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St. John's Wort reduces neuropathic pain through a hypericin-mediated inhibition of the protein kinase C γ and ε activity

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ABSTRACT

Current pharmacological treatments for neuropathic pain have limited efficacy and severe side-effect limitations. St. John's Wort (SJW) is a medicinal plant, mainly used as antidepressant, with a favourable side-effect profile. We here demonstrate the ability of SJW to relieve neuropathic pain in rat models. The antihyperalgesic profile and mechanism of action of SJW and its main components were studied in two rat models of neuropathic pain: the chronic constriction injury and the repeated administration of oxaliplatin. SJW, acutely administered at low doses (30–60 mg kg^{-1} p.o.), reversed mechanical hyperalgesia with a prolonged effect, being effective up to 180 min after injection. Further examinations of the SJW main components revealed that hyperforin and hypericin were responsible for the antihyperalgesic properties whereas flavonoids were ineffective. The effect of SJW on the PKC expression and activation was investigated in the periaqueductal grey (PAG) area by immunoblotting experiments. Mechanistic studies showed a robust over-expression and hyperphosphorylation of the PKCγ (227.0 ± 15.0% of control) and PKCε (213.9 ± 17.0) isoforms in the rat PAG area. A single oral administration of SJW produced a significant decrease of the PKCγ (131.8 ± 10.0) and PKCε (105.2 ± 12.0) phosphorylation in the PAG area due to the presence of hypericin. Furthermore, SJW showed a dual mechanism of action since hyperforin antinociception involves an opioid-dependent pathway. Rats undergoing treatment with SJW and purified components did not show any behavioural side effects or signs of altered locomotor activity. Our results indicate SJW as a prolonged antihyperalgesic treatment through inhibition of PKC isoforms and their phosphorylation.

1. Introduction

Neuropathic pain can arise from a wide variety of injuries to peripheral or central nerves, including metabolic disorders, traumatic injury, inflammation, and neurotoxicity, and is characterized by spontaneous pain, hyperalgesia and allodynia which can persist long after the initial injury is resolved [1]. Common causes of neuropathy are diabetes, herpes zoster infections, chronic or acute trauma, and neurotoxins. Neuropathic pain occurs frequently in cancer as it may result from tumor invasion of nervous tissue, surgical nerve damage during tumor removal, radiation-induced nerve damage or as a side effect of many chemotherapeutic drugs [2,3].

The underlying molecular mechanisms of neuropathic pain are still not completely understood, and as a consequence, treatment is unsatisfactory in many cases [3,4]. Despite the large number of approved analgesic drugs, effective treatment of chronic pain is still often unattainable due to the unsatisfactory performance of the available drugs and due to the negative side effects from the drugs [5,6]. Neuropathic pain is generally insensitive to non-steroidal anti-inflammatory drugs and relatively resistant to opioids, but can be treated by high opioid doses [7] with a high incidence of untoward side effects. The suggested treatment strategies for neuropathic pain management are represented by tricyclic antidepressants (TCAs) [8], that represent the first medication category proved to be effective for neuropathic pain in clinical trials [9] and still are a first choice treatment, anticonvulsants [10,11], antiarrythmics [12]. All these drugs have limited efficacy and have severe side effects. In particular, TCAs induce numerous untoward effects such as sedation, anticholinergic effects like dry mouth, constipation and postural hypotension, weight gain, greater risk to develop myocardial infarction [13,14]. Therefore, for an effective treatment of neuropathic pain, there is still a need to obtain therapeutics which possess a greater level of tolerability and safety.

Abbreviations: CCI, chronic constriction injury; CHL, chloroformic fraction; i.c.v., intracerebroventricular; i.p., intraperitoneal; MET, methanolic fraction; OXA, oxaliplatin repeated administration; p.o., per os; PAG, periaqueductal grey area; PKC, protein kinase C; PMA, phorbol-12-myristate-13-acetate; SJW, St. John's Wort; s.c., subcutaneous.

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Hypericum perforatum L., commonly known as St. John's Wort (SJW), has been proven to relieve mild-to-moderate forms of depression and tests have also shown SJW to have favourable side effects [15]. The most common SJW preparations used are hydroalcoholic extracts of the aerial portion of the plant that contain at least ten different kinds of biochemical compounds [16]. SJW interacts with the monoaminergic system through different mechanisms: the MAO-inhibitory properties of SJW were mainly due to hypericin [17,18] whereas the inhibition of serotonin, dopamine and noradrenaline synaptosomal uptake is related to the presence of hyperforin [19–22]. It has therefore been suggested that SJW may induce antidepressant activity through a mechanism similar to TCAs [23]. This evidence may suggest that similar to TCAs, SJW can also relieve neuropathic pain. The aim of the present study was to investigate the antihyperalgesic properties of SJW in different animal models, which were suffering from neuropathic pain that was induced by chronic constriction injury of the sciatic nerve, or by the repeated administration of the chemotherapeutic agent oxaliplatin. The role of the SJW main constituents, hypericin, hyperforin and flavonoids, and their cellular and molecular effects in the modulation of the pain threshold was also investigated in comparison with the effects produced by the SJW dried extract in order to better elucidate the mechanism of action in this herbal plant.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley albino rats (180–200 g) from Harlan (S. Piero al Natisone, Italy) were used. Four rats were housed per cage. The cages were placed in the experimental room 24 h before the test for acclimatization. The animals were fed a standard laboratory diet and tap water *ad libitum* and kept at 23 ± 1 °C. The rats had a 12-h light/dark cycle, light at 7 AM. All experiments were carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize animal suffering, and to reduce the number of animals used.

