

雷酚内酯的抗炎免疫药理作用[△]

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摘要 雷酚内酯(triptophenolide TN)是由雷公藤醋酸乙酯提取物中分离的一种白色片状结晶。口服TN能显著抑制二甲苯及巴豆油所致急性炎症;且可使小鼠肾上腺VitC含量明显降低。TN可明显抑制DNCB和BSA诱导的迟发超敏反应;并可使大、小鼠外周血ANAE⁺淋巴细胞明显减少,显示TN对T淋巴细胞的免疫功能有抑制作用,TN还能显著提高大、小鼠血清总补体含量及抑制循环中IgG的形成量。其对小鼠腹腔M ϕ 吞噬功能呈现小剂量促进、大剂量抑制的双向作用。

关键词 雷酚内酯 总补体 抗炎 免疫

雷公藤*Tripterygium wilfordii* Hook. f.系卫矛科雷公藤属木质藤本植物。临床上用其根的醋酸乙酯提取物治疗风湿病、肾小球疾病等具有肯定的疗效^[1]。近年来我们从其提取物中分离到一种白色片状结晶,经理化测试、波谱分析和单晶衍射确定了化学结构,证实该结晶为雷酚内酯(triptophenolide, TN)^[2]。有关其药理作用尚未见报道。本文对TN的抗炎作用及其对免疫功能的影响进行了初步探讨。

1 材料

药品与试剂: TN由广州军区武汉总医院药化室提供,用无水乙醇配成3mg/ml备用,临用前用吐温-80助溶,蒸馏水配成所需浓度供用。牛血清白蛋白(BSA),上海生物化学研究所;无色孔雀绿(leucomalachite green),SIGMA公司;RPMI-1640, GIBA公司;HEPES, MERCK公司;羊抗鼠IgG,上海生物制品研究所;环磷酰胺(CY),上海第十二制药厂。

动物: SD大鼠120 $\pm$ 20g,广州军区武汉总医院动物房提供;BA1B/C小鼠,18~21g,购自中国科学院武汉水生生物研究所动物室;昆明种小鼠,湖北省医学科学院动物中心提供;C₅₇Bl/6小鼠,20~25g,我室繁殖。

仪器: 日本岛津UV-120型分光光度计;日本Qlympus显微镜。

2 方法与结果

2.1 雷酚内酯对免疫功能的影响

2.1.1 TN对迟发超敏(DTH)反应的影响

2.1.1.1 对2,4-二硝基氯苯(DNCB)所致DTH反应的影响:昆明种小鼠,随机分组,按表1所示剂量分组灌胃给药(ig),于第7天用DNCB致敏小鼠,第11天进行攻击。48h后以左、右耳片肿胀之差作为判断反应的指标。结果显示,TN对DNCB诱发的耳廓DTH反应有明显的抑制作用($P<0.05\sim 0.01$),且呈一定的剂量依赖关系。长时间给药对胸腺、脾脏无明显影响。见表1。

2.1.1.2 对牛血清白蛋白(BSA)诱导的DTH反应的影响: DTH实验按TITUS法^[3]。取C₅₇Bl/6小鼠28只,雌雄兼用,随机分为4组按图1所示剂量分组,TN组灌胃21d;CY组于第

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表1 TN对DNCB诱发小鼠耳廓DTH反应的影响($\bar{x} \pm SD$)

组 别	剂 量 (mg/kg·d×d)	n	耳廓肿胀程度 (mg)	胸腺指数	脾 指 数
对 照	0	10	4.2±1.9	2.6±0.7	4.5±1.3
TN	2.0×16	10	2.7±0.9*	2.3±0.5	4.4±0.6
	4.0×16	7	1.5±0.7**	3.0±0.7	4.9±1.2
强的松	5.0×10	9	2.4±1.7*	2.3±0.6	5.1±0.8

