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扇贝糖胺聚糖对感染 HSV- I 小鼠免疫功能的影响研究 *

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摘要 目的:研究扇贝裙边糖胺聚糖(glycosaminoglycan from Scallop Skirt, SS-GAG)对感染单纯疱疹病毒 I 型(herpes simplex virus type, HSV- I)小鼠免疫功能的影响。**方法:**通过无菌条件下给予小鼠注射扇贝糖胺聚糖 SS-GAG,连续 11 天,并在给药第三天给小鼠腹腔注射 HSV- I 病毒悬液建立小鼠感染模型,用 MTT 等方法观察 SS-GAG 对 HSV- I 感染小鼠腹腔巨噬细胞吞噬活性的影响、对脾脏指数、胸腺指数的影响以及对脾淋巴细胞转化能力等免疫指标的影响。**结果:**与病毒对照组相比,扇贝糖胺聚糖 SS-GAG 低剂量组、中剂量组、高剂量组均能显著增强 HSV- I 感染小鼠的腹腔巨噬细胞的吞噬活性和 HSV- I 感染小鼠的脾脏指数和胸腺指数($P<0.01$),并且能促进其脾淋巴细胞转化增殖能力($P<0.01$)。**结论:**扇贝糖胺聚糖在体内有一定的抗 I 型单纯疱疹病毒作用。其抗病毒作用可能与增强机体免疫功能有关。

关键词:扇贝裙边;糖胺聚糖;单纯疱疹病毒 I 型;免疫调节

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The Effect of Scallop Skirt Glycosaminoglycan on Immune Function in Mice Infected with HSV- I *

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ABSTRACT Objective: To investigate the effect of scallop skirt glycosaminoglycan on immune function in mice infected by HSV-I.

Methods: Every mouse was treated by SS-GAG with different doses for 11 days. After 72 hours, the mice were injected by HSV-I. The effects of SS-GAG on the phagocytic activity of peritoneal macrophage and the proliferation of splenic lymphocyte were studied by MTT colorimetry. The index of spleen and thymus were used to study the influences of SS-GAG on function of the immune organs. **Results:** Compared with virus control, SS-GAG (10 mg/kg, 20 mg/kg and 40 mg/kg) could significantly enhance phagocytic activity of peritoneal macrophage in mice infected by HSV-I, and promote proliferation of splenic lymphocyte induced by concanavalina and lipopolysaccharide ($P<0.01$). Meanwhile, the index of thymus of mice treated by SS-GAG was improved significantly ($P<0.01$). **Conclusion:** The glycosaminoglycan from scallop skirt (SS-GAG) has the depressant effect on HSV-I *in vivo*, and the mechanism of anti-virus may attribute to improving the immune function of organs.

Key words: Scallop skirt; Glycosaminoglycan; HSV-I; Immune regulation

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前言

单纯疱疹病毒 I 型(herpes simplex virus type I, HSV-I)属疱疹病毒科,是一种人类最常见的病原体,人是其唯一的自然宿主^[1-4]。HSV- I 可以经口腔、呼吸道、生殖道粘膜和破损皮肤等多种途径侵入人体,潜居于人体粘膜、血液、唾液及感觉神经节细胞内。当机体免疫力下降时,如发热、妊娠、病灶感染和情绪改变时,体内潜伏的 HSV- I 被激活而发病^[5,6]。扇贝是一种海洋贝类,其裙边由于感官性状差,一直没有得到很好的开发利用。近年来,人们从中提取到了一种酸性粘多糖-扇贝糖胺聚糖

(glycosaminoglycan from Scallop Skirt, SS-GAG)。研究表明,SS-GAG 具有如抗凝血、降血脂、抗肿瘤、抗病毒及增强免疫功能等多种药理活性^[7-10],现已引起人们对这类生物高分子的高度重视。本实验旨在通过扇贝糖胺聚糖对感染 HSV-I 小鼠免疫功能的影响。

1 材料与方

1.1 实验材料

1.1.1 药品与试剂 药品:扇贝糖胺聚糖(SS-GAG):由青岛大学药理教研室 SS-GAG 课题组提供;注射用阿昔洛韦(ACV);扬

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1.1.2 病毒 标准 I 型单纯疱疹病毒(HSV-)SM44 株由清华大学北京协和医学院提供,在 HeLa 细胞中传代^[11-14]。实验用病毒浓度为 100TCID₅₀。

