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Journal of Ethnopharmacology 64 (1999) 109–115

Journal of ETHNO-PHARMACOLOGY

Stimulation of interleukin-1 and -6 production in alveolar macrophages by the neotropical liana, *Uncaria tomentosa* (Uña de Gato)

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Received 5 February 1998; received in revised form 1 June 1998; accepted 8 June 1998

Abstract

Two extracts of different collections of the traditional medicine uña de gato (*Uncaria tomentosa*) from Peru were characterized by High Pressure Liquid Chromatography as containing approximately 6 mg/g total oxindole content prior to studies with alveolar macrophages. The plant preparations greatly stimulated IL-1 and IL-6 production by rat macrophages in a dose dependent manner in the range of 0.025–0.1 mg/ml. They were also able to enhance IL-1 and -6 in lipopolysaccharide-stimulated macrophages. The results suggest a strong immunostimulant action of this plant.

Keywords: Interleukins-1 and -6; Immunostimulants; Oxindoles; Macrophages; *Uncaria tomentosa*

1. Introduction

Uña de gato (Cat's claw) is a herbal product prepared from the neotropical liana *Uncaria tomentosa* (Willd.) D.C.(Rubiaceae) which has recently become a widely sold phytomedicine in North America. The vine is found in tropical forests from Belize to Peru and its large recurved spines give rise to its common name in Spanish and English. In Peru, the root bark has been used traditionally for inflammatory conditions such as arthritis, as well as for cancer, in convalescence (as a tonic), and in the treatment of viral diseases, gastric ulcer, and menstrual disorders. (Obregon, 1995).

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0378-8741/99/$ - see front matter
PII S0378-8741(98)00113-5

Cat's claw contains a number of characteristic oxindole alkaloids (Fig. 1) (Stuppner et al. 1992), as well as quinovic acid glycosides (Cerri et al., 1988; Aquino et al., 1989), and novel polyhydroxylated triterpenes (Aquino et al., 1991). A number of steroids (Senatore et al., 1989) and other widely distributed phytochemicals have also been identified.

Antiviral activity against vesicular stomatitis virus was demonstrated with six quinovic acid

