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Involvement of 5-HT_2 receptors in the antinociceptive effect of *Uncaria tomentosa*

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Abstract

Uncaria tomentosa (Willd.) DC (Rubiaceae) is a vine that grows in the Amazon rainforest. Its bark decoctions are used by Peruvian Indians to treat several diseases. Chemically, it consists mainly of oxindole alkaloids. An industrial fraction of *U. tomentosa* (UT fraction), containing 95% oxindole alkaloids, was used in this study in order to characterize its antinociceptive activity in chemical (acetic acid-induced abdominal writhing, formalin and capsaicin tests) and thermal (tail-flick and hot-plate tests) models of nociception in mice. UT fraction given by the i.p. route dose-dependently suppressed the behavioural response to the chemical stimuli in the models indicated and increased latencies in the thermal stimuli models. The antinociception caused by UT fraction in the formalin test was significantly attenuated by i.p. treatment of mice with ketanserin (5-HT2 receptor antagonist), but was not affected by naltrexone (opioid receptor antagonist), atropine (a nonselective muscarinic antagonist), L-arginine (precursor of nitric oxide), prazosin (α1-adrenoceptor antagonist), yohimbine (α2-adrenoceptor antagonist), and reserpine (a monoamine depleter). Together, these results indicate that UT fraction produces dose-related antinociception in several models of chemical and thermal pain through mechanisms that involve an interaction with 5-HT2 receptors.

Keywords: *Uncaria tomentosa*; Antinociception; 5-HT_2 receptors; Mechanism of action

1. Introduction

Uncaria tomentosa (Willd.) DC (Rubiaceae) is a woody liana, indigenous to the Amazon rainforest. It is commonly known as cat's claw or *uña de gato* due to its morphological aspects. South American Indians have reportedly used decoctions of the barks in folk medicine as a "miraculous" remedy (Keplinger et al., 1999). Its applications include the treatment of arthritis, rheumatism, abscesses, inflammations, fever, allergy, asthma, cancer, gastric ulcer, contraception, menstrual irregularity, recovery from childbirth, and skin impurities (Laus et al., 1997).

Many biological activities have been described for numerous extracts of *Uncaria tomentosa* (UT). The anti-inflammatory activity has been assayed in vitro and in vivo. It was demonstrated that an aqueous extract of UT was able to minimize rat mucosal injury in the indomethacin-induced intestinal inflammation, depredate peroxynitrite and attenuate peroxynitrite-induced cell death, prevent the activation of the transcription factor NF-κB, inhibit the expression of inducible genes associated with inflammation—specifically negating the expression of inducible nitric oxide synthase (iNOS) and thereby attenuating NO production (Sandoval-Chacón et al., 1998; Aguilar et al., 2002; Akesson et al., 2003). Extracts and fractions of UT were also bioassayed by the carrageenan-induced oedema test in rat paw, and the

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most active fractions were isolated and analysed for their chemical constituents; however, all the resulting compounds were found to be inactive in this model at the tested doses, which suggests that the strong anti-inflammatory action of UT observed in other studies was due to a combination of compounds (Aquino et al., 1991). Decoctions of the bark of UT showed an antimutagenic effect in vitro, and were able to inhibit the epithelial cell death in response to oxidant stress (Rizzi et al., 1993; Miller et al., 2001). Antioxidant properties have also been described (Sandoval et al., 2000). Furthermore, it was found that UT extracts can have an immunostimulant action and enhance DNA repair capability (Lemaire et al., 1999; Sheng et al., 2000a,b).

Phytochemical analyses of *Uncaria tomentosa* root bark have revealed the presence of triterpenes, quinovic acid glycosides, some minor constituents and alkaloids (Aquino et al., 1989, 1997). Alkaloids are usually divided into classes according to their main chemical structure. The indole family is the most numerous plant alkaloid class, and oxindole alkaloids represent one of the major subgroups of this class (Martin and Mortimore, 1990). Six main pentacyclic oxindole alkaloids are found in UT: pteropodine and isopteropodine as major alkaloids, together with mitraphylline, isomitraphylline, speciophylline and uncarine F (Stuppner et al., 1992a,b; Laus and Keplinger, 1994; Ginkel, 1996). Other isomers – uncarines C, D and E – were also reported in lower quantities (Muhammad et al., 2001). In vitro cytotoxic activity of isolated oxindole alkaloids was observed against four human cell lines (Muhammad et al., 2001). In addition, antiviral activity of *U. tomentosa* has also been described, as some quinovic acid glycosides found in UT barks have shown an antiviral effect against cell infection with DNA viruses (Aquino et al., 1989).

