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Hypericum perforatum L. as Adjuvant to Opioid Analgesia in an Animal Model of Migraine

Nicoletta Galeotti[1*], Enrica Bianchi[2] and Carla Ghelardini[1]

[1]Department of Neuroscience, Psychology, Drug Research and Child Health (NEUROFARBA), University of Florence, Viale Pieraccini 6, 50139 Florence, Italy.
[2]Department of Medical Surgical Sciences and Neuroscience, University of Siena, Via A. Moro 6, 53100 Siena, Italy.

Authors' contributions

This work was carried out in collaboration between all authors. Author NG designed the study, performed the experiments and statistical analysis, and wrote the first draft of the manuscript. Authors CG and EB managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: Despite their high analgesic efficacy, opioids can provide only partial pain relief during a migraine attack and their tolerability profile is unsatisfactory. The aim of the present study was to investigate the potentiating properties of *Hypericum perforatum* L. (SJW) to identify a safe and tolerable adjuvant to opioid analgesia in migraine therapy.
Study Design: Experimental study.
Place and Duration of Study: Department of Neuroscience, Psychology, Drug Research, and Child Health (NEUROFARBA), University of Florence, Florence, Italy, between September 2008 and July 2009.
Methodology: A mouse model of meningeal nociception induced by administration of the nitric oxide donor sodium nitroprusside (SNP) was used. The following treatment groups were used: saline, SNP, morphine, SJW, SNP+morphine, SNP+SJW, SNP+morphine+SJW. The presence of thermal allodynia was evaluated through the cold plate test. The presence of behavioural side effects was determined by the evaluation of locomotor activity (rotarod test), spontaneous mobility and inspection activity (hole board test).
Results: SNP induced a long lasting thermal allodynia that appeared after 1 h, peaked after 3-4 h and disappeared 6 h after administration. The co-injection of a single low

**Corresponding author: Email: guy.willems@med.kuleuven.be;*

dose of SJW (1 mg/kg) with morphine (2-5 mg/kg) greatly increased the opioid analgesia (P<.05). SJW, when administered alone,was unable to counteract SNP-induced allodynia. Among the main components of this herbal drug, hypericin produced a potentiating activity comparable to that induced by SJW whereas hyperforin and flavonoids were ineffective. Animal gross' behavior and locomotor activity were not altered by co-administrationof morphine and SJW.

Conclusion: Present results showed the potentiating activity of SJW on morphine antinociception in an animal model of migraine. This herbal drug might be proposedas adjuvant to opioid agonists to produce analgesia using lower, safer doses of opioids. This combination might represent a therapeutic perspective for migraine pain.

Keywords: Migraine; opioid; analgesia; adjuvant; Hypericum perforatum; morphine.

ABBREVIATIONS

NO: nitric oxide; PKC: protein kinase C; SNP: sodium nitroprusside; SJW: St John's wort.

1. INTRODUCTION

Migraine is the most common pain syndrome and poses a heavy socio-economic impact both in term of lost productivity and burden to the health care system. Despite its high prevalence, this syndrome remain sunder treated. In recent years, the options for the management of migraine patients have expanded, but not all migraineurs respond to treatments or do not experience sustained pain relief, and many have troublesome adverse effects [1,2]. For this reason, there is still a significant need for well-tolerated new therapeutic options that provide effective, quick, and sustained relief from migraine pain [3,4,5].

Migraine therapy would greatly benefit from the elucidation of its etiopathogenesis, but, despite much research on migraine pathophysiology, the mechanisms underlying migraine attacks remain poorly understood. Recently, it has been observed an overstimulation of protein kinase C (PKC) γ and ε within dura mater in an animal model of meningeal nociception [6], suggesting the presence of an altered pattern of activation of PKC-mediated signaling pathways during a migraine attack. This imbalanced PKC activity might be related to migraine pain since the administration of a pharmacological blocker of PKC reverted the NO donor-induced hyperalgesia and allodynia [6].

