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Research Paper

Effects of intravenous Injections Paederiae and Stauntonia on spontaneous pain, hyperalgesia and inflammation induced by cutaneous chemical tissue injury in the rat

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Abstract: To study whether commercial traditional Chinese medicinal preparations Injection Paederiae (IP) or Injection Stauntonia (IS) has anti-nociceptive and/or anti-inflammatory effects, we used two persistent pain models (bee venom and formalin test) to evaluate the systemic effects of IP or IS on the chemical tissue injury-induced persistent spontaneous pain-related responses (PSPR), primary thermal/mechanical hyperalgesia and inflammation in conscious rats. Injection of bee venom (BV, 0.1 mg, 50 μl) into the plantar surface of one hind paw resulted in not only a 1-h monophasic PSPR such as flinching reflex in the injected paw and a subsequent period of 3 – 4 days primary heat and mechanical hyperalgesia, but also a marked sign of inflammation, including redness and swelling of the plantar surface in the injected paw. Intraplantar injection of formalin produced two phases of PSPR as reported previously. Systemic pre-treatment with three doses of IP (0.32, 1.6 and 9.0 ml/kg, 500%) or IS (0.32, 1.6 and 9.0 ml/kg, 250%) produced a dose-dependent suppression of the BV- or formalin-induced flinching reflex of 1 h time course as compared with the saline control group. Post-treatment with IP or IS 5 min after BV injection also produced a significant suppression of the flinching reflex in both BV test and formalin test respectively, as compared with the control group. However, neither pre- nor post-treatment with IP or IS produced any significantly suppressive effect on the BV-induced primary heat and mechanical hyperalgesia and inflammation. The analgesia produced by IP or IS was not mediated by the endogenous opioid receptors since naloxone, a non-selective opioid receptor antagonist, had no reversal effect on the IP and IS-produced analgesia in the BV-induced PSPR. Our present results suggest that IP or IS might prevent and relieve clinical persistent spontaneous pain, but without any anti-nociceptive and anti-inflammatory effects on the primary heat hyperalgesia, mechanical hyperalgesia, as well as inflammatory responses. The BV test might be a useful model of pain to evaluate and screen anti-nociceptive and anti-inflammatory effects of certain compounds of the Chinese medicinal herbs on the pathological origins of pain.

Key words: injection paederiae; injection stauntonia; bee venom test; formalin test; persistent spontaneous pain; hyperalgesia; anti-inflammation; anti-nociception

鸡矢藤注射液和野木瓜注射液对大鼠足底皮下化学组织损伤诱致自发痛、痛敏和炎症的作用

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摘　要：　研究市售中药制剂鸡矢藤注射液和野木瓜注射液有无抗伤害及抗炎作用。采用两种持续性痛动物实验模型——蜜蜂毒（bee venom，BV）模型和福尔马林（formalin，F）模型，评价鸡矢藤注射液和野木瓜注射液系统给药对持续性自发痛反应、原发性热和机械痛敏及炎症反应的作用效果。成年清醒大鼠足底皮下注射 BV（0.2%，50

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μl）不仅可诱发注射侧长达 1 h 以上的、持续的、单相性的自发痛反应（其表现为自发缩足反射行为）和之后出现的持续 3 – 4 d 的原发性热和机械痛敏现象，而且注射爪出现明显的红、肿等炎症反应。皮下注射 F（2.5%，50 μl）则产生双相性自发痛反应。与盐水组比较，致痛前系统给予 0.32、1.6 和 9.0 ml/kg 三个剂量的 500% 鸡矢藤注射液或 250% 野木瓜注射液，对 BV 或 F 诱致的 1 h 自发缩足反射次数具有剂量依赖性抑制作用；致痛 5 min 后分别给予鸡矢藤或野木瓜注射液对 BV 或 F 诱发的自发痛反应也产生显著的抑制作用。然而，致痛前或致痛后静脉注射鸡矢藤注射液或野木瓜注射液对 BV 诱致的原发性热/机械痛敏及炎症反应均无明显的抑制作用。纳洛酮（一种非选择性的阿片受体拮抗剂）不能翻转鸡矢藤或野木瓜注射液对 BV 产生的自发痛反应的镇痛作用，提示其镇痛作用不是由内源性阿片受体介导。本研究结果证实鸡矢藤或野木瓜注射液能预防和缓解临床持续性自发痛，但是对原发性热/机械痛敏及炎症反应均无抗伤害效应和抗炎作用。在中药镇痛抗炎有效成分的筛选和评价中，BV 模型是一个理想的实验动物模型。

