DOI: 10.13241/j.cnki.pmb.2014.04.016

扇贝糖胺聚糖对感染 HSV-Ⅰ小鼠免疫功能的影响研究 *

于 囡[1] 王海桃[2] 杜 鹃[1] 李 荣[1] 刘 赛[3△]

(1 青岛市第八人民医院 山东 青岛 266100;2 青岛市中心医院 山东 青岛 266042;3 青岛大学 山东 青岛 266021)

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:**通过在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

中图分类号:Q95-3,R285.5,R392 **文献标识码:**A **文章编号:**1673-6273(2014)04-668-03

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

YU Nan[1], WANG Hai-tao[2], DU Juan[1], LI Rong[1], LIU Sai[3△]

(1 Qingdao Eighth People's Hospital, Qingdao, Shandong, 266100, China;

2 Qingdaocenter medical group, Qingdao, Shandong, 266042, China;

3 Qingdao University, Qingdao, Shandong, 266021, China)

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

Chinese Library Classification(CLC): Q95-3, R285.5, R392 Document code: A

Article ID: 1673-6273(2014)04-668-03

前言

单纯疱疹病毒 I 型(herpes simplex virus typeI, 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 材料与方法

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

* 基金项目:山东省中医药发展科技项目(2005-16)

作者简介:于囡(1982-),女,硕士,药师,研究方向:抗病毒药理学,E-mail:wf-hjj@163.com

△通讯作者:刘赛,硕士生导师,E-mail:liusai5151@126.com

(收稿日期:2013-05-23 接受日期:2013-06-18)

子江药业集团有限公司生产,批号:20110609;试剂:四甲基偶氮唑(MTT):Sigma 公司生产。

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**

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。

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 PMΦ 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 μL/ 孔,置于培养箱(37℃、5% CO_2)孵育 4 小时后弃上清,每孔加 0.075%中性红溶液 100 μL并继续培养半小时,弃掉上清,每孔加入 PMΦ 溶解液 100 μL,置于 4℃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 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℃、5%CO_2)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± 标准差表示($\bar{X}$± 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, $\bar{X}$± S)

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

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

SS-GAG 在各浓度时均能提高小鼠 PMΦ 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	Proliferation activity of 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,经初步体内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。

参考文献(References)

[1] Whitley RJ, Roizman B. Herpes simplex virus infections [J]. Lancet, 2001, 357(9267): 1513-1518

[2] Taylor TJ, Brockman MA, McNamee EE, et al. Herpes simplex virus. [J]. Front Biosci, 2002, 7: d752-d764

[3] 陈爱荣. 单纯疱疹病毒的研究现状与展望 [J]. 微生物免疫学进展. 2003, 31(2): 62-65
Chen Ai-rong. Research status and Prospect of herpes simplex virus. [J]. Advances in Microbiology and Immunology, 2003, 31(2): 62-65

[4] Jameson DR, Celum CL, Manhart L, Menza TW, Golden MR. The Association Between Lack of Circumcision and HIV, HSV-2, and Other Sexually Transmitted Infections Among Men Who Have Sex With Men [J]. Sex Transm Dis, 2010, 37(3): 147-152

[5] AMsteyMS, MonifGR, NahlniasAJ, et al. Cesarean section and genital herpes virus infection[J]. Obstet Gynecol, 1979, 53: 641-642

[6] Conrady CD, Drevets DA, Carr DJ. Herpes simplex type 1(HSV-1) infection of the nervous system: is an immune response a good thing.[J]. Neuroimmunol, 2010, 220(1/2): 1-9

[7] 黄翠丽, 刘赛. 扇贝的生理活性物质和药用价值 [J]. 中国海洋药物, 2001, 20(2): 45-47
Huang Cui-li, Liu Sai. The physiological active substance and medicinal value of Scallop [J]. Chinese Journal of marine drugs, 2001, 20(2): 45-47

[8] 王长云, 管华诗. 海湾扇贝边中氨基多糖的研究 [J]. 中国水产科学, 1994, 1(2): 32-39
Wang Chang-yun, Guan Hua-shi. Study on amino polysaccharide of scallop Skirt[J]. Journal of Fisheries of China, 1994, 1(2): 32-39

