Analytical and pharmacological studies on Mahonia aquifolium

K. GALLE, B. MÜLLER-JAKIC, A. PROEBSTLE, K. JURCIC, S. BLADT and H. WAGNER

Institute of Pharmaceutical Biology, University of Munich, Germany

Summary:

Alcoholic extracts of the antipsoriatic Mahonia aquifolium drug which were analysed for their qualitative and quantitative alkaloid content, showed an in vitro inhibiting effect on cyclooxygenase (CO) of sheep seminal vesicles and 5-lipoxygenase (5-LO) of porcine leucocytes. The same extracts exhibited also immunostimulating activities in two phagocytosis assays. In contrast the major alkaloids of Mahonia aquifolium extracts berberine, palmatine, magnoflorine and jatrorrhizine were found to be inactive at all in both antiinflammatory assays, suggesting a different mechanism of action for the alkaloids or further not yet detected compounds in the extract.

Key words: Mahonia aquifolium, alkaloid content, 5-lipoxygenase, cyclooxygenase inhibition, immunostimulating activity.

Introduction

Phytopreparations of Mahonia aquifolium (Pursh) Hutt. have been used orally in American folk medicine orally especially for the treatment of various skin diseases (Duke 1985). In Europe a tincture has been introduced into homeopathy as a remedy against psoriasis (Schindler 1955, BGA-Monography 1987). Patient studies performed with a Mahonia extract containing ointment revealed an antiproliferative and antiphlogistic effect after long term therapy (Wiesenauer 1992, a, b).

Although various protoberberine-, benzylisoquinoline- and apophine-alkaloids such as berberine, jatrorrhizine, palmatine, berberine, oxyacanthine and magnoflorine have been isolated from the drug (Kostalova et al., 1981; Sla­vik et al., 1985; Kostalova et al., 1986) it was unknown to which extent and by which exact mechanism of action the Mahonia alkaloids are involved in the antipsoriatic activity of the drug. It was only established that berberine interferes with DNA synthesis and by this exerts an antiproliferative effect (Creasy, 1979).

This lack of information has prompted us to perform pharmacological investigations using antiphlogistic and immunological in vitro and in vivo models. In addition a qualitative and quantitative HPLC alkaloid analysis of the drug extract has been carried out to correlate the pharmacological activity with the chemical composition of the drug and its phytopreparations.

Methodology

HPLC-analysis: (Fig. 1)

HPLC-conditions: Merck Hitachi L 6200 and AS 200 A, photodiodenarray detector LKB Bromma 2140 Rapid; pre­column: Li ChroCart® 4 × with Li-Chrospher 100 RP 18,5 μm (Merck); column: 250 × 4 with LiChrosorb® RP-select B 7 μm (Merck); mobile phase: A = methanol Li Chrosolv gradient grade + ionpair reagent (IP), B = water + IP; prepar­ation of IP for 11 eluent: 0.94 g 1-hexansulfonic acid added to 5 ml of 0.1 N H₃PO₄; flow rate: 1.0 ml/min; gradient 20–60 % A linear in 20 min, linear 90 % at 35 min; inj. vol.

1 Rubisan DHU, Germany, prepared with Mahonia aquifolium mother tincture according to German Homoeopathic Pharmacopoe (HAB).
Table 1.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Magnoflorine</th>
<th>Jatrorrhizine</th>
<th>Berberine/Palmatine</th>
<th>Total Alkaloid Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mother tincture*</td>
<td>0.71 ± 0.02</td>
<td>0.53 ± 0.01</td>
<td>0.81 ± 0.01</td>
<td>2.05 ± 0.02 mg/ml</td>
</tr>
<tr>
<td>Methanol extract**</td>
<td>1.14 ± 0.05</td>
<td>0.89 ± 0.003</td>
<td>0.64 ± 0.03</td>
<td>2.67 ± 0.02 mg/ml</td>
</tr>
</tbody>
</table>

* 100 g drug/l 70 % alcohol  
** drug/50 ml methanol (NH₄⁺-pretreated)  
n = 6

Results

Alkaloid Quantification

As estimated by HPLC and calculated in terms of berberine, jatrorrhizine and magnoflorine respectively as reference alkaloids, the alkaloids berberine, palmatine, jatrorrhizine and magnoflorine were found to be the major alkaloids in a percental ratio of 8:5:7 in the mother tincture and 6:8:11 in MeOH-extract or rough drug, respectively (Table 1 and Fig. 1 and 2). Their total alkaloid content was estimated to be around 2.0 % in the mother tincture and 2.7 % in the MeOH extract. Berbamine, oxyacanthine and columbamine as minor alkaloids were detected only in small amounts only (< 0.1 %).

