Antinociceptive Activity of (−)-Carvone: Evidence of Association with Decreased Peripheral Nerve Excitability

Juan Carlos Ramos Goncalves, Fernando de Sousa Oliveira, Rubens Batista Benedito, Damiano Pergentino de Sousa, Reinaldo Nobrega de Almeida, and Demetrius Antonio Machado de Araujo

“Laboratory of Pharmaceutical Technology, Federal University of Paraiba; CP 5009, CEP 58051–970, Joao Pessoa, Paraiba, Brazil; and Department of Physiology, Federal University of Sergipe; CEP 49100–000, Sao Cristovao, Sergipe, Brazil. Received November 17, 2007; accepted December 26, 2007; published online February 13, 2008

(−)-Carvone is a monoterpenic ketone that is the main active component of Mentha plant species like Mentha spicata. This study aimed to investigate the antinociceptive activity of (−)-carvone using different experimental models of pain and to investigate whether such effects might be involved in the nervous excitability elicited by others monoterpenes. In the acetic acid-induced writhing test, we observed that (−)-carvone-treated mice exhibited a significant decrease in the number of writhes when 100 and 200 mg/kg was administered. It was also demonstrated that (−)-carvone inhibited the licking response of the injected paw when 100 and 200 mg/kg was administered (i.p.) to mice in the first and second phases of the formalin test. Since naloxone (5 mg/kg, s.c.), an opioid antagonist, showed no influence on the antinociceptive action of (−)-carvone (100 mg/kg), this suggested nonparticipation of the opioid system in the modulation of pain induced by (−)-carvone. Such results were unlikely to be provoked by motor abnormality, since (−)-carvone-treated mice did not exhibit any performance alteration on the Rota-rod apparatus. Because the antinociceptive effects could be associated with neuronal excitability inhibition, we performed the single sucrose gap technique and observed that (−)-carvone (10 ms) was able to reduce the excitability of the isolated spinal nerve through a diminution of the compound action potential amplitude by about 50% from control recordings. We conclude that (−)-carvone has antinociceptive activity associated with decreased peripheral nerve excitability.

Key words (−)-carvone; antinociception; compound action potential

Although a considerable number of analgesic drugs are available for the treatment of pain, the search for development of new compounds as therapeutic alternatives continues since the available analgesic drugs exert a wide range of side effects. Because of their relatively low cost and easy availability in several countries, natural active products could be used as synthesis models of more selective and powerful drugs.

The essential oils are natural products that exhibit a variety of biological properties, such as analgesic, anticonvulsant, and anxiolytic. Those effects are attributed to the monoterpenes, which are the major chemical components of these oils. Previous studies showed that some monoterpenes also possess antinociceptive and anesthetic activities in animal experiments, demonstrating their potential contribution to the development of more efficient analgesic drugs.

(−)-Carvone (p-mentha-6,8-dien-2-one) is a monoterpenic ketone (Mentha spicata), a relative of common mint distilled from this plant. Recently, our group has demonstrated that (−)-carvone derivatives, such as rotundifolone, hydroxydihydrocarvone, and epoxy-carvone appear to have analgesic properties. Such compounds decreased the writhing nociceptive response induced by acetic acid in mice, although the mechanisms underlying such properties remain unclear.

A previous study reported a central nervous system (CNS) depressive activity of citronellol, another monoterpenic, that exhibited peripheral activity, reducing nervous excitability through a diminution of the compound action potential (CAP) amplitude. Another study using monoterpenes indicated a neuroactive property affecting neuronal intracellular signaling which could be provoked through neurotransmitter-gated current modulation induced by these terpenes. For these reasons, it appeared possible that (−)-carvone could also have analgesic activity involving the central and peripheral nervous systems. Therefore our objective was to verify the antinociceptive effect of (−)-carvone using different experimental models of pain and to investigate whether such effect might be involved in nervous excitability using the single sucrose gap technique.

