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Systemic absorption of topically applied carvone: Influence of massage technique

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Synopsis

In the present study the percutaneous absorption of the cosmetically used fragrance compound (–)-(*R*)-carvone from a massage oil was investigated. The results showed that (–)-(*R*)-carvone easily penetrates the skin, exhibiting peak plasma concentrations after about 30 min. Dependent on the massage technique, blood levels and pharmacokinetic parameters significantly changed. While absorption rate constants exhibited no significant differences, the areas under the curve values and the elimination half-lives were significantly higher for the occlusion wrap administration compared to that for the normal massage (+60% and +222%, respectively). The irradiation technique however, failed to increase the penetration of (–)-(*R*)-carvone through the skin, and so the effect of cosmetic application is therefore doubted.

INTRODUCTION

The monoterpene (–)-(*R*)-carvone is found in various plants, but by far the highest concentration is in the oil of spearmint leaves (*Mentha spicata var. crispa*). Because of its characteristic spicy odor and taste, large amounts are used in cosmetic products (mouth washes, toothpastes) as well as in chewing gums. However, the enantiomer (+)-(*S*)-carvone, the main constituent in the essential oil of caraway, possesses a typical caraway aroma and is therefore mainly used in the food and fragrance industries. Due to its spasmolytic effect, the essential oil of spearmint leaves is used in many pharmaceutical formulations as a stomachic and carminative agent. In combination with other essential oils, it is applied as a massage treatment and used for baths and inhalations for nervous tension and several skin disorders (1,2). Moreover, this essential oil is also frequently used in aromatherapy massage treatments performed by cosmeticians in cosmetic institutes. In cosmetic treatment, massages have a long tradition and established their indispensable place in the natural therapy concept (3). In spite of its widespread use, no data about the absorption of (–)-(*R*)-carvone through intact human skin, resulting in measurable blood levels, have been described so far, although data from animal models and from *in vitro* techniques using cadaver skin exist (4–9), indicating a high rate of absorption for various essential oils. In previous *in vivo* experiments, we showed that

linalool and linalyl acetate, the main constituents of lavender oil, penetrate human skin, leading to detectable blood levels (10). Therefore, in continuation of our research activities about the biological fate of fragrance compounds (11), the aim of this study was to investigate the absorption of (–)-(*R*)-carvone from a massage, using three different massage techniques, and to quantify the concentration of this fragrant in the plasma after ending the cosmetic treatment.

MATERIALS AND METHODS

CHEMICALS

(–)-(*R*)-carvone (p-Mentha-6,8-dien-2-one; optical purity > 99%) and racemic piperitone (1-methyl-4-isopropyl-1-cylohexen-3-one) were obtained from Roth (Karlsruhe, Germany). Methanol (Lichrosolv®) was obtained from Merck (Darmstadt, Germany). Peanut oil was purchased from W. Pauli (Vienna) and sodium chloride infusion flask 100 ml (Na^+ 15.4 mmol, Cl^- 15.4 mmol and aqua ad injectionem ad 100 ml, pH = 5.0–7.0, 308 mosm/l) from Laevosan (Austria). All other chemicals and solvents were of analytical grade and used without further purification.

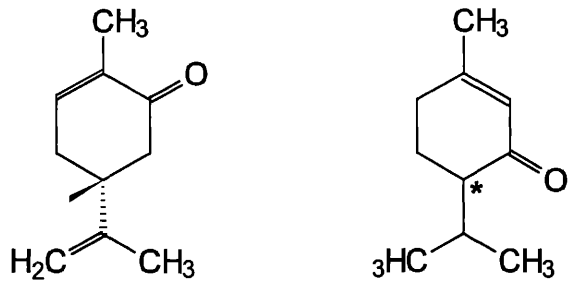

Figure 1. Structures of (–)-carvone and racemic piperitone. The chiral center is marked with an asterisk.

MATERIALS

Breathing masks (large adult face masks, cat. no. 01065, size 6, lot no. 1362H6 from King Systems Corp., Noblesville, Indiana) were obtained from B+P Beatmungsprodukte GmbH, Neunkirchen, Seelscheid, Germany). Isolute-C18 (EC) columns (100 mg) were purchased from International Sorbent Technology Ltd., Mid Glamorgan, UK. Lithium-heparine vacuum tubes (Vacutainer, 10 ml) were obtained from Becton Dickinson, Meyland Cedex, France, and the irradiation lamp Osram HX 16 (220 V, 60 W) was purchased from Osram fitted with a special filter (golden amber, transmission of 43% between the wavelengths of 620 and 660 nm; Osram, Vienna, Austria).

EXPERIMENTAL CONDITIONS

One and one-half gram of massage oil containing 20% (w:w) (–)-(*R*)-carvone in peanut

oil was spread on a defined skin area of the lower abdomen (376 cm^2) of a female subject (body weight 56 kg, height 165 cm, age 25 years, non-smoker). Starting a week prior to the massage, all food and cosmetics containing carvone were avoided. The subject put on a breathing mask before the massage to avoid a possible uptake of the fragrance compound by inhalation. To study the penetration of carvone, the following three different massage techniques were used. Each experiment was repeated three times.

