

The spectrum of inflammatory cell response to dimethyl sulfoxide

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Dimethyl sulfoxide (DMSO), depending upon the concentration and mode of application to the skin, can induce either a non-immunological immediate contact urticaria or an irritant reaction. The dermal cellular infiltrate after open application of varying concentrations of DMSO has been studied in an experimental guinea pig model. The composition of the dermal cellular infiltrate showed a spectrum dependent on the concentration and number of applications of DMSO. The immediate reaction infiltrate 3 h after application of 100% DMSO consisted of 50% granulocytes, basophils being predominant. On the other hand, 12% DMSO applied 3 × daily for 3 days (cumulative insult) caused histologically a cellular reaction in which 80% of the infiltrate consisted of mononuclear cells. The present findings are compared to the microscopic findings in 3 other cutaneous reactions previously studied in this animal model, namely, the Type I immediate hypersensitivity reaction, the Type IV delayed hypersensitivity reaction, and the irritant reaction. Differing cellular infiltrate patterns are discernible at the same time points. The study illustrates the spectrum of inflammatory reactions seen in the skin and provides background information for future clinical studies, for instance, on the role of the basophil granulocyte in immediate contact reactions.

Key words: cellular infiltrate; non-immunological immediate contact urticaria; basophil granulocytes; early phase; late phase; dimethyl sulfoxide. © Munksgaard, 2000.

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The non-immunological immediate contact reaction (NIICR) is a localized reaction, the occurrence of which depends on the concentration, mode of exposure and the chemical properties of the applied substance (1). The reaction occurs without previous sensitization. The passive transfer test is negative indicating that no specific antibodies against the provoking agents are present (2). The substances most commonly studied are benzoic acid, sorbic acid, cinnamic acid, cinnamic aldehyde and nicotinic esters (3, 4). More than 1/2 of all individuals react with local erythema and oedema within 45 min of exposure to these substances. The pathogenetic mechanisms of these reactions have not been fully established (5).

NIICR has been studied in animal models (1, 6, 7), often in the ear where the reaction is quantified by measuring changes in ear thickness. Guinea pig body skin also reacts with rapidly appearing erythema to, e.g., cinnamic aldehyde, methyl nicotinate and dimethyl sulfoxide (DMSO) (6). The reaction has been reported to be characterized micro-

scopically by a dermal inflammatory cell infiltrate comprising initially neutrophilic and eosinophilic granulocytes (6).

DMSO is an aprotic solvent which not only produces NIICR (8) but is also reported to give irritant reactions (9). That the same agent can produce different cutaneous reactions can depend, among other factors, on the provoking substances concentration and method of application (10).

One method of discerning various pathogenetic patterns is to study the time course and composition of the dermal inflammatory cell infiltrate in an experimental model. In a guinea pig model, using plastic embedding and optimal staining, the classical Type IV delayed hypersensitivity contact reaction shows a dermal cellular infiltrate composed chiefly of mononuclear cells but also a large proportion of basophil granulocytes (11–13). The time course and cell infiltrate in a Type I immediate hypersensitivity reaction, a toxic reaction, or an irritant reaction are different (13–15). For example, the neutrophil is present 1 h after provo-

cation in a Type I reaction and eosinophils are present in irritant reactions as late as 72 h. In allergic reactions, mast cells are either not discernible (Type I) or present in lower numbers (Type IV), while in toxic reactions they are commonly found (6). The NIICR reaction caused by DMSO is another reaction type that may show yet another morphological pattern.

The aim of the present study was to extend the existing material on the dermal cellular infiltrate in cutaneous reactions of differing pathogenetic types by studying the effects of DMSO at varying concentrations in single and multiple open applications in the above guinea pig model. Points of specific interest were the presence or absence of, and time course for, the appearance of basophils and mast cells in the reactions, and comparisons with previously studied reaction types.

Materials and Methods

Animals

Female albino Dunkin Hartley guinea pigs weighing around 300 g were kept in separate cages with free access to standard pellets and water.

Experimental design

In 4 groups of 10 animals the flanks were shaved with an electric razor and 5 μ l of solutions of DMSO (12, 25, 50% diluted in distilled water, and 100%) was applied with a pipette to marked test

areas 13 mm in diameter. The application was open and performed singly, 3 $\times$ daily during 1 day (1 $\times$ 3), or 3 $\times$ daily for 3 days (1 $\times$ 3 $\times$ 3). Single applications were assessed 3 and 24 h after testing. Multiple applications (1 $\times$ 3 and 1 $\times$ 3 $\times$ 3) were assessed at 24 and 72 h, respectively, after the 1st application (Fig. 1a and b). The same time points have been used in studies of other contact reaction types in the same experimental model.