与对照组比较, * $P<0.05$, ** $P<0.02$

5天每3d皮下注射1次,共6次。于第9天给小鼠尾根部皮下注射50 μ g(BSA)/20 μ l·只(BSA以完全弗氏佐剂配成乳剂,2.5mg/ml),致敏。于第18天给小鼠右足底皮下注射20 μ l热凝集的2%BSA悬液进行攻击。此后逐日观察右足肿胀程度,以攻击前、后足肿胀截面积之差作为判断反应的指标。停药后继续观察10d。结果,TN对BSA诱发的DTH反应有明显抑制作用,并呈一定的量效关系。TN3mg/kg与CY30mg/kg的作用持久,停药10d仍保持较好的治疗效果。见图。

2.1.2 TN对大、小鼠T淋巴细胞酯酶染色率的影响:SD大鼠,雌雄各半,按表2(A)所示剂量分组给药,于第9天,CY组皮下注射1次。第14天取尾血涂片,用 α -醋酸萘酯酶法^[4]进行酯酶染色,观察淋巴细胞中ANAE⁺的百分率。另取BALB/C-鼠(雌性),按表2(B)所示剂量分组给药,同法测定小鼠外周血ANAE⁺淋巴细胞的百分率。由表2可见TN可使大、小鼠外周血T淋巴细胞酯酶染色率明显降低($P<0.01$)。

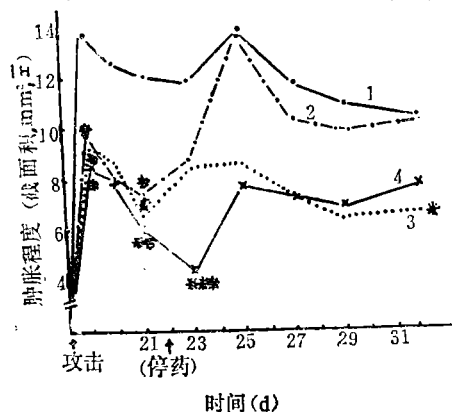


图 TN对牛血清白蛋白诱导迟发超敏反应的影响

1-对照 2-TN2mg/kg·d
3-3mg/kg·d 4-CY30mg/kg

与对照比较 * $P<0.05$ ** $P<0.02$ *** $P<0.01$

表2 TN对大小鼠T淋巴细胞酯酶染色率的影响

组别		剂 量 (mg/kg·d×d)	ANAE ⁺ (%) ($\bar{x} \pm SD$)
A	对照	0	62±5
	TN	1.0×14	36±3*
	CY	40.0×1	33±4*
B	对照	0	65±5
	TN	2.0×18	32±2*
		3.5×18	31±5*
		CY	30.0×6次
		隔2dsc一次	
			36±8*

与对照组比较 * $P<0.01$

2.1.3 TN对小鼠血清IgG、IgM含量的影响: BALB/C小鼠(雄性,8周龄),按表3所示剂量分为4组。1~3组分别灌胃水及TN(1.5及3mg/kg)18d,第4组为CY组,每隔2d皮下注射一次(30mg/kg,共6次),第19天取血,同时取胸腺及脾脏,称重,参照文献^[5]测定HcIgG及HcIgM。结果显示,TN对循环中IgG的形成有明显抑制作用,对抗体IgM的形成无明显影响。

2.1.4 TN对大、小鼠血清总补体含量的影响: 血清总补体测定采用改良的Mayer法。SD大鼠(雌雄兼用),随机分为对照组、TN组(灌胃1mg/kg·d×14d)及CY组(第10天,皮下注射40mg/kg一次),于第10天腹腔注射30% SRBC 0.5ml/只,第14天断头取血,

表3 TN对小鼠血清IgG及IgM含量的影响($\bar{x} \pm SD$)

组别	剂 量 (mg/kg·d×d)	n	HClgG	HClgM	HClg	胸腺指数	脾 指 数
对 照	0	11	373±79	106±34	481±138	11±2	78±6
TN	1.5×18	10	250±55**	124±54	374±93*	10±5	70±7*
	3.0×18	10	278±76*	101±30	388±65*	11±1	67±4**
Cy	30.0×6次	10	68±19*	48±9**	118±26**	4±1**	45±6**

