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Laboratory and preclinical studies on the anti-inflammatory and anti-oxidant properties of rosehip powder – Identification and characterization of the active component GOPO®

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Abstract

The studies presented in this short communication demonstrate that an aqueous extract of rosehip from *Rosa canina L.* inhibited the chemotaxis as well as chemiluminescence of human peripheral blood leukocytes *in vitro*. Using a bioassay-guided fraction of the extract, the active principle was shown to be a galactolipid named GOPO®. These *in vitro* studies demonstrate that rosehip powder exhibits anti-inflammatory as well as anti-oxidant activities.

Key words: Rosehip, anti-inflammatory, anti-oxidant, galactolipid, GOPO®

Introduction

Rosehip or fruits of dog rose (*Rosa canina L.*) has been used as a source of vitamin C in tea and other products for many years. The British Herbal Pharmacopoeia (1983) notes that rosehip has properties, which can be used for gastritis, diarrhea, polydipsia and vitamin C deficiency. Some undocumented claims have also been made that rosehip may reduce the pain in patients suffering from inflammatory diseases such as osteoarthritis. The present drugs such as acetylsalicylic acid, non-steroid anti-inflammatory drugs and glucocorticoids used for the alleviation of the symptoms associated with this disease are claimed to exhibit a variety of side effects such as gastric erosion and adverse effects on the kidneys and liver. Therefore, it is necessary to find drugs or remedies, which are free from side effects and help to provide these patients with relief from pain and other symptoms.

Methods

Rosehip powder. A standardized rosehip powder from *Rosa canina L.* prepared by a special drying process provided by Hyben Vital, Langeland, Denmark was used throughout these studies.

Cells. Polymorphonuclear leukocytes (PMNs) were isolated from the peripheral blood of either healthy subjects or osteoarthritis patients, suspended in appropriate culture medium and used for biological activity assays.

Chemotaxis. A chemotaxis assay was performed using a modified Boyden chamber technique. The purified PMNs were pre-incubated with different dilutions of rosehip powder, various concentrations of the active compound GOPO® or control medium for 30 min at 37°C. Following pre-incubation, the chemotaxis of the cells towards the chemotactic factor zymosan activated serum (ZAS), which contains the biologically active chemo-attractant C5a, were tested. The migrated cells were counted by a computer-assisted image analysis system. The inhibition of chemotaxis was determined as the percentage of the cell response in the control culture medium.[1]

Chemiluminescence. A chemiluminescence assay was used as a measure of oxygen radical generation by activated PMNs. The cells were pre-incubated with different dilutions of rosehip powder, various concentrations of GOPO® or control medium for 30 min at 37°C and then stimulated with opsonized zymosan. The oxidative burst response of the activated cells was then measured by a luminometer. The inhibition of chemiluminescence was determined as the percentage of response in the control culture medium.[2]

Cell viability. Cell viability was determined by a trypan blue dye exclusion method. The dead cells take up the dye and appear blue under the microscope.

Isolation of GOPO®. GOPO® was isolated from the dried and milled fruits of dog rose by sequential extraction with *n*-hexane, dichloromethane, methanol and water, and the extracts evaporated *in vacuo* to dryness. Following testing of the resulting residues in the bioassay it was found that the activity was confined to the dichloromethane extract. The active ingredient was isolated using bioassay-guided fractionation by open column chromatography on silica gel followed by preparative HPLC. The structure of the active compound was elucidated from 1D and 2D NMR data.[3]

Results and Discussion

Our group has been involved in research on rosehip powder for the past 10 years. We have shown that a powder prepared from rosehip, *Rosa canina L.*, developed in Denmark, inhibited the chemotaxis and chemiluminescence

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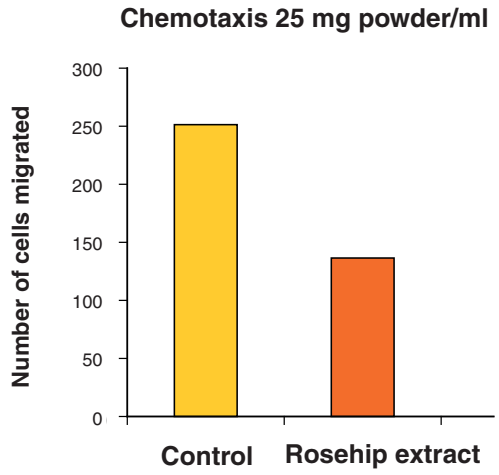

Fig. 1. The effect of rosehip powder on chemotaxis response of PMNs.

