Local therapy of herpes simplex with dried extract from Melissa officinalis

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Summary
An overt multicentric study involving 115 patients and another subsequent placebo-controlled double-blind study involving 116 patients contributed significantly to the corroborative evidence of the antiviral activity in vitro of a specially prepared dried extract from Melissa leaves (Melissa officinalis L.) against herpes simplex infections. The studies provided the proof that the ingredient gave protection against herpes simplex infections. The initiation of the treatment in the very early stages of the infection revealed itself as most effective.

Key words: Melissa officinalis extract, randomized placebo-controlled double blind study, herpes simplex.

Introduction
Ever since the antiquity the melissa plant is well known as a curative herb. The first mention of the melissa appeared already more than two millennia ago, around 300 B.C., in "Historia plantarum" compiled by Theophrastus of Ephesus, a pupil of Aristoteles. Having observed that the bees had a special liking for this herb, Theophrastus named it Melissophyllon, i.e. bee-balm. Later, between about 50 and 80 A.D., Plinius Secundus, a Roman, suggested the melissa for therapeutical use for the first time in his "Materia Medica". It had been used externally in case of insect bites and internally for the treatment of abdominal colic and uterine spasm. The medical school of Salerno passed on the tradition over the middle ages, in that by virtue of its sedative action on the vegetative system and its spasmolytic effect the old usage of melissa persists even in this day and age (Koch-Heitzmann and Schulze, 1988). The pharmacological effect of the melissa is ascribed to be attributed to the essential oils.

Recently new investigations of the dried extract from melissa leaves revealed a new potentiality for its therapeutical use. Already in the sixties, it was ascertained that the dried extract from melissa leaves exhibited inhibitory activity towards smallpox, mumps and Newcastle disease viruses (Cohen, Kucera and Herrmann, 1964; Kucera and Herrmann, 1967).

The evidence of its antitherpetic activity as well was established in the late seventies (May and Willuhn, 1978).

Manufacture and Standardization of the Dried Extract from Melissa Leaves

The melissa leaves, used for the manufacture of the extract, must conform to the specifications set forth in the current edition of DAB (German Pharmacopoeia). Fig. 1 represents the sequence of operations in the manufacturing process of the extract. The standardization of the extract in terms of the antiviral potency is ensured by the use of specially selected drugs, strict implementation of the prescribed manufacturing process, and the relevant chromatographic analyses and virological tests.

Chromatography: The identity and the chemical composition of the dried extract from melissa leaves are confirmed by the chromatographic HPLC-fingerprint analysis. Besides caffeic acid and chlorogenic acid rosmarinic acid as major component of the drug was used as leading substance.
Product Test

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Fig. 1. Criteria for the standardization of manufacture of the dried extract from melissa leaves.

Standardization of the antiviral potency by means of in vitro tests on herpes simplex viruses: The standardization of the dried extract, being the all-embracing criterion, calls for the assurance of the uniformity of antiviral potency of the dried extracts from melissa leaves by means of appropriate virological tests. A modified method of the generally known in vitro plaque inhibition test, which is also suitable for the exploration of new antiviral agents, is applied for this purpose. The principle applied throughout this method is that of a comparison of the sample being examined (e.g. drug, extract, pharmaceutical preparation, and the like) with a standard melissa extract that is preserved under strictly defined conditions of storage. It is essential that the potency derived from the plaque inhibition test on the sample being examined matches up to the potency of the standard, apart from the minor deviations that are unavoidable in such case of a natural drug despite specifically selected material.

For the plaque inhibition test, human or animal cells are cultivated in Petri-dishes, which are then infected with herpes simplex viruses. A congealing overlay medium is poured over the infected cell cultures, and a filter paper disc impregnated with the melissa extract being examined is deposited on each of the hardened overlay surfaces, followed by incubation under prescribed conditions. The cells are destroyed during incubation by the unhindered replication of the viruses. Around the filter paper disc, this so-called cytopathic effect is arrested by the action of the melissa extract. The inhibitory zones that develop during incubation by the unhindered replication of the viruses. Around the filter paper disc, this so-called cytopathic effect is arrested by the action of the melissa extract. The inhibitory zones that develop during incubation by the unhindered replication of the viruses. Around the filter paper disc, this so-called cytopathic effect is arrested by the action of the melissa extract.

Fig. 2. Plaque inhibition test using a filter paper disc impregnated with 200 µg dried extract from melissa leaves.

Clinicopathology of Herpes simplex infection

Herpes simplex viruses, types 1 and 2 (HSV-1 and HSV-2), cause as a rule local infections of the skin, transitional mucosa and the mucosa, characterized by frequent incidence and recidivation rates. The guiding symptom typical of the disease is the collective vesicular eruption occurring on the inflamed edematous skin. Whereas the active nucleoside analogues such as idoxuridine and acyclovir induce intracellular impediment to virus procreation (Wasilew, 1983, 1986), the dried extract from melissa leaves hinders dissemination of the infection to the intact cells by blocking up the host cells and the virus receptors.