关键词：鸡矢藤注射液；野木瓜注射液；蜜蜂毒试验；福尔马林试验；持续性自发痛；痛敏；抗伤害作用；抗炎作用
中图分类号：R285.5

Subcutaneous (s. c.) *injection of bee venom* (BV) solution into one hindpaw of rats has been demonstrated to be able to produce a prolonged persistent spontaneous pain-related behaviors such as flinching reflex in a monophasic manner for 1 h, followed by a subsequent period of profound, persistent primary thermal and mechanical hypersensitivity (hyperalgesia) in the injected hind paw for 3 – 4 d[1-3]. Meanwhile, s. c. BV injection *also produces a striking edema* and redness of the plantar surface for nearly the same period as the development of hyperalgesia[1-4]. Electrophysiological and c-Fos immunostaining results have shown that *the altered* or hyper-excitable state of spinal dorsal horn nociceptive neurons is responsible for the process of the tonic flinching reflex, while the c-Fos expression in the spinal dorsal horn neurons is likely to contribute to the development of the BV-induced long-term hyperalgesia or hypersensitivity[5-9]. Moreover, pharmacological studies have shown that both behavioral expressions and *the increased spinal neuronal activities following* s. c. BV could be effectively suppressed by morphine and NSAIDs (non-steroidal anti-inflammatory drugs, such as aspirin or indomethacin)[4,5,9] as well as by other anti-nociceptive drugs at both spinal and peripheral levels[2,6,10-13]. It has also been shown by our series of pharmacological studies that the BV-induced persistent spontaneous nociception, thermal and mechanical hypersensitivity in behavioral expressions involve multiple neurochemical pathways in the spinal cord including membrane ligand-gated receptors (LGRs such as glutamate *NMDA/non-NMDA*), *G-protein coupled receptors* (GPCRs such as metabotropic glutamate and neurokinin receptors), voltage-dependent calcium channels (VDCCs) and essentially several intracellular protein kinase (PK) systems (*cAMP-PKA*, DAG-PKC, NOS-PKG)[14,16]. However, the three types of the BV-induced pain and hypersensitivity are different from each other in the underlying mechanisms based on not only the spinal neuropharmacological studies[2,6,10-14], but also a genetic survey by using 11 strains of inbred mice and 12 commonly used assays of pain including the BV test[15]. The features of the BV test enable this new model to mimick multiple types of clinical pathological pain and it could therefore be used to evaluate the effective targets of a certain drug in various pain and inflammation *at the same time in the same animal*. Moreover, the above experimental results also suggest that good control of clinical pathological pain requires a mixture of drugs with blocking effects on multiple targets at the central and peripheral sites of the nervous system. Based on this presumption, only the compounds of traditional Chinese medicinal herbs could appropriately satisfy the therapeutic needs of clinical pathological pain control because multiple compounds of the medicinal herbs may have an ability to block multiple pharmacological targets involved in the inducing and maintaining processes of pathological pain[14,16]. Therefore, to see whether different traditional Chinese medicinal preparations have the same effects on persistent spontaneous nociception (PSN), primary heat hyperalgesia, mechanical hyperalgesia (or allodynia), and inflammatory responses such as edema and plasma extravasation, we used the novel BV test in the present study to examine the anti-nociceptive or anti-inflammatory effects of *two commercially available traditional Chinese medicinal* (TCM) analgesic drugs: Injection Paederiae (IP) and Injection Stauntonia (IS) which have been demonstrated to be effective in clinical pain control[17]. Mean-

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while, to see whether there is a difference in pain between animal models we also used the formalin test as a positive control in this study.

1 MATERIALS AND METHODS

1.1 *Animals.* The experiments were performed on Sprague-Dawley male albino rats weighing from 180 to 260 g. The animals were provided by Laboratory Animal Center of the Fourth Military Medical University (FMMU) and use of the animals was reviewed and approved by the FMMU Animal Care and Use Committee. The IASP's ethical guidelines for pain research in conscious animals were followed[18]. The animals were housed in plastic boxes in group of three at 22-26℃ with food and water available *ad libitum* in a colony room. A 12:12 h light dark cycle with lights on at 08:00 was maintained and testing was done between 09:00 and 18:30. The rats were acclimatized to the laboratory and habituated to the test boxes for at least 30 min each day for 5 days before testing.

1.2 *Drugs and administration.* Honeybee venom was lyophilized whole venom of *Apis mellifera* (Sigma, St. Louis, MO) dissolved in 0.9% sterile saline. A volume of 0.05 ml saline containing 0.2 mg lyophilized whole venom was subcutaneously injected into the posterior surface of one hind paw of conscious rats according to our previous studies[1-3]. The formalin test was also used as a control to examine whether the TCM analgesic drugs were effective in control of different origins of pain. Formalin (2.5% in 50 μl/saline) was also injected similarly as described for the BV test.

Injection Paederiae [Yuweiyaozhunzi (1996) No. 111020, provided by Pharmaceutical Co. Ltd. Henanguishancao, China; Batch number: 010808; 10 g/2 ml per vial] and Injection Stauntonia [ZZ-5982-Ganweiyaozhunzi (1996) No. 003064, provided by JiangXi TianShi Traditional Chinese Medicine Group, China; Batch number: 20010405; 5 g/2 ml per vial], which have been approved by the local authority of drug safety and quality control for clinical application, were injected intravenously (i. v.) via a jugular vein catheter. Three doses of IP (0.32, 1.6 and 9.0 ml/kg body weight, diluted from the original concentration 500% in 10 g/2 ml per vial) and IS (0.32, 1.6 and 9.0 ml/kg body weight, diluted from the original concentration 250%, 5 g/2 ml per vial) were administered 10 min prior to BV injection to test whether they have preventive effects on the BV-induced persistent spontaneous pain-related response. A single effective dose of the two drugs was also administered (1) 10 min prior to BV-induced persistent pain; (2) 5 min after BV when the persistent pain was well established; (3) or 2-4 h after BV injection when the thermal and mechanical hyperalgesia as well as inflammation were well established, so as to see whether they have relief effects on the BV-induced persistent nociception, and preventive and/or relief effects on the inducing and maintaining processes of the BV-induced heat or mechanical hypersensitivity (hyperalgesia) and inflammation. The methods for drug administration were described in detail in one of our previous reports[13]. The effects of a single dose of IP or IS were also studied in the formalin test. Naloxone (0.04%, 2.5 ml/kg body weight, i. v.) was used to test whether the anti-nociceptive effect of the IP or IS were mediated by endogenous opioid receptors.

1.3 *Measurement of spontaneous pain-related behavior, thermal and mechanical hypersensitivity (hyperalgesia).*

The procedures for assessment of the persistent spontaneous pain-related behavior and hyperalgesia to mechanical and thermal stimuli were described in detail in our previous reports[1-3].

Briefly, a 30 cm × 30 cm × 30 cm transparent plexiglas test box with a transparent glass floor was placed on a supporting frame of 50 cm high above the experimental table to allow the experimenters to observe the paws of the animals without obstruction. The rat was placed in the test box for at least 30 min before administration of BV and the testing drugs. The spontaneous pain-related behavior was determined by counting the number of paw flinches during each 5-min interval for 1 h.

