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顺铂联合 VEGF 抗体对食管癌移植瘤小鼠免疫调节 以及癌细胞增殖和肺转移的影响 *

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摘要 目的:研究顺铂联合血管内皮生长因子(vascular endothelial growth factor, VEGF)治疗对食管癌移植瘤小鼠免疫功能、癌细胞增殖以及肺转移的影响。**方法:**30 只 BALB/c 小鼠通过皮下注射食管癌移植瘤模型。一周后,30 只食管癌移植瘤模型小鼠被随机均分为 3 组,即模型组、顺铂组和联合组。模型组不进行治疗,顺铂组腹腔注射顺铂治疗,联合组腹腔注射顺铂联合尾静脉注射 VEGF 抗体进行治疗,共治疗 7 周。比较各组小鼠体重,食管癌移植瘤体积和重量,卵巢癌细胞肺组织转移结节数、癌细胞转移面积和转移病灶总数,以及食管癌移植瘤外周血 CD4⁺、CD8⁺ 以及 CD4⁺/CD8⁺ T 淋巴细胞比例。**结果:**(1)顺铂组和联合组小鼠体重均显著高于对照组,而联合组小鼠体重显著高于顺铂组($P<0.05$);(2)顺铂组和联合组小鼠 CD4⁺ 和 CD8⁺ 细胞比例均显著低于对照组($P<0.05$),而 CD4⁺/CD8⁺ 却显著高于对照组($P<0.05$);(3)联合组小鼠 CD4⁺ 和 CD8⁺ 细胞比例均显著高于对照组($P<0.05$),而 CD4⁺/CD8⁺ 却显著低于顺铂组($P<0.05$);(4)顺铂组和联合组小鼠食管癌肿瘤组织体积和重量,肺转移结节数、转移面积和转移病灶数均显著低于对照组($P<0.05$),而联合组小鼠显著低于顺铂组($P<0.05$)。**结论:**VEGF 抗体可以显著增强顺铂在体内对食管癌的抗癌特性,并有助于增强食管癌移植瘤小鼠免疫功能、抑制癌细胞体内增殖和肺部转移。

关键词:顺铂;血管内皮生长因子;食管癌;免疫功能;肺转移

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Effect of Cisplatin Combined with VEGF Antibody on Immune Regulation, Cancer Cell Proliferation and Lung Metastasis in Mice Transplanted with Esophageal Cancer*

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ABSTRACT Objective: To study the effect of cisplatin combined with vascular endothelial growth factor (VEGF) treatment on the immune function, cancer cell proliferation and lung metastasis of esophageal cancer transplanted mice. **Methods:** Thirty BALB/c mice were injected subcutaneously into the esophageal cancer xenograft model. One week later, 30 esophageal cancer transplanted tumor model mice were randomly divided into 3 groups, namely model group, cisplatin group and combination group. The model group was not treated, the cisplatin group was treated with intraperitoneal cisplatin, and the combination group was treated with intraperitoneal cisplatin combined with tail vein injection of VEGF antibody for a total of 7 weeks. Compare the body weight of each group of mice, the volume and weight of esophageal cancer transplanted tumor, the number of ovarian cancer cell lung tissue metastatic nodules, the area of cancer cell metastasis and the total number of metastatic lesions, and the peripheral blood CD4⁺, CD8⁺ and CD4⁺/CD8⁺ T lymph of esophageal cancer transplanted tumor Cell ratio. **Results:** (1) The weight of mice in the cisplatin group and the combination group was significantly higher than that of the control group, while the weight of the mice in the combination group was significantly higher than that in the cisplatin group ($P<0.05$); (2) CD4⁺ and CD8⁺ cells in the cisplatin group and the combination group The ratios were significantly lower than those of the control group ($P<0.05$), while the CD4⁺/CD8⁺ ratios were significantly higher than those of the control group ($P<0.05$); (3) The ratios of CD4⁺ and CD8⁺ cells in the combination group were significantly higher than those of the control group ($P<0.05$), while CD4⁺/CD8⁺ was significantly lower than that of the cisplatin group ($P<0.05$); (4) the volume and weight of esophageal cancer tumor tissue, the number of lung metastasis nodules, the area of metastasis, and the metastatic lesions in the cisplatin group and the combination group The numbers were significantly lower than those in the control group ($P<0.05$), while the mice in the combination group were significantly lower than those in the cisplatin group ($P<0.05$). **Conclusion:** VEGF antibody can significantly enhance the anti-cancer properties of cisplatin against esophageal cancer in vivo, and help to enhance the immune function of esophageal cancer transplanted mice, and inhibit the proliferation of cancer cells in vivo and lung metastasis.

