ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 Aug; 21 (8): 765 - 768
http://www.chinaphar.com

Immunosuppressive effects of rubidatum in mice

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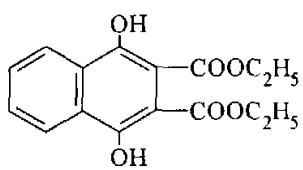
KEY WORDS rubidatum; phagocytosis; hemolysis; delayed hypersensitivity; lymphocyte transformation; immunosuppression; spectrophotometry

ABSTRACT

AIM: To study the effect of rubidatum (Rub) on immune function in normal mice. **METHODS**: Serum lysozyme concentration (SLC) was measured using micrococcus lysodiekticus as a substrate. Delayed type hypersensitivity (DTH) was determined by measuring the thickness of the right hind footpad 24 h after the injection of 1×10^8 washed sRBC (50 μL 10 % sRBC). Serum hemolysin concentration was determined by OD measuring at A_{540} after the serum was treated with 2-mercaptoethanol. Phagocytic function of peripheral leukocyte (Leu) were determined by the incorporated radioactivity of [^{3}H]TdR. The hemolytic activity of plaque forming cell (PFC) was determined by measuring the lymphocyte-mediated hemolysis of sheep red blood cell *in vitro*. T- and B-lymphocyte transformation (TLT and BLT) were induced by phytohemagglutinin (PHA) and lipopolysaccharide (LPS) respectively and measured by the incorporation of [^{3}H]TdR. **RESULTS**: Rub 125, 500, 2000 mg·kg^{-1}·d^{-1} *po* to BALB/c (or NIH) mice decreased the SLC; inhibited the phagocytosing functions of peripheral leukocytes; diminished the hemolytic activity of PFC; decreased the HC_{50}; inhibited the DTH reaction; and showed inhibitory effects on TLT and BLT. **CONCLUSION**: Rub has immunosuppressive effects on immune system in mice by affecting *MΦ*, T, and B lymphocyte, which suggests that Rub has inhibitory effects on both nonspecific and specific immune function.

INTRODUCTION

Rubia cordifolia L is one of Chinese traditional herbs clinically used to alleviate rheumatoid arthritis, hepatitis, and neurodermatitis, etc. Rubidatum (Rub), extracted from Radix *Rubia cordifolia* L, has been proved to stimulate the hemopoiesis (leukocytosis) both experimentally and clinically. Rub has scavenging effects on oxygen radicals and hydroxyl radicals produced by phorbol myristate acetate (PMA)-induced stimulation of polymorphonuclear leukocytes (PML)[1]. It is not known whether Rub has any effect on the immune functions. In the present study, the immunopharmacologic effects of Rub were studied in mice.



Rubidate (Rubidatum)

MATERIALS AND METHODS

Rub A light yellow powder, purity ≥ 99%, insoluble in water, made as a suspension by 5 % amylogen-NS before use, was provided by Shanghai № 2 Pharmaceutical Co.

Mice NIH and BALB/c inbred mice of either sex, Grade Ⅱ (№ 001), 6 - 8 wk, weighing 20 ± *s* 3 g, were from the Experimental Animal Center, Xinjiang Medical University.

Reagents Cyclophosphamide (Cy) (Shanghai № 12 Pharmaceutical Co); dexamethasone (Dex) (Wuxi Pharmaceutical Factory); lysozyme (standard sample) (Shanghai Institute of Biochemistry, Chinese Academy of Sciences); phytohemagglutinin (PHA) (Difco Lab, USA); lipopolysaccharide (Sigma Chemical Co); [^{3}H] thymidine (Shanghai Institute of Nuclear Research) were used. Medium RPMI 1640 was from J R Scientific Inc, USA; All RPMI 1640 containing media were supplemented with 10 % newborn bovine serum (NBS, from Xin-

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Phn 86-991-662-5448, ext 2366.
Received 1999-09-14 Accepted 2000-04-18

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 Aug; 21 (8)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

jiang Cattle Farm), HEPES buffer 10 mmol/L (Fluka AG, Buchs, Switzerland), benzylpenicillin 100 IU/mL (Suzhou Second Pharmaceutical Factory, Suzhou, China), and streptomycin 100 mg/L (Shanghai Fourth Pharmaceutical Factory, China). PH was adjusted to 7.2. Staphylococcus albus and Micrococcus lysodiekticus were from Department of Microbiology, Xinjiang Medical University.

Serum lysozyme concentration (SLC) Blood samples were obtained from the retroorbital plexus of BALB/c mice and allowed to clot for 1 h. SLC was measured using Micrococcus lysodiekticus as a substrate[2].

Delayed hypersensitivity (DTH) Sheep red blood cell (sRBC) 10 % 50 μL was injected into the left hind footpad sc. Hypersensitivity was measured as the increase in right hind footpad thickness 24 h after injecting an eliciting dose of 1×10^8 washed sRBC (50 μL 10 % sRBC) on d5[3].

