Assay of Contact Photosensitivity to Musk Ambrette in Guinea Pigs

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This study reports the induction of contact photodermatitis to musk ambrette, 2-methoxy-3,5-dinitro-4-methyl-t-butylbenzene, in guinea pigs. Photoallergic contact dermatitis was assayed using 2 alternative induction methods. Successful photosensitization was achieved only when the nuchal skin was stripped with scotch tape before application of musk ambrette and ultraviolet radiation. Induction methods utilizing non-stripped nuchal skin which induce photosensitivity to potent photoallergens were ineffective for musk ambrette.

Photoxicity tests to musk ambrette at concentrations between 1 and 50% and a dose of 10.2 joules/cm² from "Black Light" fluorescent tubes were all negative. Under identical irradiation conditions, anthracene at 0.9% and 8-methoxypsoralen at 1% were consistently positive.

The mechanism of photosensitivity to musk ambrette appears to be photoallergic rather than phototoxic. The requirement for skin abrasion to induce photosensitization parallels the clinical reports of photosensitivity to musk ambrette in man.

Contact photodermatitis has been reported to relatively few materials considering the thousands of compounds daily met by the skin in its role as an interface between the body and the environment [1,2]. Recently, 5 cases of contact photoallergic dermatitis to an after shave lotion were described by Raugi and Storrs [3], and Larson [4], and Taylor (Taylor J: personal communication) [7-10]. The process in laboratory animals has features consistent with a delayed hypersensitivity immunologic response. Contact photosensitivity of the photoxic type has also been induced in guinea pigs (Brodhagen H: personal communication) [7-10]. The process in laboratory animals has features consistent with a delayed hypersensitivity immunologic response. Contact photosensitivity of the photoxic type has also been induced in guinea pigs and man with many compounds including anthracene [11] and 8-methoxypsoralen [12].

The purposes of this study were: (a) to determine whether or not the photosensitivity described in man to musk ambrette could be induced in guinea pigs, and (b) if so, whether musk ambrette was a photoxic or a photoallergic agent. Previously established methods for the induction of contact photosensitivity as well as a new type of guinea pig sensitization method mimicking the "maximization" test in man were employed. A standard test for phototoxicity was utilized.

MATERIALS AND METHODS

Experimental Animals:

Hartley strain female albino guinea pigs, weighing 375 to 488 gm were used as test animals. Musk ambrette, 2-methoxy-3,5-dinitro-4-methyl-t-butylbenzene, (Figure 1) was used as a test agent (Lot #2885.78 Givaudan Corp., Clifton, N.J.). Its purity was assayed by high pressure liquid chromatography and thin-layer chromatography. Detection at 290, 300 and 360 nm indicated that impurities were less than 0.2%. 8-methoxypsoralen was obtained from Elder Chemical Co. Solvents were reagent grade. Nair (Carter-Wallace Inc.) was used as the depilating agent. Anthracene was obtained from Matheson Coleman and Bell.

Light Sources

"Sunlamp" fluorescent tubes (Westinghouse FS-40) emitting radiation predominantly in the 285-350 nm range were used. The fluence rate at 25 cm was 3.75 mw/cm². The dose used for induction gave brisk erythema in guinea pigs. "Black Light" fluorescent tubes (General Electric), emitting radiation in the 320-400 nm range were employed. The fluence rate measured at 12.5 cm through 3mm of window glass was 2.85 mw/cm². All "Black Light" irradiations used this glass filter. The dose used for induction and elicitation (10.2 joules/cm²) is non-erythrogenic without sensitizer.

Ultraviolet Absorption Spectra

The absorption spectra of musk ambrette and tetrachlorosalicylanilide were determined in ethanol using an Aminco DW-2 UV/VIS spectrophotometer.

Induction of Sensitization

Nonstripped Nuchal Skin: The nuchal area (2.5 cm x 2.5 cm) of 20 guinea pigs was either shaved and 0.1 ml of 5% musk ambrette in acetone applied or shaved and depilated with Nair (R) and 10% musk ambrette applied. After 30 min the nuchal area was irradiated at 25 cm with the "Sunlamp" fluorescent light source (dose = 6.6 joules/cm²) and subsequently at 12.5 cm with light from the "Black Light" fluorescent tubes (dose = 10.2 joules/cm²). Four treatments were given during a 9-day period.