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前言

食管癌是指原发于食管的恶性肿瘤,是最常见的消化系统恶性肿瘤,患者典型的临床特征是进行性加重的吞咽困难,以中老年男性发病率最高,综合治疗后5年生存率为20%左右^[1-3]。据世界卫生组织发布的数据^[4],2020年全球食管癌新发患者约60万人,食管癌的死亡率为54万人次。在我国^[5-7],2020年食管癌新发患者为32万人次,是我国发病率第六高的恶性肿瘤,并且呈逐年上升趋势,且死亡率仅次于肺癌、肝癌和胃癌,我国每年因食管癌死亡的患者超过30万人。此外,由于食管癌早期症状不明显,大部分患者初次确诊时已处于食管癌晚期,并且食管癌还具有复发率较高的特征^[8,9]。手术切除、化疗和放疗是食管癌患者最常见的临床治疗放射,其中以化疗联合手术切除治疗最为常见。顺铂是食管癌化疗治疗中常用的药物之一,但研究发现,我们无法通过增加顺铂剂量来提高肝癌患者化疗疗效,并且大剂量使用顺铂通常伴随着严重的不良反应^[10,11]。因此,开发能够应用于肝癌临床治疗的新的化疗方案意义重大。血管内皮生长因子(Vascular Endothelial Growth Factor, VEGF)抗体,商品名为贝伐珠单抗,是一种单克隆抗体,在血液中通过与VEGF蛋白结合而使VEGF失活,进而抑制肿瘤细胞的转移,被用于多种转移性恶性肿瘤的治疗^[12,13]。然而,目前关于VEGF抗体用于治疗食管癌的报道较少,本研究探讨顺铂联合VEGF抗体对食管癌移植瘤小鼠治疗效果,并研究联合治疗对食管癌移植瘤小鼠免疫功能、移植瘤增殖和肺转移的影响。

1 资料与方法

1.1 实验动物

30只SPF级别Balb/c小鼠(6-8周龄,雌雄比例为1:1)购买于上海杰思捷实验动物有限公司(实验动物使用许可证号:SCXK(沪)2020-0093)。

1.2 食管癌移植瘤模型的建立与治疗

30只Balb/c小鼠在实验室适应性喂养一周后,颈部皮下注射 2×10^6 个/0.2 mL的EC9706食管癌细胞以建立食管癌移植瘤小鼠模型。食管癌细胞皮下注射2周后,随机将其分为3组:模型组,腹腔注射生理盐水;顺铂组,每两天接受腹腔注射4 mg/kg顺铂注射液(每周2次,江苏豪森药业集团有限公司,

国药准字H20040813);联合组,每周腹腔注射5 mg/kg贝伐珠单抗注射液(每周2次,齐鲁制药有限公司,国药准字S20190040),每两天接受腹腔注射4 mg/kg顺铂注射液。共治疗6周。

1.3 观察指标

1.3.1 体重和抑瘤率 治疗后,每2周测量一次小鼠体重,治疗6周后颈椎脱臼安乐死各组小鼠,分离食管癌肿瘤组织,排水法量取肿瘤体积,并称重。根据公式抑瘤率=(模型组肿瘤重量-治疗组肿瘤重量)/模型组肿瘤重量 $\times 100\%$,治疗组分别指顺铂组或联合组。