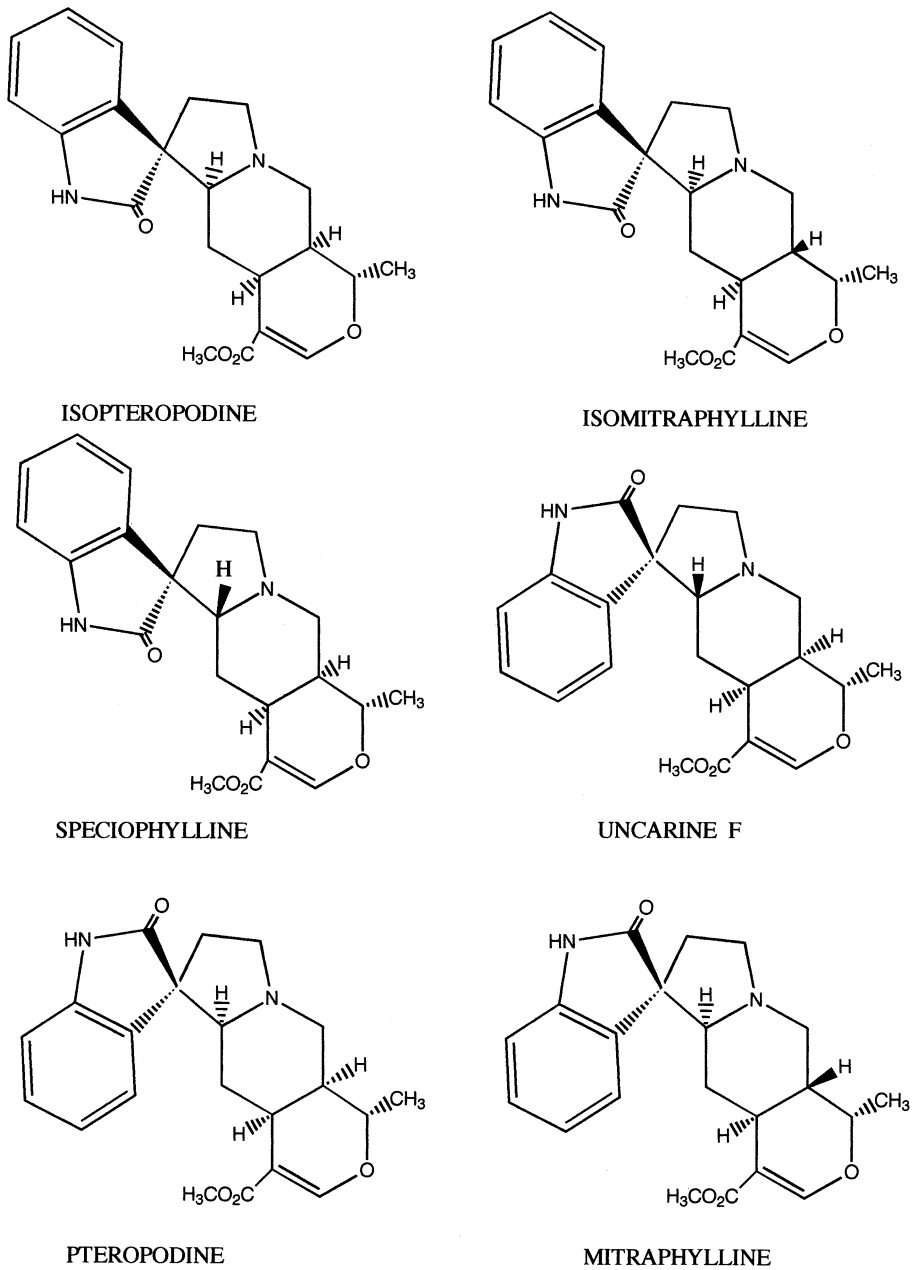

Fig. 1. Alkaloids of *Uncaria tomentosa*.

glycosides (Aquino et al., 1989) but only at concentrations close to cellular cytotoxicity. Anti-inflammatory activities have been associated with the steroids (Senatore et al., 1989) and quinovic acid glycosides (Aquino et al., 1991). Pharmacological investigations of aqueous extracts of the plant did not demonstrate any mutagenic activity in the Ames test but did show an antimutagenic activity in photomutagenesis induced by 8-methoxypsoralen and UVA in *Salmonella* spp. (Rizzi et al., 1993). In a recent study, no toxicity was observed with aqueous cat's claw extracts evaluated in several in vitro toxicity bioassays (Maria et al., 1997).

Considerable scientific interest in the plant has been generated by the demonstration of the plant's potent immunostimulant action. Four indole alkaloids, isopteropodine, pteropodine, isomitaphylline, and isorynochophylline potentiated the phagocytosis of carbon particles by white blood cells (Wagner et al., 1985). This use has now been patented (Keplinger et al., 1989, 1990) and commercial *Uncaria* products are already available. Wagner (1995) reported that an *Uncaria tomentosa* extract was among the most potent immunostimulant species examined in his laboratory. In order to better understand the effect on the immune system, we have studied the effect of this phytomedicine on cytokine production in alveolar macrophages in the present study, the results of which are reported here. In particular interleukin-1 (IL-1) and interleukin-6 (IL-6) which are known to initiate a cascade of defense activities of the immune system were examined.

2. Materials and methods

2.1. Plant material

Two commercial extracts of *Uncaria tomentosa* stem bark derived from different areas of Peru were used in the study. The Pacifico material (Liofilizadora del Pacifico, S.A. of Peru; UO # 19503) was obtained from Dr F. Cabieses, Instituto de Medicina Tradicional (INMETRA), Lima. This material was collected from the Ashaninka region of Peru. The extract was prepared by water extraction of the bark and lyophilization of the extract. The La Molina material (ARCO distributiong Co.-UO # 19502) was obtained from the Universidad La Molina and was prepared by water extraction and atomization of material derived from lowland tropical collections between Cusco and Manu National Park. A traditionally used milled dry plant powder (not an extract) which was reputed to have similar ethnobotanical properties (coded AS3-UO # 19501) was also included in the trials.

