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Anti-arthritogenic effect of Saponin-1 by alteration of Th1/Th2 cytokine paradigm in arthritic mice

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ABSTRACT

Objective & design: Investigation was carried out on Saponin 1 (SAP-1), a novel molecule isolated from *Parthenium hysterophorus*, on proinflammatory (Th1) & anti-inflammatory (Th2) cytokines in blood of arthritic balb/c mice.

Methods: Adjuvant induced developing inflammatory arthritis was induced in mice which were treated with SAP-1 in graded oral doses. The molecular markers were determined using Flow Cytometry which uses sensitivity of amplified fluorescence detection to measure soluble analytes in particle based immune assay. The T-helper (Th1) deviated cells produce detectable level of Tumor necrosis factor (TNF-alpha), interleukin-2 (IL-2) & interferon-gamma (IFN-gamma), while the Th2 deviated cells produce significant amount of interleukin-4 (IL-4) and interleukin-5 (IL-5).

Results: SAP-1 at graded oral doses inhibited expression of IFN-gamma & TNF-alpha in serum & correspondingly increased expression of IL-4 significantly. SAP-1 also inhibited IL-17 and $CD4^{+}CD25^{+}$ cell population showing to have suppressive effect on Th-17 pathway as well as T-regulatory cells. It also suppressed the increased levels of pro-inflammatory mediators like IL-1β and NO. Inhibitors of Cox-2 and MCP-1 provide effective improvements in signs and symptoms of Rheumatoid Arthritis. SAP-1 decreased the elevated concentration of both COX-2 and MCP-1 in arthritic animals.

Conclusions: SAP-1 diminishes Th1 immunity activation, a primary cause of arthritis, in favour of Th2 dominance, which reduces arthritic condition in mice displaying immune-modulatory potential.

1. Introduction

Triterpene saponins are known for a wide range of biological activities like antifungal [1], anti-leishmanial [2], antitumor [3] immunomodulatory [4] and molluscicidal activity [5]. Medicinal plants containing these have been used in folk medicine much before it was known as to which constituents were responsible for their therapeutic effectiveness. Contemporary scientific research which led to the isolation and identification of saponins revealed and confirmed to have several pharmacological effects. Demand for natural products coupled with their biological activity has led to the emergence of saponins as commercially significant compounds with expanding applications in cosmetics, and pharmaceutical sectors. The ability of committed Th1 and Th2 cells to function in altered cytokine environments is a central issue in autoimmune and immune-mediated diseases [6,7] and skewing of Th phenotype to either Th1 or Th2 is associated with pathogenicity or protection from disease. Novel immunotherapies in experimental development are designed to alter the cytokine environment, and suppress activation and/or function of the pathogenic Th1/Th2 cells [8–10]. Human rheumatoid arthritis has a strong immune mediated component and is marked by increased activated levels of Th1 cells and their cytokines IL-2, IFN-gamma with fewer Th2 cells and their cytokines like IL-4 [11]. It is possible that the beneficial effect of Saponin-1 (SAP-1) in inflammatory arthritis may be in part from immunomodulatory activity in addition to anti-inflammatory properties [12].

Patients with rheumatoid arthritis (RA) have increased expression of COX-2 which is highly regulated with rapid induction in response to inflammatory stimulus driven by the pro-inflammatory cytokines IL-1β and TNF-α [13].

Apart from T cells other cell types like macrophages in RA also secrete many inflammatory mediators and matrix metalloproteinases that play a central role in the inflammation and joint destruction characteristic of RA [14]. The number of macrophages

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in RA synovium correlates significantly with clinical symptoms and the degree of joint damage [15]. The migration of peripheral blood monocytes in the synovial macrophages is influenced by chemokines which include IL-8, monocyte chemotactic protein-1 (MCP-1), etc. To address this issue, we examined expression of MCP-1 in paw tissue homogenate of the experimental animals.

Cytokines mediate the migration, activation and proliferation of phenotypically diverse cells [16–18] and are pleiotropic and possess overlapping functions thus regulating the production of other cytokines and make-up of the cytokine milieu is often of a greater importance than the actions of an isolated one or two cytokines.

The Th1/Th2 paradigm has been updated following the discovery of a third subset of Th cells, known as Th17 cells. Th17 cells produce IL-17 as well as other pro-inflammatory cytokines such as IL-21 and IL-22. Increased levels of IL-17 have been observed in patients with RA which directly promotes cartilage and bone destruction [19].

$CD25^+$ is the transmembrane protein present on activated T cells. $CD4^+CD25^+$ T cells from the inflamed joint express higher levels of CTLA-4 (cytotoxic T-lymphocyte-associated protein 4) and GITR (glucocorticoid-induced TNF receptor family-regulated gene) and have an activated phenotype, which is characterized by the expression of CD69 and class II MHC molecules. There is ongoing inflammation and joint damage in the presence of increased numbers of $CD4^+CD25^+$ cells in the RA joint [20,21]. $CD4^+CD25^+$ subset contains both effector and regulatory T cells and only additional markers like Foxp3 can distinguish these in mice. Transcriptional factor Foxp3 serves as a lineage specification factor of regulatory T cells.