Normal massage. For ten minutes the oil was gently massaged into the skin, and the remaining oil was completely removed. Blood samples (5.0 ml) were drawn from the left cubital vein 0, 5, 10, 15, 20, 25, 30, 40, 55, 70, 85, and 100 minutes after finishing the massage and collected in heparinized tubes. Blood samples were centrifuged at 4°C (4500 rpm/5 min) and the plasma separated and stored at 4°C until chromatographic and spectroscopic investigations. Between the blood drawings a physiological sodium chloride solution was injected into the veinflow to prevent clogging of the needle. As previous experiments showed that terpenes might accumulate in body fat, only one massage per week was applied to the female subject.

Massage with an occlusion wrap. The massage oil was gently massaged into the skin for one minute. Then a plastic film (0.5 × 1 m) was tightly wrapped around the body to cover the massaged skin area. Finally, after putting a hot poultice on the lower abdomen, the area was covered with a thick woollen blanket. After 20 minutes this occlusive wrap was removed and the remaining oil was washed off. Time intervals for blood drawing were the same as described above.

Irradiation massage. After spreading the massage oil for one minute over the above-defined skin area, the stomach was irradiated with an orange light for 20 minutes. Then the oily residue was removed and blood drawing was continued as described. Cosmeticians frequently apply this method in the treatment of wrinkles and for relaxation (12). Orange light is recommended to raise the skin temperature in order to increase the absorption of different compounds (e.g., terpenes), although no evidence for this specific application exists in the literature.

EXTRACTIONS

After adding piperitone as the internal standard (200 ng/ml) 2 ml of plasma was extracted by using solid-phase extraction cartridges, equilibrated with 3 ml of methanol and 3 ml of water. The cartridges were washed with 2 ml of water and (−)-carvone, and the internal standard was eluted with 0.5 ml of methanol (recovery: >94.3 ± 2.17%). Extracted plasma samples were immediately analyzed by gas chromatography (GC) and by gas chromatography coupled with mass spectroscopy (GC/MS). All steps of the sample preparations were done on ice or in a cold room to prevent loss of the volatile compounds.

GC-ANALYSIS OF CARVONE

An HRGC Mega 2 Series instrument with a flame ionization detector (FID) was used (Fisons Instruments S.p.A., Rodano, Italy). Separation of carvone from piperitone was performed on a 10 m × 0.25 mm Permabond SE-30-DF-0.25 fused silica column (Machery & Nagel, Germany), operated with an oven temperature of 65°C. The carrier

gas was nitrogen (75 kPa) and that for the FID was hydrogen (60 kPa; air: 80 kPa; make-up gas: 70 kPa). Samples were introduced onto the column by split injection (split ratio = 1:10) whereby the injector temperature was set at 260°C and the detector temperature at 220°C, respectively. The injection volume was 1.5 µl. The limit of quantitation was 5 ± 1.23 ng/ml (n = 5) of plasma by spiking drug-free human plasma with carvone to give a concentration of 5 ng/ml.

GC/MS ANALYSIS

For GC/MS analyses, a QP-1000EX-GC/MS-system (Shimadzu, Kyoto, Japan) was used. The GC-column was a 50 m × 0.25 mm i.d. capillary with a 0.25-mm film thickness (Machery & Nagel, Germany), operated at a temperature program of 60°C for two minutes, and then increased at 3°C/min to 250°C. The helium carrier gas flow rate was 1 ml/min. Samples were introduced onto the column by splitless injection (the split was opened 30 s after the injection; injection port temperature 250°C. The column effluent was introduced directly into the ion source, which was held at 180°C. Electron impact spectras were introduced at 50–600 amu/2 s, with ionization energy of 70 eV and with a vacuum pressure of 8.10^{-6} torr. Chemical ionization spectra were recorded with ammonia as the reactant gas (prepressure 1 bar, vacuum pressure 5.10^{-5} torr, ionization energy 200 eV, ion source 180°C).

KINETIC ANALYSIS OF CARVONE

The data sets were fitted via nonlinear iterative least square regression analysis. Curve modeling was performed using a two-compartment open pharmacokinetic model with the program MW-Pharm, Version 3.0 (Mediware, Groningen, The Netherlands), in which AUC represents the area under concentration-time curve; $t_{1/2\alpha}$, $t_{1/2\beta}$, the distribution and elimination half-lives, respectively; k_a, the absorption rate constant; t_{max}, the time to peak; and c_{max}, the peak concentration. Results were expressed as the mean ± standard deviation (SD). The significance of differences ($p < 0.05$) was evaluated by the Student's *t*-test.

RESULTS AND DISCUSSION

(–)-Carvone was quantified in plasma and urine samples by GC-FID well separated from the internal standard piperitone (t_R = 7.65 min and 8.21 min, respectively). To confirm the results of the GC-FID measurements, analyses were carried out by GC/MS with retention time and mass spectra comparison exhibiting identical main fragments for carvone (m/z at 82 amu, 93 amu, 108 amu, and 150 amu) and for piperitone (82 amu, 110 amu, 137 amu, and 152 amu), respectively.