1.3.2 外周血淋巴细胞 小鼠被安乐死后,立即用注射器插入小鼠心脏收集外周血,离心以及收集血细胞,裂解红细胞后分别加入CD3-PE抗体、CD4-TITC抗体和CD8-APC抗体避光孵育半小时,通过流式细胞仪分析T淋巴细胞亚型。

1.3.3 食管癌细胞肺转移 小鼠被安乐死后,解剖小鼠,取小鼠肿瘤结节和肺组织制作石蜡切片,进行苏木精-伊红染色,然后在光学显微镜下观察癌细胞转移情况。

1.4 统计学方法

本研究数据通过SPSS20.0软件进行记录和统计学分析,以(均值 $\pm$ 标准差)表示计量资料,t检验比较两组间差异,单因素方差分析比较三组间差异。 $P < 0.05$ 表示差异显著具有统计学意义。

2 结果

2.1 三组小鼠不同时间体重

每组食管癌裸鼠异种移植瘤小鼠在皮下注射食管癌细胞后4周、6周和8周测定其体重,结果显示:顺铂组小鼠注射食管癌细胞后4周、6周和8周后体重均显著低于对照组($P < 0.05$),而联合组小鼠体重在注射食管癌细胞后4周、6周和8周后均显著高于对照组,差异均显著具有统计学意义($P < 0.05$)。具体如表1所示。

2.2 三组小鼠免疫功能比较

食管癌细胞皮下注射8周后,安乐死各组小鼠,收集小鼠外周血,检测CD4⁺和CD8⁺T淋巴细胞比例,结果显示:与对照组相比,顺铂组和联合组小鼠CD4⁺和CD8⁺细胞比例均显著下降,而CD4⁺/CD8⁺比例均显著升高($P < 0.05$);与顺铂组相比,

表1 各组小鼠体重比较(g)

Table 1 Comparison of body weight of mice in each group(g)

Groups	n	Week 4	Week 6	Week 8
Model group	10	25.15 $\pm$ 2.13	23.56 $\pm$ 2.52	21.72 $\pm$ 1.62
Cisplatin group	10	23.67 $\pm$ 2.221	21.01 $\pm$ 2.12*	18.68 $\pm$ 1.09*
Combine group	10	26.89 $\pm$ 2.08	27.65 $\pm$ 3.02 **	27.75 $\pm$ 1.69 ^{ab}
F		6.928	5.438	9.02819.672
P		<0.001	0.002	<0.001

Note: Compared with the model group, * $P < 0.05$; compared with the cisplatin group, ** $P < 0.05$.

联合组小鼠 CD4⁺ 和 CD8⁺ 细胞比例均显著上升 ($P<0.05$), 并且 CD4⁺/CD8⁺ 比例均显著下降 ($P<0.05$)。具体如表 2 所示。

表 2 各组小鼠外周血 T 淋巴细胞亚群比较

Table 2 Comparison of T lymphocyte subsets in peripheral blood of mice in each group

Groups	n	CD4 ⁺ (%)	CD8 ⁺ (%)	CD4 ⁺ /CD8 ⁺
Model group	10	45.13±5.21	37.51±5.02	1.20±0.33
Cisplatin group	10	40.41±4.82 *	13.18±2.33 *	3.06±0.89 *
Combine group	10	41.38±5.02 * [#]	26.09±3.01 * [#]	1.58±0.69 * [#]
<i>P</i>		9.167	11.235	10.917
<i>P</i>		<0.001	<0.001	<0.001

Note: Compared with the model group, * $P<0.05$; compared with the cisplatin group, [#] $P<0.05$.