1.1.3 实验动物 清洁级昆明小鼠 48 只,雌雄各半,质量 20 ± 2 g,由青岛市药检所动物实验中心提供。

1.2 实验方法

表 1 实验动物分组及药物剂量

Table 1 Animal grouping and drug dose

Groups	Drugs	Dose
Normal control	0.9%NS	0.2ml/d
Virus control	-	-
SS-GAG(40 mg/kg)	SS-GAG	40 mg/kg/d
SS-GAG(20 mg/kg)	SS-GAG	20 mg/kg /d
SS-GAG(10 mg/kg)	SS-GAG	10 mg/kg/d
ACV	ACV	0.1 g/ kg/d

1.2.3 感染 HSV- I 小鼠脾指数、胸腺指数的测定 末次给药后 24 小时采用颈椎脱臼法处死小鼠。分别剥离和称量各组小鼠的脾脏、胸腺,并计算其脾脏指数和胸腺指数。

1.2.4 腹腔巨噬细胞 PM Φ 吞噬能力的测定 将小鼠颈椎脱臼处死,严格进行皮肤消毒,用含肝素的冷 PBS 液灌洗小鼠腹腔,然后吸取腹腔灌洗液制成巨噬细胞悬液并离心 1500 r/min, 10 min。用完全 DMEM 培养基将细胞浓度调整至 4×10^6 /mL 后加至 96 孔板中,100 μ L/ 孔,置于培养箱(37 $^{\circ}$ C、5% CO₂)孵育 4 小时后弃上清,每孔加 0.075%中性红溶液 100 μ L 并继续培养半小时,弃掉上清,每孔加入 PM Φ 溶解液 100 μ L,置于 4 $^{\circ}$ C 冰箱 12 小时,次日用酶标仪测定 A570 值。

1.2.5 T、B 淋巴细胞转化活性的测定 将上述已处死小鼠的脾脏碾碎并在钢丝网上过滤,然后用 PBS 液冲洗并制成脾淋巴细胞悬液。台盼蓝染色计数使活细胞在 95%以上,将细胞数调整为 5×10^6 /mL。然后将调整好的悬液分三孔加入 96 孔培养板上,0.2 mL/ 孔。T 淋巴细胞转化试验:在孔中加入伴刀豆蛋白 A

1.2.1 建立小鼠病毒感染模型 在无菌条件下给予小鼠腹腔注射 HSV- I 病毒悬液(空白对照组除外),0.2 mL/ 只,在 30 分钟内完成。小鼠于注射病毒后第 5 天出现感染症状,并且均在发病后 1-3 天内死亡。

1.2.2 给药及分组 将小鼠按质量随机分成 6 组,每组 8 只。在接种病毒前 3 天开始给 SS-GAG,给药途径均为腹腔注射。每天固定时间段给药一次。连续给药 11 天。病毒对照组只接种病毒,不接受药物治疗。具体分组见表 1。

使每孔终浓度为 5 g/mL;B 淋巴细胞转化试验:在孔中加入脂多糖使每孔终浓度为 50 g/mL;同时做不加任何诱导剂的阴性对照孔,置培养箱(37 $^{\circ}$ C、5%CO₂)孵育 72 小时,于孵育结束前 4 小时加入 MTT 液,孵育结束后加盐酸异丙醇 1 mL/ 孔溶解,用酶标仪测定 A570 值,淋巴细胞的增殖能力 = 加伴刀豆蛋白 A 或脂多糖孔的 A570 值—不加伴刀豆蛋白 A 或脂多糖孔的 A570 值^[15]。

1.2.6 统计学方法 每组的数据以 8 个平行样本的平均数 $\pm$ 标准差表示($\bar{X} \pm S$),采用 SPSS13.0 for Windows 统计软件对结果进行方差分析和 q 检验。

2 结果

2.1 SS-GAG 对感染 HSV- I 小鼠胸腺指数、脾指数的影响

SS-GAG 各组均能显著提高感染 HSV- I 小鼠的胸腺指数和脾脏指数。与病毒对照组相比,差异具有显著性($P < 0.01$),而且提高的程度与 SS-GAG 剂量呈正性相关关系(见表 2)。

