Phytomedicine Vol. 1/1994, pp. 59–62

Analytical and pharmacological studies on Mahonia aquifolium

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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 homoeopathy as a remedy against psoriasis (Schindler 1955, BGA-Monography 1987). Patient studies performed with a Mahonia extract containing ointment[1] revealed an anti-cell-proliferative and antiphlogistic effect after long term therapy (Wiesenauer 1992, a, b).

Although various protoberberine-, benzylisoquinoline- and aporphine-alkaloids such as berberine, jatrorrhizine, palmatine, berbamine, 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 H_3PO_4; 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.

drug	magnoflorine	jatrorrhizine	berberine/palmatine	total alkaloid content
mother tincture*	0.71 ± 0.02	0.53 ± 0.01	0.81 ± 0.01	2.05 ± 0.02 mg/ml
methanol extract**	1.14 ± 0.05	0.89 ± 0.003	0.64 ± 0.03	2.67 ± 0.02 mg/ml

* 100 g drug/l 70 % alcohol
** drug/50 ml methanol (NH_3-pretreated)
n = 6

= 10 µl mother tincture or methanol extract; detection: 280 and 345 nm. The quantitative estimation was performed according to the method of external standard by peak square measurement. For berberine, jatrorrhizine and magnoflorine the calibrations were made with n = 8 conc. (3 µg–30 µg).

5-Lipoxygenase-assay: (Wagner, Fessler, 1986)
The test was carried out with porcine leucocytes. ETYA, ionophor A 23187, $CaCl_2$ and ^{14}C-arachidonic acid in the incubation medium followed by HPLC-separation of the metabolites. The 5-LO-inhibition was calculated on the basis of the reduced 5-HETE-concentration against the control.

Cyclooxygenase assay: (Wagner et al., 1986)
The test was performed with a cyclooxygenase preparation obtained from a microsome fraction of sheep seminal vesicles, incubation in EDTA-sol, with glutathione, adrenalinbitartrate and ^{14}C-arachidonic acid followed by HPLC-separation of the metabolites. The CO inhibition was calculated on the basis of the reduced PGE_2-concentration against the control.

In vitro granulocyte phagocytosis assay: (according to Jurcic et al., 1994)
Heparinized human blood was incubated with the extracts and latex particles (Fluoresbrite carboxy microspheres, Polysciences Inc. ur. 15702) and the percentages of cells phagocytized determined in the FACSan (Becton-Dickinson, Heidelberg). LPS was used as reference compound. The extracts were applied in a concentration of 0.2–0.3 µl/ml.

In vivo phagocytosis assay: (carbon clearance, Biozzi et al., 1953, modified according to Bauer et al., 1985).
The test was performed with mice. The extracts (0.3 ml/30 g mouse, 3 times daily over 2 days) were applied orally. The colloidal carbon particles (Indian ink Rotring, Art 591017 black) were injected i.p. Blood samples were taken at intervals of 3, 6, 9, 12 and 15 min from the retro-orbital plexus and the regression times (RC_{tr}/RC_c) determined spectrophotometrically at 650 nm. The Indian ink was injected 12 h after the last sample administration.

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 antipsoriatic 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 analogeous manner with a cyclooxygenase preparation from sheep seminal vesicles (Wagner et al., 1986). In the 5-LO assay 20 µl of the mother tincture equivalent to 0.3 mg dry weight showed an inhibition rate of 58,6 ± 6,3 % (IC_{50} = 0.28 mg/ml). 20 µl of the MeOH-extract equivalent to 20 mg dry weight was found to inhibit 5-LO to 16 %. For comparison nordihydroguaretic acid has an IC_{50} = 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 µl/ml–50 µl/ml with an IC_{50} of 7.0 µl/ml or 0.11 mg dry weight/ml and by this was found to be approx. 3 times more active in the CO-assay than aginst 5-LO. For comparison indometacin has an IC_{50} 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-

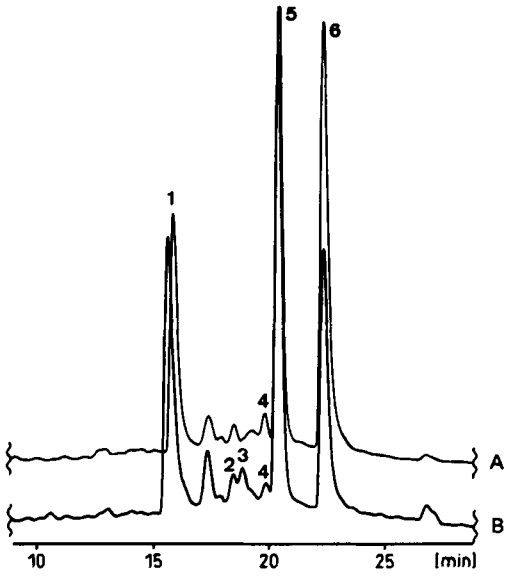
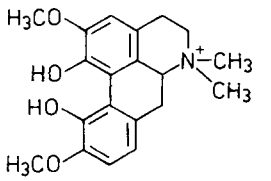


Fig. 1.
A= mother tincture (10 µl) 280 nm
B= MeOH-extract (10 µl) 280 nm
1= Magnoflorine
2= Oxyacanthine
3= Berbamine
4= Columbamine
5= Jatrorrhizine
6= Berberine/Palmatine



Magnoflorine

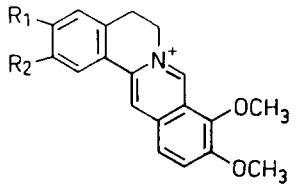


Fig. 2.
Berberine $R_1 + R_2 = OCH_2$
Palmatine $R_1 + R_2 = OCH_3$
Jatrorrhizine $R_1 = OH$; $R_2 = OCH_3$

gocytosis. The in vitro FACS test performed with human granulocytes and latex particles as challenge (Jurcic 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 RC_{tr}/RC_c[1]) = 1.7 at a concentration of 3 × daily 0.3 ml[2]/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 (tetrahydroberberine, stylopine and others) tested on soybean lipoxygenase were found to increase the oxidation of linoleic acid in the presence of this lipoxygenase (Beneytout 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.

[1] Regression coefficients: tr = treated/c = control
[2] of 0.5 ml mother tincture/30 ml NaCl sol.

Acknowledgement

We thank the DHU (Deutsche Homoeopathic Union) Karlsruhe for providing the plant drug and extract and for the financial support.

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