Opioids represent the cornerstone therapy for moderate-to-severe pain of many etiologies. Despite their high analgesic efficacy, these drugs can often provide only partial pain relief during a migraine attack and their tolerability profile is unsatisfactory [7]. It has been observed both in animals and humans that, in addition to the well-known analgesic activity, opioids can also induce hyperalgesia [8,9,10]. Interestingly, this paradoxical effect is producedvia μ-opioid receptor-mediated PKCγ stimulation [11,12]. This signalling pathway appears to play an opposing role in morphine analgesia since the silencing of PKCγ induced a potentiation in analgesic response in mice treated with a morphine analgesic dose [11]. The identification of an adjuvant to opioid analgesia endowed with PKC blocking properties might elevate opioid analgesic efficacy, thus reducing the dose and the incidence of side effects.

Hypericum perforatum L., commonly called St John's wort (SJW), has been used for centuries as medicinal plant. In the past two decades it has received attention for its antidepressant efficacy with a favourable tolerability profile [13]. Despite pharmacological studies on SJW have focused on its antidepressant activity, this herbal plant is also endowed with other bioactivities related to pain modulation. SJW showedanti-inflammatory properties following topic [14] and systemic administration [15]. More recently, the analgesic activity against acute pain [16] and the capability to relieve neuropathic pain in different animal models [17] were observed. This analgesic activity appeared after oral administration of low doses of SJW and was related to the presence of hypericin, an active constituent of SJW endowed with PKC blocking properties [18].

To identify a safe and tolerable adjuvant to opioid analgesia, we designed the present study to examine the potentiating efficacy of a SJW dried extract on morphine antinociception in an animal model of migraine.

2. MATERIALS AND METHODS

2.1 Animals

Male CD1 mice (20-22 g) from the Harlan Laboratories (Bresso, Italy) breeding farm were used. Mice were randomly assigned to standard cages, with four to five animals per cage. The cages were placed in the experimental room 24 h before behavioural tests for acclimatization. The animals were fed a standard laboratory diet and tap water ad libitum and kept at 23 ± 1 °C with a 12 h light/dark cycle, light on at 7 a.m. The experimental protocol was carried out after approval by the Animal Care and Research Ethics Committee of the University of Florence, Italy, under license from the Italian Department of Health. All studies involving animals are reported in accordance with the ARRIVE guidelines for experiments involving animals [19]. All efforts were made to minimize animal suffering, and to reduce the number of animals used.

2.2 Drug Administration

2.2.1 Animal model of meningeal nociception

The NO donor sodium nitroprusside (SNP) (1 mg/kg, Sigma, Italy), dissolved in saline, was administered intraperitoneally (i.p.) as previously reported [6] and the behavioural tests were performed 1-6 h after administration.

2.2.2 Behavioural testing

Morphine hydrochloride (SALARS, Como, Italy) was dissolved in isotonic saline solution (NaCl 0.9%) and administered intraperitoneally at the doses of 2 and 5 mg/kg, 210 min after NO donor administration. The morphine analgesic activity was detected 30 min after administration.

Hypericum perforatum L. (SJW) dried extract containing 0.32% of total hypericins (Indena Research Laboratories, Settala, Milan Italy), hypericin, hyperforin, hyperoside, quercetin, amentoflavone (Sigma, Milan, Italy) were dissolved in 1% carboxymethyl cellulose (CMC) solution immediately before use and administered by oral gavage. SJW was administered at the dose of 1 mg/kg. The doses of hypericin (2 µg/kg), hyperforin (42µg/kg), quercetin (8.3

μg/kg), amentoflavone (0.58 μg/kg), hyperoside (63.4 μg/kg) correspond to the amount of each component present in a 1 mg/kg preparation of SJW dried.

Since SJW effect peaked between 90 and 120 min after administration, to evaluate the capability of SJW to potentiate morphine analgesia, the herbal drug and its main components were administered 90 min before the opioid agonist.

Vehicles used to dissolve drugs were tested for the absence of any effect on pain threshold in comparison with naïve mice.

Doses and administration schedules of compounds used were chosen on the basis of time-course and dose-response curves performed in our laboratory.

2.3 Cold Allodynia

For assessment of cold allodynia, mice were placed on a cold plate that is maintained at a temperature of 4±0.1°C. Reaction times (s) were measured with a stopwatch before and 1, 2, 4 and 6 h after administration of the NO donor. The time between placements of a mouse on the plate and licking or lifting of a hind paw was measured with a digital timer. An arbitrary cut-off time of 60 s was adopted. Eight mice per group were tested.

