The Galactolipid GOPO Mediates Beneficial Effects of Rose Hip on Arthritis by Reducing Chemokine and Interleukin Production in Macrophages and Chondrocytes

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Introduction

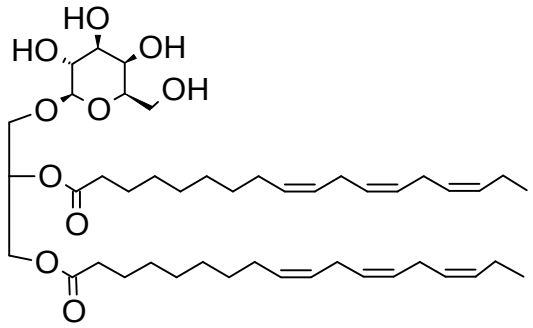
Features:

- Osteoarthritis is a disease condition that affects cartilage (erosion or insufficient regeneration of cartilage).
- Cartilage erosion and rebuilding are highly complex biological processes.
- Rose hip has been found to have beneficial effects in the treatment of OA.
- A constituent galactolipid of rose hip (*i.e.* GOPO) has effects on chemotaxis of neutrophils and thus appears to be one bioactive constituent of rose hip.

Rationale and Experimental Approach:

- Identify bio-actives that account for biological efficacy of rose hip
- Expand biological profiling of one known constituent of rose hip, GOPO
- Establish cellular mode of action of rose hip and GOPO

Structure of GOPO



Experimental *in vitro* Models

Cellular models

- Mouse macrophages
  - Permits to determine anti-inflammatory effects
- Peripheral blood leukocytes
  - Permits to measure anti-inflammatory effects on various cell populations
- Chondrocytes (normal human articulocytes)
  - Is the most appropriate target cell population to delineate anabolic and catabolic events related to osteoarthritis

Levels of action of rose hip and its constituents

- Inflammatory mediators (PGE_2, nitric oxide; interleukins, chemokines, cytokines)
- Expression of genes (inflammatory genes in macrophages, leukocytes; catabolic and anabolic genes in chondrocytes)

Effects of GOPO and RH on activated mouse macrophages (I)

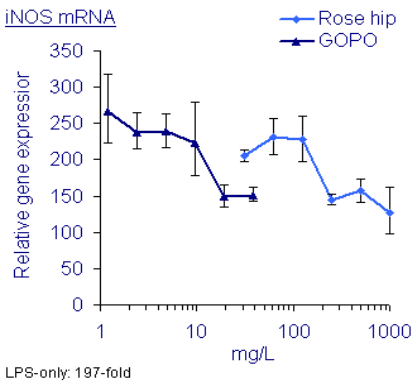
Macrophages

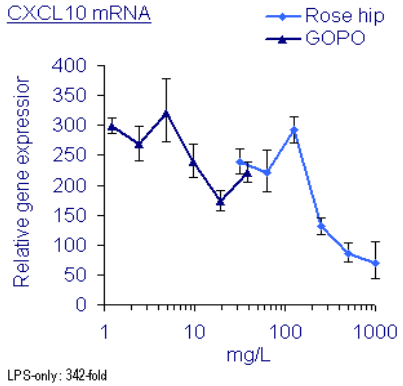
- Inflammatory mediators
  - ➢ Nitric oxide
  - ➢ PGE_2: pro-inflammatory prostaglandin

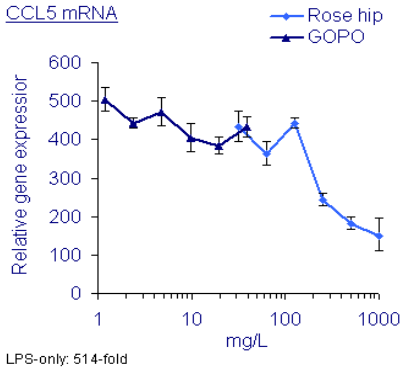
Compound	IC_{50} (mg/mL)	
	NO	PGE_2
RH powder	833 ± 40	541 ± 41
GOPO	28 ± 5	>38

Expression of genes (inflammatory genes, chemokines)

- GOPO and RH differentially modulate the expression of iNOS (→ nitric oxide), CXCL-10/IP-10, und CCL5 (→RANTES). The chemokines regulate migration of T_H lymphocytes and macrophages