2.2. Production of neuropathy

2.2.1. Chronic constriction injury

A peripheral mono neuropathy was produced in adult rats by placing loosely constrictive ligatures around the common sciatic nerve according to the method described by Bennett and Xie [24]. Rats were anesthetized with chloral hydrate. The common sciatic nerve was exposed at the level of the middle of the thigh by blunt dissection through biceps femoris. Proximal to sciatica's trifurcation, about 1 cm of the nerve was freed of adhering tissue and four ligatures (3/0 chromic tread) were tied loosely around it with about 1 mm spacing. The length of the affected nerve was 4–5 mm long. Great care was taken to tie the ligatures such that the diameter of the nerve was barely constricted when viewed with 40× magnification. In sham-operated animals, an identical dissection was performed, but the sciatic nerve was not ligated. The ligation was made on the right paws and left paws were untouched. Experiments were carried out 14 days after surgery.

2.2.2. Oxaliplatin repeated treatment

Oxaliplatin was dissolved in a 5% glucose solution at a concentration of 2.4 mg kg^{-1}. Oxaliplatin was administered i.p. for five consecutive days per week for three consecutive weeks according to a previously established protocol [25]. The control group received a 5% glucose solution. The experiments were carried out on day 21.

2.3. Paw pressure test

The instrument has a cone-shaped pusher and exerts a force which is applied at a constant rate (32 g per s) on the upper surface of the rat hind paw. The force is continuously monitored by a pointer which moves along a linear scale. The pain threshold is given by the force which first induces struggling from the rat. An arbitrary cut off value of 250 g was adopted. Those rats scoring <40 g or over 75 g in the pretest, performed before chronic constriction injury or oxaliplatin administration, were rejected (25%). The pain threshold of neuropathic animals was measured previously (pretest) and then 30, 60, 90, 120 and 180 min after administration of SJW and components.

2.4. Rota rod test

The apparatus consisted of a base platform and a rotating rod with a diameter of 3 cm and a non-slippery surface. The rod, 30 cm in length, was placed at a height of 15 cm from the base. Up to five rats were tested simultaneously on the apparatus, with a rod-rotating speed of 16 rpm. The integrity of motor coordination was assessed on the basis of the number of falls from the rod in 30 s. Those rats scoring less than three and more than six falls in the pretest were rejected (20%). Their performance time was measured previously (pretest) and then 60, 90 and 120 min after SJW, CHL and MET fractions or purified components administration. A total of 4–5 rats per group were tested.

2.5. I.c.v. injection technique

Intracerebroventricular (i.c.v.) administration was performed under ether anesthesia with isotonic saline as a solvent which has been previously described [26].

2.6. Preparation of membrane and cytosol fractions

Rat periaqueductal grey (PAG) area was dissected and homogenized in a homogenizing buffer. The homogenate was centrifuged at $9000 \times g$ for 15 min at 4 °C and the low speed pellet was discarded. The supernatant (total proteins) was centrifugated at $100{,}000 \times g$ for 60 min at 4 °C. The resulting supernatant was the cytosol fraction, and the pellet was resuspended in the homogenizing buffer containing 0.2% (wt/vol) Triton X-100. The homogenate was kept at 4 °C for 60 min with occasional stirring and then centrifugated at $100{,}000 \times g$ for 60 min at 4 °C. The resulting supernatant was used as the membrane fraction. Protein concentration of the cytosol and membrane fractions was quantified using Bradford's method (protein assay kit, Bio Rad Laboratories, Milan, Italy).

2.7. Immunoblot analysis

Membrane homogenates (10–50 μg) of naïve, untreated CCI and SWJ treated CCI rats were separated on 10% SDS-PAGE and transferred onto nitrocellulose membranes (100 min at 100 V) using standard procedures. The membranes were blocked in PBST (PBS containing 0.1% Tween) containing 5% nonfat dry milk for 90 min. Following washings, blots were incubated overnight at 4 °C with specific antibodies (Santa Cruz Biotechnology, CA, USA) against PKCγ, p-PKCγ, PKCε, p-PKCε or β-actin (1:1000 dilution). After being washed with PBST, the nitrocellulose membranes were incubated with goat anti-rabbit horseradish peroxidase-conjugated secondary antisera (1:5000) and left for 90 min at room temperature. Blots were then extensively washed according to the manufacturer's instruction and developed using enhanced chemiluminescence detection system (Pierce, Milan, Italy). The