2.4 Motor Coordination

The motor coordination was assessed by using the 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 was placed at a height of 15 cm from the base. The rod, 30 cm in length, was divided into 5 equal sections by 6 disks. Thus, up to 5 mice were tested simultaneously on the apparatus, with a rod-rotating speed of 16 r.p.m. The integrity of motor coordination was assessed on the basis of the number of falls from the rod in 30 s. Those mice scoring less than 3 and more than 6 falls in the pre-test were rejected (20%). The number of falls was measured before (pre-test) and 1, 2, 4 and 6 h after the administration of the NO donor. Eight mice per group were used.

2.5 Locomotor Activity

The locomotor activity was evaluated by using the hole-board test. The apparatus consisted of a 40 cm square plane with 16 flush mounted cylindrical holes (3 cm diameter) distributed 4 by 4 in an equidistant, grid-like manner. Mice were placed on the centre of the board one by one and allowed to move about freely for a period of 5 min each. Two photobeams, crossing the plane from mid-point to mid-point of opposite sides, thus dividing the plane into 4 equal quadrants, automatically signalled the movement of the animal (counts in 5 min) on the surface of the plane (locomotor activity). Miniature photoelectric cells, in each of the 16 holes, recorded (counts in 5 min) the exploration of the holes (exploratory activity) by the mice. Experiments were performed 4 h after administration of the NO donor. Eight mice per group were tested.

2.6 Statistical Analysis

All experimental results are given as the mean ± S.E.M. One-way or two-way analysis of variance (ANOVA) followed by Tukey or Bonferroni post hoc test, respectively, was used for statistical analysis.

3. RESULTS

3.1 Potentiation of Morphine Antinociception by Low Doses of SJW

The effect of low doses of St. John's Wort (SJW) on morphine antinociception was detected in an animal model obtained by administration of the NO donor sodium nitroprusside (SNP) in mice. SNP (1 mg/kg) produced a long lasting allodynia in the cold plate test that was significant 1 h after administration, persisted up to 4 hand disappeared after 6 h (Fig. 1).

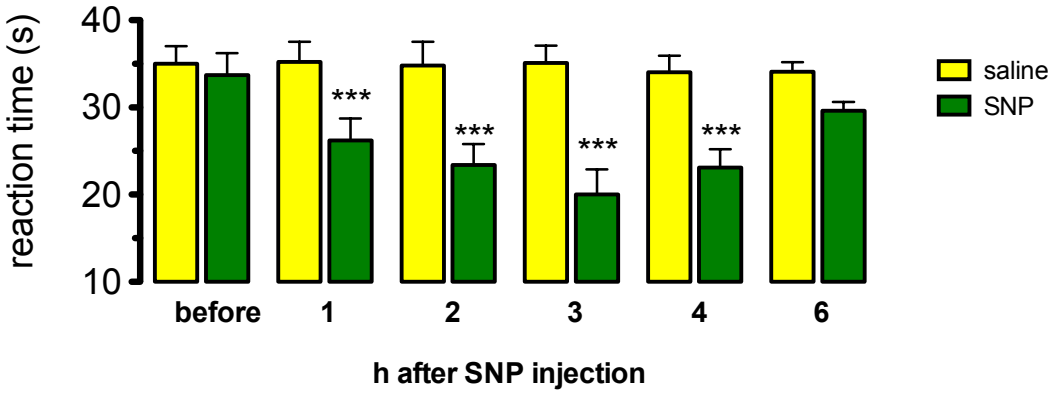

Fig. 1. Decrease of pain threshold by administration of the NO donor sodium nitroprusside (SNP) at different time intervals in the mouse cold plate test

**** P < 0.001 in comparison with corresponding saline-treated control group*

Oral administration of a SJW dried extract (1 mg/kg) alone was unable to modify pain hypersensitivity observed in the cold plate test following SNP administration. Morphine, at the dose of 5 mg/kg, significantly reversed cold allodynia showing antinociceptive properties. The co-injection of the opioid agonist with SJW significantly potentiated morphine activity (Fig. 2).

Lower doses of morphine (2 mg/kg) did not induce analgesia. The co-administration with SJW increased the reaction time values producing an antinociceptive effect (Fig. 3).

