆抗体和抗小鼠CD31单克隆抗体)。并对肿瘤标本进行HE染色。

1.4　VEGF染色结果的判定

细胞浆/膜出现棕黄色颗粒则视为VEGF染色呈阳性。每个标本在200倍镜下选取5个视野进行计数, 分别计数每个视野中阳性细胞所占的百分率, 取其平均值进行半定量计数分级: 阳性细胞>10%即判定为阳性[7]。

1.5　微血管密度(MVD)检测

CD31阳性染色表现为血管内皮细胞胞浆呈现棕黄色。参照WEIDNER等[8]计数方法, 被CD31单抗染为棕黄色的内皮细胞或幼稚内皮细胞被判定为可计数的血管。先在低倍镜下找出血管高度密集区域即"热点", 再在200倍镜下选择3个血管数目多的视野。由3位人员对这3个视野微血管分别计数, 取其平均值为该标本MVD值。

1.6　肿瘤血流情况

CDFI是利用超声多普勒效应检测肿瘤内血流的速度、方向、分布状态等, 其产生的血流图包含丰富的血流信号信息。CDFI血流信号的分级参照ADLER等的方法[9]: 0级, 肿瘤块未发现血流信号; Ⅰ级, 少量血流, 肿瘤块可见1~2个点状或细短棒状血管; Ⅱ级, 中量血流, 肿瘤块

可见3~4个点状血管或一个较长的血管(其长度可接近或超过肿块半径); Ⅲ级, 多量血流, 肿瘤块可见5个以上点状血管或2个较长的血管。

1.7　毒副反应和生存质量观察

观察用药后小鼠的皮毛光泽度、食欲、腹泻情况、活动状况、精神状态、对刺激的反应和体质量(以g为单位), 每3 d称重一次。用药后每3 d测量小鼠肿瘤体积(以mm³为单位), 取每组的平均值绘制肿瘤生长曲线。

1.8　抑瘤率

治疗结束后, 各组小鼠行彩色多普勒检查, 处死后记录肿瘤质量。以对照组平均肿瘤质量为M1, 治疗组平均肿瘤质量为M2, 通过(M1—M2)/M1×100%计算出抑瘤率。

1.9　瘤内坏死情况

在治疗用药全部结束后, 对小鼠肿瘤内坏死及血流情况行彩超检测。使用设备为Philips IU22型彩色多谱勒血流显像超声仪, 探头频率为5 MHZ。先使用二维超声观察小鼠肿瘤的大小、形状及瘤内坏死情况, 然后检测各组小鼠瘤内血流信号。肿瘤坏死率的计算参照Cavalieri法[10]。

1.10　统计学方法

除抑瘤率、VEGF评分和CDFI血流信号分级外, 其余数据均用$\bar{x} \pm s$表示。小鼠体质量、肿瘤体积、肿瘤质量、肿瘤坏死率、MVD组间比较采用ANOVA方差分析, 若上述指标总体均数不全相等, 则采用S-N-K检验; 抑瘤率和VEGF阳性率分析采用卡方检验; CDFI等级比较采用秩和检验。采用GraphPad Prism 5 Demo绘制生存曲线, 生存分析采用log-rank检验。$P<0.05$为差异有统计学意义。

2　结果

2.1　肿瘤体积、肿瘤质量及抑瘤率

由图1可见, 与对照组相比, 各治疗组肿瘤体积、肿瘤质量均减少, 联合组减少最为明显。Rg3组、5-FU组及联合组抑瘤率分别为29.96%、68.78%及73.42%, 单用Rg3对
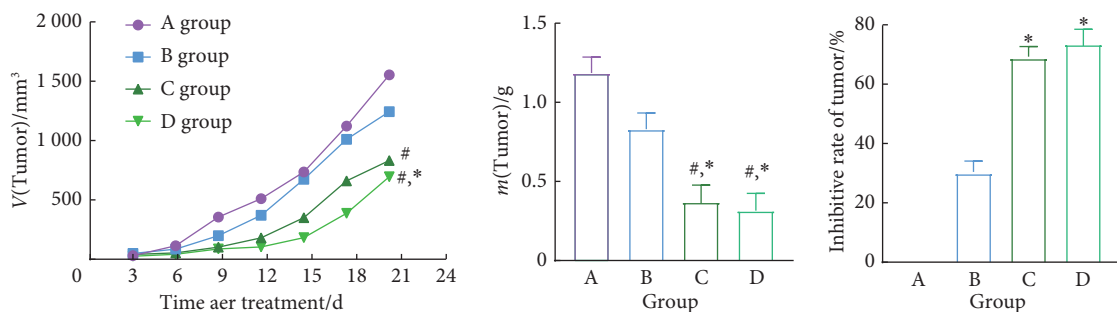

