服制剂的抗心肌缺血作用, 正常状况下, 大鼠在 iv Pit 后, 心电图有明显的变化, 实验中对照组在 iv Pit 后心电图发生明显的变化, 表现为 T 波升高, P-R, Q-T 间期延长, 而葛根素口服制剂在给药 6 d 后, 大鼠 iv Pit 后 T 波变化不明显, 证明葛根素口服制剂有抗心肌缺血作用。通过观察葛根素口服制剂在缺氧条件下对原代培养心肌细胞的作用显示, 缺氧条件下心肌细胞形态发生改变, 胞浆空泡形成, 胞浆颗粒形成, 异形心肌细胞数目增加, 同时心肌细胞分泌 CK, LDH, α-HBDH 明显增多, 给葛根素口服制剂后心肌细胞形态未发生明显改变, CK, LDH, α-HBDH 未见明显增多, 说明该药具有提高大鼠心肌细胞抗缺氧能力, 对心肌细胞有明显的保护作用。

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Antithrombotic effects of *Veratrum nigrum* var. *ussuriense* alkaloids

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Abstract: **Object** To investigate antithrombotic effects of *Veratrum nigrum* L. var *ussuriense* Nakai alkaloids (VnA) in rats. **Methods** The electrically induced rat carotid artery thrombosis model was used to examine the antiarterial thrombosis effect of VnA using occlusion time (OT) of carotid artery as pharmacologic index; the stasis-induced inferior vena cava thrombosis model was used to evaluate antivenous thrombosis effect of VnA in terms of thrombosis formation rate and decrease in thrombus dry weight. **Results** The iv administration of VnA (7.2—42.9) μg/kg to rats resulted in a dose-dependent effect and significant prolongation in OT. VnA at the dose of 30 μg/kg produced an antiarterial thrombosis effect equal to that of LAS (18.0 mg/kg). Also, a single bolus iv VnA (30 μg/kg) increased OT in a time-dependent manner; the antiarterial thrombosis effect was rapid in onset and lasted at least 80 min, and peaked at 15 min postdosing. VnA (15—45 μg/kg iv) decreased the thrombus dry weight significantly and dose-dependently. **Conclusion** VnA has powerful inhibitory effects against both arterial and venous thrombosis in rats and acts in a dose- and time-dependent manner. Its effective dose is as low as μg per kg body weight of rats. The finding of the VnA antithrombotic effeets reveal a bright future of its R & D.

Key words: thrombosis; *Veratrum nigrum* L. var *ussuriense* Nakai; alkaloid; Lilu

乌苏里藜芦碱抗血栓作用的研究

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摘 要: 目的 研究乌苏里藜芦生物碱 (VnA) 对大鼠的抗血栓作用。方法 分别应用电刺激诱发大鼠颈总动脉血栓形成模型和瘀血诱发下腔静脉血栓形成模型评价 VnA 的抗动、静脉血栓形成作用。结果 大鼠 iv 6 种不同剂量的 VnA (7.2～42.9 μg/kg) 导致血管阻塞时间 (OT) 显著延长, 且具明显的剂量依赖性, VnA 30 μg/kg 产生的抗动脉血栓形成作用与赖氨匹林 18.0 mg/kg 的作用相当。单次 iv VnA 30 μg/kg 使 OT 呈时间依赖性延长。VnA

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抗动脉血栓形成作用起效迅速, 持续至少 80 min, iv 后 15 min 达效应峰值。iv VnA (15～45 μg/kg) 显著减少下腔静脉血栓干质量, 并呈剂量依赖性。结论 VnA 具有强大的抗动、静脉血栓形成作用, 并具明显的剂量依赖性和时间依赖性。其抗血栓效能高, 作用强度大, 在大鼠的有效剂量低至 μg/kg 水平。VnA 抗血栓活性的发现为其研究开发展示了新的前景。

关键词: 血栓; 乌苏里藜芦; 生物碱; 藜芦
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Veratrum nigrum L. var *ussuriense* Nakai is a well known medicinal plant widely distributed in northeast region of China. As a source of the Chinese crude drug "Lilu", this herbal medicine has been recorded in Chinese medical books and noted for its high toxicity. In the past Chinese and foreign scientists did much research on its medicinal values, but their research was only focused on its antihypertensive effects. So far, no information concerning its antithrombotic effects has been reported in published literature.

VnA is the total alkaloid extracted from root of *V. nigrum* var *ussuriense* of Mount. Qian in Liaoning Province, China, and has been shown to contain at least eleven alkaloids featuring ester-type isosteroidal structure identified by modern analytical techniques such as HPLC/MS, NMR, *etc*. The principal components contained in VnA include verussurien, verabenzoamine, verazine, germidine, jervine, germerine, 15-*O*-(methylbutyroyl)-germine, verussurinine, neogermbudine, zygadenine and echinuline[1～4].

