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Effects of ginseng root saponins and ginsenoside Rb_1 on immunity in cold water swim stress mice and rats

LUO Yun-Man[1], CHENG Xiu-Juan, YUAN Wen-Xue[2]
(*Department of Pharmacology, Shenyang College of Pharmacy, Shenyang 110015, China*)

ABSTRACT The proliferation of splenic lymphocytes, the humoral immune response to sheep red blood cells, and the phagocytotic function of intraperitoneal macrophages were all suppressed by cold water (4℃) swim stress (CWSS) for 5 min in rats and for 3 min in mice. Meanwhile, the levels of serum corticosterone increased. Ginseng root saponins 100 $mg \cdot kg^{-1}$ or ginsenoside Rb_1 10 $mg \cdot kg^{-1}$ ip or ig completely antagonized the immunosuppression induced by CWSS, and inhibited the increase of serum corticosterone in CWSS rats, but increased the level of serum corticosterone further in CWSS mice.

KEY WORDS ginseng; saponins; swimming; stress; lymphocytes; macrophages; corticosterone; immunosuppression

Ginseng root saponins (GRS) showed many anti-stress effects related to the modulation of neuro-endocrine system in heat-stress mice and cold-stress mice and rats[1,2]. On the other hand, stress altered the responsiveness of immune system[3]. Thus we investigated the effects of GRS and ginsenoside Rb_1 on the immune functions of mice and rats under 4℃ cold water swim stress (CWSS).

MATERIALS AND METHODS

Agents GRS, a yellowish brown powder, was extracted from the root of *Panax ginseng* CA Meyer, and contained 84.2% saponins as determined by vanillin-H_2SO_4 reaction. Eleven spots of ginsenosides were shown on the silica gel thin-layer plate. Rb_1, a white powder (purity>98%), and GRS were provided by the Department of Phytochemistry, Shenyang College of Pharmacy. The dosage of GRS and Rb_1 were calculated according to the ginsenosides content. GRS and Rb_1 were dissolved in normal saline (NS). Sheep red blood cells (SRBC) were suspended in Alserver's solution. 3 (4, 5-Dimethylthiazol-2-yl) 2, 5-diphenyl terazolium bromide (MTT, Sigma). RPMI 1640 medium (Gibco). Concanavalin A (Con A, Sigma). Corticosterone (Organon).

Animals Kunming mice and $C_{57}BL/6$ mice, ♂, weighing $20 \pm s$ 1 g, and Wistar rats, ♂. Weighing $210 \pm s$ 26 g were used. The mice and rats were housed 10 per cage under 12-h light-dark cycle with free access to food and water. Stimulations of various non-experimental stressors, such as the changes of temperature and noise, were meticulously avoided.

Stress procedure For the stress groups mice and rats were put in CWSS for 3 and 5 min, respectively. Naive unstressed animals were used as the control. All experiments were performed during 8—10 AM.

Measurement of proliferation of splenic lymphocytes The MTT colorimetric assay[4] was used. Spleens were finely dissociated to single cell suspension and diluted to 1×10^7 cells $\cdot ml^{-1}$. Con A 5 $\mu g \cdot ml^{-1}$ in the splenocyte culture was the optimal concentration of mitogen. MTT 0.5 mg was added to 0.5 ml of RPMI 1640 culture during the last 4 h of a 72-h incubation.

Immunization of antigen and assay of humoral immune response Mice and rats were immunized with 4×10^8 SRBC (washed by NS for 3 times) which was injected ip. Four days later, the humoral immune response was assayed by determination of the serum hemolysin concentration (HC)[5], and by spectrophotometric quantitative determination of the function and the amount of anti-SRBC antibody secreted from the immune splenocytes[6]. It has been pointed out that using the quantitative hemolysis of SRBC-absorbance (QHS-A) and HC_{50} as the indices of humoral immune response is reliable[6,7].

The phagocytotic functions of intraperitoneal

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[1] Now in *Anhui Institute of Medical Sciences, Hefei 230061, China.*

[2] *Liaoning Institute of Materia Medica, Shenyang 110015, China.*

macrophages were assayed according to Ref 8.

Estimation of corticosterone The levels of serum corticosterone were determined by a spectrofluorometric method[9].

Statistical test employed *t* test.

RESULTS

Effects of CWSS on the immune functions in mice and rats The proliferative response of splenic lymphocytes to optimal concentration of Con A was suppressed pronouncedly within 48 h under CWSS, but returned to the level as that of the nonstress control mice 96 h after CWSS ($P>0.05$) (Tab 1, Fig 1).

The phagocytic % and the phagocytic index of intraperitoneal macrophages in rats and mice reduced after exposure to CWSS *vs* those of the nonstress control group (Tab 2). The indices of humoral immune responses (HC_{5c} and QHS-A) were also reduced under CWSS *vs* those of the nonstress control group (Tab 3).