To examine thermal hyperalgesia, the rats were placed on the surface of a 2 mm thick glass plate covered with a plexiglas chamber (20 cm × 20 cm × 25 cm) to measure the sensitivity to heat stimuli with a RTY-3 radiant heat stimulator (Xi'an Fenglan Instrumental Factory, P. R. China). The radiant heat source was a high intensity halogen lamp bulb (100 W) positioned under the glass floor directly beneath targeting area on the hind paw. The distance between the projector lamp bulb and lower surface of the glass floor was adjusted to produce a light spot on the floor surface 5 mm diameter. The heat stimuli were directed onto the injected area and the symmetrical site on the contralateral hind paw of each rat under the voltage of 9.5 V. Five stimuli were repeated and the mean paw withdrawal thermal latency (PWTL) was obtained from the later three stimuli in each test. The inter-stimulus interval

was more than 10 min for the same region and 5 min for the different region. To avoid excessive tissue injury, manual cut-off of the heat stimulus was performed if heat stimulation for 30 s failed to evoke a paw withdrawal reflex.

Mechanical stimuli were applied by using ascending graded individual monofilaments with bending forces of 0.1, 0.4, 0.7, 1.0, 2.5, 3.5, 4.0, 5.0, 6.0, 8.0, 12.0, 16.0, 25.0, 30.0, 40.0, 50.0, 62.5, 75.0 g. The rat was placed on a metal mesh floor covered with the same plexiglas chamber and von Frey filaments were applied in a upgrade intensity order from underneath the metal mesh floor to the testing sites of the bilateral hind paws. The bending force of the von Frey filament able to evoke paw withdrawal with a 50% occurrence frequency was determined to be the paw withdrawal *mechanical threshold* (PWMT).

1.4 *Assessment of inflammation.* Four hours after BV injection when hyperalgesia and inflammatory responses reach peak and stable state, the injected paw was measured by the following three methods: (1) paw ventrodorsal thickness was measured with precision calipers (MC 2616, Harbin Measure Factory, P. R. China); (2) paw volume was measured by water displacement of the hind paw immersed in a 50 ml cylinder to the rostral edge of the heel, using a 1 ml syringe to collect and measure the water (Weihai Medical High-Molecular Ltd., P. R. China); and (3) plasma extravasation was measured by the optical density (OD) of Evans blue extravasation under the skin of the injected paw, using a spectrophotometer. Evans blue (50 mg/kg) was intravenously injected via a jugular vein catheter under urethane anesthesia (0.9 g/kg, i. p.) 1 d prior to BV injection. After measuring hyperalgesia and inflammation, an overdose of pentobarbital (200 mg/kg) was given to the rats. The skin of both hind paws was dissected and placed in 4 ml of formamide and incubated for 96 h at room temperature. The dye concentration in the formamide was measured by a spectrophotometer under absorption at 620 nm. All measures of inflammation were calculated as percentage increases as compared to the paw prior to inflammation or the difference between the ipsilateral and contralateral hind paws.

1.5 *Statistical analysis.* All data were expressed as mean(SEM. ANOVA (Fisher's PLSD) was used for comparative analysis of the flinching number in 1 h time course of BV- or formalin-induced persistent pain, and for the analysis of changes in thermal (PWTL) or mechanical (PWMT) sensitivity of the injected hind paws, and inflammatory responses (difference in paw volume, paw dorsoventral thickness and optical density of Evans blue between the injected and non-injected hind paws) between the vehicle and the drug-treated groups. $P<0.05$ was considered to be statistically significant.

2 RESULTS

No signs of acute toxic reaction and severe motor dysfunction were found to be caused by i. v. application of the drugs (IP and IS) or by s. c. injection of BV or formalin except the behaviors relative to pain and inflammation described below.

2.1 Effects of systemic pre- or post-treatment of IP or IS on chemical tissue-injury-induced persistent spontaneous nociception (pain-related behaviors)

As shown in Fig. 1*A*, systemic pre-treatment of both 500% IP and 250% IS at 0.32, 1.6 and 9.0 ml/kg produced a dose-dependent inhibitory effect on the BV-induced persistent spontaneous pain-related behavior (the number of flinches per 5 min of 1 h time course) as compared with the vehicle control. The suppressive effects of both IP and IS lasted for nearly 1 h. The inhibitory rate for the three doses of IP was (26.22 ± 5.39)% (538.60 ± 39.38 flinches/1 h, $n=5$; $P<0.01$), (39.52 ± 6.13)% (441.5 ± 44.77 flinches/1 h, $n=5$; $P<0.01$) and (54.21 ± 3.07)% (334.33 ± 22.38 flinches/1 h, $n=6$; $P<0.001$) respectively compared with the control group (730.00 ± 19.86 flinches/1 h, $n=5$) (Fig. 1*a*). The inhibitory rate for the three doses of IS was (4.98 ± 9.49)% (693.67 ± 69.29 flinches/1 h, $n=6$; $P>0.05$), (40.17 ± 9.33)% (436.75 ± 68.14 flinches/1 h, $n=5$; $P<0.01$) and (63.26 ± 1.93)% (268.17 ± 14.19 flinches/1 h, $n=6$; $P<0.001$) (Fig. 1*b*). Post-treatment with IP or IS 5 min after establishment of the BV-induced flinching reflex also produced a significant suppressive effect by (30.75 ± 5.66)% (450.50 ± 36.79 flinches/h, $n=6$, $P<0.01$) and by (43.91 ± 1.39)% (354.20 ± 16.89 flinches/h, $n=7$, $P<0.001$) respectively compared with that of the saline group (650.50 ± 45.71 flinches/h, $n=5$) (Fig. 2*A*).

To test whether the anti-nociceptive effect of IP or IS has model difference, we used a single effective dose of the two drugs and found pre-treatment of IS produced significant suppression of both phase 1 and 2 of the formalin test, the inhibitory rate respectively being (37.61 ± 9.84)% (101.80 ± 16.05 flinches, $n=5$;

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$P < 0.01$) compared with that of the saline group (163.14 ± 12.26 flinches, $n = 8$) during the 1st phase and (60.72 ± 5.47)% (178.00 ± 24.78 flinches, $n = 5$; $P < 0.001$) compared with that of the saline group (453.14 ± 37.42 flinches, $n = 8$) during the 2nd phase (Fig. 1*B*). However, the same treatment of IP only produced significant *suppression of phase 1* (48.63 ± 7.35)% (83.80 ± 11.99 flinches, $n = 7$; $P < 0.01$), but *without significant inhibitory effect* on the phase 2 of the formalin test (23.20 ± 9.44)% (348.00 ± 42.76 flinches, $n = 7$; $P > 0.05$) (Fig. 1*B*). Post-treatment with IP or IS 5 min after es*tablishment* of the F-induced flinching reflex also produced a significant suppressive effect by (50.66 ± 11.58)% (223.60 ± 52.48 flinches, $n = 5$, $P < 0.01$) and (57.63 ± 3.87)% (192.00 ± 17.49 flinches/h, $n = 5$, $P < 0.001$) respectively compared with that of the saline group (453.14 ± 37.42 flinches, $n = 8$) during the 2nd phase. (Fig. 2*B*).