图1　人参皂苷Rg3和5-FU对小鼠肿瘤体积生长曲线、肿瘤质量及抑瘤率的影响

Fig 1　Effect of ginsenoside Rg3 and 5-FU on the growth curves of tumor volume, the tumor mass, and the inhibition rate of tumor in mice

A, Control; B, Rg3; C, 5-FU ; D, combination. # $P<0.05$, vs. control group; * $P<0.05$, vs. Rg3 group. n=10.

肿瘤生长具有一定的抑制作用,但5-FU单用或与Rg3联用对肿瘤的抑制作用更强,联合组抑瘤率虽然高于5-FU组水平,但差异无统计学意义($P>0.05$)。

2.2 肿瘤坏死率

在治疗用药21 d结束后,使用彩色多普勒检测小鼠肿瘤内部回声状况,肿瘤坏死区域呈低回声或无回声。如图2A所示,Rg3组和联合组小鼠肿瘤内部可见明显的多局部、较大的肿瘤坏死区域,而5-FU组和对照组仅有较小的肿瘤坏死区域,联合组的肿瘤坏死率为(55.63 ± 3.12)%,高于其他各组水平($P<0.05$)(图2B)。
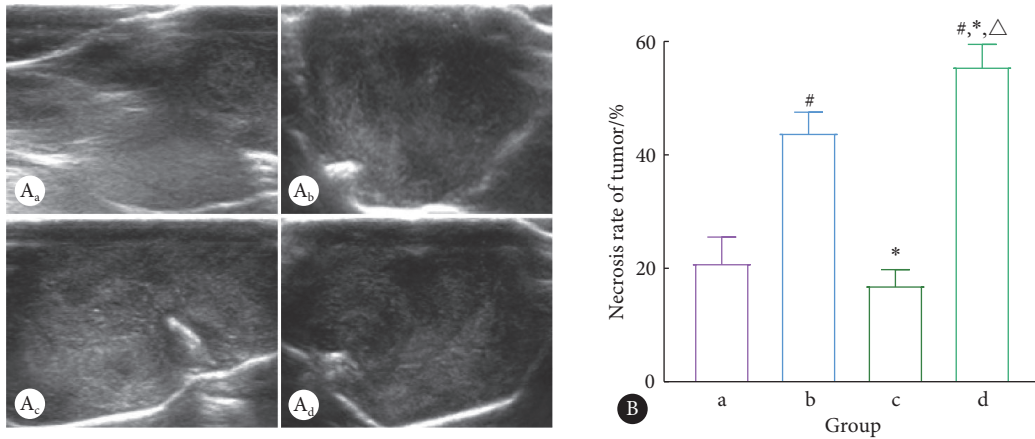

图 2 彩色多普勒超声观察用药21 d后各组小鼠肿瘤坏死情况
Fig 2 Observation of tumor necrosis in the mice by color Doppler ultrasound after treatement for 21 d
A, Areas of tumor necrosis; B, necrosis rate of tumor; a: control; b: Rg3; c: 5-FU; d: combination. [#]$P<0.05$, vs. control group; [*]$P<0.05$, vs. Rg3 group; [△]$P<0.05$, vs. 5-FU group. $n=10$.

