Homeopathy (2016) 105, 131–147

http://dx.doi.org/10.1016/j.homp.2016.02.001, available online at http://www.sciencedirect.com

ORIGINAL PAPER

Arnica montana effects on gene expression in a human macrophage cell line. Evaluation by quantitative Real-Time PCR

Debora Olioso[1], Marta Marzotto[1], Clara Bonafini[1], Maurizio Brizzi[2] and Paolo Bellavite[1,*]

[1]*Department of Medicine, University of Verona, Strada Le Grazie 8, 37134 Verona, Italy*
[2]*Department of Statistical Sciences, University of Bologna, Via delle Belle Arti 41, 40126 Bologna, Italy*

Background: ***Arnica montana*** **is a popular traditional remedy widely used in complementary medicine, also for its wound healing properties. Despite its acknowledged action in clinical settings at various doses, the molecular aspects relating to how *A. montana* promotes wound healing remain to be elucidated. To fill this gap, we evaluated the whole plant extract, in a wide range of dilutions, in THP-1 human cells, differentiated into mature macrophages and into an alternative IL-4-activated phenotype involved in tissue remodelling and healing.**

Methods: **Real-time quantitative Reverse Transcription Polymerase Chain Reaction (PCR) analysis was used to study the changes in the expression of a customized panel of key genes, mainly cytokines, receptors and transcription factors.**

Results: **On macrophages differentiated towards the wound healing phenotype, *A. montana* affected the expression of several genes. In particular CXC chemokine ligand 1 (CXCL1), coding for an chief chemokine, exhibited the most consistent increase of expression, while also CXC chemokine ligand 2 (CXCL2), Interleukin8 (IL8) and bone morphogenetic protein (BMP2) were slightly up-regulated, suggesting a positive influence of *A. montana* on neutrophil recruitment and on angiogenesis. MMP1, coding for a metalloproteinase capable of cleaving extracellular matrix substrates, was down-regulated. Most results showed non-linearity of the dose-effect relationship.**

Conclusions: **This exploratory study provides new insights into the cellular and molecular mechanisms of action of *A. montana* as a promoter of healing, since some of the genes it modifies are key regulators of tissue remodelling, inflammation and chemotaxis.** *Homeopathy* (2016) **105**, 131–147.

Keywords: *Arnica montana*; Macrophages; Real-Time PCR; Gene expression; Wound healing; Chemokines

Introduction

Arnica montana L. (*A. montana*) is a herbaceous perennial plant belonging to the Asteraceae family, native to the temperate region of Europe and widely distributed in mountainous areas. It is a popular traditional remedy widely used in complementary medicine to treat various pathological conditions such as contusion, swelling associated with trauma, pain, inflammation, wounds and post-operative clinical conditions.[1–3] However, the evidence of its clinical efficacy is subject to debate.[4–6] A recent systematic review of herbal remedies for treating

*Correspondence: Paolo Bellavite, Department of Medicine, University of Verona, Strada Le Grazie 8, 37134 Verona, Italy.
E-mail: paolo.bellavite@univr.it
Received 8 October 2015; revised 1 January 2016; accepted 1 February 2016

osteoarthritis concluded that topical *A. montana* gel probably improves pain and function in this common condition.[7] There is some experimental evidence, in laboratory animals, of an anti-inflammatory action of *A. montana* administered as crude ethanolic extract[8] or at the 6th centesimal homeopathic dilution (6c).[9,10]

The chemical composition of *A. montana* depends on the part of the plant that is used, principally the flowers and roots, and in general the most pharmacologically active compounds are sesquiterpene lactones, thymol derivatives, flavonoids, acid polysaccharides and their glycoconjugates.[11,12] One of the major components of *A. montana* with acknowledged biological activity is the sesquiterpene lactone helenalin, known for its anti-inflammatory properties. In a lymphoid cellular model, helenalin was found to inhibit the transcription factor NF-kappaB – a central mediator of human immune response – by the alkylation of p65 subunit (RelA), thus preventing its binding to DNA.[13] However the cited studies did not evaluate the contribution of the whole plant extract, nor did they evaluate the pharmaceutical properties of different doses and formulations employed in medicine,[1,3,14] ranging from crude herbal extract or low dilutions, to high dilutions (homeopathic doses).