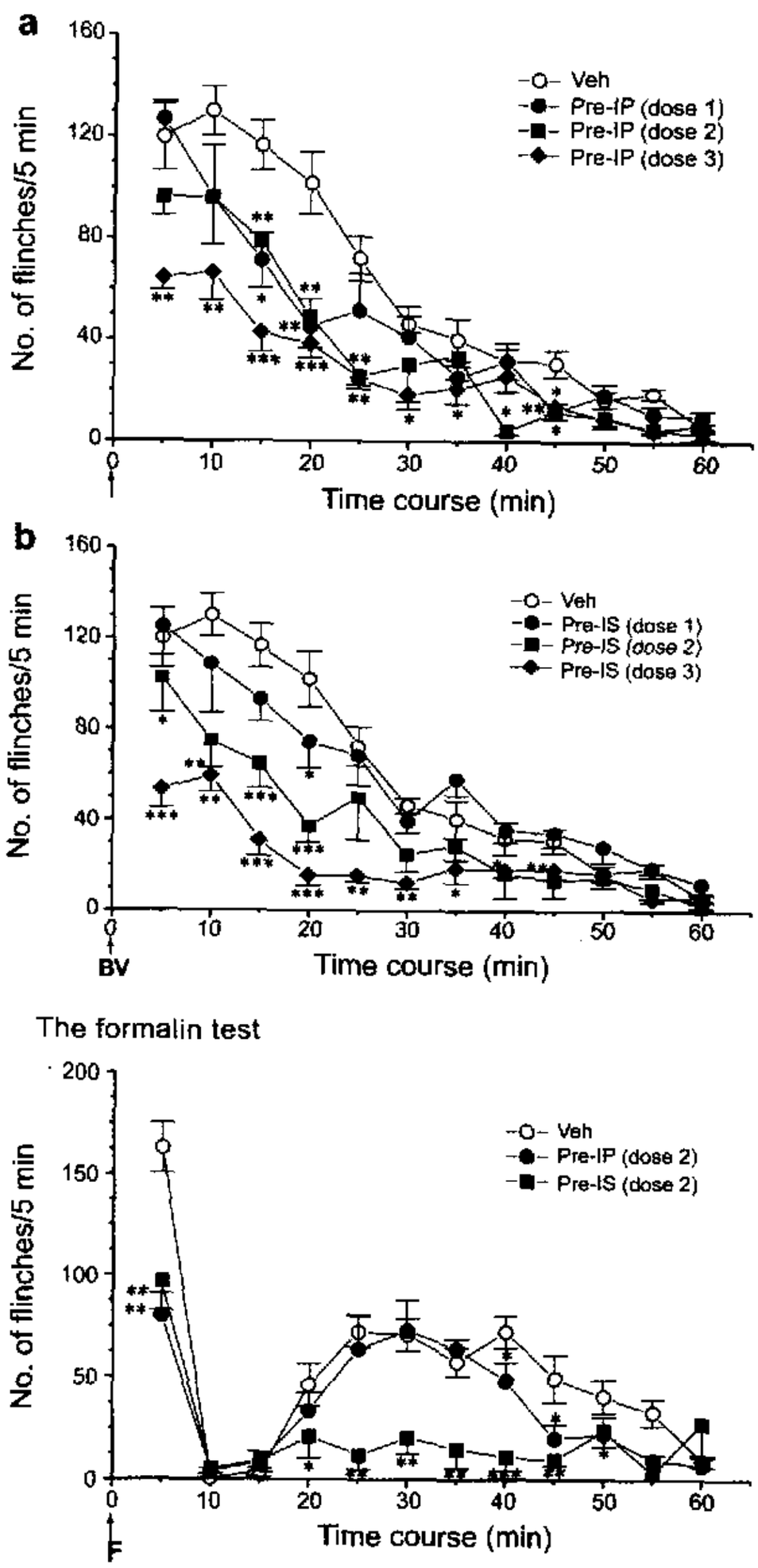

Fig. 1. Time course effects of systemic pre-treatment of Injection Paederiae (IP) and Injection Stauntonia (IS) on the persistent spontaneous pain-related responses induced by intraplantar injection of bee venom (BV, *A*) or formalin (*B*). *A*: The mean time courses of the anti-nociceptive effects of pre-IP (*a*) and pre-IS on the BV-induced flinching reflex of 1 h (*b*). *B*: The mean time courses of the anti-nociceptive effects of pre-IP or pre-IS on the formalin-induced flinching reflex. $^{***}P < 0.001$; $^{**}P < 0.01$; $^{*}P < 0.05$ compared with control experiments. Vertical bars, ± SEM. Arrows for BV and F indicate the start time of subcutaneous injection of the two chemicals.

