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Topical immunotherapy with contact sensitizers: a model to study the natural history of delayed hypersensitivity

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So-called 'topical immunotherapy' (TI) is a well-established treatment modality based on the use of potent contact sensitizers, such as squaric acid dibutylester (SADBE), that are not found in the general or occupational environment of man, and do not induce cross-sensitization to other substances. Thus, unlike patients with sensitivity to other more or less commonplace allergens, subjects submitted to this treatment modality might provide useful data for the study of the natural course of contact sensitivity. On the basis of these premises, we have retrospectively reviewed 40 patients who had consecutively received TI with SADBE for severe alopecia areata and viral warts in the period 1993–2001, and returned to visit after a 1–7-year period since the last therapeutic challenge with the sensitizer. The chronological profile of loss of contact sensitization of this case cohort has been evaluated and compared to that of other series available in the literature. Provided more standardized TI application procedures are established, aim-oriented studies of the outcome of this treatment modality may bring useful data to be employed in construction of predictive models of decay of contact sensitization under conditions of antigen avoidance.

Key words: allergic contact dermatitis; antigen avoidance; hardening; loss of contact sensitivity; squaric acid dibutylester; topical immunotherapy. ©Blackwell Munksgaard, 2002. © Blackwell Munksgaard, 2002.

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Topical immunotherapy (TI) with contact sensitizers has long been part of routine management of more severe, recalcitrant alopecia areata and warts. This treatment modality implies first a piloted induction of delayed hypersensitivity with high concentrations (2%, on average) of various agents (2,4-dinitrochlorobenzene, DNCB; squaric acid dibutylester, SADBE; and, diphenylcyclopropenone, DPC) followed by weekly applications of varied dilutions of the allergen, adjusted to the patients' reactivity in order to maintain a mild (neither blistering nor oozing) eczematous reaction at lesional level (1, 2).

Of note, TI is performed with strong sensitizers that are not found in the general or technical environment of man, and do not induce cross-sensitization to other substances. Owing to this, unlike patients with sensitivity to nickel and other commonplace allergens, subjects submitted to this treatment modality may provide a useful model for the study of the natural course of contact sensitivity.

In the light of these premises, a retrospective study of a series of patients sensitized with SADBE for therapeutic purposes was performed.

Patients and Methods

All records of patients ($N = 82$) subsequently referred to the departments of Dermatology of Bari and Foggia in the period 1993–2001 for treatment of alopecia areata (AA) and viral warts (VW) with TI were preliminary reviewed.

In all cases the contact sensitizer utilized was SADBE. The agent was synthetized via 'Dean–Stark' esterification of squaric acid. The preparation was then added with 4-Å molecular sieves maximizing stability of the solutions. Moreover, in the light of the well-known intrinsic instability of SADBE solutions, effects of any unpredictable changes in the preparation were further minimized by keeping solutions refrigerated (+4°C) when not in use, and by newly synthesizing the ester every 3 months.

Sensitization had been obtained by applying 0.1 mL of 2% SADBE solution in acetone, kept under occlusion for 48 h on the lateral aspect of the arm. Local flare-ups, confirming development of sensitization, were awaited for 2–3 weeks. Varied dilutions of the agent had subsequently been applied weekly at lesional level, ranging from 0.000001 and 1% in patients with AA, and from 0.01 and 1% in patients with VW.

Case histories of patients returning to visit for (1) disease recurrence after initial resolution, or (2) having dropped out of TI because of side-effects or personal reasons, were then reviewed with the aim of surveying cases in which new skin challenges with SADBE had again been necessary for management of the condition. Of these cases, the following data were collected: (1) time elapsed from disease resolution (corresponding to last skin challenge with SADBE); (2) outcome of attempts to rekindle skin sensitivity to SADBE. In more detail, in each case (via an open application test) restitution of eczematous reactions at lesional level was first attempted with the last concentration able to elicit a significant response during the initial therapy course; in cases in which no reaction was observed, concentrations of the agent were weekly raised as high as 1%. In the absence of responsiveness to this peak concentration, patients were considered to have lost sensitivity.

Data analysis was carried out as follows. Non-parametric tests were used to evaluate differences between continuous variables; χ^2 was used to show differences between categorical variables; χ^2 for trend was performed to assess a temporal trend in yearly percentages of patients having lost their sensitivity during the observation period. *P*-values less than 0.05 were considered significant.

Results

During the 9-year period surveyed by the present study, 40 out of 82 (48.8%) patients returned to visit for disease recurrence. Each had been successfully treated with SADBE for AA ($N = 27$) or VW ($N = 23$) after 3–18 months of therapy.

The profile of this series is summarized in Table 1. Overall, 23 out of these 40 subjects (57.5%) were judged to have lost their sensitivity (according to criteria described above) after a 1–7-year period. Moreover, when relative contribution of cases of ascertained desensitization was stratified by time, statistically significant differences were found between proportion of cases per year and time elapsed from the last therapeutic contact with SADBE (χ^2 for trend 19.99; $p < 0.001$). Of note, patients having ceased contact for 6–7 years contributed more than 50% to the total amount of desensitizations observed, while none of the subjects returning to visit during the last year failed to have eczematous reactions elicited by concentrations of no more than 1% of the agent.