Macroscopic score

Naked eye assessment according to the following scheme was performed prior to sacrifice at 3, 24 and 72 h after the 1st application of DMSO. Parentheses were used to give further gradation.

Morphological score	Numerical score
0=no change or uncertain reaction;	0
(+)	0.5
+ =redness, often slightly irregular in distribution;	1
+ (+)	1.5
++ =redness with slight palpable induration;	2
++ (+)	2.5
+++ =redness with obvious swelling.	3

The numerical score expressed the "macroscopic score" for the animals. Group means were obtained by dividing the total numerical score by the number of animals in the group.

Histological processing

After sacrifice, punch biopsies of the test sites were fixed in 10% neutral phosphate buffered formalin, embedded in glycol methacrylate and polyethylene glycol (JB-4 Plastic Embedding Kit, Polysciences, USA), cut in 3 mm sections and stained with May-Grünwald-Giemsa.

Microscopic assessment

Mononuclear cells (lymphocytes of varying size and monocytes), mast cells (a resident mononuclear cell), basophils, eosinophils and neutrophils in the upper dermis were counted. The number of cells per high power field (1000 $\times$ oil immersion) for 20 fields was averaged (12) and expressed either as cells/high power field or as a differential (in %).

Cell response

The cell response is the number of cells in tested skin minus the number of cells in normal skin (Fig.

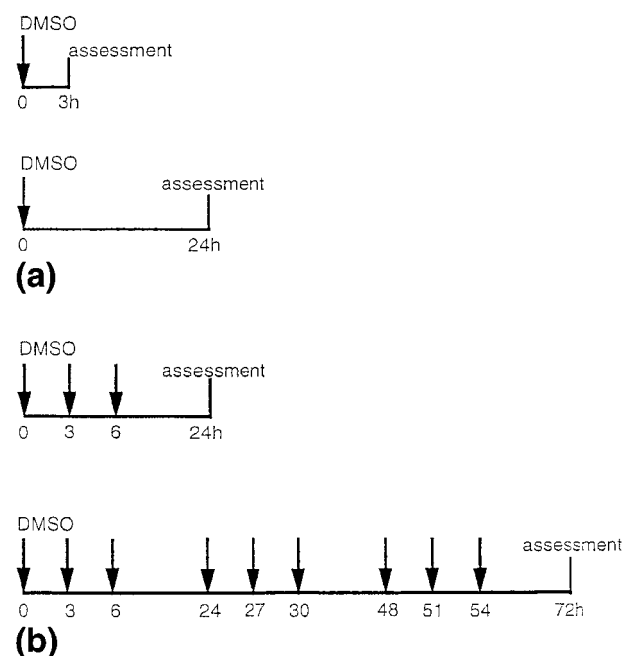


Fig. 1. (a) Experimental design. Single applications. (b) Experimental design. Multiple application.

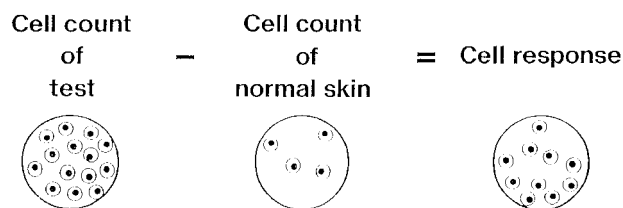


Fig. 2. Dermal cell response.

2). This procedure compensates for normally infiltrating cells and other resident cells in the dermis, allowing assessment of the cell traffic into the reaction area (12) and grading of a reactions magnitude within the same animal. In order to facilitate the comparison of reaction types, the cell response can also be expressed as a differential (in %). In some instances, however, a specific cell type may decrease in number. This can either be caused by migration of a cell out of the reaction area or a morphological change making the cell difficult to classify. An arbitrary adjustment for this phenomenon is to report the observation as "LOSS" and to thereafter exclude that cell type from the calculation of the cell response differential. In all cases "LOSS" was localized to one cell type. The cell response differential is thus a description of the cells which increase in number in a particular reaction.

Statistical analysis

The statistical significance of changes in cell counts was tested with a 2-tailed paired *t*-test analysis. A *p*-value of less than 0.05 was considered significant.