In the present paper antithrombotic effects of VnA in the rat thrombosis models are reported, so as to provide experimental basis for its clinical use and new drug development as a highly efficacious antithrombotic agent.

1 Materials and methods

1.1 Chemicals and instruments: VnA hydrochloride injection (100 μg/mL) was prepared by Department of Pharmaceutical Engineering, Dalian University of Science and Technology. It was diluted to the required concentration with sterile saline solution prior to use. Heparin sodium was a product of Shanghai Biochemical Pharmaceutical Factory, lot No. 870516; *dl*-lysine-acetylsalicylate (LAS) was purchased from Anbao Pharmaceutical Factory, Anhui Province, lot No. 950327. BT87-3 model experimental *in vivo* thrombosis instrument was purchased from Cardiovascular Research Department, Baotou Medical College.

1.2 Animals: Sprague-Dawley rats of both sexes (half to half) weighing 250—300 g were supplied by Animal Center of Dalian Medical University (Approval document No. 022, Liaoning Province, China).

1.3 Rat carotid artery thrombosis model: The carotid artery thrombosis in rats was induced by the method developed by Hladovec[5] with minor modification. Rats were randomly divided into groups and anesthetized by ip 20% urathane (1 g/kg). The unilateral cartotid artery was isolated surgically, a direct current stimulating electrode and a temperature electrode were placed at heart-proximal and heart-remote ends of carotid artery respectively. VnA-treated groups of rats received a single bolus iv administration of VnA, with positive and negative control groups given iv LAS (18 mg/kg) and equivolume saline solution respectively. The continual electric stimulation (1.6 mA, 7 min) was started in 15 min postdose. The time elapsing from the beginning of stimulation to abrupt fall in temperature of carotid artery surface was recorded by means of the temperature electrode of the instrument. This time represented the occlusion time (OT) of carotid artery injured electrically, namely, thrombus formation time. Then, the percent increase of OT, an index was used to evaluate antiarterial thrombosis effects of the drugs, VnA-treated groups were calculated in comparison with saline control group.

1.4 Rat inferior vena cava thrombosis model: The inferior vena cava thrombosis was induced by venous blood stasis in accordance with Reyer's method[6]. Briefly, rats were randomly divided into groups and anesthetized as above. The middle

abdominal wall of rats was incised, then inferior vena cava was separated surgically; different groups of rats were treated with iv varying doses of VnA (15-45 μg/kg), heparin (400 μg/kg) as positive control and equivolume saline as negative control respectively. Five minutes later, the inferior vena cava was ligated to induce stasis. Then, abdominial cavity was closed, and reopened two hours after ligation. The blood vessel of inferior vena cava was clamped at 2 cm below the ligation. The thrombus clot in the blood vessel was removed (if any) and weighed after drying at 60 ℃ for 20 min. The animal numbers of thrombus formation and thrombus drying weight, which were used to evaluate the antivenous thrombotic effects of the drug, were recorded so as to calculate thrombus formation rate (animal number of thrombosis/test animal number) and percent inhibition of thrombus weight respectively.

2 Results

2.1 Effects of VnA on rat carotid artery thrombosis: As seen in Fig. 1, iv injection VnA resulted in a dose-dependent increase in the OT. The OTs in the VnA-treated groups (n= 6, each) at iv doses of 7.2, 10.3, 14.7, 21.0, 30.0 and 42.9 μg/kg were (11.27±1.47) (P> 0.05), (12.23±1.43), (13.64±2.68), (15.43±3.59) (P< 0.05), (18.47±3.89) and (20.21±3.40) (P< 0.01) min, respectively, compared with (10.32±1.35) min in negative control group and (18.24±1.81) min in positive control group. The percent increases of OT in VnA-treated groups relative to the negative control group were 9.2%, 18.5%, 32.2%, 49.5%, 78.9% and 95.8% repectively. It was also found that iv VnA (30.0 μg/kg) produced an effect equal to that of iv LAS (18.0 mg/kg).

Also, a time-dependent increase of OT in VnA-treated group by iv dose of 30.0 μg/kg (n= 6, at each time point) was shown in Fig. 2. The OT at 2, 5, 10, 15, 20, 30, 50, 80 and 120 min postdose were (11.31±2.61), (11.74±2.16) (P> 0.05), (13.96±2.47) (P< 0.05), (17.36±3.51), (15.37±3.25) (P< 0.01), (13.36±2.87), (13.15±2.40), (12.04±2.01) (P< 0.05) and (11.16±2.52) (P> 0.05) min respectively *vs* (10.02±1.22) min in the negative control group, which corresponded to the percent OT increases of 12.9%, 17.2%, 39.3%, 73.2%, 53.4%, 33.4%, 31.2%, 20.2% and 11.3%, respectively. The time to peak effect was found to be 15 min postdose.