Effects on production of mediators by mouse macrophages (II)

Approach

- Generate culture supernatants of activated macrophages
- Determine interleukins, chemokines and cytokines by multiparametric ELISA in supernatants

Main features (in *mouse* macrophages)

- Substantial reduction of the secretion of pro-inflammatory interleukins (IL-1) and concomitant upregulation of anti-inflammatory IL-10
- Only marginal impact on murine chemokines
- No effects on murine TNF-α and INF-γ

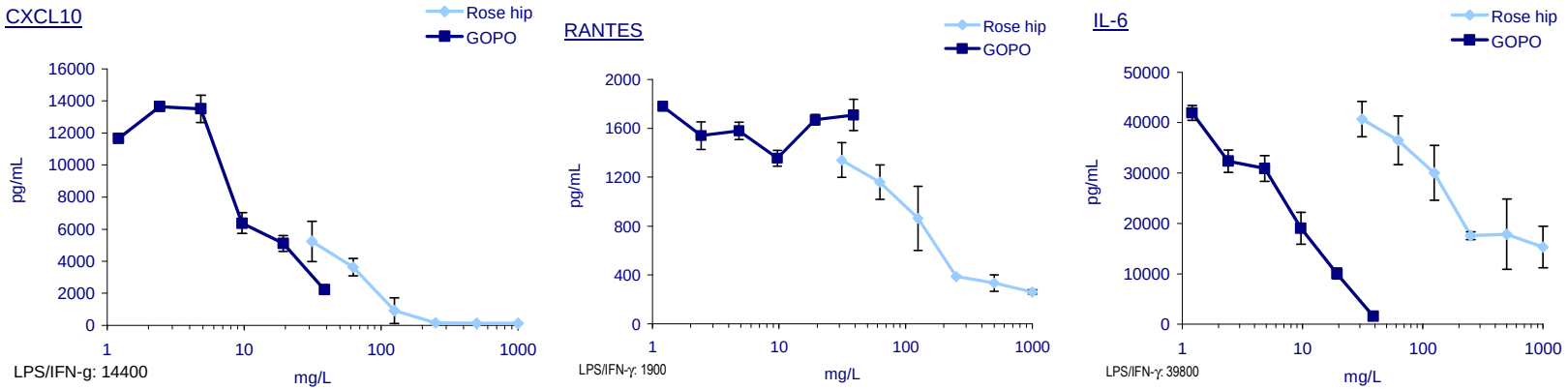
		GOPO	RHP
Chemokines	CCL5/RANTES	→	→
	MIP-1alpha	→	→
	MIP-1beta	→	→
	Eotaxin	→	↓
	MCP-1	→	↑
Interleukins	IL-1alpha	↓	↓
	IL-1beta	↓	↓
	IL4	→	→
	IL-6	→	↓
	IL-10	→	↑
	IL-12	→	→
	TNF-alpha	→	→
	IFN-gamma	→	→

Decreasing: ↓ increasing: ↑ unaltered: →

Effects on secretion of interleukins and chemokines by PBL (I)

Approach

- Generate culture supernatants of activated human peripheral blood leukocytes
- Determine interleukins/chemokines/cytokines by multiparametric ELISA



Main features

- The production of key chemokines involved in mobilisation of T_H lymphocytes are reduced by GOPO and/or rose hip
- The production of a key pro-inflammatory interleukin, IL-6 is decreased by GOPO and RH

Effects on secretion of interleukins and chemokines by PBL (II)

		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: →

Main features

- Various chemokines are robustly reduced by GOPO and to a lower extent by rose hip powder
- Both substances have inhibitory effects on a panel of pro-inflammatory interleukins and cytokines

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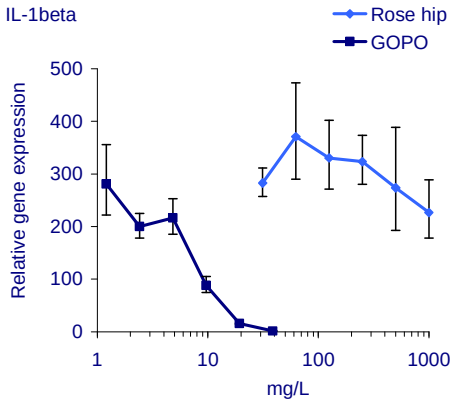
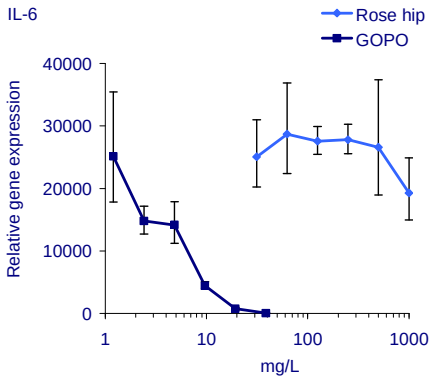
Effect on gene expression in human peripheral blood leukocytes