Moreover, in the 17 patients (42.5%) with positive open application tests, variable degrees of acquired tolerance were observed. In fact, in all instances restitution of skin reactivity was obtained with concentrations that were 10–10000 times those applied during the preceding therapy course.

As for the conditions prompting TI administration, the proportion of patients having lost sensitivity was 59.3 and 46.6 among AA and VW cases, respectively, a difference that did not reach statistical significance. No other clinical or demographical variables (sex, age, or duration of treatment) was consistently associated with occurrence of loss of sensitivity.

Discussion

This study has retrospectively shown the chronological profile of loss of contact hypersensitivity in a group of patients previously sensitized with squaric acid dibutylester (SADBE), in order to receive topical immunotherapy (TI) for severe alopecia areata and viral warts. Time was found to be the sole variable significantly associated with loss of skin responsiveness to the agent. The standardized procedures applied both in preparing/stocking the antigen solutions, and in piloting delayed skin reactions, ruled out significant sources of heterogeneity among our findings. Other variables (for instance, TI duration, or type of conditions prompting TI administration) did not appear to have played a meaningful role in this context. The study protocol implied assessment of acquired tolerance to SADBE via open application tests. In our opinion, this evaluation procedure adequately substituted for patch testing (not carried out, indeed, as outwith our aims at the time of patients' re-evaluation visit).

We are aware that our series is too small to allow the drawing of conclusions robust enough to settle all the many controversies which still lie around the natural history of loss of contact sensitization in man, which certainly deserves further investigation. A number of studies have been carried out in this area (Table 2; 3–19). However, despite the amount of new knowledge accumulated over recent decades in many fields of the pathomechanism of delayed-type hypersensitivity, the rate of decline of allergic contact sensitivity still remains an intriguing question. Obvious difficulties in collecting and comparing this type of data derive from the great heterogeneity among allergen series, concentration of test allergens, patient selection criteria,

Table 1. Profile of the series of patients submitted to topical immunotherapy with SADBE and returning for disease recurrence in the period 1993–2001. Stratification by time elapsed from the last skin challenge with the sensitizer, and proportion of cases having lost contact sensitivity

Time elapsed after the last challenge (years)	Patients returning for disease recurrence	Estimated loss of sensitivity	%[a]
1	4	0	0.0
2	4	1	25.0
3	7	1	14.3
4	5	3	60.0
5	8	6	75.0
6	6	6	100.0
7	6	6	100.0
Total	40	23	57.5

[a]χ^2 for trend = 19.99 ($p < 0.01$).

and methods of recording the response. All these circumstances leave to date unresolved the debate as to whether repeated exposure to a specific allergen may increase (or, at least, maintain) the level of sensitivity to that substance, and whether repeated exposure may also widen the spectrum of sensitization to further allergens (cross- and poly-sensitization)

In animal experiments it has been shown that frequent exposure to low doses of DNCB, a potent contact sensitizer, induces both systemic hyposensitization and local unresponsiveness within 8 weeks, and that discontinuation of skin challenges results in reversal of systemic hyposensitization within 5 weeks and local unresponsiveness within 1 week (20). Subsensitizing doses of other agents, for instance picryl chloride, chlorprothixene and phenyloxazolone, have also been found to be tolerogenic when painted once or repeatedly on mice or guinea pigs (20–23). What remains to be assessed is to what extent such experimental models for acquired tolerance may be relevant to man in the general and/or occupational setting. In fact, with only sporadic exceptions (11), the majority of investigators have provided evidence ruling out any significant role of antigen avoidance in loss/maintenance of sensitivity (4, 8, 14, 18). To cite an example in this context, Katsarou *et al.* (18), studying a cohort of 163 cement workers with positive patch tests to potassium dichromate, found a rate of loss of sensitivity and diminished reactivity of 17% and 20%, respectively, after a period of 2–6 years during which patients were told to avoid contact with the allergen and applied for workers' compensation benefits. On follow-up examination, some of the patients admitted to have continued to work with cement ('though less frequently') and, despite the absence of any obvious differences between subjects avoiding contact or not with the allergen in terms of re-patch test results, the authors themselves claimed possible sources of inaccuracy deriving, on one hand, from the risk of patients' dishonesty related to fear of loss of compensation benefits, and on the other hand, from the impossibility of grading consistently levels of allergen avoidance.

On the basis of these premises, one may consider that the study of duration of contact sensitivity to agents utilized in so-called 'topical immunotherapy' (TI) (1, 2) can help by-pass all these sources of distortion (clinical and methodological). In fact, TI is performed with strong sensitizers that are not found in the general or technical environment, and do not induce cross-sensitization to other substances. Thus, unlike patients with sensitivity to nickel and other more or less commonplace allergens, subjects submitted to this treatment modality might provide useful data for the study of the natural course of contact sensitivity. To the best of our knowledge, very few studies have to date been carried out on this matter.