Alkaloid compounds, such as morphine, have been extensively used in human history to alleviate pain. Mitragynine, an indole alkaloid from the Thai medicinal plant *Mitragyna speciosa*, had its antinociceptive activity described by Matsumoto et al., (1996, 2004), and there is an extensive literature describing the antinociceptive activity of other alkaloids (Elisabetsky et al., 1995; Ameri, 1998; Küpeli et al., 2002; Verotta et al., 2002). However, no previous correlation between the alkaloids of *Uncaria tomentosa* and antinociceptive activity has been reported so far. In this study, we have examined in detail the antinociceptive properties of UT in chemical and thermal models of nociception in mice and also some of the mechanisms that might potentially underlie this activity.

2. Materials and methods

2.1. Animals

Male Swiss mice weighing 35–40 g were used in all experiments. Animals were housed under standard light/dark cycle (12 h each) and temperature (22±2 °C) and acclimatized to the laboratory for at least an hour before the tests. Food and water were available ad libitum. The experiments were performed after approval of the protocol by the Institutional Ethics Committee and were carried out in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985) and the Ethical Guidelines for Investigations of Experimental Pain in Conscious Animals (Zimmermann, 1983). The numbers of animals and intensities of noxious stimuli used were the minimum necessary to demonstrate the consistent effects of the drug treatments.

2.2. Drugs

Formalin (Nuclear, SP, Brazil), acetic acid (Cinética Química, SP, Brazil), capsaicin (8-methyl-*N*-vanillyl-6-nonenamide; Calbiochem, San Diego, USA), atropine sulphate, chlorimipramine hydrochloride, clonidine hydrochloride, ketanserin, L-arginine (L-ARG), N^G-nitro-L-arginine (L-NOARG), naltrexone hydrochloride, phenylephrine hydrochloride, prazosin hydrochloride, reserpine, R-(−)-DOI (R-[-]-2,5-Dimethoxy-4-iodoamphetamine), yohimbine (Sigma Chemical Co., St. Louis, MO, USA), ascorbic acid and morphine hydrochloride (Merck AG, Darmstadt, Germany) were dissolved in PBS (NaCl 137 mM, KCl 2.7 mM and phosphate buffer 10 mM; Sigma Chemical Co., St. Louis, MO, USA). Capsaicin was dissolved in 1% dimethylsulphoxide (Sigma Chemical Co., St. Louis, MO, USA) and PBS. Reserpine was dissolved in 2% ascorbic acid solution. Prazosin was dissolved in 0.2% dimethylformamide (Sigma Chemical Co., St. Louis, MO, USA) solution.

An industrial standardised *Uncaria tomentosa* fraction (UT fraction) containing 95% oxindole alkaloids was used in all experiments. Plant material was collected in Pozuzo area, in Peru. The following stereoisomers were identified by HPLC method: speciophylline, uncarine f, mitraphylline, isomitraphylline, pteropodine, rhynchophylline, isorhynchophylline and isopteropodine, accounting for 95% of the sample (m/m). In order to be administered by i.p. route, the fraction was dissolved in Tween-80 (Fischer Scientific International) and then diluted with PBS in order to obtain a 10% Tween-80 solution. The final concentration of Tween-80 did not exceed 10% and did not cause any "per se" effect. Control animals received vehicle only (10 mL/kg i.p.).

2.3. The abdominal writhing test

The writhing test was carried out according to the method previously described by Koster et al. (1959). Animals were pre-treated intraperitoneally (i.p) with the UT fraction at the following doses: 3, 10, 30 and 100 mg/kg administered 30 min before the induction of nociception. 0.6% acetic acid solution was injected i.p. (10 mL/kg) and the animals were placed in an acrylic observation chamber ($12 \times 12 \times 25$ cm). The number of writhing responses