CO_2-EXTRACT FROM GERMAN CHAMOMILE-PROPOSED MEDICINAL USE

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Abstract

The CO_2-extract from German chamomile contains the lipophilic components of the flowerheads. Compared with the essential oil, its drug-product ratio is lower and its composition is slightly different. In the pharmacological model of the arachidonic acid cascade, it shows anti-inflammatory properties according to the higher drug-product ratio compared with a "normal" alcoholic extract. Its wound-healing properties should be further evaluated. In the mouse ear edema, it shows a significant, but weaker activity than hydrocortisone. Its use in a treatment scheme of the atopic eczema is promising. However, further clinical evidence is needed.

1. Introduction

CO_2-extracts from plants are well-known ingredients in the food industry. The medicinal use of the CO_2-extract from *Matricaria recutita L.* is hampered due to the strict requirements of the health authorities who consider the CO_2-extract to be a basically new extract for which the experiences with other extracts are not applicable. The composition of the CO_2-extract from German chamomile is closely related to the essential oil as has already been shown since 1978 (Stahl *et al.*, 1978; Vuorela *et al.*, 1990; Reverchon *et al.*, 1994). In order to further evaluate the medicinal use of the CO_2-extract, we have once more compared it analytically with the essential oil. In collaboration with other scientific institutions we have performed pharmacological studies about the anti-inflammatory and wound-healing properties and have initiated preliminary clinical studies in comparison to hydrocortisone.

2. Analytical comparison with the essential oil

The essential oil and the CO_2-extract were obtained from the same batch of chamomile flowerheads and analyzed by GC (glass column PS086 20 m x 3 mm i.d. 40°-200° progr., FID).

All lipophilic components of the essential oil were also part of the CO_2-extract with the exception of chamazulene. Matrizine was not determined. The quantitative composition was different due to the different drug-product ratio and the instability of some components during hydrodistillation of the essential oil. The CO_2-extract showed 4 additional peaks representing the hydrocarbons $C_{25}H_{52}$, $C_{27}H_{56}$, $C_{29}H_{60}$ and $C_{31}H_{64}$, as was shown by GC/MS. Such n-alkanes had been previously found in CO_2-extracts from other medicinal plants (Hawthorne *et al.*, 1993). (Table 1)

3. Influence on metabolites of the arachidonic acid cascade

The anti-inflammatory activity of a compound can be evaluated in the well-known model of the arachidonic acid cascade. The phospholipids of the cell wall are cleaved off

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to arachidonic acid which is further metabolized by cyclooxygenase and lipoxygenases to the true mediators of the inflammation such as hydroxyeicosatetraenic acids, leucotrienes or prostaglandins. These metabolites can be measured by HPLC and so the influence of an added compound on the formation of these metabolites can be determined. Leucotriene B_4 (LTB_4) was measured as assay for 5-lipoxygenase, 12-hydroxyeicosatetraenic acid (12-HETE) for 12-lipoxygenase and 12-hydroxy-heptadecatrienic acid (12-HHT) for cyclooxygenase (Safayhi *et al.*, 1994). (Table 2)

The results clearly show that the CO_2-extract has an anti-inflammatory effect; the potency depends on the higher concentration of the active components due to the higher drug-product ratio compared with a "normal" isopropanolic extract (Ammon *et al.*, 1996).

4. Effect of chamomile ointment 1% on wounds of partial thickness in the pig

Chamomile is known for its wound-healing properties. A study with 3 pigs was performed in order to evaluate the effect of an ointment with 1% CO_2-extract on the epithelization. Square wounds, 25 by 25 mm with a thickness of 0.4 mm were surgically placed on the back of the pigs, 6 wounds per pig. By this technique, only the epidermis and parts of the corium were removed. One third of the wounds was treated with the ointment with 1% CO_2-extract, another third with the ointment basis and the last third received no treatment. The ointments were applied twice, first immediately after placing the wounds and then after 24 h. After 66 h the pigs were sacrificed, the wounds were surgically taken out and examined histologically in order to determine the degree of epithelization. (Table 3)

The chamomile ointment showed the highest degree of epithelization, but the difference is not statistically significant. (Hoyer, 1997).