[9] Miao Ben-chun, Geng Mei-yu, Li Jing, et al. A Study on Immunopotentiating Activity of Marine Sulfated Polysaccharide 911[J]. China J Mar Drugs, 2002, 5: 1-4

[10] AK, Ferreira VF, Souza TM, et al. Synthesis and anti-HSV-1 activity of new 1 2, 3-triazole derivatives[J]. Bioorg Med Chem, 2011, 19(6): 1860-1865

[11] 曾凡力, 周敏, 许敏, 等. 贵州老鹰茶提取物体外抗单纯疱疹病毒Ⅰ型活性的实验研究[J]. 时珍国医国药, 2011, 22(2): 282-284
Zeng Fan-li, Zhou Min, Xu Min, et al. Study on the in vitroanti-HSV-Ⅰ activity of aqueous extract from Litsea coreana[J]. Lishizh Medicine and Materia Medicare Seach, 2011, 22(2): 282-284

(下转第 667 页)

Tang Ying, Fang Qi, Shi Dao-hua. Construction of eukaryotic expressing vector of FKBP12 and establishment of stable transfectant A549 Cell Line [J]. Progress in Modern Biomedicine, 2010, 10(4): 617-619

[8] Jantan I, Raweh SM, Sirat HM, et al. Inhibitory effect of compounds from Zingiberaceae species on human platelet aggregation[J]. Phytomedicine, 2008, 15(4): 306-309

[9] Li N, Liu JH, Zhang J, et al. Comparative evaluation of cytotoxicity and antioxidative activity of 20 flavonoids [J]. J Agric Food Chem, 2008, 56(10): 3876-3883

[10] Cho M, Ryu M, Jeong Y, et al. Cardamonin suppresses melanogenesis by inhibition of Wnt/beta-catenin signaling [J]. Biochem Biophys Res Commun, 2009, 390(3): 500-505

[11] Liao Q, Shi DH, Zheng W, et al. Antiproliferation of cardamonin is involved in mTOR on aortic smooth muscle cells in high fructose-induced insulin resistance rats[J]. Eur J Pharmacol, 2010, 641(2-3): 179-186

[12] Niu P, Zhang Y, Shi D, et al. Cardamonin Ameliorates Insulin Resistance Induced by High Insulin and High Glucose through the mTOR and Signal Pathway[J]. Planta Med, 2013, 79(6): 452-458

[13] Kamada Y, Yoshino K, Kondo C, et al. Tor directly controls the Atg1 kinase complex to regulate autophagy[J]. Mol Cell Biol, 2010, 30(4): 1049-1058

[14] Hosokawa N, Hara T, Kaizuka T, et al. Nutrient-dependent mTORC1 association with the ULK1-Atg13-FIP200 complex required for autophagy[J]. Mol Biol Cell, 2009, 20(7): 1981-1991

[15] Kim J, Kundu M, Viollet B, et al. AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1 [J]. Nat Cell Biol, 2011, 13(2): 132-141

[16] Ganley IG, Lam du H, Wang J, et al. ULK1.ATG13.FIP200 complex mediates mTOR signaling and is essential for autophagy [J]. J Biol Chem, 2009, 284(18): 12297-12305

[17] Chresta CM, Davies BR, Hickson I, et al. AZD8055 is a potent, selective, and orally bioavailable ATP-competitive mammalian target of rapamycin kinase inhibitor with in vitro and in vivo antitumor activity[J]. Cancer Res, 2010, 70(1): 288-298

[18] Ravikumar B, Vacher C, Berger Z, et al. Inhibition of mTOR induces autophagy and reduces toxicity of polyglutamine expansions in fly and mouse models of Huntington disease[J]. Nat Genet, 2004, 36(6): 585-595

[19] Floto RA, Sarkar S, Perlstein EO, et al. Small molecule enhancers of rapamycin-induced TOR inhibition promote autophagy, reduce toxicity in Huntington's disease models and enhance killing of mycobacteria by macrophages[J]. Autophagy, 2007, 3(6): 620-622