Valsecchi *et al.* (24) have reported the results of fresh patch testings with DNCB and SADBE (0.05–1% in acetone) performed in a series of 66 patients consecutively treated for alopecia areata during the period 1978–85. The minimum time elapsed from the last therapeutic challenge was 3 and 7 years for SADBE and DNCB patients, respectively. In 19 patients (29%), loss of contact sensitization was observed, and in only 2 of these cases was restitution of skin reactivity obtained when 'a topical provocation test was performed with the highest dose used during the previous period of treatment' (concentrations not specified by the authors). By computing the relative contribution of each negative fresh patch test/year elapsed from the last therapeutic contact, a negative correlation emerges between time and occurrence of desensitization. In fact, in Valsecchi's series more than 1/2 (52.5%) the patients had lost their sensitivity within 3–5 years over an 11-year period of examination. This data is in striking con-

Table 2. Studies on the persistence of patch test reactions

Authors	Allergen	Time elapsed since primary test (years)	Number of patients	Negative on re-testing (%)
Gomes-Orbaneja & Barrientos, 1938 (3)	Turpentine ?%[a]	1–3.5	106	3
Morgan, 1953 (4)	Nickel 10%	1.5–11	54	43
	Nickel ?%		9	22
Bülow, 1954 (5)	Balsam of Peru ?%	4.2 (mean)	18	16
	Turpentine ?%		12	66
	Nickel ?%		11	18
Nielsen & Bang, 1954 (6)	Balsam of Peru ?%	13.5 (mean)	9	44
	Turpentine ?%		29	62
	Chromate ?%		8	50
Fisher & Shapiro, 1956 (7)	Nickel 10%	2–5	40	10
	PPD 2%		50	8
Fisher *et al*. 1958 (8)	Nickel ?%	3–10	100	10
	Chromate ?%		20	45
	Benzocaine ?%		25	4
	Ragweed oleoresin ?%		18	0
Marcussen, 1959 (9)	Nickel 5%	5.5–7.6	27	4
	Nickel 2.5%		21	38
Rhodes & Warner, 1966 (10)	Rubber mix ?%	2	19	16
	tetrachlorsalicylanilide (TCS) 1%		57	
	Turpentine 25%			64
Te Lintum & Nater, 1973 (11)	Nickel 5%	2–15	105	32
	Balsam of Peru 25%			50
Meneghini & Angelini, 1977 (12)	22 allergens	< 1–3	208	50
Dooms-Goossens *et al.* 1979 (13)	24 allergens	0.25–2	50	37
Keczkes *et al*. 1982 (14)	46 allergens	10–13	100	14
	Nickel ?%	10	65	6
Motolese *et al*. 1988 (15)	Ethylenediamine ?%	3	24	29
	PPD ?%	3	15	20
Edman & Möller, 1989 (16)	Wool alcohol 30%	1–4	33	39
Carmichael *et al*. 1991 (17)	Wool alcohol 30%	0.5–5	37	59
Katsarou *et al*. 1997 (18)	Cobalt 1%	2–6	98	40
	Dichromate 0.5%		163	17
von der Werth *et al*. 1998 (19)	Cetylstearyl alcohol 5–20%	?	23	95

[a]?%, percentage unknown.

trast to our present findings, in that we found in our patients that the longer the time elapsed from the last therapeutic challenge, the greater was the contribution to the total number of desensitizations observed (Table 1). Reasons for these discrepancies remain unclear and deserve further investigation.

The second, and last, example is provided by Seo & Eun (25), who, in 18 subjects treated with DPC for alopecia areata, surveyed the quantitative profile of both systemic desensitization and local unresponsiveness during a 4-month treatment course. In particular, during this very short observation period, a weakening of patch test reactions, along with an increase in concentrations needed to produce mild contact dermatitis on the affected scalp, were observed with statistically significant differences compared to baseline. Of note, the concentration of DPC used to sensitize patients (0.1% in acetone) is significantly lower than the 2% usually applied by most dermatologists in routine therapeutic applications. This may account for the particularly rapid evolution of tolerance observed in Seo & Eun's series (in terms of both local unresponsiveness and patch test negativization rate), which are not indeed in agreement with our and others' clinical experience with DPC and other topical sensitizers in therapeutic uses (1, 2, 26)

Data reported in the 2 studies cited above and our present findings are not easily and consistently comparable to each other. One has in fact to consider, for instance, differences in the type of allergen used, and in procedures employed to pilot and assess cutaneous sensitivity. However, we maintain that case series receiving topical immunotherapy with SADBE, and other topical sensitizers which are not present in human environments, have so far been disappointingly little exploited in the assessment of persistence of contact sensitization in man. Clearly, provided more standardized TI application procedures are established, aim-oriented studies of the outcome of this treatment modality may instead provide useful data to be used in the construction of predictive models of decay of con-

tact sensitization under conditions of antigen avoidance.

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