Carvone quickly penetrated the skin of the female subject and could be detected in blood ten minutes after starting the massage, with peak plasma concentrations of 24–32 ng/ml. The resulting mean plasma levels of (–)-(*R*)-carvone are shown in Figure 2; the corresponding pharmacokinetic parameters from the fitted curves are summarized in Table I. Dependent on the massage technique, some pharmacokinetic parameters significantly changed. While the values for the absorption rate constant k_a were in a close

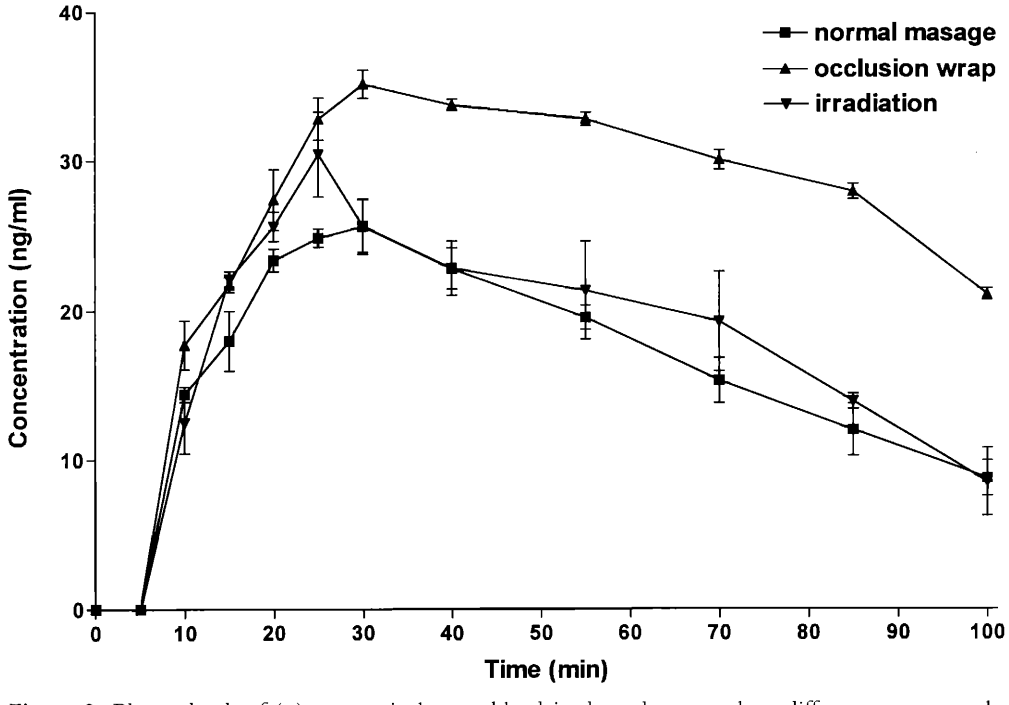

Figure 2. Plasma levels of (−)-carvone in human blood in dependence on three different massage techniques.

Table I
Pharmacokinetic Parameters for (−)-Carvone

Parameter	Normal massage	Occlusion wrap	Irradiation
C_{max} (ng)	23.92 ± 2.32	32.56 ± 3.35	28.31 ± 4.01
t_{max} (min)	25.81 ± 3.05	36.65 ± 4.87*	23.49 ± 4.35
k_a (1/h)	2.34 ± 0.53	2.23 ± 0.29	1.82 ± 0.22
$t_{1/2\alpha}$ (min)	7.80 ± 2.27	9.21 ± 1.96	6.69 ± 2.87
$t_{1/2\beta}$ (min)	33.49 ± 6.10	74.45 ± 15.62*	26.81 ± 4.93
AUC (ng/ml × min)	1713 ± 28	2746 ± 33*	1843 ± 113

Each value represents the mean ± SD of three massages.
* Significantly different from normal massage and irradiation technique ($p < 0.05$).

range from 1.82 to 2.34 h^{-1}, indicating the same half lives of absorption, the values for the time to reach the peak concentration t_{max} (23.49–36.65 min, respectively) and the blood levels from 30 to 100 min for the occlusion wrap administration, were significantly higher than those for normal massage and the irradiation technique. The distribution half-life $t_{1/2\alpha}$ was short (7.80–9.21 min). The elimination half life $t_{1/2\beta}$ was significantly longer (2.2–2.7 times) when an occlusion wrap was used.

In conclusion, (−)-(*R*)-carvone rapidly penetrates the skin of a human subject, resulting in measurable blood levels. Therefore, topical application of (−)-(*R*)-carvone should be carried out only under consideration of the high absorption rate. The irradiation method failed to increase topical absorption of (−)-(*R*)-carvone, and so the effect of cosmetic

application is doubted. To verify that the trends are truly an effect of the application technique, a larger study is planned.

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