[20] Lin CI, Whang EE, Donner DB, et al. Autophagy induction with RAD001 enhances chemosensitivity and radiosensitivity through Met inhibition in papillary thyroid cancer [J]. Mol Cancer Res, 2010, 8(9): 1217-1226

[21] Sini P, James D, Chresta C, et al. Simultaneous inhibition of mTORC1 and mTORC2 by mTOR kinase inhibitor AZD8055 induces autophagy and cell death in cancer cells[J]. Autophagy, 2010, 6(4): 553-554

[22] Willems L, Chapuis N, Puissant A, et al. The dual mTORC1 and mTORC2 inhibitor AZD8055 has anti-tumor activity in acute myeloid leukemia[J]. Leukemia, 2012, 26(6): 1195-1202

[23] Shintani T, Klionsky DJ. Autophagy in health and disease: a double-edged sword[J]. Science, 2004, 306(5698): 990-995

[24] Baumgartner W, Golenhofen N, Weth A, et al. Role of transglutaminase 1 in stabilisation of intercellular junctions of the vascular endothelium[J]. Histochem Cell Biol, 2004, 122(1): 17-25

[25] Kabeya Y, Mizushima N, Ueno T, et al. LC3, a mammalian homologue of yeast Apg8p, is localized in autophagosome membranes after processing[J]. EMBO J, 2000, 19(21): 5720-5728

（上接第 670 页）

[12] Fulci G, Passer B. Analysis of HSV oncolytic virotherapy in organotypic cultures[J]. Methods Mol Biol, 2009, 542: 75-86

[13] 梁义, 林有坤, 梁铭. I 型单纯疱疹病毒感染的细胞培养系统的建立[J]. 现代生物医学进展, 2010, 10(5): 858-860
Liang Yi, Lin You-kun, Liang Ming, et al. Establishment of HSV-1 Infected Cell Culture System [J]. Progress in Modern Biomedicine, 2010, 10(5): 858-860

[14] Barkhaleva OA, Ladyzhenskaia IP, Vorob'eva MS, et al. Vitaherpavac is the first Russian herpes simplex virus vaccine obtained on the Vero B continuous cell line[J]. Vopr Virusol, 2009, 54(5): 33-37

[15] 刘甜甜. 人工合成胸腺肽 α1 对正常小鼠和免疫抑制荷瘤小鼠的免疫调节作用[J]. 现代生物医学进展, 2012, 12(30): 5827-5830
Liu Tian-tian. Immunomodulatory Effect of the Synthetic Thymus Peptide α 1 in Normal and Immunosuppressive Tumor Bearing Mice. [J]. Progress in Modern Biomedicine, 2012, 12(30): 5827-5830

[16] Thourani VH, Brat SS, Kennedy TP, et al. Nonanticoagulant heparin-inhibits NF-kappaB activation and attenuates myocardial reperfusion injury[J]. Am J Physiol Heart Circ Physiol, 2000, 278(6): H2084

[17] Borsig L, Wang L, Cavalcante MC, et al. Selectin blocking activity of a fucosylated chondroitin sulfate glycosaminoglycan from sea cucumber. Effect on tumor metastasis and neutrophil recruitment [J].J Biol Chem, 2007, 282(20): 14984-14991

[18] Piret J, Lamontagne J, Bestman-Smith J, et al. In vitro and in vivo evaluations of sodium lauryl sulfate and dextran sulfate asmicrobicides against herpes simplex and human immunodeficiency viruses[J]. J Clin Microbiol, 2000, 38: 110-119

[19] Hayashi K, Nakano T, Hashimoto M, et al. Defensive effects of a fucoidan from brown alga Undaria pinnatifida against herpes simplex virus infection[J]. Int Immunopharmacol, 2008, 8(1): 109-116

[20] Grayson MH, Cheung D, Rohlfing MM, et al. Induction of high-affinity IgE receptor on lung dendritic cells during viral infection leads to mucous cell metaplasia[J]. J Exp Med, 2007, 204(11): 2759-2769