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Effect of brucine on mouse nonspecific immune responses[1]

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AIM: To evaluate the effect of brucine (Bru) ip at analgesic doses on the nonspecific immune responses in normal and cyclophosphamide (Cyc)-treated mice. **METHODS**: The clearance of charcoal particles, the immune organ weights, the white blood cell counts in peripheral blood, the phagocytosis to neutral red (NR) of PMØ and its IL-1 production *in vitro* were tested. **RESULTS**: In normal mice, Bru slightly enhanced the clearance of charcoal particles, the phagocytosis of PMØ, IL-1 production, the immune organ weights and the WBC counts ($P>0.05$), whereas in Cyc-induced subnormal immunity model mice, Bru greatly enhanced these nonspecific immune responses ($P<0.05$ or $P<0.01$). The effects of Bru were most marked ip at 10 mg·kg^{-1} *in vivo* or 0.1 – 10 mg·L^{-1} *in vitro*. **CONCLUSION**: Bru ip at an analgesic dosage has dose- and function-dependent immunoregulatory effects.

Most of the widely used analgesics may cause physical dependence and immunodeficiencies, especially cellular immunodeficiencies. Because most of the malignant tumors are associated with cellular immunodeficiencies, the pain-controlling for patients with advanced cancer has been a challenge to clinicians and pharmacologists.

Brucine (Bru), an alkaloid extracted from seeds of *Strychnos nux-vomica* L., was used as a chemical reagent or a central stimulant in western countries. In China, it was orally administrated in treating chronic bronchitis and locally used in curing some bacterial infections and mycosis[1]. Bru had a tumoricidal effect on sarcoma 180 *in vitro*[1], Bru at safe dosage showed analgesic effect that sustained much longer than pethidine, but caused no physical dependence[2]. Although the water-decoction or the compound prescription containing this herb had a dose-dependent immunoregulatory effect, which components in this herb took the key role remains unknown[3]. In the present study, effects of Bru on the nonspecific immune responses in normal and cyclophosphamide (Cyc)-treated mice were tested.

MATERIALS AND METHODS

Mice Kunming mice, BALB/c mice, ♂ ♀, weighing 20± *s* 2 g, were purchased from Laboratory Animal Center of Henan Medical University. The ♀ were housed apart from the ♂. The mice were given rodent chow and tapwater *ad lib*.

Chemicals Bru purchased from Serva Co, USA was prepared into sulfate solution before use. Cyc was produced by Shanghai Twelfth Pharmaceutical Works, and was dissolved in normal saline (NS). Concanavalin A (Con A), lipopolysaccharides (LPS) and RPMI 1640 medium were bought from Sigma Chemical Co, USA. 3-(4,5-dimethyl thiazoal-2-yl)-2,5-diphenyl tetrazolium (MTT) was from Fluka Co, Switzerland. Recombined human interleukin-1 (rhIL-1) was from Academy of Military Medical Sciences, Beijing. Yidege ink was from Beijing Yidege Ink Factory.

Clearance of charcoal particles The assay was performed[4] with modification. Kunming mice were injected iv with 1:10 diluted Yidege ink 10 mL·kg^{-1}. At 0 min (T_1) and 15 min (T_2), blood 30 μL was taken from retro-ocular venous plexus and resolved in 0.1 % Na_2CO_3 solution 3 mL. The absorbance (A) was measured at 680 nm in 721 spectrophotometer. The clearance rate K and clearance index α were calculated:

$K=(\lg A_1-\lg A_2)/(T_2-T_1)$; $\alpha=\sqrt[3]{K}\cdot W/WLS$

(W: weight of mouse; WLS: liver weight + spleen weight)

WBC count WBC in blood taken from retro-ocular venous plexus were counted[5].

Immune organ weights After blood was taken from retro-ocular venous plexus, mice were killed by cervical vertebral dislocation. Weights of spleen, thymus, and liver were used for

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calculation of spleen index (SI), thymus index (TI), and clearance index (α)[4]

Phagocytosis to neutral red (NR) of PMØ The assay was performed[16], and the phagocytosis to NR was evaluated by the absorbance at 492 nm (A_{492}).

Production of IL-1 by PMØ The production and assay of IL-1 were performed[16].

RESULTS

Clearance of charcoal particles In normal mice, Bru increased the clearance rate K ip at 5 or 10 mg·kg^{-1} ($P < 0.01$) whereas it had insignificant influence on the clearance index α ip at 5, 10, or 20 mg·kg^{-1} ($P > 0.05$). Cyc slightly decreased the K value ($P > 0.05$), greatly decreased the α value, which were dose-dependently diminished by Bru at 5, 10, or 20 mg·kg^{-1} ($P < 0.01$) (Tab 1).

Tab 1. Effects of brucine (Bru) on clearance of charcoal particles in normal and Cyc-treated mice. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* control; $^fP < 0.01$ *vs* Cyc.

mg·kg^{-1} qd×7	n	K	α
Saline	8	0.043±0.008	7.0±0.3
Bru 5	8	0.054±0.012^c	7.3±0.6^a
Bru 10	8	0.047±0.005^c	7.4±0.6^a
Bru 20	8	0.041±0.007^a	7.2±0.7^a
Cyc (25×3)	7	0.036±0.007^a	6.4±0.6^b
Cyc + Bru 5	8	0.054±0.007^f	7.4±0.6^f
Cyc + Bru 10	8	0.056±0.016^f	7.4±0.8^f
Cyc + Bru 20	8	0.053±0.008^f	7.3±0.4^f

Immune organ weights and WBC In normal mice, Bru had insignificant influence on SI, TI, and WBC ($P > 0.05$). On the SI which was slightly ($P > 0.05$) decreased by Cyc, Bru had insignificant influence, but on the greatly decreased TI ($P < 0.05$) and WBC ($P < 0.01$), Bru reduced them at 5 and 10 mg·kg^{-1}, respectively ($P < 0.05$ or $P < 0.01$) (Tab 2).