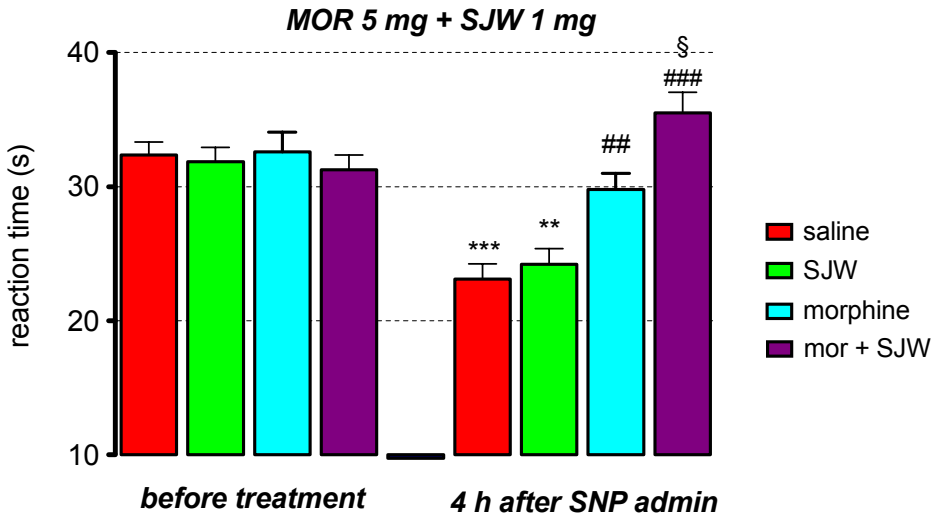

Fig. 2. Potentiation by SJW of morphine analgesic effect in a mouse model of meningeal nociception induced by SNP administration

*** P < 0.01, *** P < 0.001 in comparison with corresponding before treatment values; ## P < 0.01, ### P < 0.001 in comparison with SNP+saline; § P < 0.05 in comparison with SNP+morphine*

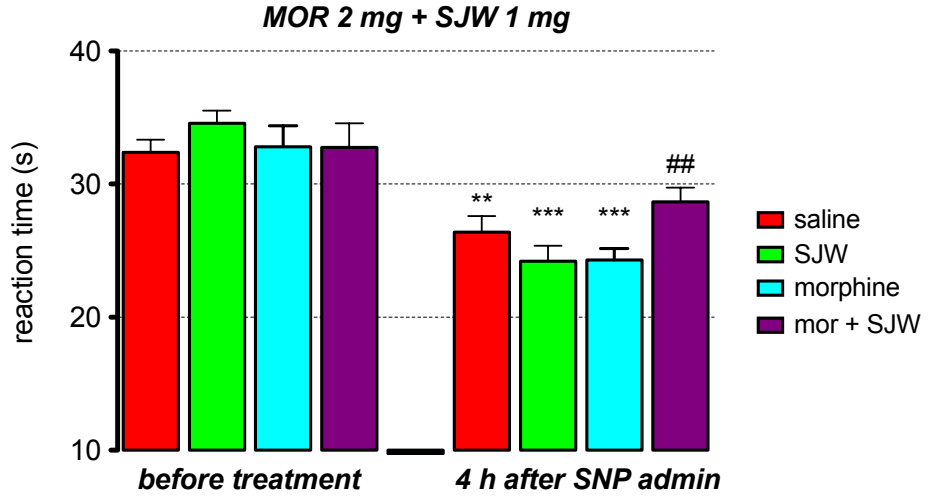

Fig. 3. Potentiation by SJW of an ineffective morphine dose in a mouse model of meningeal nociception induced by SNP administration

*** P < 0.01, *** P < 0.001 in comparison with corresponding before treatment values; ## P < 0.01, in comparison with SNP+morphine*

3.2 Differential Involvement of SJW Components in Morphine Potentiation

To elucidate the mechanism of the SJW potentiating activity, we investigated the effects produced by some of the main components of this herbal drug. The administration of hypericin (hyp) augmented the morphine analgesia produced by a dose of 5 mg/kg and rendered analgesic an ineffective dose of the opioid agonist (2 mg/kg). The efficacy profile

was similar to that showed by SJW. Conversely, oral administration of hyperforin (hyf), or the flavonoids quercetin (quer), amentoflavone (ame), hyperoside (hys) was devoid of any effect. Co-administration of hypericin with the other components did not produce any additive effect. In Fig. 4 are reported the results obtained with morphine 2 mg/kg.