2.2. HPLC analysis of plant material

The method used was essentially similar to that of Stuppner et al. (1992). Injection volume was 20 μl and chromatography was performed at 23°C with a certified Beckman reversed-phase system equipped with System Gold software, a module 168 diode array detector, module 502 autosampler, and module 126 solvent delivery system. HPLC standards were obtained from Dr Yuan Chun Ma, Vancouver, BC.

2.3. Macrophage assays

2.3.1. Alveolar macrophage culture and stimulation

Alveolar macrophages (AM) were obtained by bronchoalveolar lavage of rats as described in our previous studies (Lemaire, 1991). For cytokine measurement, AM (0.5×10^6/ml) were incubated in RPMI 1640 medium supplemented with 0.5% dialysed FBS, 0.005% gentamycin and 0.8% Hepes in the presence or absence of *Uncaria tomentosa* (LaMolina and Pacifico genotypes; 0.025–0.5 mg/ml), AS3 (0.025–0.5 mg/ml), and lipopolysaccharide (LPS) (1 μg/ml) for 24 h at 37°C in a humidified atmosphere containing 5% CO_2. After incubation, cell-free supernatants were collected by centrifugation and kept at −80°C.

2.3.2. Sample preparations

Uncaria tomentosa (LaMolina and Pacifico products) and AS3 powder were dissolved in phosphate buffered saline (PBS), pH 7.4. Except for the AS3 powder, all other preparations were dissolved easily and were used without further

Table 1
Oxindole alkaloid analysis of two preparations of *Uncaria tomentosa* with HPLC retention time of compound and concentrations determined in plant extracts

Alkaloid	t_r (min)	LaMolina accession (mg/g)	Pacifico accession (mg/g)
Uncarine	15.16	0.13	0.16
Speciophylline	15.60	0.03	0.04
Mitraphylline	17.04	0.16	0.96
Isomitraphylline	18.90	0.26	2.04
Pteropodine	19.23	2.30	0.70
Isopteropodine	25.05	2.70	3.08
Total		5.57	6.98

treatment to stimulate the cells. No attempt was made to filter AS3 powder and after careful vortexing, the partly dissolved solution was added to AM. Therefore, it is difficult to assess the relative activity of such AS3 preparation on cytokine production.

2.3.3. IL-l assay

IL-1 activity was measured using Dl0 (N4)M cells as described in our previous studies (Lemaire and Ouellet, 1996). D10 (N4)M cells (10^4/well) were cultured in 96-well round-bottom microculture plates in a final volume of 200 μl of complete medium [RPMI 1640 containing 10% dialysed FBS and 0.005% gentamycin, 5×10^{-5} M mercaptoethanol, HEPES (0.8%), concanavalin A (Con A, 5 μg/ml), and recombinant IL-2 (30 U/ml)]. Serial dilutions of each AM supernatant (1/400, 1/200, 1/100, 1/50) were incubated with cells (10^4) in triplicate ($n = 12$) at 37°C. Appropriate controls contained medium, IL-2, Con A, or LPS alone or in combination. Cultures were incubated for 66 h, pulsed with 1 μCi/well [^{3}H]thymidine (Dupont/NEN) and harvested at 72 h. Bioassay data were calculated as net cpm by the following formula: net cpm = (cpm of DlO cells + Con A + IL-2 + sample) − (cpm of DlO cells + Con A + IL-2). Incorporation of [^{3}H]thymidine in the presence of sample dilutions was compared with that in the presence of dilutions of a standard recombinant IL-1 preparation (Genzyme, 10^8 U/mg) and IL-1 units were calculated by probit analysis.

2.3.4. IL-6 assay

IL-6 activity in AM culture supernatants was measured by the B9-cell proliferation assay as described in our previous studies (Lemaire et al., 1996). B9 cells (0.5×10^4) were incubated in 200 µl of Iscove's Modified Dulbecco's Medium (IMDM; (Grand Island Biological), supplemented with 5% dialysed FBS,100 U/ml penicillin, 100 μg/ml streptomycin, 0.8% Hepes and 5×10^{-5} M 2-mercaptoethanol. Various dilutions of each AM supernatants (1/800, 1/400, 1/200, 1/100, 1/50) were incubated with B9 cells in triplicate ($n = 15$). Tritiated thymidine (1 μCi/well) was added after 66 h of incubation and cultures were harvested at 12 h with a Skatron filtration apparatus. Sample-dilution curves were related to a standard curve generated with recombinant murine IL-6 (Genzyme, 10^8 U/mg) and IL-6 levels were calculated by probit analysis.

