

Photoepicutaneous testing

Influence of the vehicle, occlusion time and concentration of the test substances on the results

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The effects of various vehicles, the occlusion time and the concentration of the test substances on the phototoxic reactions were studied using the Finn Chamber method in photoepicutaneous testing, with methoxsalen and coal tar as test substances. Petrolatum proved to be a suitable base for methoxsalen and carbowax for coal tar. The optimal concentration of coal tar was 5 % and that of methoxsalen 0.03-0.05 %. The optimal occlusion time for methoxsalen was 1-2 hours and for coal tar 24 hours. It was concluded that in order to obtain the most reliable results, these parameters should be determined separately for every photosensitizing compound to be tested.

Key words: Occlusion time - skin tests, photoepicutaneous - testing concentration - vehicles.

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There are many variables which influence the results of the photoepicutaneous tests, such as the vehicle, the occlusion time and the concentration of the test substances. Kaidbey & Kligman (1974) appear to be the only authors who have studied the influence of various vehicles on the test results. The recommended occlusion time for photoepicutaneous testing with different photosensitizers varies from "immediate" to 48 hours (Epstein 1972; Epstein 1964, 1966; Kligman & Reinhard 1968; von Meffert 1974; Osmundsen 1969; Willis & Kligman 1969). The correct concentrations of photosensitizing substances vary according to the author too (Cripps & Enta 1970; Epstein 1964, 1966; Harber et al. 1968; Kligman & Reinhard 1968; Stott et al. 1970; Willis & Kligman 1969).

The aim of this work was to study these parameters using coal tar and methoxsalen as test substances.

Material and Methods

Methoxsalen and coal tar were used as phototoxic test substances.

Five different vehicles were chosen for comparison: hand cream¹, foundation cream², petrolatum, wool fat, and carbowax (Ung. Macrogoli(DF) containing Macrogolum 3000 40 % and Macrogolum 400 60 %). The concentrations of photosensitizers in this part of the study (vehicles) were as follows: methoxsalen 0.03% and coal tar 5 %. In the second part of the study, in which the influence of the occlu-

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|----------------------|----|------------|--------|
| 1. Hand cream: | | | |
| cetylalcohol | 10 | non-ionic | |
| glycerol | 8 | emulsifier | 15 |
| | | water | ad 100 |
| 2. Foundation cream: | | | |
| petrolatum | 8 | non-ionic | |
| Macrogol 400 | 20 | emulsifier | 10 |
| Macrogol 1000 | 24 | water | ad 100 |

sion time was investigated, the different occlusion times were ½ h, 1 h, 2 h and 24 h. The concentrations of the test substances and the vehicles were the same as those used in the first part of the study.

In the third part of the work the effect of various concentrations of the test substances was studied using methoxsalen 0.0005 %, 0.001 %, 0.005 %, 0.001 % and 0.05 %, and coal tar 0.1 %, 0.5 %, 1 %, 5 % and 10 % in petrolatum and carbowax. The occlusion time for methoxsalen was 2 hours and for coal tar 24 hours, chosen according to the results of the second part of the study.

The Finn Chamber method was used for epicutaneous testing (Pirilä 1975, Suhonen et al. 1976). No filter papers were used in the chambers in the present study. The proper amount of test substance (20 µl) was measured with a Mantoux syringe. The dosage of the test substance was controlled by weighing 20 µl doses of petrolatum. In 20 consecutive measurements the mean weight of 20 µl petrolatum was 17.2 mg, with a range of 15.8–19.1 mg. In the first

part of the study the tests were made in duplicate – one for irradiation and one for dark control. For the other parts of this study the split-control method was used (Suhonen et al. 1976).

After the chambers were removed, the skin was cleaned by being pressed several times with blotting paper. No chemicals were used to remove remnants of test substances in order to avoid any nonspecific irritation of the skin.

Immediately after the cleaning procedure the skin was irradiated with an apparatus consisting of six Philips TL 20W/08 black light tubes. The irradiation time was 5 min at a distance of 5 m, giving a total dose of approximately 170 mJ/cm² UVA, with an emission peak at 365 nm. During irradiation the control area was covered with black adhesive tape.