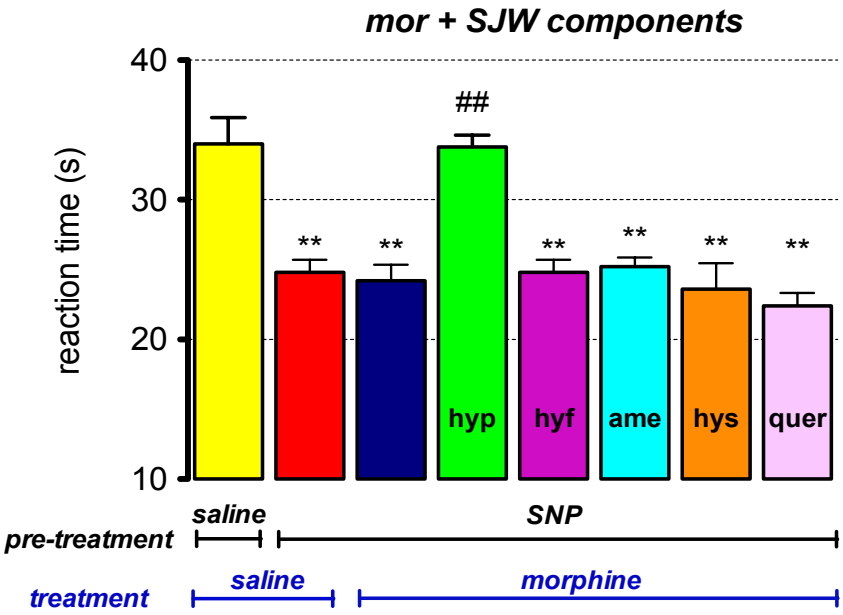

Fig. 4. Evaluation of the morphine potentiating effect of SJW components in a mouse model of meningeal nociception induced by SNP administration

*Morphine (2 mg/kg); hyp: hypericin; hyf: hyperforin; ame: amnetoflavone; hys: hyperoside; quer: quercetin. ** P < 0.01 in comparison with saline-treated control group; ## P < 0.01 in comparison with SNP+morphine*

3.3 Lack of Induction of Behavioral Side Effects

The combined administration of morphine with a low dose of SJW resulted in a significant augmentation of the thermal antinociceptive response. This augmentation was not associated with any visible sign of toxicity. Neither morphine nor SJW, when administered alone, altered locomotor activity of treated animals, as indicated by the rota rod test results (Fig. 5). Similarly, the spontaneous mobility (Fig. 6) and exploratory activity (Fig. 7) of mice treated with morphine or SJW were unmodified in comparison with the control group. Co-administration of the above-mentioned compounds did not alter mouse locomotor activity (Figs. 5,6,7).

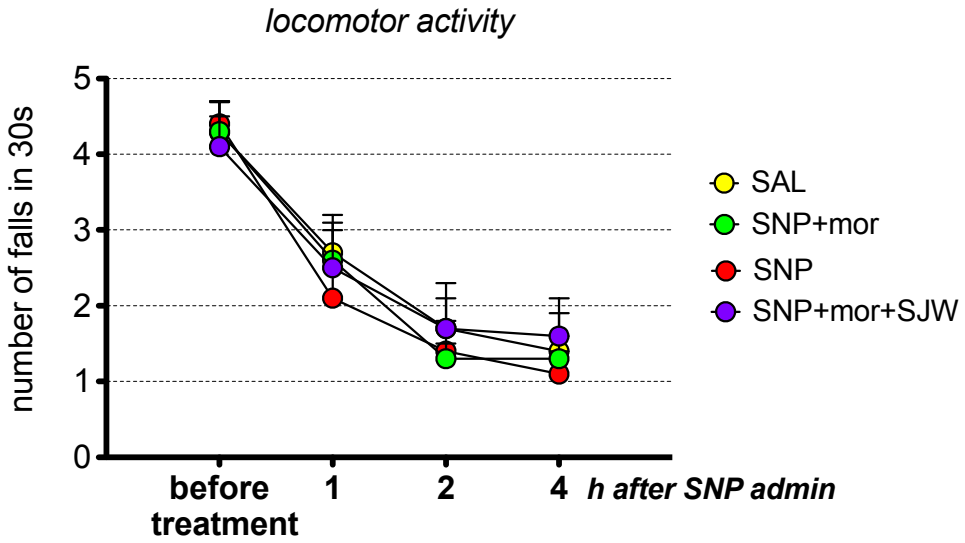

Fig. 5. Lack of locomotor impairment by co-administration of morphine and SJW at different time intervals in the mouse rotarod test