Thus, the analysis and quantification of cytokines in biological fluids is clearly important in furthering our understanding of many immunological functions of the disease as well as the effect of the test material on the disease. The objective of the present study was to explore the anti arthritic effect of SAP-1 and to determine its possible effect on Th1/Th2/Th17 balance.

Table 1
^1H and ^{13}C NMR chemical shifts (ppm) of aglycone and sugar moieties of **SAP-1** in pyridine (D_5).

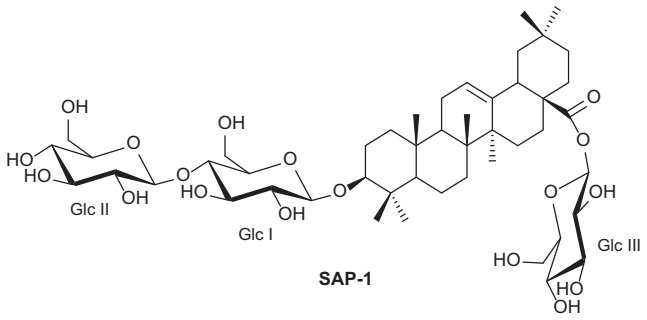
Chemical shifts (ppm) for aglycone moieties			Chemical shifts (ppm) for sugar moieties			
Carbon	SAP-1			Carbon	SAP-1	
	δ_C	δ_H			δ_C	δ_H
1	38.7	1.73, 1.21	C-3			
2	25.6	2.12, 1.78	Glc I	1′	105.7	4.86 (*d*, 7.5)
3	88.3	3.34 (*dd*, 11.6, 3.3)		2′	74.5	4.02
4	33.3	–		3′	75.7	4.05
5	55.8	0.77 (*d*, 12)		4′	80.6	4.15
6	17.8	1.42		5′	76.1	3.87
7	32.4	1.86, 1.71		6′	61.6	4.55, 4.30
8	38.0	–	GlcII	1″	104.2	5.2 (*d*, 7.6)
9	48.9	1.61		2″	73.3	4.09
10	31.8	–		3″	77.4	4.17
11	23.0	1.94		4″	70.2	4.49
12	124.8	5.43		5″	78.1	3.86
13	145.8	–		6″	61.5	4.58, 4.36
14	41.0	–				
15	28.4	2.34	C-28			
16	23.9	2.16, 1.90	Glc III	1‴	91.0	6.3 (*d*, 8.0)
17	45.5	–		2‴	74.1	4.21
18	40.8	3.18 (*dd*, 13.7, 3.8)		3‴	77.6	4.26
19	46.1	1.76, 1.26		4‴	70.4	4.36
20	30.1	–		5‴	78.5	4.03
21	33.2	1.44		6‴	62.6	4.45, 4.38
22	32.1	2.14, 1.96				
23	27.8	1.31				
24	16.3	1.04				
25	14.9	0.85				
26	16.8	1.11				
27	25.4	1.27				
28	179.1	–				
29	22.9	0.92				
30	32.5	0.89				

2. Materials and methods

2.1. Extraction and isolation

The shade dried aerial portion of the plant material including the flowers of *Parthenium hysterophorus* (1 kg) were extracted with MeOH (5 L) in a soxhlet extractor. The crude MeOH extract after concentration (97 g) was defatted with n-hexane (2 L) and the remaining extract was further extracted with chloroform (2.5 L) and re-extracted with MeOH. The crude chloroform extract after concentration (33 g) was subjected to hot water extraction (8×50 ml) for the isolation of parthenin (2.1 g, 0.21%). The left over extract combined with re-extracted MeOH extract was subjected to column chromatography over a silica gel (BDH, 60–120 mesh) column. Elution was carried with chloroform:MeOH (99:1 to 65:35) in increasing proportions. The eluants were collected in fractions of 50 ml each. Resolution of the components in the mixture was monitored on TLC. The column chromatography was repeated for impure fractions using the same solvent system leading to the isolation of SAP-**1** (0.0030%). SAP-1 was characterized by various spectroscopic techniques like Mass, IR, ^1H, ^{13}C and 2D NMR [12].

2.2. Analytical data



SAP-1

2.2.1. 3-O-[β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl]-28-O-β-D-glucopyranosyl-oleanolic acid (SAP-1)

White solid, mp 192–194 °C; $[\alpha]_D + 3.0$ (*c* 0.25, MeOH); ^1H and ^{13}C NMR in Table 1. Anal Calcd for $C_{48}H_{78}O_{18}$: C, 61.13; H, 8.34%. Found: C, 61.34; H, 8.61. ESI-MS (*m*/*z*): 965 $[M+Na]^+$.

2.3. Animals

Male adult balb/c mice weighing 20–26 g, 12–14 week old were employed in groups of six for the study. All the animals were maintained in cages at 22 ± 2 °C with 12 h light/dark cycle and free access to pellet food (Lipton India Ltd.) and water. According to ethical regulations on animal research all animals used in experimental work received humane care. Drugs were prepared as a homogenized suspension in gum acacia (1% w/v) and were administered orally daily once a day for the duration of experiment. All experimental procedures used in present study were in accordance with institutional guidelines for animal research (CPCSEA, 2003). The study protocols were approved by the Institutional Animal Use and Care Committee of Indian Institute of Integrative Medicine, Jammu.