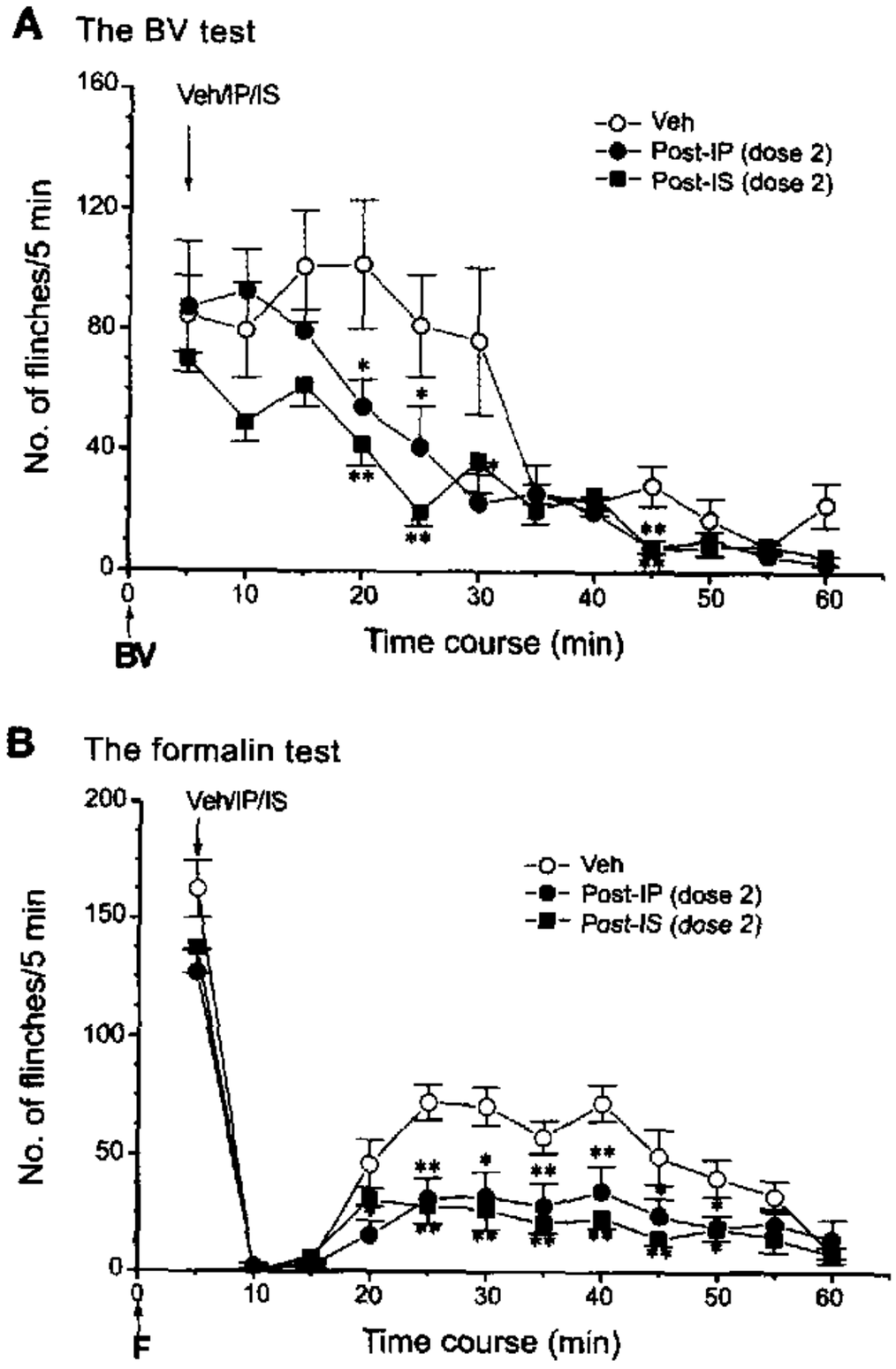

Fig. 2. Time course effects of systemic post-treatment of a single dose of Injection Paederiae (IP) and Injection Stauntonia (IS) on the persistent spontaneous pain-related responses induced by intraplantar injection of bee venom (BV, *A*) or formalin (*B*). $^{**}P < 0.01$; $^{*}P < 0.05$ compared with control experiment. Vertical bars, ± SEM. Arrows for BV and F indicate the start time of subcutaneous injection of the two chemicals.

2.2 Effects of systemic pre- or post-treatment of IP or IS on BV-induced thermal and mechanical hyperalgesia (allodynia)

As our previous reports[1-3], s. c. BV produced a dramatic reduction in both PWTL and PWMT measured 2 *h after injection of* BV, suggesting an occurrence of both heat and mechanical hyperalgesia (Fig. 3*A*, *B*). As shown in Fig 3, neither systemic pre- nor post-treatment of a single effective dose of IP or IS produced preventive or relief effects on the BV-induced thermal (Fig. 3*A*) and mechanical hyperalgesia (Fig. 3*B*) ($P > 0.05$, as compared with the vehicle control).