Mor: morphine 5 mg/kg; SJW 1 mg/kg

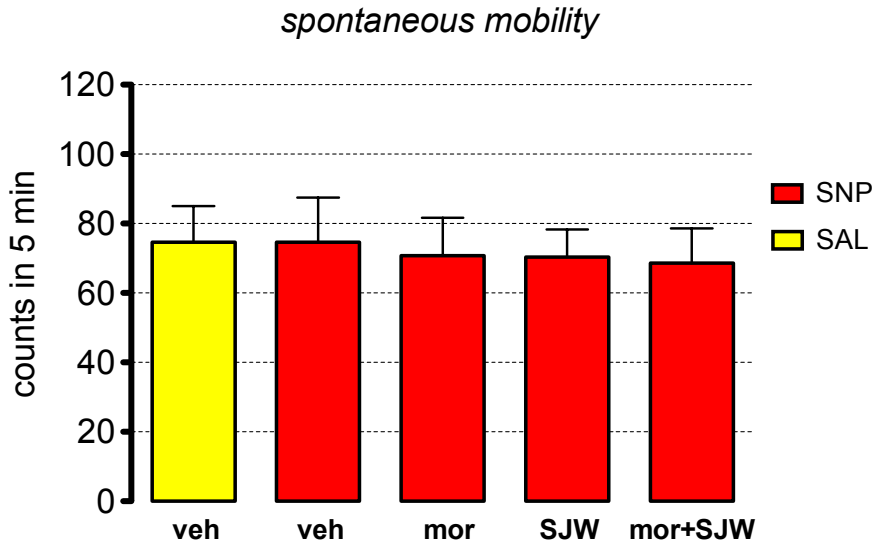

Fig. 6. Lack of impairment of spontaneous mobility by co-administration of morphine and SJW in the mouse hole-board test

Mor: morphine 5 mg/kg; SJW 1 mg/kg

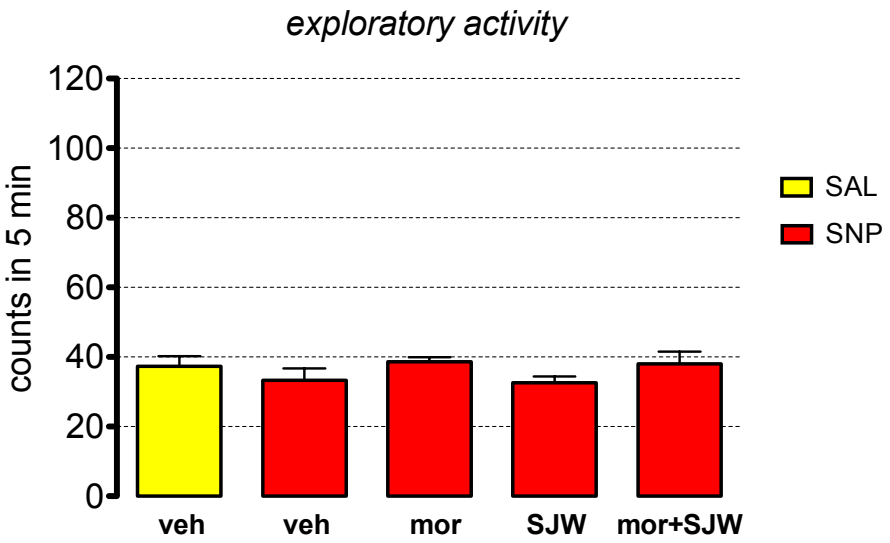

Fig. 7. Lack of impairment of exploratory by co-administration of morphine and SJW in the mouse hole-board test
Mor: morphine 5 mg/kg; SJW 1 mg/kg

4. DISCUSSION

Opioids are the cornerstone therapy for the treatment of moderate to severe pain. However, the efficacy of these drugs in the abortion of a migraine attack is surprisingly unsatisfactory and accompanied by the induction of untoward side effects [7]. In the absence of a well-tolerated therapy for the treatment of migraine able to produce a complete and quick relief from pain, migraineurs would greatly benefit from the possibility to potentiate opioid analgesic efficacy. In the present study we identify SJW as an adjuvant to opioid analgesia able to increase the antinociceptive properties of morphine in an animal model of meningeal nociception. Furthermore, co-administration of SJW with an ineffective dose of morphine increased the pain threshold up to values showed by naïve animals, drastically reducing the dose of the alkaloid required to abolish the NO donor-induced allodynia.