After irradiation the test area was covered with light-proof plastic carbon paper fixed on the skin with porous adhesive tape (Scanpor®, Norgesplaster A/S, Oslo). The results were evaluated in all tests at 24, 48

Table 1. Mean reaction indices with 5 % coal tar in different vehicles. The occlusion time was 24 hours. The number of tests subjects was 48

Vehicle	Hours after irradiation					
	24		48		72	
	test	dark control	test	dark control	test	dark control
hand cream	1.0*	0.2	0.8	0.1	0.7	0.1
foundation cream	1.4	0.2	1.3	0.1	1.1	0.1
petrolatum	0.8	0.1	0.7	0.0	0.5	0.0
wool fat	0.4	0.1	0.3	0.1	0.2	0.0
carbowax	1.3	0.1	1.1	0.0	1.0	0.0

* 0 = no reaction

1 = erythema

2 = erythema with moderate oedema

3 = erythema with beginning vesiculation

4 = uniform bulla

Table 2. The number of positive reactions with 5% coal tar in different vehicles. The occlusion time was 24 hours. The number of test subjects was 48

Vehicle	Hours after irradiation					
	24		48		72	
	test	dark control	test	dark control	test	dark control
hand cream	36	6	32	3	28	3
foundation cream	44	6	40	4	35	2
petrolatum	27	3	27	2	22	2
wool fat	17	2	13	2	10	2
carbowax	41	3	39	2	32	2

and 72 hours after irradiation using a 0-4 scale, where 0 means no reaction and 4 a uniform bulla.

The subjects were volunteers, mostly dermatologic patients without any suspicion of abnormal sensitivity to light and without any photosensitizing medication.

Results

1. Vehicles

Marked differences in the reactions were found when using different vehicles. For coal tar phototesting, foundation cream and carbowax were the most reliable vehicles

Table 3. Mean reaction indices with different occlusion times using methoxsalen 0.03 % as the test substance

Vehicle	hours after irradiation	Occlusion time (h)				dark control
		1/2	1	2	24	
hand cream	24	0.6*	0.9	0.7	0.4	0.0
	48	1.5	1.8	1.9	1.4	0.0
	72	1.9	2.2	2.4	1.6	0.0
foundation cream	24	0.7	0.9	0.7	0.4	0.0
	48	1.6	1.9	1.9	1.0	0.0
	72	2.0	2.3	2.3	1.4	0.0
petrolatum	24	0.5	0.8	0.8	0.1	0.0
	48	1.1	1.7	1.9	0.4	0.0
	72	1.5	2.1	2.3	0.8	0.0
wool fat	24	0.1	0.1	0.1	0.1	0.0
	48	0.2	0.5	0.4	0.2	0.0
	72	0.2	0.7	0.5	0.6	0.0
carbowax	24	0.0	0.0	0.1	0.1	0.0
	48	0.0	0.0	0.0	0.1	0.0
	72	0.0	0.1	0.0	0.0	0.0

* 0 = no reaction

1 = erythema

2 = erythema with moderate oedema

3 = erythema with beginning vesiculation

4 = uniform bulla

The number of test subjects was 30.

Table 4. The number of positive reactions after different occlusion times using 0.03 % methoxsalen as the test substance

Vehicle	hours after irradiation	Occlusion time (h)				dark control
		1/2	1	2	24	
hand cream	24	14	21	18	10	0
	48	23	27	28	22	0
	72	24	26	29	20	0
foundation cream	24	15	20	18	8	0
	48	27	29	28	17	0
	72	27	29	27	22	0
petrolatum	24	12	16	16	3	0
	48	18	24	28	7	0
	72	22	27	29	15	0
wool fat	24	1	3	3	2	0
	48	3	12	9	3	0
	72	4	12	11	8	0
carbowax	24	0	0	2	1	0
	48	0	0	0	1	0
	72	0	0	0	0	0

The number of test subjects was 30.

Table 5. Mean reaction indices with 5 % coal tar after different occlusion times

Vehicle	hours after irradiation	Occlusion time (h)				dark control
		1/2	1	2	24	
hand cream	24	0.0*	0.3	0.3	1.0	0.1
	48	0.0	0.3	0.4	0.7	0.0
	72	0.0	0.2	0.2	0.7	0.0
foundation cream	24	0.2	0.5	0.7	1.4	0.1
	48	0.1	0.5	0.7	1.3	0.0
	72	0.1	0.5	0.6	1.2	0.0
petrolatum	24	0.1	0.2	0.4	0.8	0.0
	48	0.1	0.2	0.5	0.7	0.0
	72	0.1	0.1	0.3	0.6	0.0
wool fat	24	0.0	0.1	0.1	0.3	0.1
	48	0.0	0.0	0.0	0.2	0.0
	72	0.0	0.0	0.0	0.1	0.0
carbowax	24	0.0	0.0	0.0	1.2	0.0
	48	0.0	0.0	0.0	1.0	0.0
	72	0.0	0.0	0.0	1.0	0.0

* 0 = no reaction

1 = erythema

2 = erythema with moderate oedema

3 = erythema with beginning vesiculation

4 = uniform bulla

The number of test subjects was 25.