Effects of GRS and Rb_1 on immune functions of mice and rats under CWSS GRS 100 mg·kg^{-1} ip 20 min before CWSS exhibited a protective action on the proliferation of splenic lymphocytes in CWSS mice ($P<0.01$, Fig 1), while ip GRS 100 mg·kg^{-1} or Rb_1 10 mg·kg^{-1} 30 min prior to CWSS exposure prevented the CWSS-induced suppression of the proliferation of splenic lymphocytes (PSL) in rats. As shown in Tab 1, the PSL-absorbance increased from 0.30±0.08 to 0.66 ±0.13 or 0.61±0.11 ($P<0.01$), approched the control level ($P>0.05$). But the addition of various concentration of GRS to the splenocyte cultures showed no protective effects on the suppression of the splenic lymphocytes mitogenic responsiveness.

To avoid the effects of ip on the intraperitoneal macrophages, rats and mice were given ig GRS 100 mg·kg^{-1} 45 and 30 min before CWSS, respectively. The reductions of the phagocytic % and the phagocytic index of macrophages in CWSS rats and mice were completely eliminated by GRS (Tab 2).

Tab 1. Effects of ginseng root saponins (GRS) and ginsenoside Rb_1 (Rb_1) on proliferation of splenic lymphocytes (PSL) in cold water swim stress (CWSS) rats. $n=6$, $\bar{x}\pm s$. [a]$P>0.05$, [b]$P<0.05$, [c]$P<0.01$ *vs* NS−CWSS. [d]$P>0.05$, [f]$P<0.01$ *vs* NS + CWSS.

Drug	ip mg·kg^{-1}	*In vitro* μg·ml^{-1}	CWSS	PSL-absorbance
NS	—		−	0.69±0.08
NS	—		+	0.30±0.08[c]
GRS	100		+	0.66±0.13[af]
Rb_1	10		+	0.61±0.11[af]
GRS		0.1	+	0.39±0.07[bd]
		1	+	0.41±0.09[bd]
		10	+	0.38±0.10[bd]
		100	+	0.39±0.10[bd]

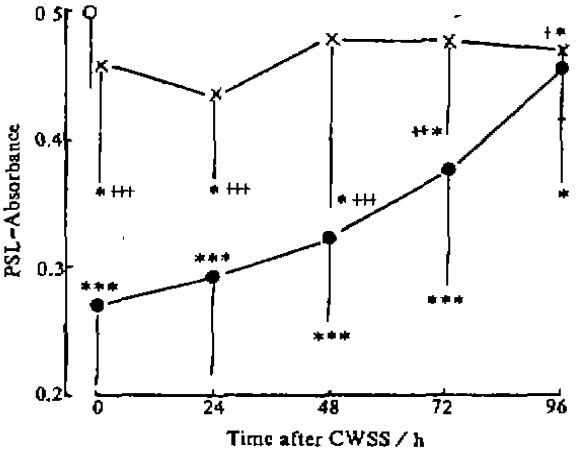

Fig 1. Effects of ip ginseng root saponins 100 mg·kg^{-1} (×) on proliferation of splenic lymphocytes (PSL) in CWSS mice. $n=9$, $\bar{x}\pm s$. *$P>0.05$, ***$P<0.01$ *vs* Saline+nonstress (○). +$P>0.05$, ++$P<0.05$, +++$P<0.01$ *vs* Saline+CWSS (●).

The suppressions of humoral immune responses induced by CWSS partly attenuated by ip GRS 50 mg·kg^{-1} (in mice) and completely prevented by ip GRS 100 mg·kg^{-1} (in mice and rats) and by ip Rb_1 10 mg·kg^{-1} (in mice). Drugs were given 30 min in rats and 20 min in

Tab 2. Effects of ginseng root saponins (GRS, 100 mg·kg^{-1}, ig) on phagocytic function of intraperitoneal macrophages in cold water swim stress (CWSS) rats and mice. $n=10$ mice or 8 rats. $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* NS−CWSS. $^dP<0.05$, $^eP<0.01$ *vs* NS+CWSS.

Animal	Drug	CWSS	Phagocytosis Rate/%	Phagocytosis Index
Mice	NS	−	11.6±3.9	0.17±0.06
	NS	+	6.3±2.1^c	0.10±0.04^c
	GRS	+	10.4±2.3^{ad}	0.16±0.05^{ae}
Rats	NS	−	15.3±5.5	0.22±0.07
	NS	+	8.7±3.9^b	0.11±0.05^c
	GRS	+	14.9±5.5^{ae}	0.25±0.12^{ad}

mice before CWSS (Tab 3).