A common clinical feature of an untreated migraine attack is hyperalgesia and allodynia affecting the scalp, face, and contiguous regions of the neck and torso [20]. Allodynia has been recognized in about 75% of migraine attacks [20]. It is a clinical reflection of sensitization, and both central and peripheral sensitization are important insofar as they both influence attacks and perhaps disease progression [21,22]. Allodynia is not only a clinical marker for sensitization of central pain pathways, since differences in treatment efficacy during migraine attacks have been demonstrated based on the presence or absence of allodynia [23], showing allodynia as an important marker for treatment efficacy. The antiallodynic activity showed by the co-administration of morphine and SJW appears to be of particular relevance for the treatment of migraine pain.

SJW is endowed with antinociceptive properties in animal models of acute and chronic pain [16,17]. Furthermore, very recently it has been reported that a single oral administration of a SJW dried extract prevented pain hypersensitivity and neuronal activation in a mouse model of meningeal nociception induced by NO donors [24,25]. However, the potentiating effect

showed by this herbal medicine was not secondary to its antinociceptive and antimigraine activity, since the increase of the morphine analgesia was produced at very low doses devoid of any capability to modulate the pain threshold. Furthermore, the dose of SJW used was largely lower than those required to induce antidepressant activity (1.8-2.7 mg/die of total hypericins) [13]. Based on these data, the SJW-induced potentiation of morphine antinociception does not appear to be secondary to its antidepressant property.

SJW dried extract contains numerous active components [26]. The effects produced by the main constituents were investigated in order to identify the component responsible for the augmentation of morphine antinociception and to better elucidate its mechanism of action. Among the numerous SJW components, naphtodiantrones (hypericins), fluoroglucinols (hyperforins) and flavonoids represent the most effective compounds. Purified hypericin increased morphine antinociception with a similar efficacy and time course of SJW whereas hyperforin and flavonoids were devoid of any effect when administered alone or in combination.Present results indicate hypericin as a main constituent of the herbal drug responsible for the augmentation of morphine antinociception. Morphine produces a paradoxical hyperalgesic effect viaμ-opioid receptor-mediated PKCγ stimulation [11,12]. We can hypothesize that the capability of hypericin to increase morphine antinociception underlies its PKC blocking properties. Specifically, hypericin can block the signalling pathway that counteracts morphine analgesia producing a potentiation in the analgesic response.

Substantial drawbacks to the use of opioids are several serious side effects associated with their acute administration, including constipation, sedation, respiratory depression, and nausea [27,28]. Among the strategies proposed to minimize the adverse effects of opioids is the administration an adjuvant drug with synergistic analgesic effects in order to minimize the dose of the opioid, and hence its side effects, while maintaining acceptable levels of analgesia. One example is the combination of non-steroidal anti-inflammatory drugs or anticonvulsants with opioids [29,30]. In the present study we show an innovative approach to augmenting analgesic efficacy of morphine through the blockade of the PKC-mediated opioid-induced pronociceptive pathway. Furthermore, SJW is endowed with a favourable tolerability and safety profile [31]. We further demonstrated the tolerability of SJW alone or in combination with morphine. Recently, interactions of SJW with prescription drugs have been reported. SJW, at antidepressant doses, is a potent inducer of cytochrome P450 enzymes resulting in decrease plasma concentration of a number of drugs used in co-medication [32]. Recent studies showed that the degree of enzyme induction by SJW correlates strongly with the amount of hyperforin found in the product [33]. We observed that SJW potentiated morphine antinociception at very low doses containing an amount of hyperforin unable to produce clinical significant interactions [32,33].

5. CONCLUSION

The results of this study indicate that SJW potentiates morphine antinociception in an animal model of migraine. These data propose the use of opioid agonists in combination with low doses of this herbal drug as a therapeutic perspective to obtain intense analgesic efficacy using lower, and then safer, doses of opioid. The use of a natural compound as adjuvant to opioid analgesia can meet a good compliance by the patients improving the management of migraine pain.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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