Clinical studies: Proof of concept - study, materials and methods study design

The first study for the assessment of the clinical benefit was conducted as an overt, controlled, multicentrical study that was carried out at three dermatological clinics (viz. Dermatological Clinic of the Town Hospital of D-64297 Darmstadt, Dir.Prof Dr.med. E. Landes, Dermatological Clinic of the Town Hospital of D-34125 Kassel, Dir. Prof Dr.med. J. Petres and the Centre of Dermatology and Venerology, Johann Wolfgang Goethe University, D-60596 Frankfurt/Main, Dir. Prof Dr. med. R. Milbradt) alongside one Frankfurt-based dermatological private practice (Dr.med. K. Rapprich).

Altogether, 115 patients, comprised of 45 male and 70 female subjects, were involved in the said study at the four test centres.
Inclusion Criteria: patients with herpes simplex infection of the skin or transitional mucosa; the clinical symptoms lasting for not more than 72 hours until admission to the study; patients of both sexes and of all age groups; patients' consent.

Exclusion Criteria: any known hypersensitivity to the ingredients of the preparation being examined; other treatment of viral infection; limited legal capacity.

Test Preparation: Lomaherpan® Cream, containing 1% dried melissa extract from melissa leaves (drug/extract 70:1) in a cream base consisting of white soft paraffin, ethylene glycol mono(di)-stearate (Tegin G), polyethylene glycol 1000 monoacetyl ether (Cetomacrogol 1000), 1-chloroacetamide/sodium benzoate (7:3), and water for injections.

Each patient was given one tube of the test preparation containing 5 g of the cream.

Duration of the Clinical Study
For the clinical study involving medication kept at home, each patient was instructed to apply the drug 5 times daily until healing of the lesion was complete, but not beyond 14 days at the maximum. The clinical symptoms were documented at the beginning of the study (day 0) and subsequently on the day 4, 6, and 8 of the treatment. Global assessment of the efficacy of the drug with regard to every individual patient was undertaken by the physician and the patient concerned on the occasion of the patient's last visit.

Clinical Assessment
A thorough clinical anamnesis was obtained from each patient during the first consultation, based on which the diagnosis was determined. The following data were recorded for the purpose of evaluation: Date of the first examination, site of the lesion, the onset of the clinical symptoms (in hours), healing of the vesicular eruption, side-effects.

Results (Course of Healing)
The healing was complete in 96% of the patients until day 8 of the treatment, whereby it was 60% and 87% on the day 4 and 6 respectively.

Tolerance
Three of the 115 patients treated with the test preparation complained of a sensation of burning and paresthesia. Any conclusive correlation between these side-effects and the application of the test preparation being examined could not be established since such symptoms are also characteristic of the herpes simplex infection itself.

Assessment
It can be inferred from the results of the study that the application of the melissa extract incorporated in a cream base brought about a speedy healing of the herpetic lesions. Natural recovery from herpetic infection of the skin or transitional mucosa usually occurs within 10 to 14 days (Nasemann and Braun-Falco, 1970).

The present study revealed that the healing in 96% of the patients was complete on day 8 already. The evidence that melissa cream proved propitious for a faster healing was evaluated as a significant therapeutical effect by all participating test centres.

The impressive results of the study led, in consequence, to the conception of the following placebo-controlled double-blind study.

Clinical study – Material and Methods – Study design
Randomized placebo-controlled double-blind study, carried out at two dermatological centres (Clinic and Polyclinic of the Technical University, D-80802 Munich, Dir. Prof. Dr. Dr. S. Borelli, and Dermatological Clinic of Charité, D-10117 Berlin, Dir. Prof. Dr. sc. med. N. Sönntuchsen).

Formulation and Dosage
Drug: 5 g of Lomaherpan® cream, containing 1% dried extract from melissa leaves (drug/extract 70:1) in a cream base consisting of white soft paraffin, ethylene glycol mono(di)-stearate (Tegin G), polyethylene glycol 1000 monoacetyl ether (Cetomacrogol 1000), benzyl alcohol, and water for injections.

Placebo: 5 g of the cream base as used for the drug, without the melissa extract as the active substance.

The patients were instructed to apply the drug or the cream, as received, 2 to 4 times daily on the affected sites of the skin over a period of 5 to maximum 10 days. Each patient was given one tube containing 5 g of either drug or placebo.

Inclusion Criteria: patients with herpes simplex infection of the skin or transitional mucosa; the clinical symptoms lasting for not more than 72 hours until admission to the study; patients of both sexes and of all age groups; patient’s consent.

