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Chronic resveratrol treatment exerts antihyperalgesic effect and corrects co-morbid depressive like behaviors in mice with mononeuropathy: Involvement of serotonergic system

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ABSTRACT

Patients suffering from chronic neuropathic pain are at high risk of co-morbid depression, which burdens healthcare. This work aimed to investigate the effects of resveratrol, a phenolic monomer enriched in red wine and grapes, on pain-related and depressive-like behaviors in mice with mononeuropathy, and explored the mechanism(s). Mice received chronic constriction injury (CCI) of sciatic nerves, and sequentially developed pain-related and depressive-like behaviors, as evidenced by sensory hypersensitivity (thermal hyperalgesia in Hargreaves test and mechanical allodynia in von Frey test) and behavioral despair (prolonged immobility time in forced swim test). Chronic treatment of neuropathic mice with resveratrol (30 mg/kg, p.o., twice per day for three weeks) normalized their thermal hyperalgesia (but not mechanical allodynia) and depressive-like behaviors, and these actions were abolished by chemical depletion of central serotonin (5-HT) but potentiated by co-treatment with 5-HTP, a precursor of 5-HT. The anti-hyperalgesia and anti-depression exerted by resveratrol may be pharmacologically segregated, since intrathecal (i.t.) and intracerebroventricular (i.c.v.) injection of methysergide, a non-selective 5-HT receptor antagonist, separately abrogated the two actions. Furthermore, the anti-hyperalgesic action of resveratrol was preferentially counteracted by co-administration of the 5-HT_7 receptor antagonist SB-258719, while the anti-depression was abrogated by 5-HT_{1A} receptor antagonist WAY-100635. These results confirm that chronic resveratrol administration exerts curative-like effects on thermal hyperalgesia and co-morbid depressive-like behaviors in mice with mononeuropathy. Spinal and supraspinal serotonergic systems (coupled with 5-HT_7 and 5-HT_{1A} receptors, respectively) are differentially responsible for the antihyperalgesic and antidepressant-like properties of resveratrol.

1. Introduction

Neuropathic pain is caused or initiated by a primary lesion or a disease of the somatosensory system (Merksey and Bogduk, 1994). It is a chronic and disabling disease that commonly incorporates emotional deficits, such as depression (Gustorff et al., 2008; Kim et al., 2012; Yalcin et al., 2011). In fact, emotional aspect is an intrinsic construct of chronic pain, and co-morbid depression is extensively observed in individuals who suffer from chronic neuropathic pain, with the co-occurrence up to 34% (Gustorff et al., 2008). Reciprocally, pain is one of the most common complaints in patients with depression (Arnow et al., 2006; Demyttenaere et al., 2006) and depressive symptoms may exacerbate or predict chronic pain (Burke et al., 2010; Tunks et al., 2008; Wang et al., 2012). The high prevalence of this co-morbidity intensifies the disamenity of neuropathic patients and deteriorates their overall life qualities (Gustorff et al., 2008), which necessitates improved treatment. Of especial note, although amounting researches have

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been recently performed to unveil the relationship and mechanisms for this pain-depression dyad (Bravo et al., 2012; Burke et al., 2010; Kim et al., 2012; Yalcin et al., 2011), relative few studies evaluated the effects of drugs, especially that used to palliate neuropathic pain, on the co-morbid emotional disabilities such as depression.

Clinically, chronic neuropathic pain is frequently unresponsive to canonical analgesics, such as opioids and non-steroidal anti-inflammatory drugs (Arnér and Meyerson, 1988; Vo et al., 2009). In the guidelines of pharmacological treatment against neuropathic pain, antidepressants together with anticonvulsants are the first choice in the management of this chronically refractory disease (Attal et al., 2010). In spite of being ranked as first-line drugs, most antidepressants can not fully satisfy the clinical need of mitigating pain in neuropathic patients, because of inadequate efficacy, extensive limitation, undesirable side effects and poor patient compliance (Finnerup et al., 2005; Micó et al., 2006), thus indicating a need to validate novel analgesics including herb and complementary medicine.