Approach

- Stimulate human PBL in the presence of graded amounts of GOPO or RH
- Determine expression of genes by quantitative RT-PCR



LPS/IFN-g-only: 277-fold



LPS/IFNg-only: 35960-fold

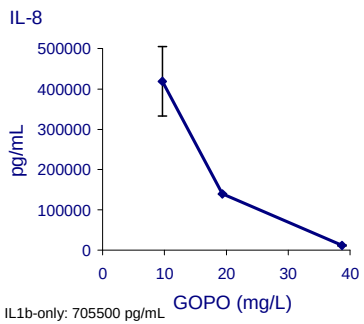
Main features

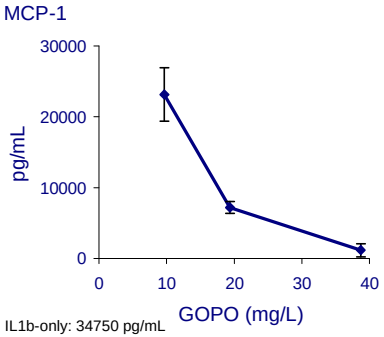
- GOPO differentially modulate the expression of the pro-inflammatory interleukin IL1-β and IL-6.
- The effect of rose hip on the expression of these genes is much weaker.

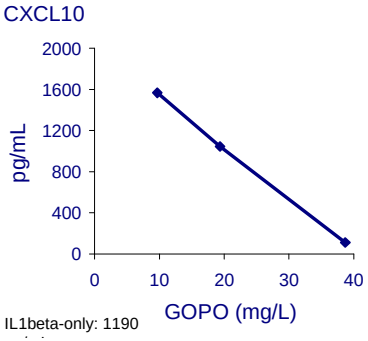
Chondrocytes: effect of GOPO on protein expression

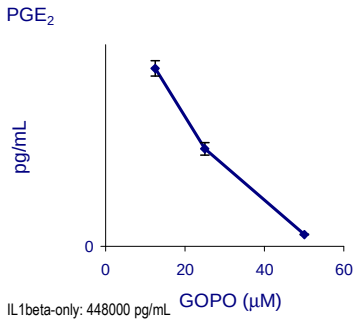
Approach

- NHAC-kn were activated with IL-1β (representing ‚diseased' joints) in the presence of RHP or GOPO for 24 hours. The chemokine or PGE_2 production was determined by multtiparametric RT-PCR