表 2 SS-GAG 对小鼠胸腺指数、脾脏指数的影响($n=8, \bar{X} \pm S$)

Table 2 The effects of SS-GAG on the indexes of spleen and thymus($n=8, \bar{X} \pm S$)

Groups	Index of spleen (mg/10g)	Index of thymus (mg/10g)
Normal control	31.37 $\pm$ 6.94	76.23 $\pm$ 8.68
Virus control	17.50 $\pm$ 1.83**	48.29 $\pm$ 10.66**
SS-GAG(10mg/kg)	31.37 $\pm$ 2.32*	93.64 $\pm$ 6.57*
SS-GAG(20mg/kg)	36.22 $\pm$ 7.13*	106.41 $\pm$ 6.69*
SS-GAG(40mg/kg)	46.18 $\pm$ 5.59*	114.13 $\pm$ 9.31*
ACV	34.64 $\pm$ 7.43*	90.79 $\pm$ 17.76*

Note:* $P < 0.01$, Compared with virus control **: $P < 0.01$, Compared with normal control.

2.2 SS-GAG 对感染 HSV- I 小鼠腹腔巨噬细胞(PM Φ)功能的影响

SS-GAG 在各浓度时均能提高小鼠 PM Φ 的吞噬能力。与

病毒对照组相比,差异有统计学意义($P < 0.05$)(见表 3)。

2.3 SS-GAG 对感染 HSV- I 小鼠脾淋巴细胞功能影响

SS-GAG 各组均能够提高感染 HSV- I 小鼠的脾淋巴细胞

表 3 SS-GAG 对小鼠腹腔巨噬细胞功能的影响 (n=8, $\bar{X} \pm S$)Table 3 The effect of SS-GAG on the phagocytic activity of peritoneal macrophage (n=8, $\bar{X} \pm S$)

Groups	Phagocytic activity of MΦ
Normal control	0.213± 0.057
Virus control	0.102± 0.033**
SS-GAG(10mg/kg)	0.279± 0.045*
SS-GAG(20mg/kg)	0.303± 0.056*
SS-GAG(40mg/kg)	0.357± 0.062*
ACV	0.257± 0.039*

Note: * P<0.05, Compared with virus control ** P<0.05 Compared with normal control.

表 4 SS-GAG 对小鼠脾淋巴细胞功能影响 (n=8, $\bar{X} \pm S$)Table 4 The effect of SS-GAG on the proliferation activity of splenic lymphocyte (n=8, $\bar{X} \pm S$)

Groups	Proliferation activity of	
	T lymphocyte	B lymphocyte
Normal control	0.172± 0.056	0.198± 0.035
Virus control	0.098± 0.033**	0.102± 0.038**
SS-GAG(10 mg/kg)	0.256± 0.060*	0.252± 0.042*
SS-GAG(20 mg/kg)	0.263± 0.074*	0.277± 0.036*
SS-GAG(40 mg/kg)	0.270± 0.076*	0.288± 0.041*
ACV	0.227± 0.042*	0.229± 0.037*

Note: P<0.01, Compared with virus control ** P<0.01 Compared with normal control.

的转化增殖活性,转化指数升高明显,与病毒对照组相比有显著性差异 (P<0.01)(见表 4)。

3 讨论

在本实验中,扇贝裙边糖胺聚糖 SS-GAG (10 mg/kg, 20 mg/kg, 40 mg/kg)能显著增强 HSV- I 感染小鼠腹腔巨噬细胞的吞噬活性,促进其脾淋巴细胞转化增殖能力以及提高 HSV- I 感染小鼠的脾脏指数和胸腺指数。结果表明 SS-GAG 能提高机体的免疫机能,这与有关报道相一致^[16]。

近几年来,在寻找有效抗病毒药物的过程中,针对多种多糖的抗病毒生物学活性研究大量展开^[17-20]。海洋生物多糖的抗病毒、抗肿瘤等生物学效应被大量报道。本研究中糖胺聚糖自扇贝裙边中经纯化提取,经初步体外抗病毒实验发现具有抗 HSV-I 活性。目前尚未见相关文献报道。后续将对 SS-GAG 抗病毒作用机制进行深入研究探索。

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