Table 6. The number of positive reactions with 5% coal tar after different occlusion time

Vehicle	hours after irradiation	Occlusion time (h)				dark control
		1/2	1	2	24	
hand cream	24	0	6	7	18	3
	48	0	6	8	15	1
	72	0	4	3	15	1
foundation cream	24	3	11	15	23	3
	48	2	11	14	21	1
	72	2	11	11	19	1
petrolatum	24	2	5	8	14	1
	48	2	5	10	14	1
	72	1	3	7	13	1
wool fat	24	0	3	3	6	2
	48	0	1	1	4	1
	72	0	0	0	3	1
carbowax	24	0	1	5	20	1
	48	0	0	1	20	1
	72	0	0	4	17	1

The number of test subjects was 25.

and the results were nearly equal (Table 1). The reactions with carbowax as the vehicle were better demarcated and tended to last longer than those with foundation cream. The reactions were strongest at 24 hours. Hand cream and petrolatum gave satisfactory results, but wool fat gave only weak and inconsistent responses. The reactions in non-irradiated test areas were few and weak with all vehicles (Table 1). The number of positive reactions with coal tar in foundation cream was 44 out of 48 subjects tested (92%) and that with carbowax 41 out of 48 (85%) (Table 2).

For methoxsalen the best vehicles were found to be petrolatum, foundation cream and hand cream, with insignificant differences in the results. Wool fat as the vehicle was weak and carbowax still weaker. No reactions were seen in the control areas (Table 3). The number of positive reactions was 29 out of 30 cases tested for both petrolatum and foundation cream (Table 4).

2. Occlusion time

Tests with graded occlusion times showed

that occlusion for 2 hours was suitable for methoxsalen (Tables 3, 4) and 24 hours for coal tar (Tables 5, 6). The results were uniform with all vehicles.

3. Concentration

As a whole, the reaction indices were greater with increasing concentration (Tables 7, 9). Coal tar (5%) in carbowax was found to be the optimal concentration. The increase of the concentration to 10% did not increase the number of positive reactions (Table 8).

Methoxsalen 0.05% failed to elicit a reaction in only one of 30 cases. With lower concentrations the incidence of positive reactions markedly decreased.

Discussion

In addition to the properties of the photosensitizing substance itself, those of the vehicle also play an important role in a photosensitivity reaction. The final result may depend, for example, on the chemical and physical properties of the vehicle, its water content, its ability to change the bar-

Table 7. Mean reaction indices using coal tar in petrolatum and carbowax at given concentrations

Concentration of test substances		Hours after irradiation					
		24		48		72	
		test	dark control	test	dark control	test	dark control
petrolatum	0.1 %	0.1	0.0	0.0	0.0	0.1	0.0
	0.5 %	0.1	0.0	0.1	0.0	0.0	0.0
	1.0 %	0.3	0.0	0.1	0.0	0.1	0.0
	5.0 %	0.7	0.0	0.7	0.0	0.5	0.0
	10.0 %	1.2	0.0	1.0	0.0	0.7	0.0
carbowax	0.1 %	0.1	0.0	0.0	0.0	0.0	0.0
	0.5 %	0.3	0.0	0.1	0.0	0.1	0.0
	1.0 %	0.5	0.0	0.3	0.0	0.2	0.0
	5.0 %	1.2	0.0	1.1	0.0	0.8	0.0
	10.0 %	1.2	0.0	1.3	0.0	1.1	0.0

The number of test subjects was 30.

The occlusion time was 24 hours.

rier effect of the epidermis, its possible interaction with the photosensitizing compound and, in some instances, its own sensitizing properties (Hannuksela et al. 1976). This is why we are in principle against the use of vehicles containing emulsifiers for photoepicutaneous testing.