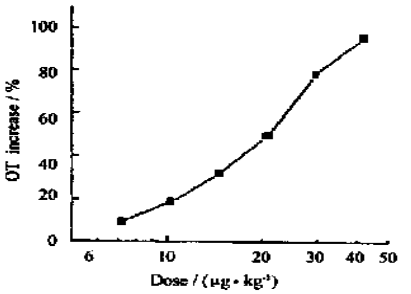

Fig. 1 Dose-effect curve of VnA in rats after administration

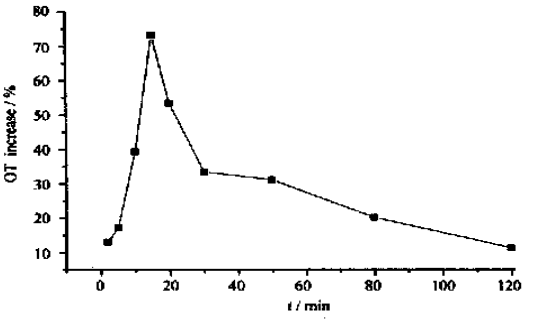

Fig. 2 Time-effect curve of VnA in rats receiving an iv administration of 30 μg/kg

2.2 Effects of VnA on rat inferior vena cava thrombosis: From Table 1 it could be found that all iv doses of VnA produced a reduction in thrombus formation rate (e.g. 8/10, 8/12 and 6/12 at 15, 30 and 45 μg/kg respectively *vs* 10/12 in the negative control), although there was no statistically significant difference (P> 0.05). However, compared with negative control group all above three doses of VnA decreased the dry weight of thrombus dose-dependently and significantly [(1.81±1.40), (1.50±0.61) and (0.71±0.18) mg in VnA-treated group at ascending order of doses *vs* (2.87±0.75) mg in negative control) (P< 0.05 at middle and large doses)]. It was evident that the three doses employed here produced the effect

potency slightly lower than that produced by iv heparin 400 μg/kg.

Table 1 Effect of VnA on stasis-induced venous thrombosis in rats ($\bar{x}\pm s$)

Drug	n	Dose /(μg·kg^{-1})	Thrombus formation rate	Dry weight of thrombus/mg	Inhibition of dry weight/%
NS	12	–	10/12	2.87±0.75	–
VnA	10	15	8/10	1.81±1.40	36.9
	12	30	8/12	1.50±0.61*	47.7
	12	45	6/12	0.71±0.18**	73.9
Heparin	10	400	5/10	0.50±0.21**	81.9

* $P<0.05$ ** $P<0.01$ *vs* NS control group

3 Discussion

In this study the antithrombotic effects of VnA were investigated using rat carotid artery thrombosis model and rat inferior vena cava thrombosis model. In the former model electric stimulation of carotid artery led to endothelia injury, followed by platelet adhesion, aggregation and formation of a platelet-rich thrombus which caused occlusion of the artery. So, the OT was used to examine the inhibitory effect of VnA against arterial thrombosis. In the later model inferior vena cava was ligated for two hours and thus rendered blood stasis, consequently leading to activation of blood clotting system and formation of thrombus. Above stated models are simple, reproducible and reliable for evaluating antithrombotic effects of drugs, including Aspirin and Heparin, which were used as positive controls, and VnA, which was examined in the present experiment.

The findings in this study strongly demonstrate that VnA has powerful inhibitory effects against both arterial and venous thrombosis, manifested by high antithrombotic efficacy and high antithrombotic potency. VnA was found to be effective antithrombotically at an extremely low doses (μg/kg level). VnA of (30 μg/kg) iv administration produced antiarterial thrombosis effect which equals that produced by 18.0 mg/kg LAS. VnA (42.9 μg/kg) caused percent increases in OT of 95.8%, VnA (45 μg/kg) significantly inhibited venous thrombosis with inhibitory rate of 73.9%, compared with 81.9% for heparin (400 μg/kg).

The duration of antithrombotic effect of VnA was found short (about 80 min), suggesting that repeated dosing may be required. It was also found that the antithrombotic effect peaked at 15 min after iv administration, indicating that there was a lag phenomenon of effect relative its blood concentration. The mechanism of this phenomenon is to be elucidated.

In rat venous thrombosis model when thrombus formation rate as an index of effect was used there was no statistically significant difference between VnA-treated group and saline-treated group. This is because thrombus formation rate is a quantal response index, more animal numbers are needed to produce significant difference than dry weight of thrombus, a graded response index.

VnA is the total alkaloid extracted from *V nigrum*, which has been used as a source of traditional Chinese medicine "Lilu" and thought to have potent hypotensive effect. The present article is the first report on the antithrombotic effects of VnA in the medical literature to date. This new finding provides a experiment support for the use of "Lilu" in the treatment of apoplexy[7].

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