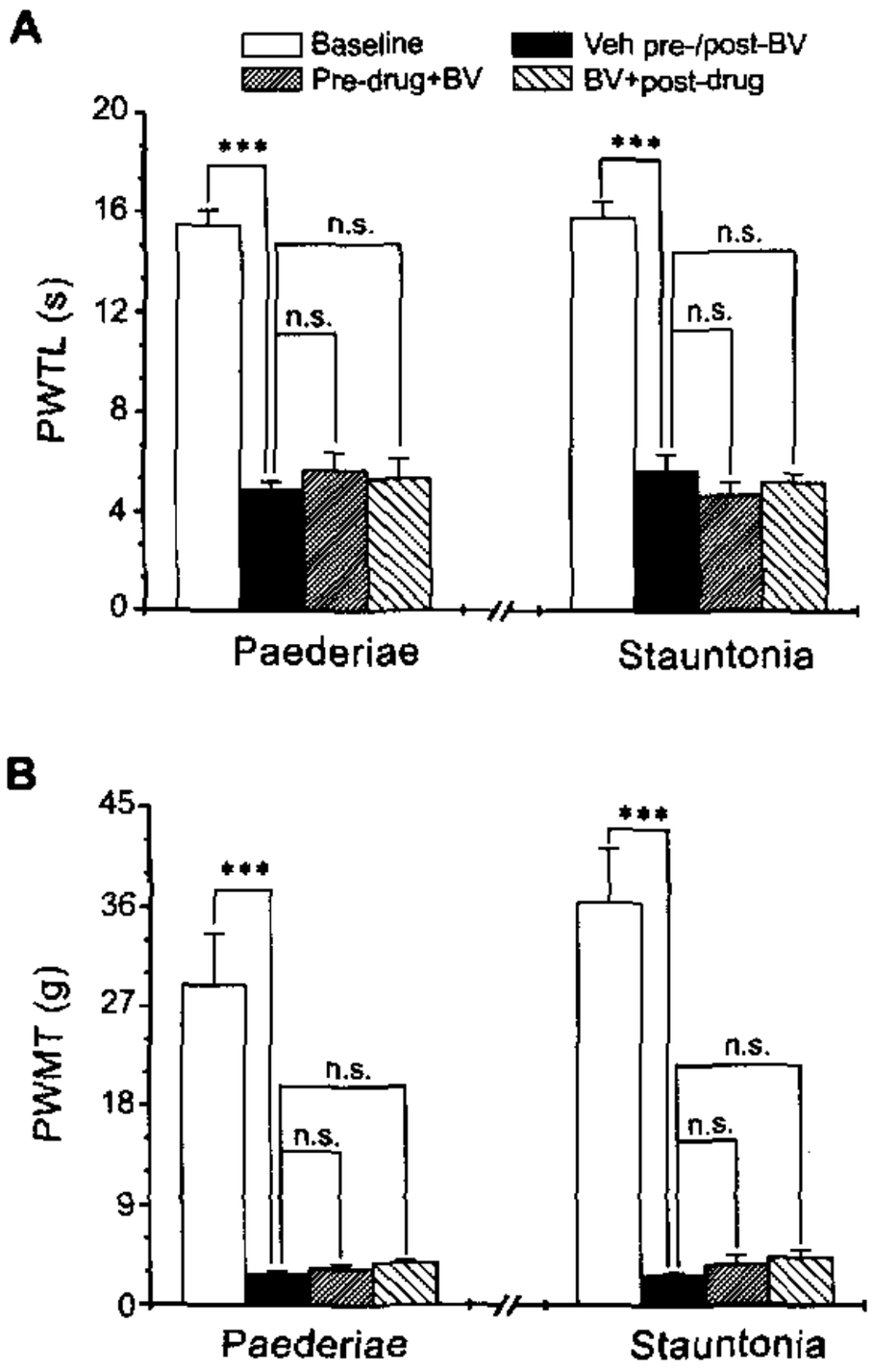

Fig. 3. Effects of systemic pre- or post-treatment of a single dose of Injection Paederiae (IP) and Injection Stauntonia (IS) on the bee venom (BV)-induced thermal (*A*) and mechanical (*B*) hyperalgesia. ***$P < 0.001$; n. s., no significant compared with control experiment. Vertical bars, ± SEM. PWTL, paw withdrawal thermal latency; PWMT, paw withdrawal mechanical threshold.

2.3 Effects of systemic pre- or post-treatment of IP or IS on BV-induced inflammatory responses

Intraplantar injection of BV also produced an increase in paw volume and dorsoventral thickness of paw (swelling or edema), as well as an increase in OD value of Evans blue in the skin of the injected hind paw (plasma extravasation) in the pre- or post-saline control groups (Fig. 4). Neither pre- nor post-treatment of IP and IS produced any significant influence upon the BV-induced inflammatory responses (Fig. 4) ($P > 0.05$, compared with that of the saline group).