Exclusion Criteria: any known hypersensitivity to the ingredients of the drug or control preparation being applied; other treatment of viral infection; limited legal capacity.

Former and Concomitant Treatments
In all groups taken together, 85 patients had not received any medical treatment so far as distinguished form 31 patients who had tried some former treatment. The previous treatments prior to the present study involved the medication with indifferent and antibiotic or antiseptic ointments in 23 patients compared to 8 patients who were treated...
with topical preparations containing an antiviral agent such as acyclovir or idoxuridine, and were statistically identical in both groups (p = 0.401; CHI²-test). Any concomitant treatment, whether external or internal, with some other antiviral preparation was not permitted during the entire period of the study.

Duration of the Study

For the present clinical study, each patient was instructed to apply the cream or the placebo, as received, over a period of 5 to 10 days subject to the healing of the lesion, the medication being terminated within this period if healing was complete. The clinical symptoms were documented after 2 days and on termination of the treatment, usually after 5 days on the average. The global evaluation of the results of each case was carried out by the physician and the patient concerned on the occasion of the patient’s last visit.

Clinical Assessment

A thorough clinical anamnesis was obtained from each patient during the first consultation, based on which the diagnosis was determined. The following data were recorded for the purpose of evaluation:

- Date of the first examination
- Site of the lesion
- Prodromal sensations (pain, burning, itching, prickling, and others)
- The onset of the prodromal sensations (in hours)
- The appearance of the symptoms on the skin (in hours)
- Beginning of the treatment (date)
- Former treatment (no, yes, which?)

The clinical symptoms were categorized as follows:

- Rubor (none, slight, moderate, severe)
- Swelling (none, slight, moderate, severe)
- Vesicles (none, few and superficial, many, many and deep)
- Scabs (none, few, many, falling off)
- Pain (none, slight, severe, extremely severe)
- Healing (complete, parital, no)

The symptoms were rated by a score system ranging from 1 to 4. Additionally, the dimensions of the lesions, mm in diameter, were recorded. The global assessment of the results pertaining to each patient was carried out by the physician and the patient concerned, using a rating score ranging between 1 and 5 (1 = very good; 5 = very bad).

Statistical Method

For the statistical evaluation of the non-parametrical data the method of two-tailed t-test was applied, whereas for other variables the method of CHI²-test was used. The differences were indicated as statistically significant if p was less than 0.05. For the sake of lucidity of the diagrams, mean values of the verbal scores were plotted against the intervals of observation of the symptoms. The inferential statistical test, however, was not based upon these mean values of scores.

Results

Case reports of 116 outpatients had been taken into account for the evaluation of the study. The average age of the patients in the placebo group was 33.2 + 14.8 years (mean value, standard deviation), compared to 40.3 + 14.8 years in the melissa group. The difference in age is statistically significant with p = 0.009, which is evident from the fact that the placebo group included three patients aged 4, 5 and 6 years respectively (Charité, Berlin).

The statistical distribution of sex was almost identical in both groups (p = 0.675). The placebo group comprised of 44 females and 14 males, compared to 41 females and 17 males in the melissa group. Altogether, the females constituted 73.7% of the patients in the entire study, compared to 26.3% by males.

Clinical Symptoms

The duration of the prodromal signs was virtually the same in both groups (p = 0.497), viz. 21.2 ± 19.0 hours and 21.3 ± 19.0 hours in the melissa group and placebo group respectively.

The clinical symptoms in the placebo group appeared 14.0 ± 12.6 hours before the treatment, compared to 18.5 ± 17.5 hours in the melissa group. A statistically significant difference did not exist (p = 0.117).

Also with regard to the pre-study treatments there was no statistical difference between the two groups (p = 0.401).

In the placebo group, 40 patients did not have any former treatment and 18 patients had undergone a treatment before the study; in the melissa group, the corresponding figures were 25 and 13 respectively.

As to the sites of the herpes infections, there was likewise a conformity in both trial groups (p = 0.3719).

The lesion on the lips represented the highest incidence with 34 and 33 cases respectively in the two trial groups. Genital herpes was diagnosed in 4 and 6 patients respectively in the two groups.

Results of Treatment

The distribution of the symptom rubor was statistically identical in both groups at the beginning (day 0) of the treatment (p = 0.708).

In contrast to the placebo group, the decline of this symptom in the melissa group after two days of the treatment revealed itself as statistically highly significant (p = 0.0055) (see fig. 3).

On day 5, i.e. on the day of final examination, no symptoms were noticed in 24 patients of the melissa group as distinguished from 15 patients of the placebo group.
In case of the symptom manifested as swelling, no significant statistical differences existed on day 0 in the two groups ($p = 0.301$). On day 2, however, the decline of the symptom as observed in the melissa group was statistically more significant than in the placebo group ($p = 0.025$) (see Fig. 4).

