In Vivo Antitumor Activity of Meretrix meretrix Glycopeptide

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Abstract.MGP_{0501}, one low-molecular-weight glycopeptide extracted from the sea mollusk Meretrix meretri, was investigated in this study for its effects on the growth of the tumor tissue and immune organs in xenografted sarcoma 180(S_{180}), Ehrlich's ascitic carcinoma (EAC) and Hepatocellular carcinoma (Heps). Cyclophosphamide was choose as the comparator for the immunologic function analysis. The results indicate that MGP_{0501} has not only treatment effect on S_{180}, EAC and Heps but also immunopotentiation effect on S_{180}, suggesting MGP_{0501} has a stable and broad spectrum antitumor activity.

Introduction

Among the molecular entities with antitumor activity that extracted from marine natural bioactive products[1,2], glycopeptides isolated from marine mollusk are of particular concern. Significant tumor inhibitation effects of the glycoproteins extracted from Aplysia, Patinopecten yessoensis and Meretrix meretrix had been reported previously[3-5], And the mechanism of action is complex and diverse, and the immune mechanism is particularly prominent in [6-12], so it is important to strengthen the anti tumor immunity in tumor prevention and treatment. In this study, we we explored the in vivo antitumor activity and stability of MGP_{0501}, one kind of glycopeptides which we firstly extracted from Meretrix meretrix in our laboratory. The results may serve as the basis for future antitumor research of this glycopeptide.

Materials and methods

MGP_{0501} was prepared by us in our laboratory. SPF grade ICR mice, 18~22 g (no limit of male or female), were purchased from Nanjing Bio-pharmaceutical Factory of QYH Biotech Co., Ltd(Certificate No.: SCXK Su 2002-0030). Pellets feed was provided by the animal laboratory of China Pharmaceutical University. S_{180}, Heps and EAC were provided by Jiangsu Cancer Hospital. Cyclophosphamide powder for injection, 200 mg/bottle, was manufactured by Jiangsu HengRui pharmaceutical Co., LTD, (lot: 020804). 10% formalin, HE staining fluid.YJ-875 Medical Clean Bench (Suzhou Purification Equipment Factory); XSZ-D Inverted microscope (Chongqing Optical Instrument Factory); TG-729B Lightning Analytical Balance (Shanghai Tianping Instrument).

Inhibitory effect of MGP_{0501} on xenografted sarcoma 180, Heps and EAC

50 ICR mice, no limit of male or female, were used for each tumor strains of S_{180}, Heps and EAC. The inoculation of these strains were performed as per the methods reported previously[13]. MGP_{0501} was administered via intravenous injection 24 hours after the inoculation then at a 2-day interval for another 4 times. All mice were sacrificed the second day of the last administration. The body weight, tumor weight and weight of immune organs (thymus, spleen and liver) were collected and the inhibitory effect, immunity index were calculated.[note]

Note: The effect of the drug on tumor was presented by inhibitory rate and calculated under the following formula: tumor weight inhibitory rate %＝(1－T/C)×100%; The effect on ascitic carcinoma was presented by increase of life span and calculated under the following formula: increase of life span %＝(T/C－1)×100%; immune organ index=immune organs weight(g)/body weight(g). All data were analyzed with T test.(T: treatment，C: control)

Inhibitory effect of MGP_{0501} on the mitotic figure of S_{180}

The seperated sarcoma got in the above were operated with a sequential process including fixing in 10% formalin, sampling, dehydration, wax infusion, embedding, cutting into 4-um slide and HE staining. The mitotic figure was evaluated with Nilcon E600 optical microscope (400*).

Results and Discussions

Inhibitory effect of MGP_{0501} on the xenografted sarcoma S_{180}

Compared with the control (natural saline), MGP_{0501} (6 and 2mg/kg) and Cy (20 mg /kg) could significant inhibit the growth of the xenografted sarcoma S_{180}. MGP_{0501} had no obvious influence on the body weight increase of the animals but Cy can significant decrease the body weight increase. The results were presented in Table 1.

Tab1. Effect of MGP_{0501} on S_{180} in mice by iv route（$\overline{X} \pm SD$）（n=10）

Groups	Dose mg·kg-1	Wt. of mice/g		Wt.of tumor /g	Inhibitory rate /%
		Pre dose	After dose		
Control		19.1±0.22	24.6±1.81	1.09±0.12	/
MGP_{0501}	6	19.0±0.17	24.1±1.24	0.33±0.07**	69.72
	2	19.4±0.23	24.4±1.53	0.50±0.20**	54.13
	0.6	19.2±0.15	24.9±1.71	0.75±0.16	31.19
Cy	20	19.3±0.10	20.4±1.58**	0.42±0.12**	61.47

*P<0.05 **P<0.01 compared with control

MGP_{0501} could increase the thymus index and spleen index of the animals, but had no obvious influence on liver index. The results were presented in Table 2.