2.3 肿瘤内血流信号

采用CDFI检测小鼠肿瘤内血流情况(图3,表1),结果显示联合组肿瘤内血流信号多为0~Ⅰ级,对照组血流信号最丰富,多为Ⅱ~Ⅲ级,Rg3组和5-FU组肿瘤内血流信号丰富程度介于对照组和联合组之间。联合组小鼠肿瘤内血流信号丰富程度与Rg3组、5-FU组及对照组相比,差异均有统计学意义($P<0.05$)。表明Rg3与5-FU联用比两者单用具有更强抑制肿瘤血管生成的作用。
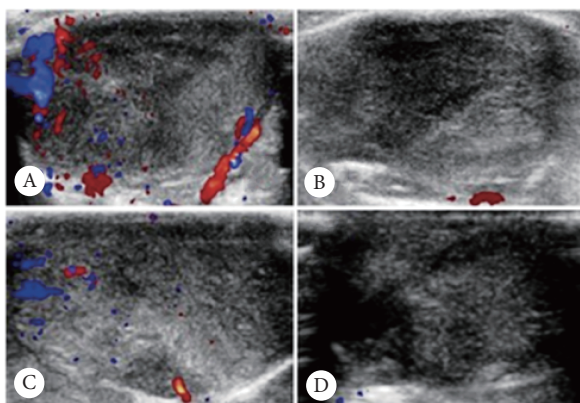

图 3 用药21 d后CDFI检测各组小鼠肿瘤内部血流信号
Fig 3 Observation of blood flow signals in the tumors by color Doppler flow imaging (CDFI) after treatement for 21 d
A, Control group; B, Rg3 group; C, 5-FU group; D, combination group.

2.4 CD31和MVD

CD31免疫组化结果见图4A。MVD结果见图4B,与对照组MVD(37.25 ± 1.71)相比,Rg3组(17.50 ± 1.29)、5-FU组(28.25 ± 0.96)和联合组(11.75 ± 1.71)肿瘤组织MVD均降低,其中联合组降低最为显著($P<0.05$)。提示Rg3与5-FU联用具有更明显的抗肿瘤血管生成作用。

表 1 治疗21 d后各组小鼠血流信号水平
Table 1 Blood flow signal levels in each group after 21 days of treatment

Group	n	Classification of blood flow by CDFI			
		0	Ⅰ	Ⅱ	Ⅲ
Control	9	0	1	2	6
Rg3	10	2	3	3	2
5-FU	9	1	2	3	3
Combination[*]	9	4	4	1	0

[*] $P<0.05$, the abundance of blood flow signals in the combination group was compared with those in the Rg3 group, 5-FU group, and control group.

2.5 VEGF

VEGF免疫组化结果见图5。VEGF评分结果见表2,与对照组(88.89%)相比,Rg3组(40.00%)、5-FU组(66.67%)和联合组(11.11%)肿瘤VEGF阳性表达均降低($P<0.05$),尤以联合组降低最为明显($P<0.05$),提示Rg3与5-FU联用可明显抑制肿瘤VEGF表达从而发挥抗肿瘤血管生成作用。
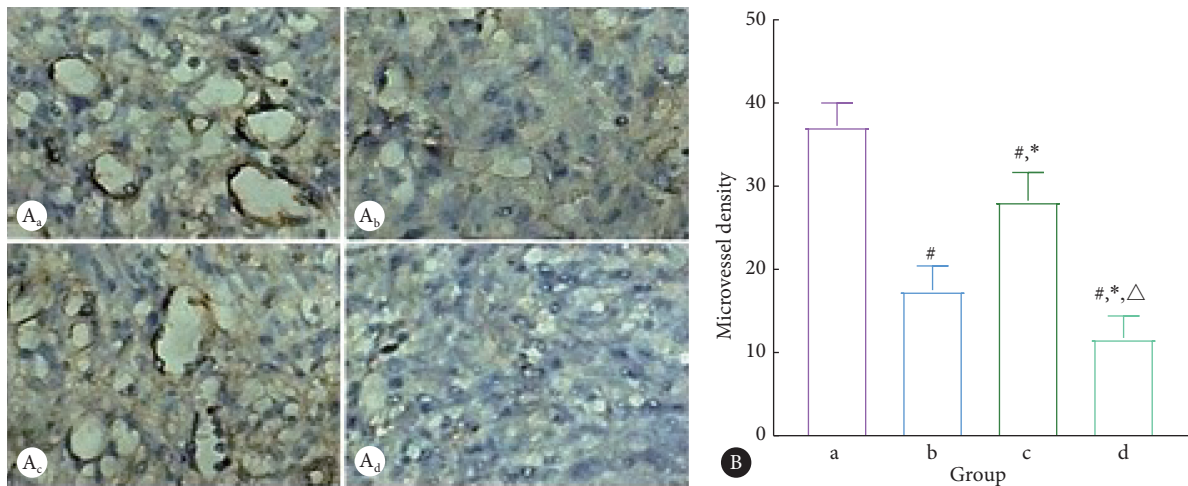
2.6 肿瘤标本的HE染色