The results pertaining to the other symptoms such as vesication, scabbing, erosion and pain revealed no significant difference in the two trial groups under the conditions of the study design. Nonetheless, it was observed that scabbing in patients of the melissa group was less than in patients of the placebo group, indicating a reduced damage of the cells in the melissa group (see Fig. 5).

In order to assess the course and extension of the lesions, the area of the nearest approaching rectangle was estimated. This procedure did not bring about any statistical difference between the trial groups in the first instance since the standard deviations were too high (see Fig. 6). This phenomenon was ascribed to the large areas of herpetic infections in the region of buttock and thigh in some of the patients. The comparison of the planar differences, however, produced the evidence that on the second day of the treatment the limit of the statistical significance in the t-test was only slightly exceeded ($p = 0.082$).

The use of the adequate procedure of multiple analysis of
Assessment of the results related to the cases of herpes labialis

The subgroup pertaining to herpes labialis infection comprised a total of 67 patients (n = 67), 33 being allocated to the melissa group and 34 to the placebo group.

The statistical analysis applied to this subgroup was the same as applied to the entire group. Judged by the decline of the area of lesions, the healing in patients of the melissa group was faster than in those of the placebo group. And, even if those patients who had to undergo a therapeutical treatment within six hours from the appearance of the symptoms are singled out, the decline of the area of lesion until the termination of treatment was still significantly faster in the melissa group as distinguished from the placebo group (p = 0.012) (see Fig. 9), although the group experienced a reduction in size.

Side-effects and withdrawals

As regards the side-effects, there was no statistical difference in the two trial groups at any time of the two examinations. In the placebo group, irritation developed in 2 patients and 2 patients also complained of a sense of burning. In the melissa group, 1 patient complained of irritation at the time of the second follow-up examination. 1 patient of each of the melissa and placebo groups complained of irritation at the time of the second follow-up examination. Altogether, three withdrawals were registered: 2 patients from the melissa group and 1 patient from the placebo group. Of
the two patients of the melissa group, the patient no. 11 displayed an exacerbation of the symptoms and the patient no. 112 remained absent from the second follow-up examination without giving any reason whatsoever. The third case of withdrawal was registered in the placebo group: the patient concerned complained of a persistent itching that led to a premature termination of the treatment after 2 days.

**Discussion**

The manifestation of the primary syndrome rubor constitutes the cardinal characteristics of the acute phase of a herpes simplex infection of the skin. In the present study, the melissa cream revealed itself as being significantly and highly superior to the placebo during the very critical initial stage of the treatment. Also the decline of swelling on the second day of the treatment with melissa was significantly more pronounced than with the placebo. It is thereby to be taken into account that the duration of the clinical symptoms until the beginning of the treatment was longer in the melissa group by 4.5 hours on the average as distinguished from the placebo group. In other words, the melissa group was handicapped right from the beginning of the study. The score related to scabbing was higher in the placebo group than in the melissa group, though it was statistically not significant.

In the global assessment of efficacy, the melissa was judged as conclusively superior to the placebo by physician and patient alike. The evidence of superiority of the melissa is also reflected in the comparison of the differences of the mean areas of lesions between the day 0 and day 2. The results of the present study are more remarkable in that the studies carried out in the USA with idoxuridine (Nasemann and Braun Falco, 1970), acyclovir (Spruance, Schnipper, Overall et al., 1982; Fiddian, Yeo, Stubbings and Dean, 1983; Spruance, Crumpacker, Schnipper et al., 1984), and 5-ara-AMP (Spruance, Crumpacker, Haines et al., 1979) had revealed no benefits distinguishable from the placebo, except that in two subgroups the drug proved statistically superior to placebo with regard to the clinical symptoms. Only one clinical study, carried out in the UK (Fiddian, Yeo, Stubbings and Dean, 1983), brought about the evidence that acyclovir was superior to placebo. Moreover, it is worth remarking that in one of these studies (Spruance, Crumpacker, Schnipper et al., 1984) neither the decline of virus titre in the vesicular fluid due to the treatment with acyclovir 10% was significantly different from that due to placebo. The results of the study with melissa extract, in contrast to the above studies, provide the convincing argument that it is of value in the treatment of herpes simplex diseases.

As regards the side-effects, no allergic contract reactions were noticed. The minor untoward effects characterized by irritation were similar in both groups.

**Inference**

The effect of melissa cream in the topical treatment of herpes simplex infections of the skin and transitional mucosa is statistically significant. To be effective the treatment must be started in the very early stages of the infection. The achieved acceleration of healing particularly in the first two days of the treatment adds corroborative evidence to this phenomenon. A further merit of the melissa extract in topical use is displayed by its virtue of inducing no viral resistance.

**References**


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