MATERIALS AND METHODS

Drugs For all in vivo experiments the following drugs were used: (−)-carvone (Aldrich, U.S.A.), morphine hydrochloride (Merck, U.S.A.), naloxone hydrochloride (Research Biochemical, U.S.A.) 37% formaldehyde (Vetec, Brazil), diazepam, acetylsalicylic acid (ASA), and acetic acid (Sigma, U.S.A.). Vehicle was 5% Tween 80 (Sigma, U.S.A.) dissolved in 0.9% saline solution and used to dilute the test drugs. The physiologic solution used for the in vitro tests was composed of (in mEq): NaCl 150; KCl 4; CaCl2 1; MgCl2 1; glucose 10; and [N-(2-hydroxyethyl)piperazine-N’-2-ethane sulfonic acid] (HEPES) 10, adjusted to pH 7.4 with NaOH.

Fig. 1. Chemical Structure of (−)-Carvone

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**Animals**  
Adult (3-month-old) male albino Swiss mice (25—35 g) and Wistar rats (350 g) were randomly housed in appropriate cages at 21±1°C with a 12/12-h light/dark cycle (light from 06:00 to 18:00), with free access to food (Purina, Brazil) and tap water. All experimental observations were conducted between 12:00 and 17:00. The animals were killed by cervical dislocation, and all procedures were carried out in accordance with Ethical guidelines (CEPA/LTF-UPPB, process number 0208/06).

**Acetic Acid-Induced Writhing**  
Initially the mice were divided into five groups (*n* = 8). Subsequently, (−)-carvone (50, 100, 200 mg/kg), vehicle (control), and morphine (6 mg/kg) were administered intraperitoneally (i.p.) 30 min before an injection of 0.8% acetic acid (10 ml/kg). Each animal was isolated in an individual observation chamber and 10 min after acetic acid injection the cumulative number of writhing responses was recorded during 10 min.\(^1,15\)

**Formalin-Induced Nociception**  
This test was performed according to the model described by Vaz et al.,\(^1,16\) which represents a modification of the original model described by Hunskaar et al.\(^17\) The animals were divided into six groups (*n* = 8) and treated i.p. with vehicle (control), (−)-carvone (50, 100, 200 mg/kg), morphine (6 mg/kg), and 100 mg/kg of ASA. After 30 min, 20 μl of 2.5% formalin solution (0.92% formaldehyde in 0.9% saline) was injected into the subplantar area of the right hindpaw. The duration of paw licking was measured at 0—5 min (first phase) and 15—30 min (second phase) after formalin administration.

**Possible Antagonism of the (−)-Carvone Antinociceptive Effect by Pretreatment with Naloxone**  
Mice were subcutaneously (s.c.) pretreated (*n* = 8) with 5 mg/kg of naloxone, a nonselective opioid antagonist, 15 min before the i.p. administration of vehicle (control), (−)-carvone (100 mg/kg), or morphine (6 mg/kg). Subsequently, the acetic acid-induced writhing test was performed as described above.

**Rota-Rod Test**  
Initially, the mice able to remain on the Rota-rod (Ugo Basile, Model 7750, Italy) longer than 180 s (7 rpm) were selected 24 h before the test.\(^18\) Then the selected animals were divided into five groups (*n* = 8) and treated i.p. with vehicle (control), (−)-carvone (50, 100, 200 ml/kg), and diazepam (4 mg/kg). Thirty minutes later, each animal was tested on the Rota-rod and the time (s) they remained on the bar for up to 180 s was recorded after 30, 60, and 120 min.