Tab2. The index of internal organs in S_{180} mice（$\overline{X} \pm SD$）（n=10）

Groups	Dose $mg \cdot kg^{-1}$	Thymus index （g/g，$\times 10^{-3}$）	Spleen index （g/g，$\times 10^{-3}$）	Liver index （g/g，$\times 10^{-2}$）
Control		1.99±0.50	7.59±1.43	6.17±0.75
MGP_{0501}	6	3.52±0.80**	10.13±1.67**	5.82±0.39
	2	3.50±0.58**	9.95±1.65*	5.96±0.27
	0.6	3.24±0.81**	7.88±1.27	5.93±0.68
Cy	20	2.18±0.79	6.89±0.86	6.11±0.67

*P<0.05 **P<0.01 compared with control

Inhibitory effect of MGP_{0501} on the xenografted Heps

Compared with the control (natural saline), MGP_{0501} (6 and 2mg/kg) and Cy (20 mg /kg) could significant inhibit the growth of the xenografted Heps. MGP_{0501} had no obvious influence on the body weight increase of the animals but Cy can significant decrease the body weight increase. The results were presented in Table 3.

Tab3. Effect of MGP_{0501} on Heps in mice by iv route ($\overline{X} \pm SD$) (n=10)

Groups	Dose $mg \cdot kg^{-1}$	Wt.of mice/g		Wt.of tumor /g	Inhibitory rate /%
		Pre dose	After dose		
Control		20.46±0.18	27. 79±3.35	1.43±0.37	/
MGP_{0501}	6	20.08±0.11	27.18±1.80	0.48±0.21**	66.43
	2	20.44±0.15	25.30±1.20	0.60±0.19**	58.04
	0.6	20.08±0.09	26.74±1.44	0.72±0.29**	49.65
Cy	20	20.26±0.20	24.40±1.36*	0.56±0.14**	60.84

*P<0.05，**P<0.01 compared with control

MGP_{0501} could increase the thymus index and spleen index of the animals, but had no obvious influence on liver index. The results were presented in Table 4.

Tab 4. The index of internal organs in Heps mice ($\overline{X} \pm SD$) (n=10)

Groups	Dose mg·kg^{-1}	Thymus index （g/g，×10^{-3}）	Spleen index （g/g，×10^{-3}）	Liver index （g/g，×10^{-2}）
Control		2.42±0.36	6.97±2.07	7.25±1.47
	6	3.67±0.40**	8.95±1.58*	6.51±0.65
MGP $_{0501}$	2	3.51±0.91**	8.73±0.97*	6.27±0.61
	0.6	3.50±0.74**	8.98±1.69*	6.28±0.34
Cy	20	2.54±0.56	5.63±1.19	6.13±0.76

*P<0.05 **P<0.01 compared with control

Inhibitory effect of MGP$_{0501}$ on the life span increase of EAC mice

Compared with the control (natural saline), MGP$_{0501}$ (6 and 2mg/kg) and Cy (20 mg /kg) could modestly inhibit the growth of the xenografted Heps. MGP$_{0501}$ had no obvious influence on the body weight increase but could modestly increase the life span of the animals, while Cy could significant decrease the body weight increase. The results were presented in Table 5.

Tab 5. Life elongation effect of MGP$_{0501}$ on EAC mice by iv route($\overline{X} \pm SD$) (n=10)

Groups	Dose mg·kg^{-1}	Wt. of mice/g		Survival of days	Life elongation rate/%
		Pre dose	After dose		
Control		18.48±0.34	29.90±2.50	13.2±1.81	/
	6	18.40±0.19	30.18±2.54	15.3±1.95*	15.91
MGP$_{0501}$	2	18.52±0.17	29.77±2.64	14.3±13.4	8.33
	0.6	18.54±0.24	29.59±1.38	13.7±1.25	3.79
Cy	20	18.52±0.20	30.39±1.89	15.0±1.80*	13.64

*P<0.05 compared with control

Effect of MGP$_{0501}$ on the mitotie figure of the xenografted S$_{180}$ sarcoma cells

The mitosis process of the cell were consisted with a series of sequential process, including: the chromatins curve and condense into the chromosomes, the nuclear membrane disintegrates, the chromosomes move to the equatorial plane (metaphase plate), the sister chromatids of each chromosome separated and move to the opposite poles of the cell, the chromosomes decondense into chromatins, and the nuclear membrane integrates. Cell underthrough such mitosis process that observed under optical microscope is called a “mitotiefigure”, as showed in Figure 4-1.