In fibroblast-like cells, whole ethanolic extract of *A. montana* showed antioxidant activity and a cytoprotective effect against oxidative damage.[15] Defence against oxidative stress was reported in studies where a 30th centesimal dilution (30c) of *A. montana*, administered orally to laboratory rats, decreased oxygen consumption of isolated liver mitochondria and protected from oxidative damage caused by lipid peroxidation.[16] Only one paper reported an inhibitory action of *A. montana* on nitric oxide and TNF-α production by murine macrophages.[17]

Although the action of *A. montana* on wound healing is regarded as a promising therapeutic property of this plant, the current knowledge of the effects of *A. montana* in laboratory models of wound healing is scant. A commercial homeopathic complex containing a low dilution (4th decimal, 4x) of *A. montana*, *Calendula* and *Hypericum* promoted fibroblast growth in a scratch model of cellular wound closure[18]; *A. montana* 3x as a topical gel improved the healing of surgically-induced wounds in Wister rats, but significant differences were noted only when the drug was delivered together with microcurrent application.[19]

Wound healing involves different cell types such as fibroblasts, leukocytes, and monocytes/macrophages, as well as endothelial and epidermal cells which cooperate to restore the damaged tissue through hemostasis, inflammation, angiogenesis and remodelling of the new tissue. The delicate balance between inflammation – with its potentially destructive phases – and tissue repair depends on a number of local and systemic factors and can be pharmacologically influenced. In the early inflammatory phase, a prominent role is played by cytokines derived by epithelial cells and macrophages such as IL8, IL-1α, IL-1β, IL-6, TNF-α. A pivotal role in immune defence and repair is played by Interleukin-4 (IL-4), whose pleiotropic effects on leukocytes include TH2 differentiation[20] and class switch of naïve B cells to IgE,[21] immunological events that increase antibody defence. On macrophages, IL-4 is essential for 'alternative' polarization, by which these cells take on characteristic properties functional to immune regulation, wound healing and tissue remodelling.[22–24] In the subsequent phase, additional factors are important to healing, including CXC chemokine ligand 1 (CXCL1) and 2 (CXCL2), CC chemokine ligand 2 (CCL2, also known as monocyte chemotactic protein-1, MCP-1),

Table 1 List of genes of THP-1 macrophages included in the panel

Gene full names	*Gene code*	*Gene symbol and abbreviation*
Chemokine (C–C motif) ligand 1	NM_002981	CCL1
Chemokine (C–C motif) ligand 2; MCP-1	NM_002982	CCL2
Chemokine (C–C motif) ligand 3; MIP-1α	NM_002983	CCL3
Chemokine (C–C motif) ligand 4; MIP-1B	NM_002984	CCL4
Chemokine (C–C motif) ligand 5; RANTES	NM_002985	CCL5
Chemokine (C–C motif) ligand 7; MCP-3	NM_006273	CCL7
Chemokine (C–C motif) ligand 17; TARC	NM_002987	CCL17
Chemokine (C–C motif) ligand 22	NM_002990	CCL22
Chemokine (C–C motif) ligand 23	NM_005064	CCL23
Chemokine (C–X–C motif) ligand 1; GRO-a	NM_001511	CXCL1
Chemokine (C–X–C motif) ligand 2; GRO-b	NM_002089	CXCL2
Chemokine (C–X–C motif) ligand 13; BCA-1	NM_006419	CXCL13
Interleukin 1 – beta	NM_000576	IL1B
Interleukin 1, receptor antagonist	NM_000577	IL1RN
Interleukin 8	NM_000584	IL8
Interleukin 10	NM_000572	IL10
Tumour necrosis factor – alpha	NM_000594	TNFA
Vascular endothelial growth factor A	NM_003376	VEGFA
Bone morphogenetic protein-1	NM_001200	BMP2
Colony-stimulating factor 1	NM_000757	CSF1
Chemokine (C–C motif) receptor 5	NM_000579	CCR5
Coagulation factor XIII, A1 polypeptide	NM_000129	F13A1
Mannose receptor, C type 1	NM_002438	MRC1
Matrix metallopeptidase 1 (collagenase)	NM_002421	MMP1
V-rel reticuloendotheliosis viral oncogene homolog A (p65)	NM_021975	RELA
Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 (p50)	NM_003998	NFKB1
Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha	NM_020529	NFKBIA
Inhibitor of kappa light polypeptide gene, kinase beta	NM_001556	IKBKB