**Electrophysiologic Assays**  
The single sucrose gap technique was used as described in a previous papers.\(^13,19\) Briefly, the sciatic nerves from Wistar rats were carefully removed and desheathed. One nerve bundle was positioned across the five compartments of the experimental chamber, which contained solid petroleum jelly at the partitions to isolate them electrically. Compartments 1 and 2 at one end of the nerve bundle were used to apply supramaximal stimulation, which consisted of 100-μs isolated rectangular voltage pulses delivered by a stimulator (CF Palmer, Model 8048, U.K.) triggered manually. These parameters were chosen to stimulate fast-conducting myelinated fibers (Aβ) selectively. All compartments were filled with physiologic solution, except for the fourth compartment, which was filled with isotonic sucrose (280 mN in ultralitrified water) solution that was continuously renewed to isolate the neighboring recording com-partsments electrically. (−)-Carvone (10 mM), diluted in physiologic solution with 0.5% Tween 80, was introduced into the test compartment. The potential difference between the test and the fifth (last) compartment was recorded every 10 min. Data were converted to digital form by a microcomputer-based 12-bit A/D converter at a rate of 10.5 kHz and later analyzed using a suite of programs (Lynx, Brazil). To quantify the effects of (−)-carvone, we used the CAP amplitude as a parameter, measured by the potential difference between the baseline and the maximal voltage of the CAP.

**Statistical Analysis**  
The in vivo and in vitro experimental data obtained were evaluated using one-way analysis of variance (ANOVA), followed by Dunnett’s test. Differences were considered to be statistically significant when *p*<0.05.

**RESULTS**

In the acetic acid-induced writhing test, the antinociceptive effect, represented by writh reduction, elicited by 100 mg/kg of (−)-carvone (2.6±1.1) in mice was similar to that of morphine 6 mg/kg (3.3±1.4), a standard opioid drug,\(^1\) when both groups were compared with control (14.4±2.7). The nociceptive response was almost extinguished when 200 mg/kg of (−)-carvone was administered (0.3±0.1), although a dose of 50 mg/kg did not produce any significant effect (Fig. 2A).

(−)-Carvone inhibited the licking response to the inject-paw when 100 mg/kg (63.5±6.4 s; 83.9±39.4 s) and 200 mg/kg (40.7±8.5 s; 16.8±16.4 s) were administered i.p. in mice compared with the control group (119.7±9.9 s; 296.4±31.8 s) in the first and second phases of the formalin

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Fig. 2. Antinociceptive Activity of (−)-Carvone (−)-CV Using Different Experimental Models of Pain

(A) Effects of (−)-CV in the acetic acid-induced writhing test in mice. Vehicle (control), (−)-CV (50, 100, 200 mg/kg), or morphine (MOR) were administered i.p. 30 min before acetic acid injection. (B) Effects of (−)-carvone (50, 100, 200 mg/kg), MOR (6 mg/kg), and acetylsalicylic acid (ASA, 100 mg/kg) on formalin-induced nociception in mice. Each column represents mean±S.E.M. (*p*<0.01 vs. control; *p*<0.01 vs. 1st-phase control, *p*<0.01 vs. 2nd-phase control (ANOVA followed by Dunnett’s test).
Fig. 3. Effects of Naloxone (NAL) in (−)-Carvone (−)-CV) and Mor- phine (MOR) Antinociceptive Activity

The acetic acid-induced writhing test was performed as described in the text. Pre-treatment with NAL (5 mg/kg, s.c.) was performed 15 min before treatment (i.p.) with vehicle (control), (−)-CV (100 mg/kg), or MOR (6 mg/kg). The number of writhes was counted over a period of 10 min. Each column represents mean ± S.E.M. (n = 8).

Table 1. Time (s) on the Rota-Rod Observed in Mice after i.p. Treatment with Vehicle (Control), (−)-Carvone (50, 100, 200 mg/kg), or Diazepam (4 mg/kg)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg, i.p.)</th>
<th>Time (s) on Rota-rod/180 s</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 min</td>
<td>Period (observation)</td>
</tr>
<tr>
<td>Vehicle (control)</td>
<td>—</td>
<td>177.0±3.0</td>
</tr>
<tr>
<td>(−)-Carvone</td>
<td>50</td>
<td>179.6±0.4</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>161.3±16.7</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>162.1±17.9</td>
</tr>
<tr>
<td>Diazepam</td>
<td>4</td>
<td>32.0±7.3*</td>
</tr>
</tbody>
</table>

The motor response was recorded for the following 180 s after drug treatment. Statistical differences vs. control group were calculated using ANOVA, followed by Dunnett’s test (n = 8). *p < 0.01.