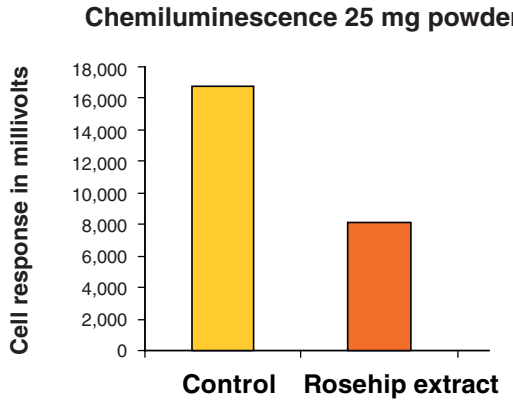

Fig. 2. The effect of rosehip powder on chemiluminescence response of PMNs.

of human PMNs *in vitro* as shown in Figs 1 and 2.[4,5] Daily intake of the powder for four weeks by healthy volunteers and patients suffering from osteoarthritis resulted in decreased chemotaxis of PMNs and a reduced level of the inflammatory marker acute phase C-reactive protein (CRP) in these subjects.[4,5]

Several double-blind, placebo-controlled clinical studies (presented in the following article) showed that rosehip powder exhibited several beneficial effects such alleviating pain and improving general wellbeing, sleep quality and mood in these patients.[6–8]

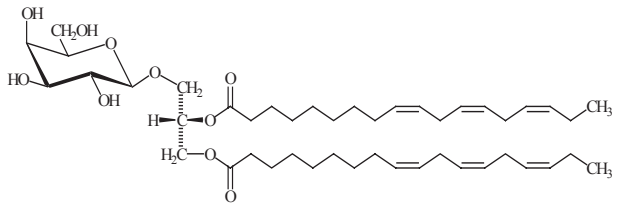

Fig. 3. The structure of GOPO®.

Studies from other groups have also shown that the extract of *Rosa canina L.* inhibited the respiratory burst of human PMNs[9] and exhibited an inhibitory effect on inflammatory cytokines such as interleukin-1 and tumor necrosis alpha.[10]

Using a bioassay-guided fraction we have identified the active anti-inflammatory and anti-oxidant principle in the rosehip. Using NMR this compound was identified as a galactolipid (2S)-1,2-di-*O*-[(9Z,12Z,15Z)-octadeca-9,12,15-trienoyl]-3-*O*–*B*-D-galactopyranosy glycerol, which was named GOPO®. The molecular weight of the compound is 775 g/mol.[3] The structure of the compound is shown in Fig. 3.

The content of GOPO® varies considerably in different subtypes of rosehip. The content of GOPO® in the standardized Hyben Vital rosehip used in our studies is quite high. This is due to a patent-protected drying process, which has been used to prepare the Hyben Vital rosehip in order to ensure that the active component GOPO® is preserved and is available in the finished product. Other methods for processing the rosehip powder might destroy this essential compound, which means that commonly available rosehip products might not produce the same effects as have been illustrated in the studies using a specially processed and standardized rosehip powder from *Rosa canina L.*

As shown in Table I GOPO® inhibited the chemotaxis and oxidative burst response of PMNs at microgram concentrations, demonstrating the anti-inflammatory and anti-oxidant properties of the compound. Cell viability tests showed that the PMNs were viable even at the highest concentration used in these studies indicating that the inhibition of cell migration and the oxidative burst response was not related to toxicity.

These data indicate that GOPO® has the potential to be developed into a new anti-inflammatory drug for the treatment of inflammatory diseases, such as rheumatoid arthritis and osteoarthritis. Studies are in progress to develop GOPO®-enriched new products.

Table I
The effect of GOPO® on chemotaxis and chemiluminescence response of PMNs

GOPO® concentrations (µg/ml)	Chemotaxis (% Inhibition)	Chemiluminescence (% Inhibition)	Cell viability (% Viable cells)
50	99	37	99
25	52	19	100
10	62	13	99
1	64	NC	NC
0.1	7	NC	NC
0	0	0	100

NC: not completed.

The recent withdrawal of Vioxx, one of the major drugs for osteoarthritis, by Merck, Sharpe and Dohme due to serious side effects, raised concerns about the whole group of synthetic Cox-2 inhibitors, which rofecoxib belongs to. It is therefore important that in contrast to NSAIDs and acetylsalicylic acid, rosehip powder does not inhibit platelets and does not influence fibrinolysis or the coagulation cascade. This is a significant benefit for patients with cardiovascular conditions who are often limited in the availability of safe and effective pain medications due to the increased cardiovascular disease risk of long-term rofecoxib usage.

In conclusion, the specially processed and standardized rosehip powder from *Rosa canina L.* might provide a safer alternative for millions of patients suffering from osteoarthritis and other inflammatory diseases.

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