与对照组比较, * $P<0.05$ ** $P<0.01$

在420nm波长处测OD值,计算样品的 CH_{50} ,组间进行统计学处理。另取BA1B/C小鼠(雄性2月龄),随机分为对照组、TN组(1.5及2.5mg/kg·d两个剂量组)及强的松组(5mg/kg·d×10d),同法测定血清总补体含量。结果,TN可明显提高大、小鼠血清总补体含量($P<0.05\sim0.01$),见表4。

2.1.5 TN对小鼠腹腔巨噬细胞吞噬功能的影响:采用孔雀绿比色法^[6]略加改良,测定巨噬细胞的吞噬功能。BA1B/C小鼠,用3%硫代乙醇酸钠2.5ml/只,分2次腹腔注射(第1天1.5ml/只,第2天1ml/只)诱导产生腹腔巨噬细胞(M ϕ);用光径0.5cm比色杯于波长620nm处测OD值。每一剂量作4个平行管。见表5。

表4 TN对大小鼠血清总补体含量的影响

组别	剂 量 (mg/kg·d×d)	n	总补体含量(CH_{50}) (u/ml, $\bar{x} \pm SD$)
(大鼠)			
对照	0	8	3.55±2.28
TN	1.0×14	9	9.23±3.17**
CY	40.0×1次	8	12.75±8.10**
BA1B/C			
对照	0	6	2.91±0.57
TN	1.5×12	6	5.59±2.67*
(小鼠)			
	2.5×12	8	4.89±1.67**
强的松	5.0×10	8	5.85±2.39

与对照组比较, * $P<0.05$, ** $P<0.01$

表5 TN对小鼠腹腔M ϕ 吞噬功能的影响

组 别	剂量(μ g/ml)	吸收度(OD)
对照	0	0.289±0.03
TN	20.0	0.091±0.01***
	10.0	0.131±0.02***
强的松	250.0	0.007±0.01***
对照	0	0.254±0.02
TN	5.0	0.303±0.03*
	2.5	0.318±0.04**
强的松	100.0	0.164±0.02

与对照组比较 * $P<0.05$ ** $P<0.02$ *** $P<0.001$

2.2 雷酚内酯的抗炎作用

2.2.1 TN对二甲苯诱发小鼠耳廓炎症的影响:BA1B/C小鼠(雌雄各半),随机分为4组。TN组(1.5及3.0mg/kg2个剂量)连续给药18d;CY组(30mg/kg)每隔2d皮下注射1次,共6次。第18天用二甲苯致炎,1h后取耳片称重,以左、右耳片重量之差作为判断肿胀程度的指标,见表6(A)。

2.2.2 TN对巴豆油合剂诱发大鼠耳廓炎症的影响:SD大鼠(雌雄各半),按表6(B)所示剂量随机分组给药。末次给药后1h,用巴豆油合剂诱发耳廓炎症,3h后取耳片称重。观察TN对炎症的影响,见表6(B)。TN对2种致炎剂诱发的耳廓水肿均有明显的抑制作用($P<0.05\sim0.01$)。

2.2.3 TN对小鼠肾上腺VitC含量的影响:BA1B/C小鼠(雄性,2月龄),随机分为对照组、TN组(1.5及2.5mg/kg·d两个剂量)及强的松组(50mg/kg·d)。采用2,4二硝基苯肼法,稍加改良,测定肾上腺VitC含量。结果显示,TN2.5mg/kg组肾上腺VitC含量明

显降低 ($P<0.05$), 见表7。

表6 TN对二甲苯(A)及巴豆油合剂(B)诱发鼠耳廓炎症的影响△

组别	剂 量 (mg/kg·d, × d)	n	耳肿胀程度 (mg) ($\bar{x} \pm SD$)
(A) 对照	0	10	7.0 ± 4.0
TN	1.5 × 18	10	2.1 ± 1.2**
	3.0 × 18	10	3.2 ± 1.0*
CY	30.0 × 6次	9	2.2 ± 1.3**
(B) 对照	0	9	11.9 ± 5.6
TN	1.0 × 14	8	5.6 ± 3.6*
	7.0	7	4.8 ± 2.7**