Results

Macroscopic score

The effect of different concentrations and single or multiple application of DMSO was assessed by the naked eye.

Single application. A light erythema (mean score 1.0) was noted in all 10 animals 3 h after application of 100% DMSO. The reaction was concentration dependent – no reaction was seen in any animal to 12% DMSO. The 24-h reactions to all concentrations were less pronounced.

Multiple applications. At 24 h, 1×3 applications of 100, 50 and 25% DMSO concentrations gave a higher macroscopic score than the single application of the same concentrations assessed at 24 h. In addition, an erythema was observed for the 12% DMSO concentration in 5 of 10 animals, resulting in a mean score of 0.25. At 72 h, 1×3×3 applications showed increased erythema for all concentrations of DMSO compared to the 1×3 application. Erythema was seen in all animals at all concentrations of DMSO except for 12% DMSO where only 5 of 10 animals reacted (mean score 0.35). 100% DMSO (1×3×3 applications, mean score 3) caused difficulty in the visual scoring due to scaling at the test site. This was not seen for the other concentrations.

Cellular infiltrate

Single application. Table 1 shows the mean of macroscopic scores and the dermal cell responses for the different concentrations of DMSO. All DMSO applications except 12% DMSO gave a significant ($p < 0.01$) total cell response when compared to normal skin. The reaction caused by 100% DMSO after 3 h was characterized by a cellular infiltrate comprising mononuclear cells and polymorphonuclear cells. Basophils were the most common polymorphonuclear cell. There were fewer mast cells in the test area than were found in normal skin (this is shown as a negative value). At 24 h, the proportion of mononuclear cells had increased and the number of neutrophils decreased. Mast cells were slightly more numerous than in the normal skin. DMSO concentrations of 50 and 25% gave an infiltrate in which mono-

Table 1. The mean cell response after 3 and 24 h of a single application of different DMSO concentrations

Test site time	DMSO % conc.	Macro. score	Cell response per high power viewfield					
			tot cell	mono	mast	baso	eos	neutro
3 h	100	1.00	10.7***	5.8	−0.2	2.1	1.3	1.6
	50	0.80	5.3**	3.5	0.1	1	0.5	0.2
	25	0.80	8.4***	6.2	0.1	1.1	0.8	0.2
	12	0.00	−0.2	−0.4	0.1	0	0.1	0
24 h	100	0.40	12.7***	8.5	0.1	2.7	1.3	0.2
	50	0.25	9.5***	6.2	0.1	1.4	1.6	0.1
	25	0.20	8.1***	5.2	0.1	1.1	1.5	0
	12	0.00	1	0.6	0	0.2	0.1	0

n=10 for each concentration and time point. A minus (−) indicates fewer cells found in the test reaction than in normal skin. ** $p < 0.01$; *** $p < 0.001$.

Table 2. The mean cell response for DMSO applied 3 × during 1 day (1×3) or 3 × daily for 3 days (1×3×3)

Test site appl.+time	DMSO % conc.	Macro. score	Cell response per high power viewfield					
			tot cell	mono	mast	baso	eos	neutro
1×3 24 h	100	0.60	11.4***	7.8	0.3	2.2	0.8	0.4
	50	0.50	10.8***	7.7	0.3	1.7	0.9	0.2
	25	0.50	10.4***	7.8	0.4	1.3	0.8	0.1
	12	0.25	8.6***	7.1	0.3	0.7	0.2	0.3
1×3×3 72 h	100	3.00	13.4***	6.2	0	2.9	4.1	0.3
	50	0.95	9.8***	5	0	2.4	2.4	0
	25	0.85	10.6***	5.2	0.2	2.3	2.9	0
	12	0.35	6.2***	4.9	0.2	0.4	0.3	0.3

The tests were examined 24 or 72 h respectively after the first application of DMSO. $n=10$ for each concentration and time point. A minus (−) indicates fewer cells found in the test reaction than in normal skin. ***= $p<0.001$.

Table 3. The mean cell response in irritant reaction types

1×3×3×reaction agent	Macro score	Cell response total cell	Cell response/high power view field				
			mono	mast	baso	eos	neutro
SLS 2% ($n=76$)	2.1	11.38	9.56	0.22	0.28	0.34	0.97
SLS 1% ($n=26$)	1.5	7.29	5.65	0.16	0.49	loss ^{a)}	1.07
DMSO 12% ($n=10$)	0.4	6.16	4.93	0.19	0.41	0.31	0.33

The irritant reaction is elicited by repetitive open applications 3 × daily for 3 days (1×3×3) of 2% and 1% SLS (from ref. 13) and 12% DMSO.