Serum hemolysin concentration 50 % (HC_{50}) Serum samples from all the mice were obtained from clotted samples, and serially diluted (1:80) in normal saline (NS). Serum of mice 0.5 mL, 5 % sRBC 0.5 mL were mixed with serum of guinea pig (1:5) 0.5 mL and NS 0.5 mL. HC_{50} was calculated by A_{540}[4].

Phagocytic function of peripheral leukocyte (Leu) An overnight sub-culture of Staphylococcus albus was diluted in RPMI 1640 medium and the optical density was adjusted to 0.5 at 620 nm in a spectrophotometer, then it was diluted(1:200). Heparinized venous blood were obtained from NIH mice. Each tube contained 0.1 mL staphylococci, 0.1 mL peripheral blood, and 0.8 mL RPMI 1640 medium (without benzylpenicillin and streptomycin). The tubes were incubated for 2 h at 37 ℃. [^{3}H] Thymidine 37 KBq per tube was added during the last hour. The cultures were then harvested and the incorporated radioactivity was determined by a liquid scintillation counter[5]. The data were calculated as Phagocytosis Index (PI). PI (%) = 1 − (Experimental tubes Bq/Control tubes Bq).

Hemolytic activity of plaque forming cell (PFC) Using a spectrophotometer[6] 0.5 mL of an appropriately diluted spleen cell suspension was mixed with an equal volume of thrice washed sRBC (0.2 %) and 0.5 mL of 1/10 diluted guinea pig serum. The mixtures were incubated at 37 ℃ for 1h in RPMI 1640 medium, then centrifuged, and the supernatant was analysed at 413 nm.

T-lymphocyte transformation (TLT) After giving Rub *po*, 0.1 mL peripheral blood was obtained from each of the BALB/c mice and was dispensed into a 96-well flat bottom plate containing PHA (0.5 g/L) in a total volume of 0.2 mL in RPMI 1640. The plate was incubated for 56 h at 37 ℃ in 5 % CO_2 and [^{3}H] TdR 37KBq per well was added during the last 8 h. All experiments were performed at least twice. Lymphocyte proliferation was expressed by the incorporated radioactivity of [^{3}H] TdR. The data were then described as Stimulation Index (SI = experimental well Bq/PHA-free well Bq).

B-lymphocyte transformation (BLT) Lymphocyte proliferation assay was performed as described above. Only LPS, instead of PHA, was used.

Statistical analysis Data were expressed as $\bar{x} \pm s$. Evaluation was accomplished using *t*-test.

RESULTS

Effects of Rub on SLC, DTH, and HC_{50} Rub 500 and 2000 $mg \cdot kg^{-1} \cdot d^{-1}$ decreased SLC in BALB/c mice, inhibited DTH to sRBC in mice. Rub 150 and 750 $mg \cdot kg^{-1} \cdot d^{-1}$ abated the production of HC_{50} in sRBC of NIH mice. The inhibitory effects increased in a concentration-dependent manner (Tab 1).

Tab 1. Effects of *po* Rub and Dex (or Cy) given daily for 7 d on SLC, DTH and HC_{50} in mice. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* ANS; $^dP > 0.05$, $^eP < 0.05$, $^fP < 0.01$ *vs* Dex (or Cy). ANS = 5 % amylogen, 0.5 mL per mouse in control.

	Dose/ $mg \cdot kg^{-1} \cdot d^{-1}$	*n*	$\bar{x} \pm s$
	Serum lysozyme concentration/ $mg \cdot L^{-1}$		
ANS		8	14 ± 5
Dex	5	8	5 ± 2^c
Rub	125	8	11 ± 5^{af}
	500	8	7 ± 3^{cd}
	2000	8	6 ± 2^{cd}
	Increased thickness of footpad/mm		
ANS		8	0.19 ± 0.04
Dex	5	9	0.10 ± 0.06^c
Rub	125	9	0.16 ± 0.06^{ae}
	500	7	0.12 ± 0.06^{cd}
	2000	10	0.12 ± 0.07^{cd}
	50 % Hemolytic concentration of serum hemolysin		
ANS		8	336 ± 103
Cy	10	8	19 ± 6^c
Rub	30	8	254 ± 73^{af}
	150	8	238 ± 111^{bf}
	750	8	117 ± 71^{cf}

Effect of Rub on ability of leukocyte phagocytosis None of blood groups' Bq value was greater than that of ANS group's, showing the Leu phagocytosing staphylococcus activity was high. Rub group's Bq value were between ANS and Cy, indicating that Rub inhibited the Leu phagocytosis (Tab 2).

