Anti-inflammatory and chondro-protective effects of Rose hip powder and its constituent galactolipid GOPO

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Introduction

Cartilage erosion and rebuilding are highly complex biological processes that reflect interactions of cells and mediators produced by different cell types. These might efficiently be modulated by the concomitant action of a variety of constituents such as they occur in natural extracts and substances that might provide relief for joint diseases like osteoarthritis (OA). Indeed, a rose hip powder (RHP) has been found to have beneficial effects in the treatment of OA as shown in several randomized controlled clinical trials. The rose hip powder tested was produced by Hyben Vital, Denmark, with a standardized process ensuring the preservation of its active constituents and is commercially available in Europe as Litozin™ and in the rest of the world as i-flex™. A constituent of RH, the galactolipid GOPO inhibits chemotaxis of neutrophils and is thus one of several bioactives contained in RH powder, that has a putative chondroprotective and/or cartilage-regenerating effect in OA.

We present experimental approaches for identifying RH bioactives. The anti-inflammatory and chondroprotective effects of GOPO has been evaluated at different levels *in vitro* in three relevant cellular systems.

RHP and GOPO inhibit the production of nitric oxide (NO) and prostaglandin E_2 (PGE_2) in murine macrophages (RAW264.7 cells)

RAW264.7 cells were activated with lipopolysaccharide (LPS) in the presence or absence of RHP and GOPO for 24 hours. The amount of PGE_2 and NO was measured in the cultured supernatants.

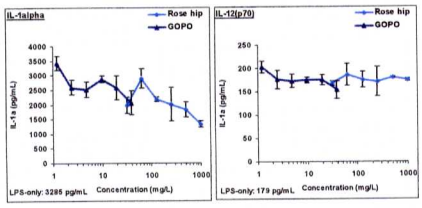
Compound	IC_{50} (mg/L)	
	NO	PGE_2
RHP	833±40	541±41
GOPO	28±5	>38

RHP and GOPO inhibit the production of inflammatory mediators in murine macrophages

RAW264.7 cells were activated with lipopolysaccharide (LPS) in the presence or absence of RHP and GOPO for 24 hours. The production of chemokines, interleukins and cytokines was measured in the supernatants by multiparametric analysis (Luminex technology).

		GOPO	RHP
Chemokines	CCL5/RANTES	→	→
	MIP-1alpha	→	→
	MIP-1beta	→	→
	Eotaxin	→	↓
	MCP-1	→	↑
Interleukins	IL-1alpha	↓	↓
	IL-1beta	↓	↓
	IL4	→	→
	IL-6	→	↓
	IL-10	→	↑
	IL-12	→	→
	TNF-alpha	→	→
	IFN-gamma	→	→

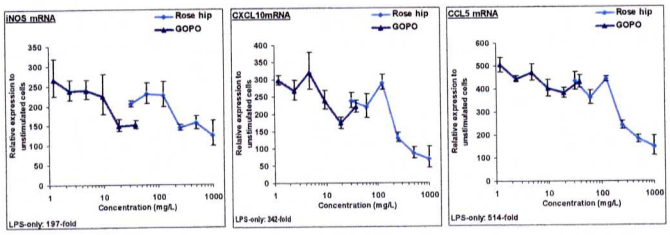
Decreasing: ↓ increasing: ↑ unaltered: →



RHP and GOPO have different effects on the secretion of interleukins. For example they dose-dependantly reduce the IL-1alpha production whereas they have no effect on the IL-12 production.

RHP and GOPO decrease the expression of inflammatory genes in murine macrophages

RAW264.7 cells were activated with LPS in the presence or absence of RHP and GOPO for 4 hours. The gene expression was measured by quantitative RT-PCR.

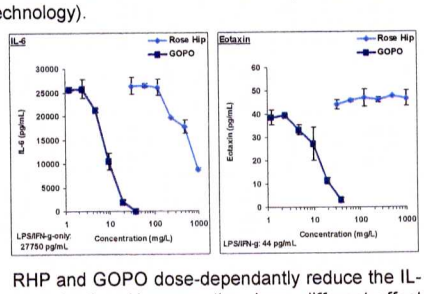


RHP and GOPO inhibit the secretion of chemokines and interleukins in human peripheral blood leukocytes (PBL)

PBLs were activated with lipopolysaccharide (LPS) / Interferon-gamma (IFN-γ) in the presence or absence of RHP and GOPO for 24 hours. The production of chemokines, interleukins and cytokines was measured in the cultured supernatants by multiparametric analysis (Luminex technology).