HE染色结果(图6)表明,治疗21 d后,对照组小鼠肿

图4 各组小鼠肿瘤组织中CD31表达情况和微血管密度

Fig 4　CD31 expression and microvessel density in the tumor tissues of the mice in each group

A, Immunohistochemical staining of CD31 (original magnification ×200); B, microvessel density; a, control; b, Rg3; c, 5-FU; d, combination. #$P<0.05$, vs. control group; *$P<0.05$, vs. Rg3 group; △$P<0.05$, vs. 5-FU group. $n=10$.
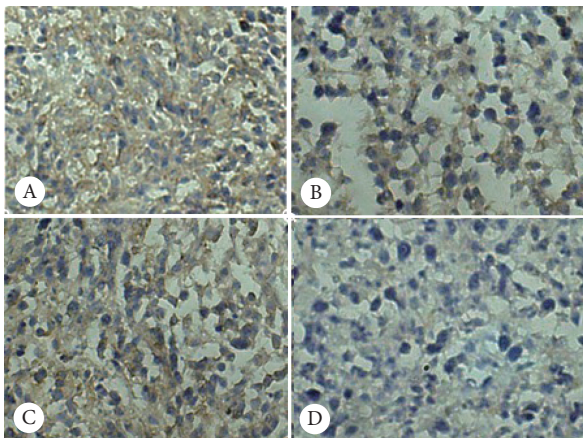

图5 各组小鼠肿瘤组织中VEGF表达情况（免疫组化染色 ×400）

Fig 5　VEGF expression in tumor tissue of the mice in each group (immunohistochemical staining, original magnification×400)

A, Control group; B, Rg3 group; C, 5-FU group; D, combination group.

表2 各组小鼠肿瘤的VEGF评分

Table 2　VEGF scores of the tumors in mice in each group

Group	n	VEGF				Positive rate/%
		−	+	++	+++	
Control	9	1	1	2	5	88.89
Rg3	10	6	2	1	1	40.00#
5-FU	9	3	1	2	3	66.67#,*
Combination	9	8	1	0	0	11.11#,*,▲

VEGF staining was scored semiquantitatively as the intensity of the immunoreactive reaction and the positive rate of tumor cells. Score 0 to 1 was represented as negative (−), 2 to 3 as weak expression (+), 3 to 4 as moderate expression (++), and above 5 as strong expression (+++). #$P<0.05$, vs. control group; *$P<0.05$, vs. Rg3 group; ▲$P<0.05$, vs. 5-FU group.

瘤的坏死情况明显, 血管较多; Rg3组、5-FU组血管较少, 肿瘤内出现空隙状坏死; 联合组的肿瘤组织血管最少, 可见条索状坏死。

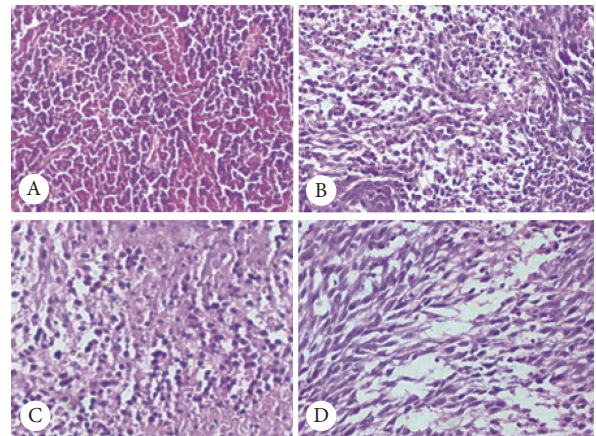**图6 各组小鼠肿瘤组织HE染色 (×200)**

Fig 6　HE staining of the tumor tissues (original magnification×200)

A: Control group; B: Rg3 group; C: 5-FU group; D: combination group.