2.3 三组小鼠肿瘤增殖比较

食管癌细胞皮下注射 8 周后, 安乐死各组小鼠, 收获食管癌肿瘤组织并检测其体积和肿瘤, 结果显示: 与对照组相比, 顺铂组和联合组小鼠食管癌肿瘤组织体积和重量均显著降低

($P<0.05$); 并且联合组小鼠食管癌肿瘤组织体积和重量均显著低于顺铂组 ($P<0.05$); 联合组小鼠抑瘤率也显著高于顺铂组 ($P<0.05$)。具体如表 3 所示。

表 3 各组小鼠肿瘤体积、肿瘤和抑瘤率比较

Table 3 Comparison of tumor volume, tumor and tumor inhibition rate in each group of mice

Groups	n	Volume(mm ³)	Weight(g)	Tumor inhibition rate(%)
Model group	10	23.24±2.08	2.92±0.18	---
Cisplatin group	10	21.19±3.36*	1.92±0.19 ^a	34.24±7.55*
Combine group	10	19.73±2.01* [#]	1.53±0.12* [#]	47.60±10.08* [#]
<i>F</i>		3.016	8.937	15.628
<i>P</i>		0.023	<0.001	<0.001

Note: Compared with the model group, * $P<0.05$; compared with the cisplatin group, [#] $P<0.05$.

2.4 三组小鼠癌细胞肺转移比较

食管癌细胞皮下注射 8 周后, 安乐死各组小鼠, 收获小鼠肺组织, 通过 HE 染色检测食管癌细胞肺转移情况, 结果显示: 与对照组相比, 顺铂组和联合组小鼠食管癌肺周围淋巴结转移

数目、肺组织癌细胞转移面积百分比以及肺组织食管癌转移病灶均显著降低 ($P<0.05$); 联合组小鼠食管癌肺周围淋巴结转移数目、肺组织癌细胞转移面积百分比以及肺组织食管癌转移病灶均显著低于顺铂组 ($P<0.05$)。具体如表 4 所示。

表 4 各组小鼠食管癌肺转移结节数、肺转移面积和转移灶总数比较

Table 4 Comparison of the number of esophageal cancer lung metastasis nodules, lung metastasis area and total number of metastases in each group of mice

Groups	n	Number of metastatic nodules(n)	Metastasis area(%)	Number of metastases(n)
Model group	10	6.58±1.02	8.23±2.31	3.62±0.59
Cisplatin group	10	5.38±1.31 *	7.18±1.35 *	2.54±0.38 *
Combine group	10	2.62±0.83 * [#]	3.12±0.98 * [#]	1.38±0.29 * [#]
<i>F</i>		10.003	11.328	16.759
<i>P</i>		<0.001	<0.001	<0.001

Note: Compared with the model group, * $P<0.05$; compared with the cisplatin group, [#] $P<0.05$.

3 讨论

据 2020 年全球最新癌症负担数据显示^[4]: 2020 年全球癌症死亡病例 996 万例, 其中中国癌症死亡人数 300 万, 占癌症死亡总人数 30%; 2020 年, 中国新发癌症中, 男性食管癌新发病例 223044 例, 占男性癌症新发病例比例为 9.0%, 因食管癌死亡男性患者 206780, 占男性癌症死亡病患比例为 11.4%; 女

性食管癌新发病例 101378 例, 占女性癌症新发病例比例为 4.8%, 因食管癌死亡女性患者 94355, 占女性癌症死亡病患比例为 8.0%。由此可见中国不仅是一个 "食管癌大国", 而且是一个因食管癌死亡的大国, 2020 年中国癌症死亡人数前十的癌症分别是: 肺癌, 肝癌, 胃癌, 食管癌, 大肠癌, 胰腺癌, 乳腺癌, 神经系统癌症, 白血病, 宫颈癌, 其中食管癌患者死亡 301135 例, 占 10.0%。在中国, 食管癌而个体化差异较大, 所有

多采用个体化综合治疗,治疗手段包括手术治疗、化疗药物治疗、放疗、免疫治疗和靶向药物治疗^[14,15]。此外,由于食管癌患者因吞咽困难而造成营养较差,所以食管癌患者治疗期间要注意营养支持治疗,尤其是放化疗引起患者免疫功能下降时^[16,17]。