Mitotie figure has been demonstrated an indicators which can reflect in some degree the proliferation level of the sarcoma[14]. In this study, we did an intuitionistic investigation to the inhibitory effect of MGP$_{0501}$ on the proliferation of the sarcoma S$_{180}$ through the counting to the mitotie figure under the optical microscope (400*) to the HE stained slide prepared from fresh tumor tissue of the xenografted S$_{180}$ sarcoma mice The results were presented in Table 6 and Figure 1&2. The results indicated that mitotic figure number in the tumor tissue slides of the MGP$_{0501}$ group was in significant negative correlation with the dose levels of MGP$_{0501}$, that is, with the decrease of the dose level, the number of the mitotic figure increased accordingly. Compared with that of the control/comparator cyclophosphamide, MGP$_{0501}$ showed an superior inhibitory effect, suggesting MGP$_{0501}$ can significantly inhibit the proliferation of the S$_{180}$ sarcoma.

Tab 6. Mitotiefigure counting of S$_{180}$ tissue section

Groups	Dose	Mitotiefigure
Control	mg·kg^{-1}	9
	6	0
MGP$_{0501}$	2	2
	0.6	4
Cy	20	2

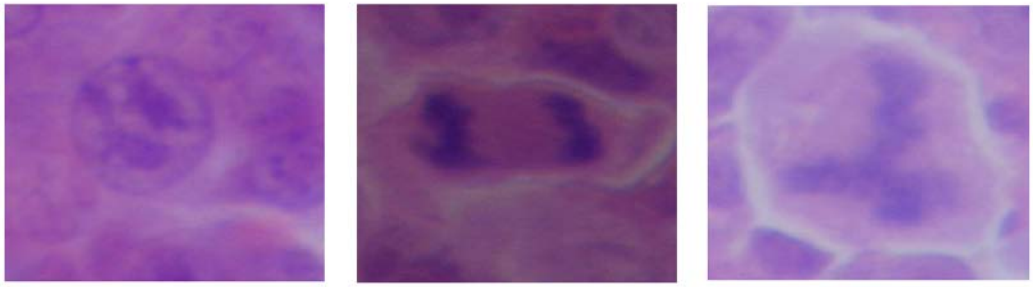

Fig 1. The mitotiefigure photograph by optical microscope

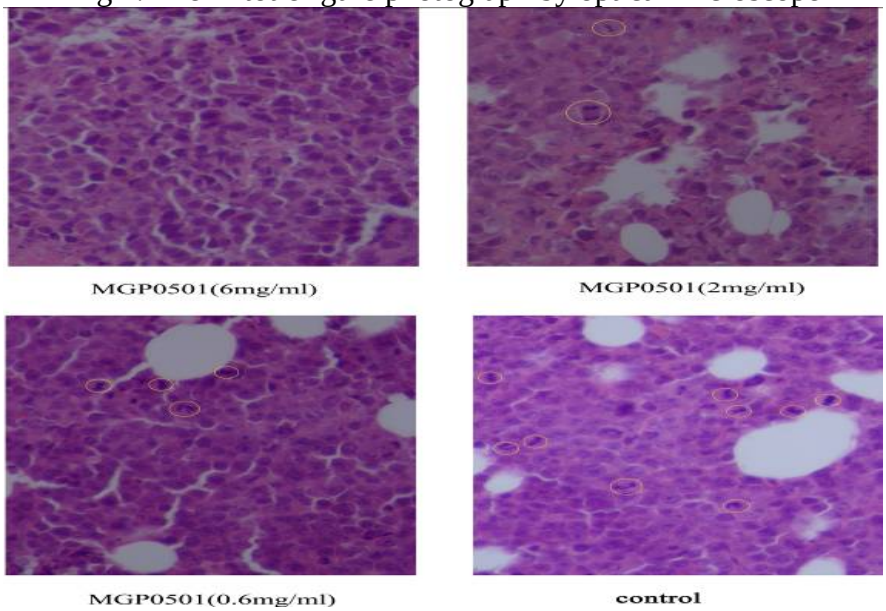

Fig 2. The mitotiefigure photograph of MGP_{0501} group by optical microscope

Conclusions

Through the above indicators, it can determine whether animals could inhibit under the tolerance dose drugs the role of the tumor. This experiment adopts the method of animal experiment to observe MGP_{0501} for solid tumor S_{180} and EAC Heps and ascites tumor growth in mice and the effect of the test results show that the MGP_{0501} (6, 2 mg/kg) group and Cy group have significant inhibition effect on S_{180} and Heps tumor growth, including MGP_{0501} 6mg/kg dose groups of S_{180} and Heps inhibitory rate respectively reached 69.72% and 66.43%. It showed that MGP_{0501} can inhibit the growth of S_{180} mice and Heps sarcoma is superior to the Cy group, but the EAC ascites tumor effect is not obvious. The prompt MGP_{0501} has strong antitumor effect, but different sensitivity to different nature of the tumor. MGP_{0501} on S_{180} tumor cell proliferation, meanwhile, the experimental results show that MGP_{0501} group can obviously restrain S_{180} tumor cell nucleus division, and the number of the fission like and give the dose was significantly negative correlation, the existing literature[15]confirmed split like can reflect the level of the proliferation of tumor cells to some extent, the way MGP_{0501} can inhibit tumor cell proliferation play a significant anti-tumor effect.