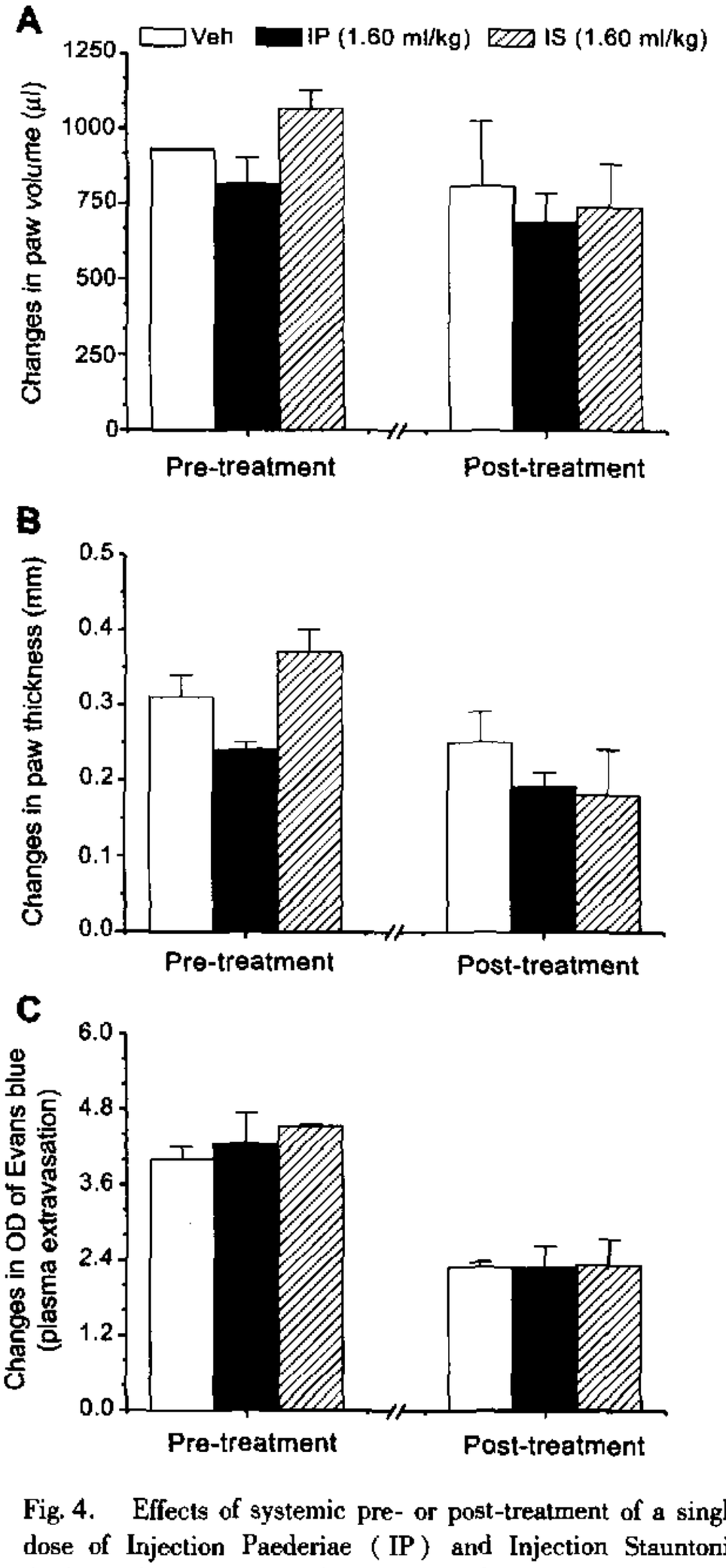

Fig. 4. Effects of systemic pre- or post-treatment of a single dose of Injection Paederiae (IP) and Injection Stauntonia (IS) on the bee venom (BV)-induced inflammatory responses. Graph shows the changes in paw volume (*A*), paw ventrodorsal thickness (*B*) and the optical density (OD) value of Evans blue in the skin of the injected hind paw (plasma extravasation) (*C*) after bee venom (BV) injection. n. s., no significant compared with control experiment. Vertical bars, ± SEM.

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2.4 Effects of systemic naloxone on the IP- or IS-produced anti-nociception

As described above, systemic treatment of IP and IS could produce anti-nociceptive actions upon the chemical tissue injury-induced spontaneous pain-related behaviors, although the BV-induced hyperalgesia and inflammation were not influenced. To further investigate whether endogenous opioid receptors play a role in the anti-nociception of IP and IS in the BV test, naloxone, a non-selective opioid receptor antagonist was systemically administered (0.04%, 2.5 ml/kg) which was effective to reverse morphine-induced analgesia in the BV test[5, 9]. The results showed that naloxone could not reverse the anti-nociceptive effects of both IP (Fig. 5*A* and *B*) and IS (Fig. 5*C* and *D*) for the whole time course (per 5 min for 1 h) and the total mean number of the BV-induced spontaneous pain-related response.

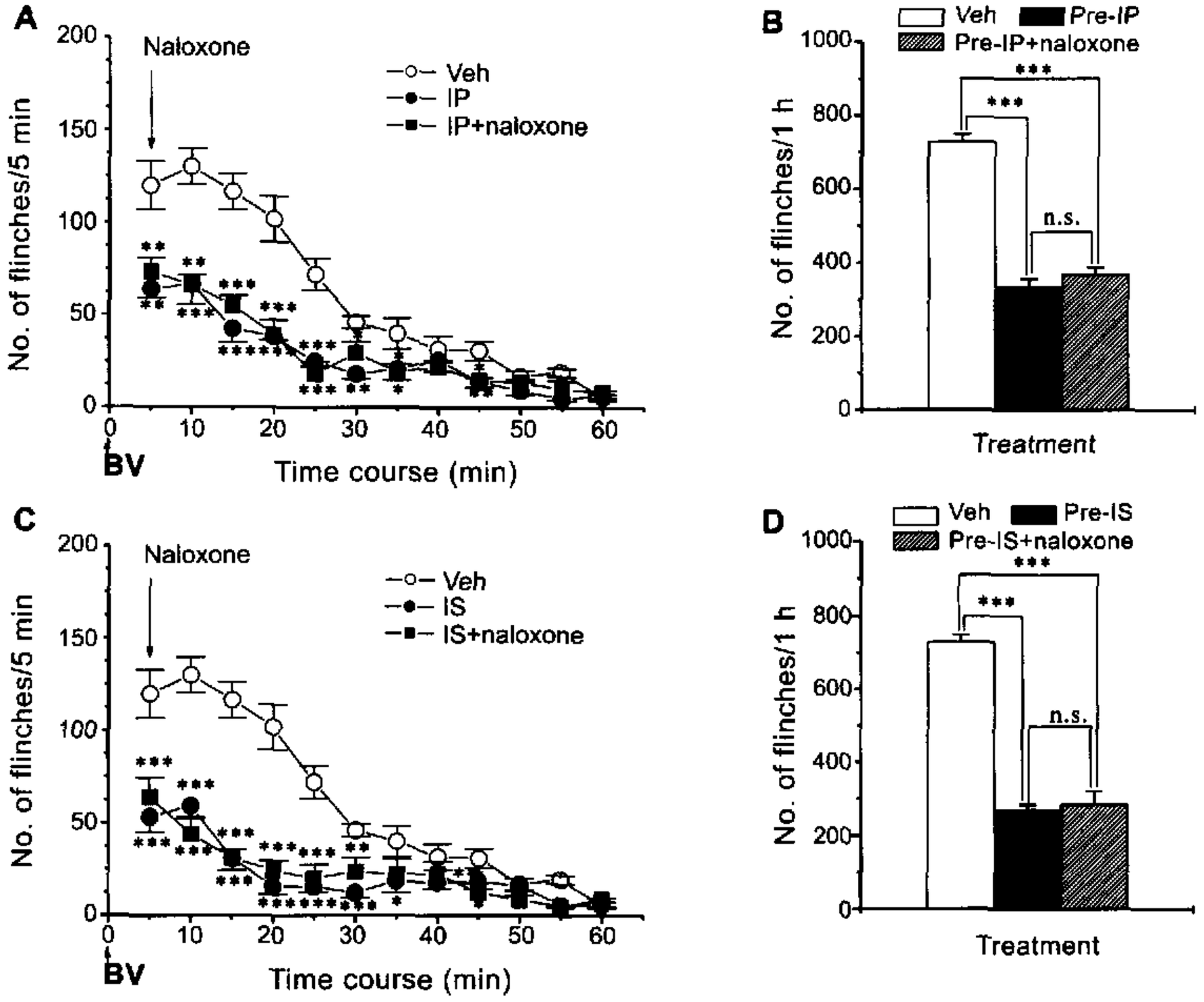

Fig. 5. Time course effects of systemic naloxone, a non-selective opioid receptor antagonist, on the analgesia produced by Injection Paederiae (IP) (*A* and *B*) and Injection Stauntonia (IS) (*C* and *D*) in the bee venom-induced persistent spontaneous pain-related responses. $^{***}P < 0.001$; $^{**}P < 0.01$; $^{*}P < 0.05$ compared with control experiment; n. s., no significant. Vertical bars: ± SEM. Arrows for BV indicate the start time of subcutaneous injection of the chemical. The doses for IP (500%, 9 ml/kg) and IS (250%, 9 ml/kg) are the highest ones among the three used in the present experiment. The dose for naloxone (0.04%, 2.5 ml/kg) is effective to reverse morphine analgesia tested in the BV test[5,9].