顺铂,又名顺式-二氯二氨合铂,是一种含铂的抗癌药物,临床上被发现多种实体瘤治疗中具有显著疗效,但由于顺铂具有较大的细胞毒性对癌症患者免疫功能会产生影响,尤其是在对营养缺乏的食管癌患者进行治疗时,要重视对患者免疫功能的影响^[18-20]。本文通过检测顺铂治疗食管癌移植瘤小鼠期间体重和外周血淋巴细胞亚型变化表征其免疫功能时发现:与模型组食管癌移植瘤小鼠相比,经顺铂治疗的食管癌小鼠体重显著降低,而经顺铂联合 VEGF 抗体治疗的食管癌小鼠体重却显著高于模型组小鼠,并且联合组小鼠 CD4⁺ 和 CD8⁺ 细胞比例均显著高于对照组($P<0.05$),而 CD4⁺/CD8⁺ 却显著低于顺铂组($P<0.05$),表明顺铂治疗会影响食管癌移植瘤营养状态和免疫功能,而联合 VEGF 抗体治疗却可以显著减低顺铂的影响。王子卿^[21]和陈晓波^[22]等人的研究均发现,顺铂治疗肿瘤小鼠模型时会降低小鼠的 CD4⁺ 和 CD8⁺ 细胞比例,而升高 CD4⁺/CD8⁺ 比例,而通过药物联合顺铂治疗有助于降低顺铂毒性,与本研究结论类似,不同之处在于:本研究使用 VEGF 抗体作为辅助药物减低顺铂毒性,而王子卿和陈晓波等人中药汤剂或作为辅助药物降低顺铂毒性。此外,本次研究中,与顺铂组相比,联合组小鼠食管癌肿瘤组织体积和重量均显著降低,而抑瘤率显著升高($P<0.05$),表明 VEGF 抗体显著增强顺铂对食管癌移植瘤的抗肿瘤作用,这与蔡霄月^[23]等研究结果一致,其研究通过药物降低食管癌移植瘤小鼠外周血 VEGF 含量有助于增强顺铂在体内对食管癌细胞的抗癌作用,与本次研究不同的是蔡霄月等人使用中药汤剂降低小鼠外周血 VEGF 含量,而本研究使用的是 VEGF 抗体,相比而言 VEGF 抗体作用效果更好。分析其原因可能在于:肿瘤组织与正常组织类似,其生长发育也需要血液的供应,并且由于肿瘤细胞快速生长的特性,其血管生成也是大幅度加快,而 VEGF 抗体作为抗血管生成药就是作用在关键位点,因此具有更佳的肿瘤抑制效果^[24-26]。

由于食管癌早期症状不明显,筛查作用有限,所以 70 % 左右的食管癌患者在确诊时已经处于晚期,即出现食管癌细胞转移^[27,28]。侵袭转移是恶性肿瘤细胞最主要的生物学特征之一,也是约 90 % 以上的恶性肿瘤患者死于癌细胞转移,在食管癌中癌细胞转移同样是造成患者死亡和术后复发的主要原因之一,其中以肺转移最为常见^[29,30]。本文研究发现,顺铂治疗可以显著降低食管癌细胞转移结节数,肺组织癌细胞转移面积百分比和病灶数,并且联合 VEGF 抗体治疗效果更好。VEGF 是一种高度特异性的促血管内皮细胞生长因子,具有促进血管通透性增加、细胞外基质变性、血管内皮细胞迁移、增殖和血管形成等作用^[14],已经被广泛用于多种转移性恶性肿瘤的治疗^[24-26]。

综上所述,本文研究表明:VEGF 抗体可以增强顺铂在体内抗食管癌效果,并可降低顺铂治疗对小鼠免疫功能的伤害,另外该抗体联合顺铂治疗对食管癌肺转移的抑制作用更强,从而为食管癌的治疗提供动物实验研究思路,为其临床的进一步研究奠定基础。

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