3. Results and discussion

The HPLC analysis of the two Peruvian samples of *U. tomentosa* revealed six characteristic alkaloids of the species, thereby confirming with certainty the identity of the samples. Profiles were similar to those described previously by Stuppner et al. (1992). The analysis (Table 1) revealed a total alkaloid content that was slightly higher in the Pacifico sample as compared with the La Molina sample.

U. tomentosa (LaMolina sample) stimulated IL-1 (Fig. 2) and IL-6 (Fig. 3) production by

macrophages in an approximately dose dependent manner in the range of 0.025–0.1 mg/ml. At its maximum, IL-1 production was stimulated to 10.0 × control levels, while IL-6 increased 7.5 × . At higher concentrations, production of IL-1 and IL-6 began to fall in a concentration-dependent manner to control levels or below, probably due to the cytotoxicity of the alkaloids.

Without *U. tomentosa* treatment, LPS treatment induced production of IL-1 and IL-6 to levels over 11–35-fold over previous LPS-control values. However, even in the LPS-stimulated cells, the *U. tomentosa* treatment was able to potentiate IL-1 (Fig. 4) and IL-6 (Fig. 5) production above the LPS control in the 0.025–0.1 mg/ml concentration range. Stimulation of IL-1 was 5.2 × greater than the LPS control and IL-6 was > 2 × the LPS control at maximum stimulation. At higher concentrations (> 0.1 mg/ml), production fell below LPS control values.

When similar trials were undertaken with the Pacifico genotype of *U. tomentosa*, comparable levels of stimulation and trends in results were obtained. These results suggest that the effects observed are not confined to specific genotypes or

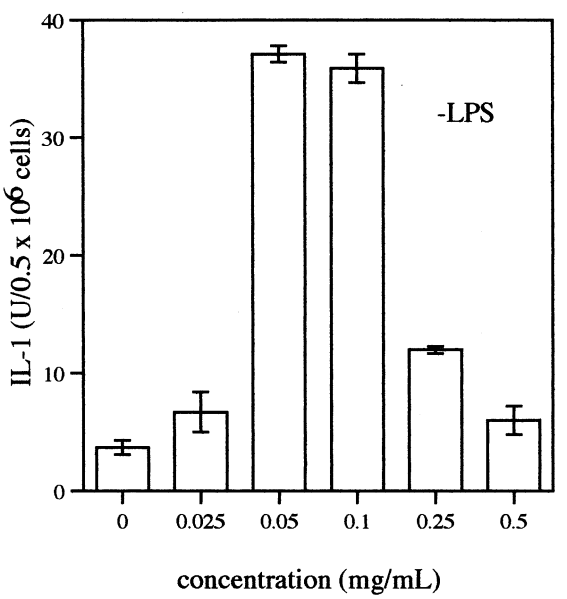

Fig. 2. Effect of *Uncaria tomentosa* on IL-1 production by alveolar macrophages. Means (S.E.M.) of two separate experiments measured in triplicate.

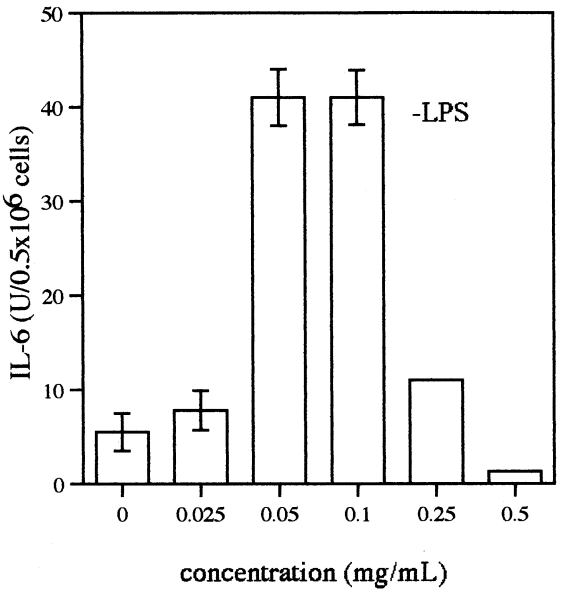

Fig. 3. Effect of *Uncaria tomentosa* on IL-6 production by alveolar macrophages. Means (S.E.M.) of two separate experiments measured in triplicate.