Resveratrol (trans-3, 4′,-5-trihydroxystilebene, $C_{14}H_{12}O_3$, for the chemical structure to see Fig. 1A), a red wine and grapes-derived phytoalexin, possesses a variety of pharmacological properties, including antioxidant, anti-inflammatory, anti-hypertensive, cancer chemo-preventive and neuroprotective activities (Csiszar et al., 2009; Farris et al., 2013; Kumar et al., 2013; Lee et al., 2009; Panaro et al., 2012). Previously, we demonstrated that resveratrol exerts antidepressant-like effect in mice and rats (Xu et al., 2010; Yu et al., 2013b). Moreover, resveratrol has been reported to exert analgesic effects in some experimental studies (Bazzo et al., 2013; Bertelli et al., 2008). The concurrent antinociceptive and antidepressant-like activities from resveratrol tempted us to investigate, in this study, whether it possesses therapeutic potentials in mitigating the co-morbidity of chronic pain and depression. Furthermore, we explored the mechanisms underlying the actions of resveratrol in the context of neuropathic pain. We hypothesized the involvement of monoaminergic system, since it is not only substantially implicated in the descending pain regulation (Millan, 2002), but is responsible for the actions of resveratrol against impairments evoked by chronic stress (Yu et al., 2013b).

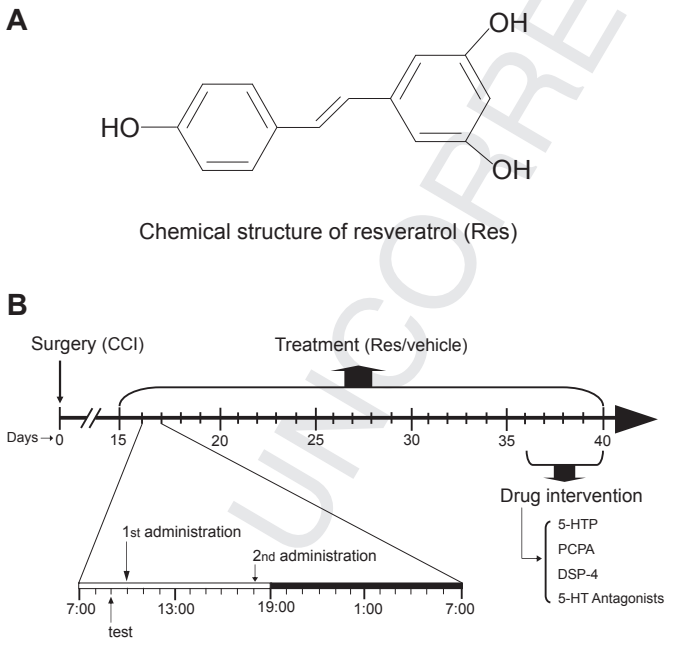

Fig. 1. Chemical structure of resveratrol (Res) and schematic of resveratrol treatment. (A) Chemical structure of resveratrol. (B) Fifteen days after the surgery of chronic construction injury (CCI), we started chronic treatment with vehicle or resveratrol (3, 10 and 30 mg/kg, p.o., twice a day, at 10:00 and 18:00 respectively). After 3 weeks of resveratrol treatment (when the thermal hyperalgesia and depressive-like behaviors of CCI mice were corrected by resveratrol), 5-HTP, PCPA, DSP-4 or 5-HT antagonists were co-administered with vehicle or resveratrol.

2. Materials and methods

2.1. Animals

Adult male C57BL/6J mice (6–7 weeks old upon arrival) from Animal Center of Chinese Academy of Sciences (Shanghai, China) were used in this study. Animals were housed in groups (4–6 per cage) with chow and water available *ad libitum*, in a temperature-controlled environment with a light/dark cycle of 12:12 h (lights on 7:00 AM). The experiments were performed in a quiet room where the temperature was maintained at 22 ± 0.5 °C with a relative humidity of 60 ± 2%. All animal procedures were approved by the Ningbo University Committee on Animal Care and Use and complied with the European Communities Council Directive of 24 November 1986 (86/609/EEC). The authors further ensure here that all efforts were made to minimize animal suffering and the number used.

