Available online at www.sciencedirect.com
ScienceDirect
Journal of Ethnopharmacology 109 (2007) 312–317
Journal of ETHNO-PHARMACOLOGY
www.elsevier.com/locate/jethpharm

Treatment of THP-1 cells with *Uncaria tomentosa* extracts differentially regulates the expression if IL-1β and TNF-α

Lisa Allen-Hall [a,b], Pablo Cano [a], John T. Arnason [c], Rosario Rojas [d], Olga Lock [e], Robert M. Lafrenie [a,b,f,*]

[a] *Regional Cancer Program, Sudbury Regional Hospital, 41 Ramsey Lake Road, Sudbury Ont., Canada P3E 5J1*
[b] *Program in Biomolecular Sciences, Laurentian University, 935 Ramsey Lake Road, Sudbury, Ont., Canada P3E 2C6*
[c] *Department of Biology, University of Ottawa, 150 Louis Pasteur Priv., Ottawa, Ont., Canada K1N 6N5*
[d] *Facultad de Ciencias y Filosofía, Universidad Peruana Cayetano Heredia, Lima, Perú*
[e] *Pontificia Universidad Catolica Del Peru, Avenida Universitaria, Cuadra 18, San Miguel, Lima, Peru*
[f] *Northern Ontario School of Medicine, 935 Ramsey Lake Road, Sudbury, Ont., Canada P3E 2C6*

Received 16 February 2006; received in revised form 14 July 2006; accepted 31 July 2006
Available online 3 August 2006

Abstract

Uncaria tomentosa, commonly known as cat's claw, is a medicinal plant native to Peru, which has been used for decades in the treatment of various inflammatory disorders. *Uncaria tomentosa* can be used as an antioxidant, has anti-apoptotic properties, and can enhance DNA repair, however it is best know for its anti-inflammatory properties. Treatment with *Uncaria tomentosa* extracts inhibits the production of the pro-inflammatory cytokine, TNF-α, which is a critical mediator of the immune response. In this paper, we showed that treatment of THP-1 monocyte-like cells with *Uncaria tomentosa* extracts inhibited the MAP kinase signaling pathway and altered cytokine expression. Using ELISA assays, we showed that treatment with *Uncaria tomentosa* extracts augmented LPS-dependent expression of IL-1β by 2.4-fold, while inhibiting the LPS-dependent expression of TNF-α by 5.5-fold. We also showed that treatment of LPS-stimulated THP-1 cells with *Uncaria tomentosa* extracts blocked ERK1/2 and MEK1/2 phosphorylation in a dose-dependent manner. These data demonstrate that treatment of THP-1 cells with *Uncaria tomentosa* extracts has opposite effects on IL-1β and TNF-α secretion, and that these changes may involve effects on the MAP kinase pathway.

Keywords: Pro-inflammatory cytokines; THP-1 cells; MAP kinase pathway; *Uncaria tomentosa*

1. Introduction

Uncaria tomentosa (Willd.) DC (Rubiaceae), commonly known as Uña de gato or cats claw, is a medicinal plant that was traditionally used by the Ashaninka First Nation of Amazonian Peru. Because of the high regard with which it is held by traditional healers, Peruvians have used it in a bark infusion for decades to treat disorders such as inflammatory diseases, arthritis, cancer, gastric ulcers, recovery from childbirth, and control of inflammation (Akesson et al., 2003a; Heitzman et al., 2005) The medicinal extract has been commercialized and is currently in widespread use across North America.

Uncaria tomentosa extracts can act as an antioxidant and are able to enhance DNA repair (Sandoval et al., 2000; Miller et al., 2001; Akesson et al., 2003a; Goncalves et al., 2005; Mammone et al., 2006; Pilarski et al., 2006). *In vitro* studies have shown that *Uncaria tomentosa* may have an anti-apoptotic activity in some cells, such as lymphocytes, following treatment with apoptotic inducers such as hydrogen peroxide, diphenyl-2-picrylhydrazyl, and peroxynitrite (Sheng et al., 1998; Miller et al., 2001; Akesson et al., 2003a,b). This increase in cell viability may be due to an enhancement of DNA repair in damaged cells (Lamm et al., 2001; Sheng et al., 2001; Mammone et al., 2006) and could be very significant for treatment of patients undergoing chemotherapy. In fact, patients treated with combination chemotherapy including *Uncaria tomentosa* showed accelerated recovery in white blood cell counts as well as decreased side effects such as hair loss, weight loss, nausea, and secondary infections (Steinberg, 1995). However, the effects of *Uncaria*

* Corresponding author at: Regional Cancer Program, Sudbury Regional Hospital, 41 Ramsey Lake Road, Sudbury, Ont., Canada P3E 5J1.
Tel.: +1 705 522 6237; fax: +1 705 523 7326.
E-mail address: rlafrenie@hrsrh.on.ca (R.M. Lafrenie).

0378-8741/$ – see front matter © 2006 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.jep.2006.07.039

tomentosa depend on the cell type studied. For example, Raji cells treated with *Uncaria tomentosa* are protected from apoptosis, whereas *Uncaria tomentosa* induces apoptosis in some human leukemia cell lines (Akesson et al., 2003a).