与对照组比较* $P<0.05$, ** $P<0.01$
△短时间给药抗炎作用不明显

表7 TN对小鼠肾上腺VitC含量影响

组别	剂 量 (mg/kg·d × d)	n	VitC含量 (μg/100mg组织) ($\bar{x} \pm SD$)
对照	0	8	176 ± 19
TN	1.5 × 12	8	163 ± 17
	2.5 × 12	7	155 ± 23*
强的松	5.0 × 12	7	128 ± 22*

与对照组比较* $P<0.05$

3 讨论

TN对DNCB和BSA诱导的迟发超敏反应有明显的抑制作用 ($P<0.05\sim0.01$)。停药10d, 对BSA所致DTH反应仍有治疗效果, 而且能使大、小鼠外周血T淋巴细胞的酯酶染色率明显降低, 表明TN对T细胞的免疫功能有抑制作用。

一些免疫复合物疾病患者的血清补体含量往往低于正常人, 而体液免疫功能表现亢进。因此, TN能提高大、小鼠血清总补体含量及抑制循环中IgG形成的作用, 有利于促进患者循环免疫复合物(IC)的清除及阻断IC的致炎作用, 加速炎症的恢复。

采用孔雀绿比色法测定Mφ功能的结果显示, TN对Mφ的吞噬功能具有小剂量促进、大剂量抑制的双向作用。

本文对TN的抗炎免疫药理作用初步研究结果表明, TN可能是雷公藤主要有效成分之一, 值得深入研究。

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出版消息

新书《中药研究与文献检索》已由上海远东出版社出版。全书100万字, 精装。每册定价40元, 邮购免收挂号寄费。一次购买5本或5本以上者, 八折优惠。购书写信至200041上海市延安中路420号王恒芬收即可。本书由王筠默教授等主编, 是在《中药药理学》大学教材后撰著的增补本。每药药名下, 先有概述, 下附百年来的全部论文文献, 检索极为方便。可用最短时间, 最快速度掌握研究信息, 是广大医药工作者的必备书。

Rhizome of *Rehmannia glutinosa* Libosch, attention was paid to reveal the chemical changes occurred in the course of processing. In order to find some clues for the process control, changes in the TLC spectrograms were examined and one of the component peak was found changing gradually as the processing went on. Phytochemical separation and identification revealed that the component peak features 5-hydroxy-methyl-furfural (5-HMF). TLCS spectrometric estimation of the 5-HMF contents was developed and used to monitor the process. It was found that the 5-HMF content at the end of processing was 20 times higher than that at the start.

Biological assay indicated that 5-HMF possesses marked antiplatelet activity, which supports the use of Dihuang as a blood activating agent in traditional Chinese medicine.

(Original article on page 13)

The Effects of Injection Stauntoniae on the Conduction of the Saphenous Nerve in Rat

Ye Wenbo, Liu Qiang, Ye Qing, et al

Injection Stauntoniae (IS) was applied on a part of the saphenous nerve of rat 4mm in length. With 50% IS, amplitudes of Aαβ, Aδ and C components of the compound action potential were reduced and their latencies were delayed. In particular, the amplitudes of Aδ and C components reduced to $47.0\% \pm 23.0\%$ and $44.0 \pm 20.0\%$ (MEAN $\pm$ SD, $n=8$, $P<0.01$) of the control's, respectively. With 10% IS, the amplitudes of all components did not reduce significantly, but the latencies of Aαβ, Aδ and C were delayed $0.05 \pm 0.01\text{ms}$ ($P<0.05$), $0.28 \pm 0.07\text{ms}$ ($P<0.05$) and $6.99 \pm 1.48\text{ms}$ ($P<0.01$) ($n=5$) respectively. There was dose-dependency between concentration of IS and its effect on nerve conduction. The effects on Aδ and C components were greater than that on Aαβ component. The effects of blocking conduction of nerve fibers were reversible.