Stripped Nuchal Skin: The nuchal area of 37 guinea pigs was shaved, depilated with Nair (R) and "stripped" with cellophane tape for 3 or 4 of the 5 treatments. Musk ambrette (0.1 ml of a 10% solution in acetone) was applied within 15 min after stripping. The animals were restrained on Eberbach restraining boards and irradiated with the same doses of light as the nonstripped animals. The sensitization treatment was performed 5 times during a period of 10 or 11 days.

Tests for Elicitation of Contact Photosensitivity to Musk Ambrette

Seventeen to 22 days following the last sensitization exposure the guinea pigs were challenged. The shaved and depilated lumbar area which had received no previous exposure to musk ambrette or light was demarcated into 4 or 6 sites (2.5 cm x 3.5 cm) with masking tape. The animals previously exposed to 5% musk ambrette in their nuchal region were challenged with 5% and 2.5% concentrations in ethanol on their dorsal lumbar skin. The guinea pigs in which the sensitization technique involved 10% musk ambrette were challenged with 5% and 7% solutions. Each concentration (0.1 ml) was applied to symmetrical sites on the left and right sides of the animal. The right side was shielded with light-opaque material. A nonerythrogenic dose (10.2...
Musk ambrette, 2-methoxy-3,5-dinitro-4-methyl-t-butylbenzene. A synthetic compound mimicking the odor of musk obtained from the preputial gland of the Tibetan musk deer.

joules/cm²) from “Black Light” fluorescent tubes was administered 30 min after application of the challenge solutions. Evaluation of Test Results: Erythema on the test sites was evaluated at 24 and 48 hr following irradiation using a scoring system ranging from 0 to 4. A score of one or higher was considered a positive response. Control Studies for Photoallergy: Photoallergy to 3,3',4',5-Tetra-chlorosalicylanilide (TCSA): Eleven guinea pigs received 6 sensitization treatments to their nonstripped nuchal area and 4 animals received 5 sensitization treatments on stripped nuchal skin. TCSA was applied to the nuchal site in a 3% acetone solution and the irradiations were performed as for the musk ambrette sensitization.

Control Studies for Phototoxicity: Phototoxicity to Musk Ambrette, Anthracene and 8-Methoxypsoralen: The dorsal lumbar area of 20 guinea pigs with no previous nuchal sensitization to musk ambrette was shaved and depilated. Musk ambrette solutions (0.1 ml) were applied to 2.5 cm × 3.5 cm areas of lumbar skin at 60%, 20%, 10%, 5%, 3% and 1% concentrations acetone. The right side of each animal was shielded from light. Thirty minutes after the test materials were applied, the animals were exposed to nonerythrogenic radiation (10.2 joules/cm²) from the “Black Light” fluorescent tubes. In 12 of the animals tested for phototoxicity to musk ambrette, anthracene (0.9% in ethanol) or 8-methoxypsoralen (1% in ethanol) was applied to the most caudal site on the right side and the irradiation performed as described above. An additional 5 animals treated similarly with musk ambrette received irradiation from the “Sunlight” fluorescent tubes (0.9 joules/cm²).

RESULTS
Contact Photoallergy to Musk Ambrette

Photosensitization to musk ambrette using a conventional induction technique was unsuccessful. None of the 20 animals in which sensitization was attempted on unstripped nuchal skin responded to challenge doses of musk ambrette and ultraviolet light (Table I). In contrast, stripping of the nuchal skin as part of the induction process produced a significant number of positive responses to musk ambrette and ultraviolet light (Table I). None of the animals responded to the musk ambrette in the absence of radiation.

Phototoxicity to Musk Ambrette

None of the 25 animals tested with musk ambrette (1 to 50% in acetone) and ultraviolet light (UVA or UVB) showed erythema at 24 hr. Under the same conditions anthracene and 8-methoxypsoralen were phototoxic (Table II).