Effects of GRS and Rb_1 on serum corticosterone in CWSS mice and rats The levels of serum corticosterone of mice and rats exposured to CWSS were higher than those of nonstress control group ($P<0.01$). GRS 100 mg·kg^{-1} or Rb_1 10 mg·kg^{-1} ip 20 min before CWSS increased the levels of serum corticosterone further in CWSS mice. The corticosterone level in the GRS (ip 100 mg·kg^{-1}) group in CWSS rats showed a significant decrease *vs* CWSS control group ($P<0.01$). However, it was still higher than that in the nonstress control group ($P<0.05$) (Tab 4).

Tab 4. Effects of ip ginseng root saponins (GRS) and ginsenoside Rb_1 (Rb_1) on serum corticosterone in cold water swim stress (CWSS) mice and rats. $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* NS−CWSS. $^dP>0.05$, $^eP<0.01$ *vs* NS+CWSS.

Animal	Drug	n	CWSS	mg·kg^{-1}	Corticosterone/ μmol·L^{-1}
Mice	NS	10	−	—	0.99±0.15
	NS	8	+	—	1.51±0.43^c
	GRS	8	+	100	2.54±0.90^{ad}
	Rb_1	10	+	10	2.55±0.96^{ad}
Rats	NS	7	−	—	1.15±0.37
	NS	7	+	—	2.71±0.48^c
	GRS	8	+	100	2.10±0.28^{ad}

DISCUSSION

It has been noted that stress induced some diseases and exacerbates the progression of physical illness. Experimental and clinical studies demonstrated that it is due to the alterations of immune system induced by both laboratory and natural stressors[10]. The present study demonstrated that administration of

Tab 3. Effects of ip ginseng root saponins (GRS) and ginsenoside Rb_1 (Rb_1) on humoral immune responses in cold water swim stress (CWSS) mice and rats. $n=8$ rats or 10 mice. $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* NS−CWSS. $^dP>0.05$, $^eP<0.05$, $^fP<0.01$ *vs* NS+CWSS. HC=hemolysin concentration. QHS-A=quantitative hemolysis of SRBC-absorbance.

Animal	Drug	mg·kg^{-1}	CWSS	HC_{50}	QHS-A
Mice	NS	—	−	249±76	0.34±0.12
	NS	—	+	117±33^c	0.14±0.05^c
	GRS	50	+	146±23^{be}	0.23±0.10^{be}
	GRS	100	+	218±40^{af}	0.30±0.16^{af}
	Rb_1	10	+	256±90^{af}	0.32±0.12^{af}
Rats	NS	—	−	312±81	0.38±0.12
	NS	—	+	147±49^c	0.16±0.05^c
	GRS	100	+	283±65^{af}	0.35±0.11^{af}

GRS or Rb_1 completely prevented the immune suppression induced by CWSS in mice and rats. Our results suggested that GRS and ginsenoside Rb_1 can be used clinically to treat the immunosuppression induced by some psychological or physiological stressors.

Previous studies have shown that the stress-induced suppression of antibody responses and function of blood lymphocytes was due to the release of corticosterone in stressed mice and rats[11,12]. Similar results were observed in our experiments. Our results also indicated that the inhibition of the serum corticosterone release played an important role in the protective effects of GRS and Rb_1 on the immunosuppression in CWSS rats. However, in stressed mice, GRS and Rb_1 failed to attenuated the increase of serum corticosterone, on the contrary the hormone level was further elevated. It is suggested that the action of GRS on the release of corticosterone is not its only mechanism of regulating the immune function. Although corticosteroides have long heen considered to be the primary mediator of stress-induced modulation of immunity, we postulated that a corticosteroid-independent mechanism may be responsible for the protective effects of GRS and Rb_1 on the suppressive immune function in CWSS mice.

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人参根总皂甙和人参皂甙 Rb_1 对冷水游泳应激鼠免疫功能的作用

罗运满，程秀娟，袁文学 R965.2

(沈阳药学院药理教研室，沈阳110015，中国)

A **摘要** 在4℃冷水中，大鼠游泳5 min，小鼠游泳3 min，两者的脾 T 淋巴细胞增殖反应，腹腔巨噬细胞吞噬功能和抗绵羊红细胞抗体反应均被显著抑制；同时血清皮质酮升高。人参根总皂甙 ip 或 ig 100 $mg \cdot kg^{-1}$ 或人参皂甙 Rb_1 ip 10 $mg \cdot kg^{-1}$，完全拮抗了应激所致的免疫功能抑制，并降低应激大鼠血清皮质酮，但进一步升高应激小鼠血清皮质酮水平。

关键词 人参；皂甙；游泳；应激；淋巴细胞；巨噬细胞；免疫抑制；皮质酮