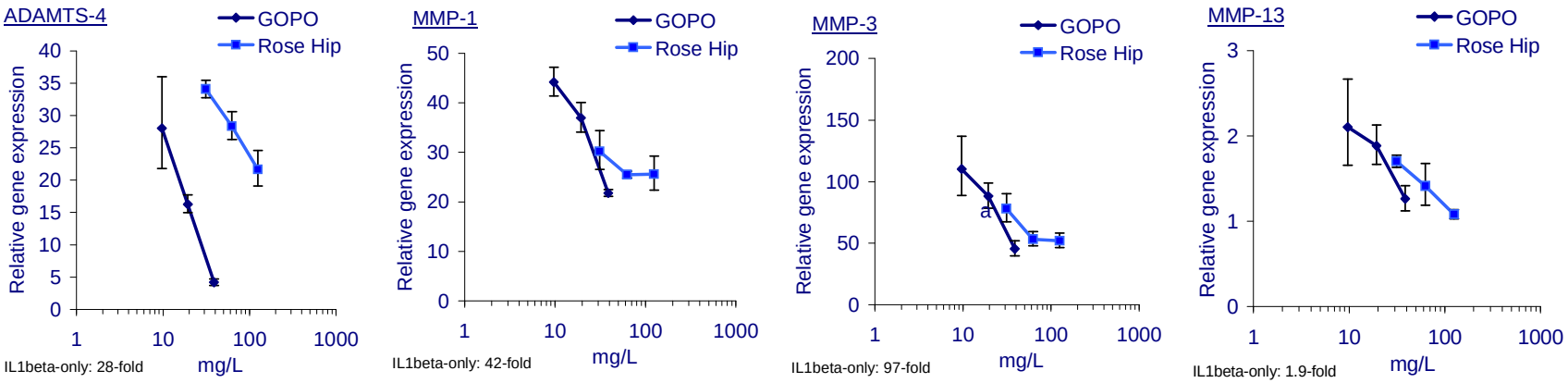
Main features

- GOPO dose-dependently inhibits the synthesis of three chemokines
- GOPO dosedependently inhibits the production of the pro-inflammatory PGE_2

Effect of GOPO and Rose Hip on catabolic genes in chondrocytes

Approach

- NHAC-kn were activated with IL-1β (representing ‚diseased' joints) in the presence of RHP or GOPO for 4 hours. The gene expression was measured by quantitative RT-PCR.



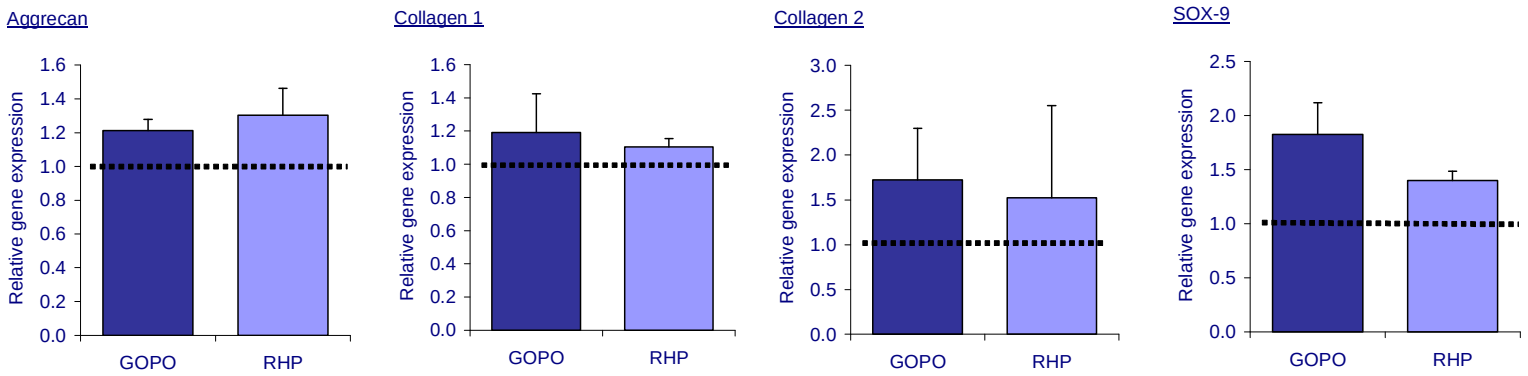
Main features

- Expression of aggrecanase and matrix metalloproteinase that trigger erosion of joints are dose-dependently reduced by GOPO and RH

Effect of GOPO and Rose Hip on anabolic genes in chondrocytes

Experimental approach

- NHAC-kn were cultured in the presence of RHP (50 mg/L) or GOPO (19 mg/L) for 4 hours. The gene expression was measured by quantitative RT-PCR (in comparison to unstimulated chondrocytes, representing „healthy“ joints).



Main features

- Anabolic genes that contribute to the expression of the extracellular matrix proteins (*e.g.* aggrecan and collagen) are activated by GOPO and RH
- Similarly, a transcription factor (SOX-9) that is involved in chondrocyte proliferation and differentiation is up-regulated by the compounds

Summary and Conclusions

- Bio-actives have been identified in rose hip powder by monitoring anti-inflammatory parameters and multi-parametric profiling in three cellular systems.
- Mouse macrophages were convenient for identifying first effects, while the heterogenous PBL populations (containing mononuclear and polymorphonuclear cells) permitted to monitor more diverse *i.e.* pleiotropic effects.
- GOPO has been identified as a potent effector molecule that modulates various facets of the inflammatory processes and cell migration mediated by chemokines.
- The importance of GOPO in cartilage protection is suggested 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.