2.7　小鼠的生活质量及生存分析

通过对用药后小鼠的精神状态、活动状况、食欲、腹泻、体质量减轻幅度、脱毛情况和对刺激的反应等观察, 发现Rg3组和联合治疗组小鼠的生存质量较好, 明显优于5-FU组和对照组。

自治疗开始后第18天小鼠开始出现死亡, 对照组小鼠在42 d全部死亡, 此时Rg3组、5-FU组和联合组分别还有3只、5只、7只小鼠存活, 存活率分别为30%、50%、70%。治疗开始后第60天时, 其余各组小鼠全部死亡(图7)。各治疗组小鼠存活率高于对照组水平($P<0.05$), 联合组存活率高于Rg3组、5-FU单药组水平($P<0.05$)。
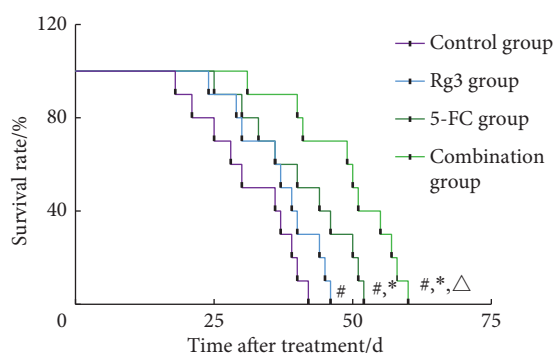
图7　荷瘤小鼠生存曲线

Fig 7　Effect of ginsenoside Rg3 and 5-FU on the survival curves of the tumor-bearing mice

[#] $P<0.05$, vs. control group; [*] $P<0.05$, vs. Rg3 group; [△] $P<0.05$, vs. 5-FU group. $n=10$.

3　讨论

人参皂苷Rg3是一种从人参中微量提取的中药单体成分, 目前已证实Rg3不仅是一种具有明显效果的血管生成抑制剂, 且能够降低化疗药物带来的巨大毒副反应、增强机体的免疫力等[11-13]。

本研究结果显示: 单用Rg3对肿瘤生长具有一定的抑制作用, 而5-FU单用或与Rg3联用对肿瘤抑制作用更强。彩色多普勒数据显示, 联合治疗导致多局部、较大范围的肿瘤坏死, 而5-FU组仅出现小范围的肿瘤坏死, Rg3组和联合组肿瘤坏死率均明显高于5-FU组, 提示Rg3单用或联合5-FU均能更有效促进肿瘤坏死。CDFI、MVD及VEGF结果表明人参皂苷Rg3与5-FU联用具有更明显的抗肿瘤血管生成作用, 而其抗肿瘤血管生成作用与其明显抑制肿瘤VEGF表达有关。

人参皂苷Rg3联合化疗药物不仅有增加疗效的作用, 还能够明显降低化疗带来的毒副反应[14-15]。本实验结果也证实, 人参皂苷Rg3与5-FU联用可明显减轻荷瘤鼠毒副反应, 提高荷瘤鼠生存质量。因此, 中药人参皂苷Rg3与化疗药5-FU联用不仅在抑制肿瘤生长、致肿瘤坏死和抑制肿瘤新生血管生成等方面均表现出较好的抗瘤效果, 同时还具有显著的减轻毒性的效果。笔者推测可能与Rg3广泛的药物作用如免疫调节[16-18]、抗氧化[19]、抗应激[19]等有关, 这也赋予了Rg3不同于其他血管生成抑制剂的优势。

肿瘤的血管形成是一个与多因素相关的复杂的生物过程, Rg3被发现对肿瘤的增殖、迁移等都有抑制作用, 其能够抑制与肿瘤增殖相关的Eph/ephrin通路, 以及阻断细胞周期从而抑制肿瘤。研究表明, Rg3在多个体内外模型中对肿瘤细胞的血管生成均有抑制作用, 其可能通过