DISCUSSION

The present study demonstrated the antinociceptive activity of (−)-carvone through two distinct in vivo models of analgesia, the acetic acid-induced writhing and formalin tests. The latter method is believed to resemble clinical pain more closely in comparison with other tests that employ mechanical or thermal stimuli.19,20)

The writhing behavior in mice produced by an i.p. injection of acetic acid is used to evaluate central and peripheral analgesic activities.1) The acetic acid-induced algesia promotes endogenous substance release and many other effects stimulating nervous termination of pain.22,23) Based on the percentage of writhing inhibition obtained with (−)-carvone administration, in the different doses tested, the analgesic effect was similar to that of morphine, a standard drug (Fig. 2A).

The formalin test is a valuable tool for assessing the analgesic properties of drug candidates.24) This test is a model of nociceptive response in two distinct phases involving different mechanisms. The first phase (neurogenic pain) results from the direct chemical stimulation of myelinated and unmyelinated nociceptive afferent fibers, mainly C fibers, which can be suppressed by opioid analgesic drugs like morphine.1) The second phase (inflammatory pain) results from the release of inflammatory mediators in the peripheral tissues and of functional changes in the neurons of the spinal dorsal horn that, in the long term, promote facilitation of synaptic transmission at the spinal level.25,26) This latter phase was reported to be sensitive to the action of the majority of nonsteroidal antiinflammatory drugs (NSAIDs), including ASA, indomethacin, and naproxen.20,27)

In this model, (−)-carvone inhibited the licking response of mice in both phases of the formalin test (Fig. 2B), sug-
gesting this monoterpenone could exert its antinociceptive effects connected with central or peripheral mechanisms. However, naloxone, an opioid antagonist, showed no influence on the antinociceptive action of (−)-carvone (100 mg/kg, i.p.) in the acetic acid-induced writhing test (Fig. 3). This suggests the nonparticipation of the opioid system in the modulation of pain provoked by (−)-carvone. Because (−)-linalool, another monoterpenone, is involved in a central antinociceptive effect associated with glutamate-NMDA receptors,38—30 it might be possible that (−)-carvone-like effects could be involved in such a nonopioid central mechanism.

Previous studies suggested that the CNS depression and the nonspecific muscle relaxation effect can reduce the formalin test results.31—33 We observed that (−)-carvone-treated mice did not show any performance alterations in the Rota-rod test with the doses used (Table 1). We did not see any interference with the motor coordination of the animals, therefore eliminating a nonspecific muscle relaxation effect of (−)-carvone at the doses used.

It was reported that lidocaine, a local anesthetic blocker of fast voltage-gated Na+ channels,34 was efficient in producing alagic-stimuli reduction in the two phases of the formalin test.35,36 This result suggests that (−)-carvone might also produce antinociceptive effects by voltage-gated Na+ channel blockade.

From the information that inhibition of neuronal excitability is associated with blockade of the voltage-dependent Na+ channels,13,34 we used the single sucrose gap method and observed that (−)-carvone was able to reduce the excitability of the isolated nerves through a diminution of CAP amplitude (Fig. 4). This supports the voltage-gated Na+ channel blocking hypothesis that would influence the observed antinociceptive response of (−)-carvone in the experimental models of pain used in this study. Since we have support for a peripheral anesthetic action, the possibility that a voltage-gated Na+ channel blocking action of (−)-carvone occur in both central and peripheral nervous systems cannot be disregarded.37 However, more specific methodologies are required to confirm that.

Based on our results, (−)-carvone, a monoterpenone ketone commonly founded in Mentha plant species, has antinociceptive activity that may be associated with decreased peripheral nerve excitability.

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REFERENCES