In addition, MGP_{0501} (concentration of 150 g/mL on normal mice spleen lymphocyte stimulation index was 1.21. It indicates that MGP_{0501} has tissue specificity, can selectively inhibit tumor cells, but had no effect on normal cells. At the same time, the mice immune organ index of examining the experimental results shows that different MGP_{0501} tumor-burdened mice dose group, the spleen (thymus)/weight index increased significantly, and there is a dose response relationship, the experimental results with the literature"Marine shellfish of polysaccharides and glycoprotein antitumor activity associated with the body's immune regulating function", explain MGP_{0501} relatively significant antitumor activity may be related to its improve immune function in mice.

References

[1] Schwarlsmann G. Marine organisms and other novel natural sources of new cancer drugs, J. Annals of Oncology. 11 (2000) 235.

[2] Ter H.E., Kowalski R.J., Hamel E, *et al.* Discodermolide, a cytotoxic marine agent that stabilizes microtubules more potentiall than taxol, J. Biochemistry. 35 (1996) 243.

[3] Kisugi J. Biopolymers from marine invertebrate, X. mode of action of an antibacterial glycolprotein, aplysianin E, from eggs of a sea hare, Aplysia kurodal, J. Chem Pharm Bull. 37 (1989) 3050.

[4] Takuma S, Hiroyki U, Noriko A. Antitumor activeity and immuneomodulatory effect of glycolprotein fraction from scallop Pationpecten yessoensis, J. Nippon suisan Gakkaishi. 53 (1987) 267-272.

[5] Huang Qingwen, Cheng Fangjin. Active material of marine shellfish research and progress[A]. The fifth china marine limnetic drugs academic development conference[C]. 1998.225.

[6] Simmons TL, Andrianasolo E, McPhail K, *et al*. Marine natural products as anticancer drugs. Molecular Cancer Therapeutics. 4 (2005) 333–342.

[7] Horti J, Juhasz E. Phase I study of TZT-1027, a novel synthetic dolastatin 10 derivative,for the treatment of patients with non-small cell lung cancer. Cancer Chemother Pharmacol. 62 (2008) 173-180.

[8] Akashi Y, Okamoto I. The novel microtubule-interfering agent TZT-1027 enhances the anticancer effect of radiation in vitro and in vivo. Br J Cancer. 96 (2007) 1532-1539.

[9] Hashiguchi N, Kubota T. TZT-1027 elucidates antitumor activity through direct cytotoxicity and selective blockade of blood supply. Anticancer Res. 24 (2004) 2201-2208.

[10] Shackelford R.E., Kaufmann W.K.. Cell cycle control,checkpoint mechanisms, and genotoxic stress. Environ Health Perspect. 107 (1999) 5-24.

[11] Maria D.F., Antonio D.L.. Cell cycle as a target of antineoplastic drugs. Curr Pharm Design. 16 (2010) 1417-1426.

[12] Burhans W.C., Heintz N.H.. The cell cycle is a redox cycle:linking phase-specific targets to cell fate. Free Radic Biol Med. 47 (2009) 1282-1293.

[13] Xu Shuyun, Bian Rulian,Chen Xiu. Pharmacological experirment method[M]. Beijing: People's Medical Publishing House(PMPH),1990:1424.

[14] LiHua, Meng Guang,Gong Xiyu, *et al.* Nasopharyngeal tumor cell proliferation and apoptosis, J. Acta Universitatis Medicinalis Anhui. 35(2000).

[15] Li Hua, Meng Gang, Xi Xiyu, *et al.* Relationship between proliferation and apoptosis in nasopharyngeal carcinoma cells, J. Journal of Medical University Of Anhui. 35(2000) 91-93.