下调基质金属蛋白酶9(MMP-9)、上皮细胞激酶(Epha2)以及血管内皮细胞钙黏蛋白(VE-cadherin)抑制肿瘤血管的形成, 并且它与5-FU联用能够增强抗肿瘤作用, 更有效地降低关键因子VEGF的表达, 并且能够降低细胞的耐药性[20-21]。由此可见, Rg3与5-FU联用能够更好地发挥抗肿瘤作用, 在肿瘤治疗领域具有一定的临床应用前景。

综上所述, 本研究结果为结肠癌的中西医结合治疗提供了一定的实验依据, 并为扩大人参皂苷Rg3的临床使用奠定了基础。但有关人参皂苷Rg3与化疗药物联用增效减毒的确切机制尚有待于进一步深入研究。

<p align="center">*　　　　*　　　　*</p>

<p align="center">**参　考　文　献**</p>

[1] SUNG H, FERLAY J, SIEGEL R L, *et al*. Global Cancer Statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin, 2021, 71(3): 209–249. doi: 10.3322/caac.21660.

[2] 徐梦圆, 单天昊, 曾红梅. 2020年全球结肠癌和直肠癌发病死亡分析. 江苏预防医学, 2023, 34(1): 12–16. doi: 10.13668/j.issn.1006-9070.2023.01.003.

XU M Y, SHAN T H, ZENG H M. Global incidence and mortality of colon cancer and rectal cancer in 2020. Jiangsu J Prev Med, 2023, 34(1): 12–16. doi: 10.13668/j.issn.1006-9070.2023.01.003.

[3] KAMRAN S, SINNIAH A, CHIK Z, *et al*. Diosmetin exerts synergistic effects in combination with 5-fluorouracil in colorectal cancer cells. Biomedicines, 2022, 10(3): 531. doi: 10.3390/biomedicines10030531.

[4] SHOARI A, KHODABAKHSH F, AHANGARI COHAN R, *et al*. Anti-angiogenic peptides application in cancer therapy; a review. Res Pharm

Sci, 2021, 16(6): 559–574. doi: 10.4103/1735-5362.327503.

［5］ 刘明锐, 赵洪瑜. 抗血管生成药物在结直肠癌治疗中的应用进展. 癌症进展, 2022, 20(7): 664–667.
LIU M R, ZHAO H Y. Progress in the application of antiangiogenic drugs in the treatment of colorectal cancer. Oncol Progress, 2022, 20(7): 664–667.

［6］ HU S, ZHU Y, XIA X, et al. Ginsenoside Rg3 prolongs survival of the orthotopic hepatocellular carcinoma model by inducing apoptosis and inhibiting angiogenesis. Anal Cell Pathol (Amst), 2019, 2019: 3815786. doi: 10.1155/2019/3815786.

［7］ 陈迪, 倪劲松, 王心蕊, 等. 20(S)-人参皂苷Rg3对乳腺癌MCF-7细胞的诱导凋亡作用. 吉林大学学报(医学版), 2008(212): 610–614. doi: 10.13481/j.1671-587x.2008.04.048.
CHEN D, NI J S, WANG X R, et al. MCF-7 cell apoptosis induced by 20 (S)-ginsendoside Rg3 in mammary carcinoma. J Jilin Univ (Med Edi), 2008(212): 610–614. doi: 10.13481/j.1671-587x.2008.04.048.

［8］ WEIDNER N, SEMPLE J P, WELCH W R. et al. Tumor angiogenesis and metastasis. Correlation in invasive breast carcinoma. New Engl J Med, 1991, 324: 1. doi: 10.1056/NEJM199101033240101.

［9］ ADLER D D, CARSON P L, RUBIN J M, et al. Doppler ultrasound color flow imaging in the study of breast cancer; Preliminary findings. Ultrasound Med Biol, 1990, 16(6): 553–559. doi: 10.1016/0301-5629(90)90020-D.