2.2. Chronic constriction injury (CCI) procedure

A chronic constriction injury (CCI) of ipsilateral sciatic nerve was performed in mice (7–8 weeks old at the time of surgery designated as day 0) as described originally by Bennett and Xie (1988) with minor modifications (Zhao et al., 2012). In brief, the mice were anesthetized by 60 mg/kg intraperitoneal (i.p.) injection of pentobarbital sodium. The right common sciatic nerve was exposed at the mid-thigh level and, proximal to the sciatic nerve trifurcation, three ligations (4/0 silk threads, with 1 mm spacing) were loosely tied around it until a brief twitch was seen in the respective hind limb. The surgical area was dusted with streptomycin and the incision was sutured. In sham-operated animals, the sciatic nerve was exposed without ligation. All surgical procedures were performed by the same researcher.

2.3. Drugs and pharmacological treatments

The drugs used in this study were: resveratrol, 5-hydroxytryptophan (5-HTP, a precursor of 5-HT), methysergide (non-selective 5-HT receptor antagonist), ondansetron (5-HT_3 receptor antagonist), ritanserin (5-$HT_{2A/2C}$ receptor antagonist), SB-258719 (5-HT_7 receptor antagonist) and WAY-100635 (5-HT_{1A} receptor antagonist). Methysergide and SB-258719 were purchased from Tocris Bioscience and the other drugs were from Sigma-Aldrich. All the drugs were dissolved or diluted in sterile saline with the exception of resveratrol, whose vehicle was saline containing 0.5% sodium carboxymethyl cellulose.

The treatment with resveratrol (p.o., via gavage, with a volume of 10 ml/kg) began 15 days after the surgical procedure (CCI or sham-operation), when the neuropathic mice displayed obvious co-morbidity of pain-related and depressive-like behaviors. Fresh resveratrol was prepared and diluted to the desired concentration on the day of testing. For chronic treatment, the sham and CCI mice received two oral administrations (morning and evening, as shown in Fig. 1B) of resveratrol or vehicle per day for 21 consecutive days (i.e. from day 15 to day 35 after CCI injury). After 3 weeks of resveratrol or vehicle treatment, the mice were co-administered with 5-HTP or one of the 5-HT receptor antagonists (Fig. 1B). These antagonists were: methysergide (non-selective 5-HT receptor antagonist), WAY-100635 (5-HT_{1A} receptor antagonist), ritanserin (5-$HT_{2A/2C}$ receptor antagonist), ondansetron (5-HT_3 receptor antagonist) and SB-258719 (5-HT_7 receptor antagonist). The doses of these drugs were selected on the basis of our previous studies (for WAY-100635, ritanserin and ondansetron, see Zhao et al., 2012) and other literature (for 5-HTP, see Liang et al., 2004; for methysergide, see Ide et al., 2006; for SB-258719, see Brenchat et al., 2010) with appropriate revisions to ensure paucity of intrinsic activity for these antagonists *per se* in the present behavioral tests. For i.p. injection, these agents were administered in a volume of 10 ml/kg. For repeated co-administered with resveratrol, these agents were injected twice per day, 30 min before resveratrol administration (Yalcin et al., 2009).

2.4. Chemical depletion of central noradrenaline (NA) and serotonin

After treatment with resveratrol or vehicle for 3 weeks, pharmaceutical manipulations were performed in mice to deplete central NA or serotonin. For chemical depletion of central NA, the mice were injected i.p. with 40 mg/kg selective NA neurotoxin N-(2-chloroethyl)-N-ethyl -2-bromobenzylamine hydrochloride (DSP-4) as reported by Boyce-Rustay et al. (2008). DSP-4 was dissolved in saline. To ablate central serotonin, p-chlorophenylalanine (PCPA, an inhibitor of serotonin synthesis), suspended in 0.5% gum acacia/physiological saline, was administered i.p. for five consecutive days at a dose of 300 mg/kg/day (Tanabe et al., 2007).

2.5. Intrathecal and intracerebroventricular injection

To localize the monoamine receptors possibly involved, intrathecal (i.t.) or intracerebroventricular (i.c.v.) injection of one of 5-HT receptor antagonists

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