Pharmacological Assays

The suggestion that the anti psoriatic efficiency of the drug might be caused by an inhibition of prostaglandin metabolism, has prompted us to investigate the influence of the extract and single alkaloids on the enzyme activities of 5-lipoxygenase and cyclooxygenase. The 5-lipoxygenase assay was carried out according to a modified in vitro method using porcine leucocytes as source for 5-lipoxygenase (Wagner and Fessler, 1986). The cyclooxygenase assay was performed in an analogous manner with a cyclooxygenase preparation from sheep seminal vesicles (Wagner et al., 1986). In the 5-LO assay 20 III of the mother tincture equivalent to 0.3 mg dry weight showed an inhibition rate of 58.6 ± 6.3 % (IC₅₀ = 0.28 mg/ml). 20 III of the MeOH-extract equivalent to 20 mg dry weight was found to inhibit 5-LO to 16 %. For comparison nordihydroguaretic acid has an IC₅₀ = 0.45 μg/ml. In the CO assay surprisingly a much higher inhibition effect was measured for the mother tincture. The mother tincture showed a dose-dependent effect in a concentration range of 2,5-50 μl/ml with an IC₅₀ of 0.11 mg dry weight/ml and by this was found to be approx. 3 times more active in the CO-assay than against 5-LO. For comparison indometacin has an IC₅₀ of 0.36 μg/ml. All alkaloids, however, were found to be inactive in both assays at a concentration of 50 and 100 μM.

Since some inflammatory skin diseases are caused by an imbalance of the immune system, we investigated the influence of the Mahonia extract on in vitro- and in vivo pha-
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Fig. 1. A= mother tincture (10 µl) 280 nm
B= MeOH-extract (10 µl) 280 nm
1= Magnoflorine
2= Oxyacanthine
3= Berbamine
4= Columbamine
5= Jatrohrrhizine
6= Berberine/Palmatine

Fig. 2. Berberine R1 + R2 = OCH3
Palmatine R1 + R2 = OCH3
Jatrohrrhizine R1 = OH; R2 = OCH3

Phagocytosis. The in vitro FACS test performed with human granulocytes and latex particles as challenge (Juricic et al., 1994) revealed a moderate dose-dependent immunostimulating effect of about 30% at a concentration of 0.2-0.3 µl/ml. In the in vivo carbon clearance test (Biozzi et al., 1953) however, the extract showed a very good phagocytosis-enhancing effect of RCc/RCc1 = 1.7 at a concentration of 3 x daily 0.3 ml/30 g mouse after oral administration.

Discussion

We have shown that the Mahonia extract has an inhibiting activity on both key enzymes of the arachidonic acid pathway, whereas the various Mahonia alkaloids have no effects even at relatively high concentrations. This leads to the conclusion that either not yet determined compounds of the drug might be responsible for the LO- and CO-inhibiting effect of the extract or that the alkaloids act antiphlogistic via other mechanisms.

In this context it is worth mentioning, that alkaloids of the structurally related benzophenanthridine type such as chelerythrine show a good 5-LO-inhibiting effect (Müller-Jakic, 1994). In contrast some isoquinoline alkaloids (tebrahydroberberine, stylopine and others) tested on soybean lipoxygenase were found to increase the oxidation of linoleic acid in the presence of this lipoxygenase (Bennetout et al., 1986).

Berberine has been found to exhibit an antiinflammatory effect in the cotton pellet, oil-granuloma pouch and punch methods (Otsuka et al., 1981) and in the TPA induced inflammation assay in mice (Yasuhawa et al., 1991). It may, therefore, be possible that other mechanisms of actions are responsible in part for this in vivo effect. Our finding that the Mahonia extract showed a phagocytosis-enhancing effect is in agreement with the observation that e.g. some bisbenzylisoquinoline alkaloids (berbamine, tetrandrine) inhibit the production of interleukin 1 and tumor necrosis factor (TNF α) by monocytes and macrophages as well as TNF β production by lymphocytes (Seow et al., 1992).

Altogether our first investigations of Mahonia extract clearly show that the known antiproliferative activity of berberine alkaloids cannot be the only mechanism responsible for the antipsoriatic efficacy of Mahonia extracts. It may be possible that the Mahonia alkaloids act either as oxygen radical scavengers and exhibit an antioxidative effect or they exert an antiproliferative and antiphlogistic effect by modulation of the unspecific immune system. Furthermore it will be necessary to clarify which other compounds of the mother tincture may be responsible for its high inhibition effect on CO as well as 5-LO.
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References


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Address

Prof. Dr. H. Wagner Institute of Pharmaceutical Biology University Munich Karlstraße 29 D-80333 München, Germany