Tab 2. Effects of *po* Rub and Cy given daily for 7 d on ability of peripheral Leu phagocytosing Staphylococcus albus with [^{3}H] TdR incorporation in NIH mice. $n = 10$ mice. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* ANS. $^dP > 0.05$, $^fP < 0.01$ *vs* Cy.

	mg·kg^{-1}·d^{-1}	10^{-6} × Radioactivity/Bq	Phagocytosis index
No blood		19.4 ± 2.2	
ANS		8.6 ± 1.2	55
Cy	10	14.4 ± 1.1^c	26^c
Cy + Rub	10 + 500	10.6 ± 1.4^{bf}	46^{bf}
Rub	500	11.9 ± 1.6^{cf}	39^{cf}
	2000	13.0 ± 1.1^{cd}	33^{cd}

Effect of Rub on the PFC response to sRBC in mice Rub inhibited the hemolytic ability of spleen PFC response to sRBC in mice (Tab 3).

Effect of Rub on LT in BALB/c mice Rub showed inhibitory effects on lymphocyte proliferation induced by PHA or LPS in a dose-dependent manner (Tab 4).

Tab 3. Effect of *po* Rub and Cy on hemolytic ability of spleen plaque forming cells in mice. $n = 10$ mice. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* ANS. $^fP < 0.01$ *vs* Cy.

	mg·kg^{-1}·d^{-1} × 7	10^{-1} × Absorbance
ANS		7.9 ± 0.4
Cy	10	3.5 ± 2.4^c
Rub	125	7.8 ± 0.2^{af}
	500	7.5 ± 0.4^{bf}
	2000	7.0 ± 0.9^{cf}

DISCUSSION

As macrophage activation has been associated with an elevation in serum lysozyme, the present studies were designed to evaluate the macrophage function by detecting SLC. Rub decreased SLC and inhibited the phagocytosing function of peripheral leukocytes probably by scavenging $\cdot O_2^-$ and $\cdot OH^-$ produced in polymorphonuclear "breath burst"[1]. In the present study, Rub diminished the hemolytic activity of PFC and the HC_{50}; inhibited DTH; showed inhibitory effects on [^{3}H] TdR incorporation during murine peripheral T- and B-LT, indicating that Rub inhibited the specific immune function. In conclusion, Rub showed immunosuppressive effects on immune system in mice by affecting MΦ, Leu, T and B lymphocytes. This immunosuppression may be related to the phenolic structure of Rub. Phenolic antioxidants reportedly have immunosuppressive effects on the immune response[7]. Therefore, Rub may be the main component of *Rubia cordifolia* L which alleviates immune diseases such as rheumatoid arthritis, hepatitis, and neurodermatitis, etc.

Tab 4. Effects of Rub on PHA (0.5 g/L) or LPS (0.1 g/L) induced murine peripheral blood lymphocyte transformation of [^{3}H] TdR incorporation *in vitro*. $n = 8$ mice. $\bar{x} \pm s$. $^cP < 0.01$ *vs* control.

	mg·kg^{-1}·d^{-1}	PHA	LPS/g·L^{-1}	10^{-6} × Radioactivity/Bq	Stimulation index	Suppression/%
ANS				0.7 ± 0.2^c		
ANS		0.5		17.2 ± 0.5	21.6	
Rub	125	0.5		12.8 ± 1.2^c	16.2	25
	500	0.5		11.7 ± 1.7^c	14.2	34
	2000	0.5		10.2 ± 1.1^c	12.9	40
ANS				2.1 ± 1.2^c		
ANS			0.1	13.7 ± 0.5	6.6	
Rub	125		0.1	11.4 ± 0.7^c	5.5	16
	500		0.1	9.2 ± 1.4^c	4.5	32
	2000		0.1	8.1 ± 0.9^c	3.9	41

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 Aug; 21 (8)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

ACKNOWLEDGMENTS To Mr QIN Fei, Xinjiang Medical University, for providing us Staphylococcus albus and Micrococcus lysodiekticus.

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茜草双酯对小鼠免疫功能的抑制作用

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关键词 茜草双酯; 吞噬作用; 溶血; 迟发性超敏感性; 淋巴细胞转化; 免疫抑制; 分光光度测定法

目的: 研究茜草双酯对正常小鼠免疫功能的影响. **方法**: 采用免疫药理学常用方法即血清溶菌酶含量的测定, 迟发型超敏反应的测定, 血清溶血素的测定, [^{3}H]TdR 参入的小鼠全血白细胞吞噬能力的测定, 鼠脾空斑形成细胞溶血能力的测定, T 和 B 淋巴细胞转化能力的测定. **结果**: 茜草双酯 125, 500, 2000 mg·kg^{-1}·d^{-1}降低血清溶菌酶含量和全血白细胞吞噬功能; 使 PFC 溶血能力和 HC_{50}产生减少; 抑制 DTH 反应; 体内给药体外测定, 抑制[^{3}H]TdR 参入的 PHA 与 LPS 诱导的淋巴细胞转化. 以上作用呈一定的剂量依赖性. **结论**: 茜草双酯对正常小鼠特异和非特异性免疫功能均有不同程度的抑制作用.

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