5. Anti-inflammatory activity in the mouse ear edema

The anti-inflammatory activity of an ointment with 1% CO_2-extract was tested in vivo in comparison to hydrocortisone 0.25% in the same ointment basis. In the mouse ear, edemas were provoked by three compounds: arachidonic acid (AA), phorbol myristate acetate (PMA) and oxazolone (Ox.). The inhibition was determined 1 h after the induction of the edema for AA, 6 h, 24 h and 48 h for PMA and 24 h for Ox. (Gohlke, 1988). (Table 4)

The results show that both ointments are almost equally effective in the AA and PMA model, but not in the oxazolone model. This model represents a more chronic-immunological inflammation and it is therefore not surprising that hydrocortisone shows a positive effect whereas chamomile does not (Hirschelmann *et al.*, publication in preparation).

6. Clinical study: chamomile 1 % vs. hydrocortisone 1 %

A confirmatory clinical trial was performed as a randomized, double-blind, parallel-grouped study with 1% CO_2-extract and hydrocortisone 1% in the same ointment basis. The patients (40 per group) suffering from neurodermatitis in the stage of a subacute eczema, were treated with the ointments twice daily for two weeks. The effect of the treatment was judged in each case as the global clinical impression by the responsible physician. (Table 5)

The average of the rating shows that hydrocortisone 1% was significantly ($p<0.001$) more effective than chamomile 1%. As nearly all the patients had been pretreated

topically with steroids, the influence of such a pretreatment on the global clinical impression was evaluated by classifying the potency of the steroids used. (Table 6)

In cases of pretreatment with weak or moderate potent steroids, hydrocortisone 1% was much better effective than chamomile 1%, but not in patients pretreated with potent or very potent steroids (Hempel, 1996).

7. Conclusions

The CO_2-extract from German chamomile is a modern phytotherapeutic agent which contains the lipophilic components of the flowerheads. It shows anti-inflammatory properties in vitro and in vivo according to its drug-extract ratio. No wound-healing properties could be shown so far. A clinical study in neurodermatitis revealed that the CO_2-extract could be a promising agent for the topical treatment of certain inflammatory skin diseases. It could lead to a substitution of weak steroids in the treatment scheme of the subacute eczema after the pretreatment with potent steroids.

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Table 1 - Comparison of the essential oil with the CO_2-extract (mg per g)

Component	Essential oil	CO_2-extract
Trans-β-Farnesene	128	97
Spathulenol	8	2
Bisabolol oxide B	89	25
Bisabolone oxide	19	5
(-)-α-Bisabolol	151	43
Chamazulene	67	-
Bisabolol oxide A	157	71
Herniarin	-	8
Cis-En-in-dicycloether	48	132
Trans-En-in-dicycloether	20	52
Drug-Product Ratio	approx. 200 : 1	approx. 50 : 1

Table 2 - Inhibitory concentration 50% (mg per ml)

	Drug-Extract Ratio	LTB_4	12-HHT	12-HETE
Isopropanolic extract	1 : 2	0.8	1	No effect
CO_2-extract	50 : 1	0.006	0.015	0.028

Table 3 - Degree of epithelization after 66 h in % of total wound area

Treatment	Epithelization
Chamomile ointment 1%	89 ± 11
Ointment without chamomile	82 ±10
Untreated	78 ±22

Table 4 - Inhibition of the mouse ear edema in % against control (n = 6)

Ointment	AA	Phorbol myristate acetate (PMA)			Ox.
	1 h	6 h	24 h	48 h	24 h
Chamomile 1%	45	72	66	73	0
Hydrocortisone 0.25 %	31	69	76	82	23

Table 5 - Efficacy: global clinical impression

Rating	Chamomile 1%	Hydrocortisone 1%
Very good (1)	7	18
Good (2)	17	18
Moderate (3)	11	4
Poor (4)	5	-
Average	2.35	1.65

Table 6 - Global clinical impression: rating according to the pretreatment of the patients with steroids

Rating	Weak or moderate steroids		Potent or very potent steroids	
	Chamomile 1%	Hydrocortis. 1%	Chamomile 1%	Hydrocortis. 1%
Very good (1)	2	14	5	4
Good (2)	13	8	4	10
Moderate (3)	5	1	6	3
Poor (4)	4	-	1	-
Average	2.46	1.43	2.19	1.94