		GOPO	RHP
Chemokines	CXC10/IP-10	↓	↓
	CCL5/RANTES	→	↓
	MIP-1alpha	↓	→
	MIP-1beta	↓	→
	IL-8	↓	→
	Eotaxin	↓	→
	MCP-1	↓	↑
Interleukins	IL-1beta	↓	↓
	IL4	↓	→
	IL-6	↓	↓
	IL-10	↓	→
	IL-12	↓	↓
	TNF-alpha	↓	↓
	IFN-gamma	↓	↓

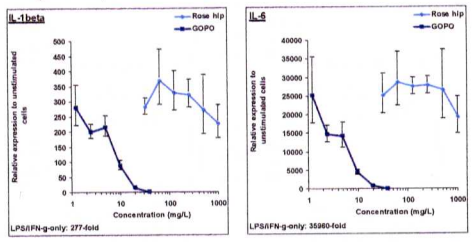
Decreasing: ↓ increasing: ↑ unaltered: →



RHP and GOPO dose-dependantly reduce the IL-6 production. However, they have different effect on *e.g.* the eotaxin secretion, since GOPO inhibits it whereas RHP has no effect.

RHP and GOPO decrease the expression of chemokine and interleukin genes in PBL

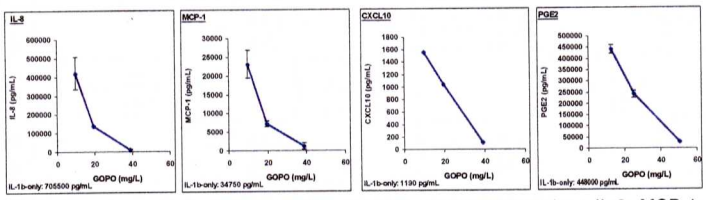
PBLs were activated with LPS/IFN-γ in the presence or absence of RHP and GOPO for 12 hours. The gene expression was measured by quantitative RT-PCR.



GOPO and RHP decrease the expression of IL-6 gene. However, they have different effect on the IL-1beta gene expression; GOPO inhibits it whereas RHP has no effect.

GOPO modulates the secretion of inflammatory mediators in Normal Human Articular Chondrocytes from knee (NHAC-kn)

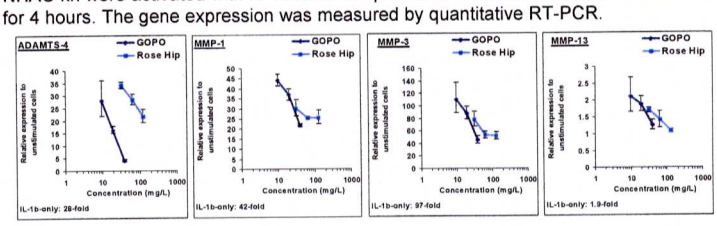
NHAC-kn were activated with IL-1beta in the presence or absence of GOPO for 24 hours. The production of chemokines, interleukins and cytokines was measured in the cultured supernatants by multiparametric analysis (Luminex technology).



GOPO dose-dependantly decreases the secretion of chemokines (*e.g.* IL-8, MCP-1, CXCL10) and PGE_2.

RHP and GOPO reduce the expression of catabolic genes in NHAC-kn

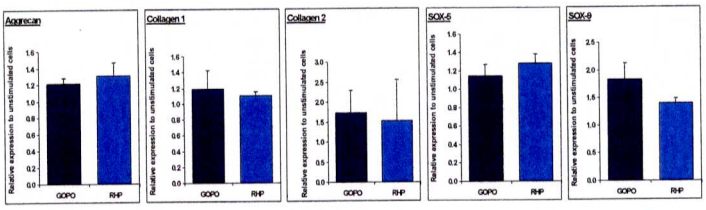
NHAC-kn were activated with IL-1beta in the presence or absence of RHP and GOPO for 4 hours. The gene expression was measured by quantitative RT-PCR.



RHP and its constituent galactolipid GOPO dose-dependantly inhibit the expression of genes that are involved in the destruction of cartilage extracellular matrix.

RHP and GOPO increase the expression of anabolic genes in NHAC-kn

NHAC-kn were cultured in the presence of RHP (50 mg/L) or GOPO (19 mg/L) for 4 hours. The gene expression, compared to unstimulated cells, was measured by quantitative RT-PCR.



RHP and GOPO increase the expression of anabolic genes (*e.g.* Aggrecan, Collagen 1 and 2) that are involved in the rebuilding of the extracellular matrix.

Summary and conclusions:

Bioactives have been identified in RH extracts initially by using anti-inflammatory parameters and subsequently multi-parametric profiling in three cellular systems. Murine macrophages were convenient for identifying first effects, while the heterogenous PBL populations (containing mononuclear and polymorphonuclear cells) permitted to monitor more diverse effects. GOPO has been identified as a potent and pleiotropic effector molecule that modulates various facets of the inflammatory processes and cell migration mediated by chemokines. Its importance in cartilage protection has been evidenced by its effect on the chemokine production by chondrocytes and the expression of catabolic and anabolic genes by human articulocytes. Although RH contains significant quantities of GOPO (<0.1%), the contents cannot account for the whole biological activity of RH. Consequently, other constituents contribute to, and might act in concert to reduce the erosion of the extracellular matrix in joints or favor the rebuilding of cartilage.

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