PMØ phagocytosis to NR *in vitro* *In vitro*, Bru had insignificant influence on the PMØ phagocytosis to NR at 0.1, 1, 10, or 100 mg·L^{-1} ($P > 0.05$), but greatly inhibited the phagocytosis at 1 g·L^{-1} ($P < 0.01$) (Tab 3).

IL-1 production of PMØ *in vitro* Bru had insignificant influence on IL-1 production of PMØ at 0.1, 1, 10, 100, or 1000 mg·L^{-1} ($P > 0.05$) (Tab 3).

Tab 2. Effects of Bru on immune organ weights and WBC in normal and Cyc-treated mice. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* control; $^dP > 0.05$, $^eP < 0.05$, $^fP < 0.01$ *vs* Cyc.

mg·kg^{-1} qd×7	n	SI (g·kg^{-1})	TI (g·kg^{-1})	$10^{-9}\times$ WBC·L^{-1}
Saline	8	4.9±0.8	2.8±0.9	10.0±2.4
Bru 5	8	5.2±0.6^a	3.1±0.7^a	9.3±4.3^d
Bru 10	8	5.5±0.7^d	3.2±0.7^a	9.2±1.9^e
Bru 20	8	4.6±0.3^a	3.0±1.0^d	12.1±3.5^d
Cyc (25×3)	7	4.2±1.0^a	1.7±0.6^b	5.1±1.5^c
Cyc + Bru 5	8	4.1±0.6^d	2.3±0.4^e	7.0±2.3^d
Cyc + Bru 10	8	4.8±1.3^d	2.4±0.8^d	8.0±1.4^f
Cyc + Bru 20	8	3.9±0.5^d	2.2±0.7^d	8.2±3.7^d

Tab 3. Effect of Bru on phagocytosis to NR of PMØ and its IL-1 production *in vitro*. $n = 8$, $\bar{x} \pm s$. $^aP > 0.05$, $^cP < 0.01$ *vs* control.

Bru mg·L^{-1}	Phagocytosis ($A_{492-630}$)	IL-1 production (A_{570})
Control	0.39±0.08	0.23±0.02
0.1	0.37±0.10^a	0.22±0.06^a
1	0.47±0.10^a	0.27±0.06^a
10	0.43±0.10^a	0.26±0.04^a
100	0.32±0.08^a	0.26±0.05^a
1000	0.18±0.03^c	0.26±0.05^a

DISCUSSION

The present results showed that Bru at an analgesic dosage had function- and dose-dependent regulatory effects on mouse nonspecific immune responses. In normal mice, Bru had insignificant influence on the immune organ weights and WBC counts, had only few influences on the clearance of charcoal particles. Bru + Cyc had insignificant influence on the spleen index that was not decreased by Cyc, either. Bru greatly increased the Cyc-decreased clearance rate, clearance index, thymus index, and WBC counts. This suggested that Bru had a function-dependent regulatory effect on the nonspecific immune responses. The regulatory effects are also dose-dependent, because they were most significant except on WBC count when Bru was injected ip at 10 mg·kg^{-1}. The analgesic ED_{50} of Bru (ip) is 7 – 11

mg·kg^{-1}[7], hence the immunoregulatory doses were in the range of its analgesic doses.

The present results also showed that *in vitro*, Bru had insignificant influences on macrophage functions. This indicated that perhaps Bru had no direct influences on the nonspecific immune responses. Further studies are needed to determine whether the indirect effects *via* other modulatory cells (eg T helper cells, T suppressor cells) or cytokines (eg interleukins, TNF) in immune system, or *via* the neuroendocrineimmune network.

In conclusion, at an analgesic dosage, Bru has function- and dose-dependent regulatory effects on mouse nonspecific immune responses. The precise mechanism should be further studied.

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布鲁生对小鼠非特异免疫功能的影响[1]

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关键词 10,11-二甲氧基士的宁; 免疫佐剂类; 天然免疫; 白细胞介素-1; 非麻醉镇痛药; 腹腔巨噬细胞; 吞噬作用 布鲁生 免疫功能

目的: 观察镇痛有效剂量的布鲁生(Bru)对正常小鼠及 Cyc 所致免疫低下小鼠非特异免疫功能的影响. **方法**: 测定小鼠碳粒廓清能力、免疫器官重量及外周血 WBC, 小鼠 PMØ 吞饮中性红及产生 IL-1 能力. **结果**: Bru 对正常鼠碳粒廓清能力、免疫器官重量、外周血 WBC、PMØ 吞噬功能及产生 IL-1 能力仅有轻微促进作用($P > 0.05$), 而对被 Cyc 降低的上述非特异免疫反应, Bru 呈剂量依赖性的增强作用($P < 0.05$ 或 $P < 0.01$), 作用以 10 mg·kg^{-1}时最好. **结论**: Bru 在镇痛有效剂量 ip 时, 对小鼠非特异免疫功能有剂量依赖性及功能依赖性的调节作用.