This study shows that the results of photoepicutaneous tests are highly dependent on the vehicle, but also on the occlusion time and the concentration of the test substances. With coal tar we obtained good results using carbowax (polyethyleneglycols 400/3000) as the vehicle with an occlusion time of 24

Table 8. The number of positive reactions using coal tar in petrolatum and carbowax

Concentration of test substances		Hours after irradiation					
		24		48		72	
		test	dark control	test	dark control	test	dark control
petrolatum	0.1 %	2	1	1	0	2	0
	0.5 %	4	1	2	0	1	0
	1.0 %	9	1	4	0	3	0
	5.0 %	20	1	17	0	21	0
	10.0 %	16	1	21	0	18	0
carbowax	0.1 %	4	1	1	0	1	0
	0.5 %	10	1	3	0	3	0
	1.0 %	16	0	8	0	5	0
	5.0 %	28	1	22	0	17	0
	10.0 %	26	0	25	0	22	0

The number of test subjects was 30.

The occlusion time was 24 hours.

Table 9. Mean reaction indices using methoxsalen in petrolatum and carbowax

Concentration of test substances		Hours after irradiation					
		24		48		72	
		test	dark control	test	dark control	test	dark control
petrolatum	0.0005 %	0.0*	0.0	0.0	0.0	0.1	0.0
	0.001 %	0.0	0.0	0.2	0.0	0.2	0.0
	0.005 %	0.1	0.0	0.5	0.0	0.7	0.0
	0.01 %	0.4	0.0	1.0	0.0	1.1	0.0
	0.05 %	0.5	0.0	1.4	0.0	1.9	0.0
carbowax	0.0005 %	0.0	0.0	0.0	0.0	0.0	0.0
	0.001 %	0.0	0.0	0.0	0.0	0.0	0.0
	0.005 %	0.0	0.0	0.0	0.0	0.0	0.0
	0.01 %	0.0	0.0	0.0	0.0	0.0	0.0
	0.05 %	0.0	0.0	0.1	0.0	0.1	0.0

* 0 = no reaction

1 = erythema

2 = erythema with moderate oedema

3 = erythema with beginning vesiculation

4 = uniform bulla

The number of test subjects was 30.

The occlusion time was 2 hours.

Table 10. The number of positive reactions using methoxsalen in petrolatum and carbowax in different concentrations

Concentration of test substances		Hours after irradiation					
		24		48		72	
		test	dark control	test	dark control	test	dark control
petrolatum	0.0005 %	0	0	0	0	3	0
	0.001 %	0	0	6	0	7	0
	0.005 %	4	0	13	0	17	0
	0.01 %	10	0	22	0	23	0
	0.05 %	13	0	27	0	29	0
carbowax	0.0005 %	0	0	0	0	0	0
	0.001 %	0	0	0	0	0	0
	0.005 %	0	0	0	0	0	0
	0.01 %	0	0	0	0	0	0
	0.05 %	0	0	3	0	3	0

The number of test subjects was 30.

The occlusion time was 2 hours.

hours. However, if the occlusion time was limited to 2 hours, the reactions were rather weak and inconsistent. On the other hand, coal tar in foundation cream and hand cream showed earlier responses.

In contrast to coal tar, methoxsalen in carbowax did not show phototoxicity at all, regardless of the occlusion time and the concentration of methoxsalen. Hand cream, foundation cream and petrolatum were good vehicles for methoxsalen in respect of both the strength and reproducibility of the reactions. With these vehicles the importance of proper concentrations of test substances was obvious: some subjects elicited strong, even bullous reactions in higher test concentrations. On the other hand, a lowering of the concentration tended to make the reactions weaker and inconsistent (Tables 9, 10). We believe that the differences between the phototest results to methoxsalen and coal tar principally depend on:

1. The different times of exposure for methoxsalen and coal tar before irradiation. Coal tar has more time to accumulate in the epidermis than methoxsalen, 24 hours as opposed to 2 hours. The well-known fact that methoxsalen loses its phototoxicity with longer occlusion times makes the use of a longer application time impossible. This is probably due to the rapid metabolism of methoxsalen (Pathak et al. 1974).

2. The solubility of the active substance in the vehicles. Methoxsalen did not show reactions when incorporated in carbowax, in which methoxsalen is soluble. However, the reactions were strong using, for example, foundation cream, which in addition to carbowax contains 38% water, in which methoxsalen is practically insoluble. Studies to explain this phenomenon are in progress.

This work provides insight for further studies of the physico-chemical features of the vehicles, which play a role in the libe-

ration of active substances from the base. In addition to photoepicutaneous testing, the information thus obtained might find applications in epicutaneous testing in general and in planning preparations for topical treatment.

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