<table>
<thead>
<tr>
<th>Test material</th>
<th>Concentration</th>
<th>Erythema with UVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Musk ambrette</td>
<td>50%</td>
<td>0/9</td>
</tr>
<tr>
<td></td>
<td>20%</td>
<td>0/9</td>
</tr>
<tr>
<td></td>
<td>10%</td>
<td>0/16</td>
</tr>
<tr>
<td>Anthracene</td>
<td>5%</td>
<td>0/9</td>
</tr>
<tr>
<td></td>
<td>3%</td>
<td>0/9</td>
</tr>
<tr>
<td></td>
<td>1%</td>
<td>0/2</td>
</tr>
<tr>
<td>8-methoxypsoralen</td>
<td>0.9%</td>
<td>12/12</td>
</tr>
<tr>
<td></td>
<td>1.0%</td>
<td>12/12</td>
</tr>
</tbody>
</table>

Control Photoallergy Assay to 3,3',4',5-Tetra-chlorosalicylanilide

Eight of the 11 guinea pigs receiving induction treatments to nonstripped nuchal skin, had positive responses upon challenge (Table III). Animals receiving induction treatments on stripped nuchal skin were also strongly positive upon challenge.

DISCUSSION

Musk is a reddish brown secretion obtained from the Asian musk deer [13]. It has a strong odor, reputed to function as a sexual attractant among musk deer. Muscone, the major odoriferous agent, is found in a gland located in the abdominal skin of the male deer. This secretion was formerly used as a scent in perfumes and as a fixative. Because of its extreme rarity and high cost, the natural muscone has been replaced by the plant oil obtained from the seed of ambrette (Hibiscus abelmoschus) as well as by synthetic agents.

The experimental data concerning contact photosensitivity to musk ambrette in guinea pigs confirms the recent clinical reports in man. These reports are exceedingly sparse in view of the fact that more than 100,000 pounds of musk ambrette are consumed annually [14]. However, it is equally true that only relatively few centers have done photopatch tests with this material. In addition, there may be other reasons why musk ambrette photosensitivity has not been noted until recently, such as change in the perfuming habits of men. In the first case reported by Rauji and Storrs [3] and Larsen [4], photosensitivity occurred following the use of an after shave lotion. Additional cases confirmed that the contact photosen-
sitivity occurred in men following their use of an after shave cologne. All reported cases to date have occurred on the face of men. These clinical findings suggested that facial shaving might be a factor contributing to photosensitization.

This view was strengthened by our findings in guinea pigs which showed no evidence of contact photosensitivity to musk ambrette when the attempts to induce photosensitization were performed on nuchal skin treated in the conventional manner. However, after “stripping” with cellophane tape, contact photosensitivity was induced in relatively high numbers. This induction procedure is very similar to that used by Willis and Kligman [6] in man in which the skin is stripped to the glistening layer prior to the first exposure to chemical and combined UVA and UVB radiation. In man, the exposure, but not the stripping is repeated at 48 hr intervals for a total of 5 exposures. In this study the skin was stripped before each exposure until it became too crusted. Five exposures were administered over 10 or 11 days. Whether the increased incidence of photosensitization was due to trauma or to increased percutaneous absorption as suggested by the sodium lauryl sulfate studies of Horio [15] remains to be established. In either case, the abrasive effects of shaving may be considered as mimicking a maximization procedure in man.

The purity of the sample of musk ambrette used in these studies was determined by chromatographic analyses which suggest that if contaminants were present, their concentrations were exceedingly low. It is therefore felt that the data in the guinea pig demonstrates contact photosensitivity to musk ambrette per se. However, in this animal model the index of photosensitization of musk ambrette appears to be much lower than that of any of the halogenated salicylanilides. Photosensitization to musk ambrette required stripping of the skin whereas a greater number of positive responses were observed to tetrachlorosalicylanilide without stripping.

In consonance with contact photoallergy to other compounds, the action spectrum for elicitation of a response to musk ambrette in both guinea pigs and man can be measured in the UVA range (320–400 nm). Absorption spectra data indicate that musk ambrette absorbs much less radiation than tetrachlorosalicylanilide in the range (320–400 nm) used to elicit the photoallergic response. The difference in sensitizing potential may be partially due to this fact along with numerous other factors such as variations in percutaneous absorption and genetic potential to respond. That musk ambrette undergoes photochemical changes has been well known since 1969 when Arctander reported color changes induced in the molecule when it was exposed to ordinary daylight [16].

In summary, these studies in guinea pigs have shown that musk ambrette is a contact photosensitizer when applied to cellophane stripped skin. The mechanism of photosensitivity appears to be photoallergic rather than phototoxic.

REFERENCES

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16. Arctander S: Perfume and Flavour Chemicals (aroma chemicals) II. Published by the author, Montclair, N.J. 1969