3 DISCUSSION

The present study, for the first time, evaluated the experimental effects of IP and IS on persistent spontaneous nociception, heat and mechanical hyperalgesia, and inflammatory responses in a new animal model of pathological pain — the BV test and a conventional model of persistent pain — the formalin test. It was found that systemic administration of both IP and IS could produce a significant anti-nociceptive (analgesic) effect on both induction and maintenance of the persistent spontaneous pain-related behavior regardless of the pain models used. We further confirmed that the IP- or IS-produced analgesia was not mediated by endogenous opioid receptors since naloxone, a non-selective opioid receptor antagonist has no reversal effect. However,

neither of the two drugs could produce any significantly suppressive effects on the whole processes of the BV-induced primary heat and mechanical hyperalgesia, and inflammation. Taken together, the present results suggest that the commercially available TCM analgesics IP and IS might only be effective in prevention and relief of clinical spontaneous pain, but not stimulus-induced hyperalgesia (or allodynia). Moreover, both IP and IS are not likely to be effective in control of clinical inflammatory response.

Regarding the effects of IS, a previous experimental animal study showed that oral administration of Stauntonia could increase baseline pain threshold of mice to hot plate test and suppress the pain-related behaviors observed in other pain models, such as acetic acid writhing model, trigeminal neuralgia model and inflammatory response model[19]. Since the spontaneous expressions of mice pain-related behaviors such as vocalization and body writhing induced by i. p. injection of acetic acid could also be significantly suppressed by IS, it is suggested that the anti-nociceptive actions of IS on spontaneous pain are not different between rat and mouse test. However, there is a discrepancy in the effects of IS on inflammatory responses between our rat and their mouse studies. The reason is not clear, but is likely due to the difference in animal models used between the previous (visceral pain model) and our current (somatic pain models) studies. With regard to the anti-nociceptive or anti-inflammatory effects of IP, since so far there has been no experimental study available before our current one, comparative study between different models and animal species needs to be further carried out.

The mechanisms underlying the anti-nociceptive actions of IP or IS on the BV- or formalin-induced spontaneous pain are not clear. However, based on the negative effect of naloxone on the IP- and IS-produced analgesia, the mediation of endogenous opioid receptors can be ruled out. This result strongly supports the non-abuse characteristic of the two drugs observed in clinical trial (material provided by the pharmaceutical makers), however experimental studies are still required to clarify this viewpoint at the cellular and molecular level of biomedicine.

It has been demonstrated that the BV-induced spontaneous pain-related behaviors are dependent upon ongoing primary afferent impulses originating from the injury site and maintained by both peripheral and central hyper-excitability of primary and secondary somatosensory neurons[3,5-8]. Therefore, IP and IS might have a direct suppressive action on heat nociceptors such as vanilloid receptor (VR1) which has been shown to be involved in the BV-induced pain and hyperalgesia[3] and/or peripheral glutamate NMDA and non-NMDA receptors[6,8]. It was also found that IS could block impulse conduction and destroy peripheral nerve myelin in bullfrogs and rats[20,21].

By means of i. t. administration of various drugs against the membrane LGRs and GPCRs and VDCCs and intracellular PKs, NOS and COX2 5 – 10 min prior to or 2 – 4 h after BV, it was found that in the spinal cord the persistent spontaneous nociception involves multiple pharmacological targets of spinal trans-synaptic, transmembrane and intracellular signal transduction pathways, including NMDA/non-NMDA/NK1-2/VDCC-DAG-PKC/cAMP-PKA/NO-PKG/COX2-PGs, while the primary heat hyperalgesia only involves NK1/2/mGluRI-III/VDCC-DAG-PKC pathway and the primary mechanical hyperalgesia only involves mGluR I-cAMP-PKA/NO-PKG pathways[14]. Therefore, the direct actions of IP and IS on the spinal cord dorsal horn should be taken into account based on the above results in the BV test. In the spinal cord, IP and IS might have direct blocking actions on the central terminals of primary nociceptive afferent and inhibit release of excitatory amino acids such as glutamate/aspartate, ATP and neuropeptide substance P whose receptors have been shown to be involved in both development and maintenance of the BV-induced spontaneous pain-related responses[2,10,12-14,16]. Since IP and IS have no anti-nociceptive effect on the primary heat and mechanical hyperalgesia, they are not likely to act on the targets along DAG-PKC and cAMP-PKA pathways. But actions of both drugs on the spinal NOS-PKG and COX2 are likely because these two kinds of enzymes are shown to be involved in both persistent spontaneous nociception and primary hyperalgesia[11,14,16].

Taken together, the action sites of IP and IS for analgesia might be multiple targets and remains to be further studied at both peripheral and spinal levels by means of various neurobiological methods.

IP and IS might not have peripheral effects on the pro-inflammatory mediators (such as IL-1β, tumor-necrosis factor and NGF) as well as inflammatory mediators (such as histamine, 5-HT, prostaglandins and bradykinin, etc.), since in the present study the BV-induced inflammatory processes were not significantly affected by i. v. IP and IS.

Injection Paederiae was refined sterile water solution partly extracted from Chinese fevervine herb which contains various major chemical components, e. g. asperuloside, paederoside, scanderoside, etc. Injection Stauntonia was extracted from Stauntonia chinensis DC which mainly contains flavonoid glycoside, triterpene saponin, phenol, etc. We still do not know which components of IP and IS are the most effective ones in analgesia, it will be much more interesting to examine which are the major components of IP or IS to produce anti-nociception in our future study.

* * *

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