(original article on page 20)

Studies on the Chinese Drug Shasheng

V. Comparison of Antitussive and Expectorant Activities

Tu Pengfei, Zhang Hongbin, Xu Guojun, et al

Shasheng is a common Chinese drug used as antitussive and expectorant. A survey showed that the original plants of Shasheng on the current market consist of more than 30 species. To appraise the qualities of Shasheng scientifically, the antitussive and expectorant activities of ten species of Shasheng were compared. Results showed that the EtOH extracts of the roots of *Glehnia littoralis*, *Adenophora stricta* subsp. *henanica*, *A. potaninii* and *A. liliifolioides* are very effective as antitussive, while those of *A. liliifolioides*, *A. stricta* subsp. *sessilifolia* and *A. potaninii* are very effective as expectorant.

(Original article on page 22)

Studies on Antiinflammatory and Immune Effects of Triptophenolide

Yang Jun, Yu Dongfang, et al

Triptophenolide (TN) is a colorless crystalline plate isolated from ethyl acetate extracts of *Tripterygium wilfordii*. It can obviously inhibit the edema caused by xylol and mixture croton oil, and decrease the concentration of Vitamin C in the adrenal gland of mice. TN can remarkably inhibit the delayed type hypersensitivity (DTH) reaction induced by DNCB and BSA, and diminished the peripheral blood ANAE+lymphocytes in rats and mice. More-

over, TN can dramatically increase the amount of total serum complement and significantly decrease the serum antibody products (IgG) of rats and mice. The phagocytosis of peritoneal exudate macrophages in mice present double effects in vitro. TN can promote the phagocytosis of macrophages at the small dosage, but at larger dosage, it showed inhibition.

The results mentioned above showed that triptophenolide may be one of the main effective component of *T. wilfordii* for treating auto-immune diseases.

(Original article on page 24)

Studies on the Effect of Forcing Culture on the Growth of Thunberg Fritillary (*Fritillaria thunbergii*) Introduced from South China to the Northern Region

Li zhiliang, Cao Juchang, Zhang Daming

Owing to climate influence, mean daily temperature in north China differs greatly from that of the southern regions during the same season. The introduction of *Fritillaria thunbergii* to Beijing for cultivation was hampered by delayed emergence of seedling and a shortened period for their growth, resulting in degeneration with the underground bulb becoming smaller year after year. With forcing culture as described in this paper, the emergence of seedling came earlier and growth period prolonged. The blossom and fruit became normal. By combining the method with formalin treated seed, diseases and pest index of the bulb were reduced distinctly and the yield increased with increased proportion of larger bulb year after year.

(Original article on page 31)

Studies on the Traditional Chinese Medicine Shouwu II. Botanical Origins and Applied Drugs

Li Jun, Xu Guojun, Xu Luoshan, et al

A survey on drug resources and identification of the commercial drugs showed that Heshouwuwu was derived from *Polygonum multiflorum* and the roots of another two species of the same family were misused as Heshouwuwu. The roots of 6 species of *Cynanchum* genus, *Asclepiadaceae* were used as Baishouwuwu in different regions. The index of botanical identification was listed. Botanical origins, distribution, production and sale of "Shouwuwu" were investigated.

(Original article on page 33)

An Appraisal of the Traditional Chinese Medicine Amber and Its Counterfeit

Wang Jiguang

Distinction between the traditional Chinese medicine amber and its counterfeit was studied by polarizing microscope and electron microscope, and trace elements analysed by plasma spectroscopy. Results showed that the counterfeit of amber is opal, which contains the same thirty-five kinds of trace elements as coal amber. The total amount of Ca, Mg, Al, and Fe contained in opal is 10 to 70 times more than that contained in coal amber, which can provide a basis for the appraisal of coal amber and opal.

(Original article on page 36)