［10］ CRUZ-ORIVE L M. Precision of Cavalieri sections and slices with local errors. J Microsc, 1999, 193: 182–98. doi: 10.1046/j.1365-2818.1999.00460.x.

［11］ ULLAH H M A, SABA E, LEE Y Y, et al. Restorative effects of Rg3-enriched Korean Red Ginseng and Persicaria tinctoria extract on oxazolone-induced ulcerative colitis in mice. J Ginseng Res, 2022, 46(5): 628–635. doi: 10.1016/j.jgr.2021.07.001.

［12］ SUN D, ZOU Y, SONG L, et al. A cyclodextrin-based nanoformulation achieves co-delivery of ginsenoside Rg3 and quercetin for chemo-immunotherapy in colorectal cancer. Acta Pharm Sin B, 2022, 12(1): 378–393. doi: 10.1016/j.apsb.2021.06.005.

［13］ LV S, CHEN X, CHEN Y, et al. Ginsenoside Rg3 induces apoptosis and inhibits proliferation by down-regulating TIGAR in rats with gastric precancerous lesions. BMC Complement Med Ther, 2022, 22(1): 188. doi: 10.1186/s12906-022-03669-z.

［14］ JIANG J W, CHEN X M, CHEN X H, et al. Ginsenoside Rg3 inhibit hepatocellular carcinoma growth via intrinsic apoptotic pathway. World J Gastroenterol, 2011, 17(31): 3605–3613. doi: 10.3748/wjg.v17.i31.3605.

［15］ HONG H, MILDRED Y, KEVIN H L, et al. Involvement of G proteins of the Rho family in the regulation of Bcl-2-like protein expression and caspase 3 activation by gastrins. Cell Signal, 2008, 20(1): 83–93. doi: 10.1016/j.cellsig.2007.08.018.

［16］ XIA J, ZHANG S, ZHANG R, et al. Targeting therapy and tumor microenvironment remodeling of triple-negative breast cancer by ginsenoside Rg3 based liposomes. J Nanobiotechnology, 2022, 20(1): 414. doi: 10.1186/s12951-022-01623-2.

［17］ LI M, WANG X, WANG Y, et al. Strategies for remodeling the tumor microenvironment using active ingredients of Ginseng-A promising approach for cancer therapy. Front Pharmacol, 2021, 12: 797634. doi: 10.3389/fphar.2021.797634.

［18］ PENG X, DING C, ZHAO Y, et al. Poloxamer 407 and hyaluronic acid thermosensitive hydrogel-encapsulated Ginsenoside Rg3 to promote skin wound healing. Front Bioeng Biotechnol, 2022, 10: 831007. doi: 10.3389/fbioe.2022.831007.

［19］ WANG J, ZENG L, ZHANG Y, et al. Pharmacological properties, molecular mechanisms and therapeutic potential of ginsenoside Rg3 as an antioxidant and anti-inflammatory agent. Front Pharmacol, 2022, 13: 975784. doi: 10.3389/fphar.2022.975784.

［20］ 江昌, 缪雨青, 周文丽, 等. 人参皂苷Rg3的抗肿瘤作用及研究进展. 临床肿瘤学杂志, 2017, 22(7): 664–667. doi: 10.3969/j.issn.1009-0460.2017.07.019.
JIANG C, MIAO Y Q, ZHOU W L, et al. Research progress of ginsenoside Rg3 in anticancer activities. Chin Clin Oncol, 2017, 22(7): 664–667. doi: 10.3969/j.issn.1009-0460.2017.07.019.

［21］ 丁文波, 宁青, 夏智, 等. 双丹胶囊联合5-FU对肝癌细胞Huh-7及荷瘤小鼠协同抗肿瘤作用研究. 中国中药杂志, 2020, 45(23): 5762–5769. doi: 10.19540/j.cnki.cjcmm.20201102.401.
DING W B, NING Q, XIA Z, et al. Study on synergistic anti-tumor effect of Shuangdan Capsules combined with 5-FU on hepatocellular carcinoma cells Huh-7 and xenograft mice. China J Chin Mat Med, 2020, 45(23): 5762–5769. doi: 10.19540/j.cnki.cjcmm.20201102.401.