collections. Another traditionally used Peruvian plant coded AS3 and reputed to have properties similar to uña de gato failed to produce the potent interleukin-stimulating properties observed with the currently used *U. tomentosa* extracts. This may be related to the type of plant material or to the preparation as a crude plant powder rather than soluble extract. Therefore, further studies would be necessary to assess this area.

The current study shows that *U. tomentosa* has a potent stimulant action on alveolar macrophages which complements earlier studies by Wagner et al. (1985), and suggests it to be a promising candidate for further study. A major priority is the confirmation of the identity of the active principles responsible for the cytokine stimulation and their precise mode of action. In addition, the effect on other interleukins should be examined.

Blumenthal (1995) noted that there has been a considerable increase in interest in this herb in both North America and Europe including observations with HIV and cancer patients. However, the point is also made in his article that ethnobotanical information is scant and there is little

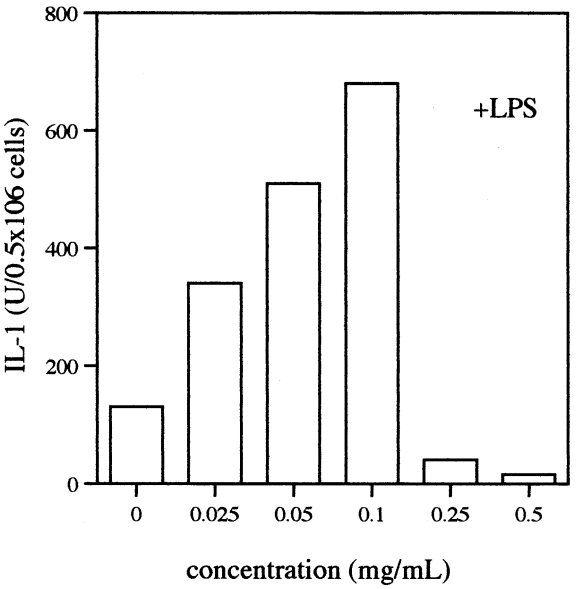

Fig. 4. Effect of *Uncaria tomentosa* on LPS induced IL-1 production by alveolar macrophages. Data are means of triplicate determinations in a representative experiment.

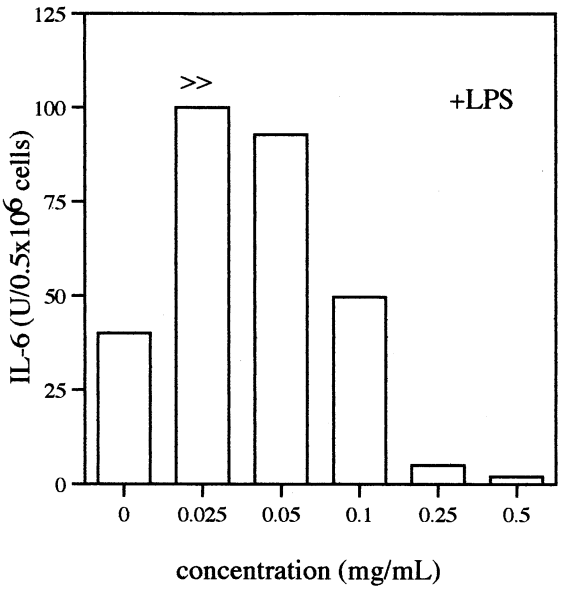

Fig. 5. Effect of *Uncaria tomentosa* on LPS induced IL-6 production by alveolar macrophages. Data are means of triplicate determinations in a representative experiment.

literature to document long-term safety. Peer-reviewed toxicity, and in vivo animal studies as well as clinical trials are required.

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