Treatment with *Uncaria tomentosa* extracts also inhibits the production of tumor necrosis factor-α (TNF-α) (Sandoval et al., 2000; Setty and Sigal, 2005). TNF-α is a potent pro-inflammatory cytokine and is a critical mediator of chronic inflammatory conditions including rheumatoid arthritis (Piscoya et al., 2001). TNF-α is cytotoxic to lymphocytes and in some situations can act to suppress the immune system. The release of TNF-α from monocytes is stimulated by treatment with various chemical agents, including bacterial lipopolysaccharide (LPS) (Aguilar et al., 2002). LPS is a potent bacterial endotoxin that is shed from the outer membrane of gram-negative bacteria and that acts as an immune system stimulant. In cells pre-treated with *Uncaria tomentosa* and stimulated by treatment with LPS, TNF-α production was suppressed by 65–85% compared to cells treated only with LPS (Sandoval et al., 2000; Sheng et al., 2000a; Piscoya et al., 2001; Aguilar et al., 2002). The ability of *Uncaria tomentosa* extracts to suppress TNF-α release occurs at concentrations four orders of magnitude lower than its anti-oxidant potential (Sandoval et al., 2000). This suggests that the medicinal effect of *Uncaria tomentosa* may be mediated via alteration in immune responsiveness.

It is thought that the inhibition of TNF-α expression following treatment with *Uncaria tomentosa* extracts is controlled by the regulation of the transcription factor NFκB (Sandoval et al., 2000; Akesson et al., 2003b; Mammone et al., 2006; Pilarski et al., 2006). NFκB can be activated by several different signals, and can regulate the expression of several pro-inflammatory cytokines including TNF-α, IL-1, IL-2, IL-6, and IL-8 (Akesson et al., 2003b). NFκB activation also promotes the expression of anti-apoptosis genes and the proliferation of lymphocytes. However, treatment with *Uncaria tomentosa* has contradictory activities since it can inhibit the activation of NFκB and can also activate anti-apoptosis mechanisms that protect some cell types, such as lymphocytes, from apoptosis (Akesson et al., 2003a,b). In this report, we showed that treatment with *Uncaria tomentosa* extracts inhibited LPS-dependent TNF-α secretion but enhanced LPS-dependent IL-1β secretion. Further, treatment with *Uncaria tomentosa* extracts strongly inhibited activation of the MAP kinase pathway but did not enhance cell death.

2. Materials and methods

2.1. Plant material

Bark was collected from wild *Uncaria tomentosa* plants grown in the lowland Peruvian Amazon Region, Iquitos. A voucher has been placed in the herbarium at the University of Ottawa. The bark was dried and ground, producing 253.7 g of plant material. The component compounds were extracted by exhaustive percolation with 95% ethanol. The solvent was then evaporated at reduced pressure with a rotavapour at a temperature less than 40 °C. The yield was 41.7 g of ethanol-extracted product. The stock solution was then diluted in RPMI media prior to use in tissue culture.

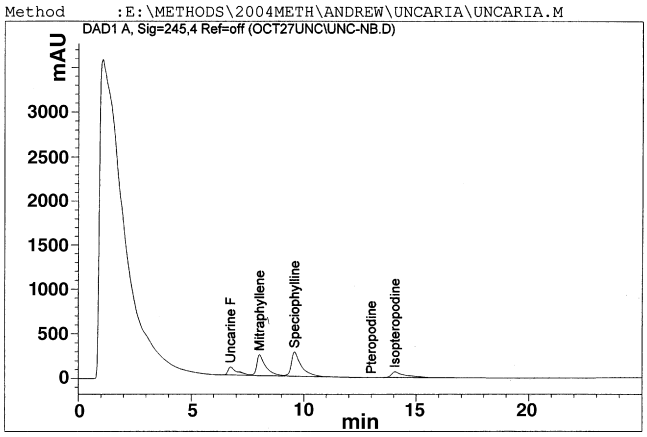

Fig. 1. HPLC analysis of *Uncaria tomentosa*.

2.2. HPLC analysis

Uncaria tomentosa extracts were filtered with a 0.2 μm filter prior to HPLC analysis. Separation was achieved using a 125 mm × 4.6 mm × 18.5 μm Superspher column with a 4 mm guard. The oven temperature was set at 45 °C with a flow rate of 1 ml/min. The solvents used were; A: 5 nM Na_2HPO_4, 5 nM KH_2PO_4, pH 6.6, and B: 1:1 MeOH:MeCN. The solvent program was 40–70% B in 30 min, 70–80% B in 2 min, hold at 80% B for 10 min, 80–40% B in 3 min and equilibrate 15 min. The detection wavelength was 245 nm (Fig. 1). Standards were obtained from our collection and from Cerilliant (Round Rock, TX) (Table 1).

2.3. Experimental protocol

THP-1 cells (ATCC, Rockville, MD) were grown at 37 °C in 5% CO_2 in RPMI media supplemented with 10% fetal calf serum (Hyclone, Logan, UT) and 100 μg/ml penicillin and 100 μg/ml streptomycin (Invitrogen, Burlington, ON) to 1×10^6 cells/ml. Cells were collected by centrifugation and resuspended at a concentration of 2×10^6 cells/ml in serum-free RPMI media. Cells were treated in the presence or absence of *Uncaria tomentosa* extracts and incubated at 37 °C in 5% CO_2 for 24 h. Samples were then treated in the presence or absence of *Uncaria tomentosa* extracts and/or 5 μg/ml of *Escherichia coli* LPS (serotype 0127, Sigma Chemical, St. Louis, MO), and incubated for 24 h.

Table 1
Compounds found by HPLC in *Uncaria tomentosa* extract used experimentally

Compound name	Retention time (min)	Peak area	Amount (μg/ml)
Uncarine F	6.75	1927	145.4
Mitraphyllene	8.04	6621	387.9
Speciophylline	9.59	8617	469.6
Isomitraphyllene	0	0	0
Pteropodine	12.99	3	0.3
Isopteropodine	14.06	2571	185.1