^{a)} Loss indicates a lesser amount of cells found in the reaction than that found in normal skin.

nuclear cells predominated at 3 and 24 h. The 12% DMSO caused no cellular infiltration.

Multiple applications. As seen in Table 2, all concentrations of DMSO assessed 24 h after application 3 × during 1 day (1×3) gave a macroscopic reaction and a highly significant ($p<0.001$) total cell response. Mast cells are seen to be increased at all concentrations in contrast to the 24 h single application. Basophils are still the predominating granulocyte. Although eosinophils are present, there are fewer than at 24 h after a single application. The cellular infiltrate caused by 12% DMSO differed from the reactions to the higher DMSO concentrations in that a larger proportion of mononuclear cells was found. 100% DMSO, after 1×3×3 applications assessed at 72 h, gave a pronounced macroscopic score, a higher total cell response, less mast cells at the higher concentrations and an increase in eosinophils compared to the 24 h 1×3 application. For the 50% and 25% DMSO concentrations, total cell counts were similar in the 1×3 application test site but the macroscopic score was greater.

Discussion

In this study, a rapidly appearing erythema characteristic of NIICR reactions (5) was seen 3 h after provocation of a single application of DMSO. The

reaction was concentration dependent with a threshold between 12% and 25%. Microscopically, the 100% DMSO reaction showed a dermal cellular response comprising eosinophils and neutrophils, as found by Lahti (6). The additional finding in our study is the predominant occurrence of basophil granulocytes, a cell type which can only be identified when plastic embedding, thin sectioning, and optimal staining are preformed (11, 16, 17). Basophils have generally been assumed to be indicative of an "allergic" mechanism. Earlier reports (18, 19) speculated that DMSO, at a concentration of 70% or higher, caused mast cell degranulation. Lahti et al. (20), however, found no sign of mast cell degranulation at 40 min nor 24 h after provocation in the guinea pig ear. The present study found a loss of mast cells only at 3 h after provocation. Loss of mast cells is presumably caused by degranulation, which renders morphological classification impossible (21, 22). Mast cell degranulation implies release of histamine and other preformed mediators stored in mast cell granules (23) and may explain the mechanism of the chemotaxis observed. DMSO is also reported to produce morphological changes in keratinocytes (18, 20, 24), providing another possible source of chemoattractants (25, 26). Thus, at least 2 cell types may be able to produce inflammatory mediators which not only can activate the endo-

Table 4. The mean cell response differential in procent in different reaction types 3 h after testing

3 h after test	Macro	Cell response	% of cell response				
agent	score	total cell	mono	mast	baso	eos	neutro
ovalbumin ^{a)} (<i>n</i> =13)	1.3	3.67	loss ^{c)}	loss	1.6	21.3	77.2
DMSO 100% (<i>n</i> =10)	1.0	10.70	54.2	loss	20.0	12.4	15.2
oxazolone ^{b)} (<i>n</i> =5)	1.0	7.95	78.3	2.5	6.7	14.7	0.9

^{a)} 10 µg overbumin injected intradermally in sensitized animals causes the immediate allergic reaction (14).

^{b)} 0.2% oxazolone in sensitized animals elicits the delayed allergic reaction type (12).

^{c)} Loss indicates a lesser amount of cells found in the reaction than that found in normal skin.

Table 5. The mean cell response differential in procent in different reaction types 24 h after testing

24 h after test		Macro	Cell response	% of cell response				
agent	applied	score	total cell	mono	mast	baso	eos	neutro
ovalbumin ^{a)} (<i>n</i> =13)	1×	2.0	11.99	55.0	loss ^{c)}	16.8	21.1	6.8
DMSO 100% (<i>n</i> =10)	1×	0.4	12.70	66.7	0.6	20.9	9.9	1.9
oxazolone ^{b)} (<i>n</i> =52)	1×	2.0	21.40	64.5	1.2	29.8	3.8	0.6
croton oil ^{c)} (<i>n</i> =53)	1×	1.7	12.10	85.1	3.1	8.6	3.3	1.6
SLS 2% ^{d)} (<i>n</i> =10)	3×1	0.4	4.34	83.0	4.1	8.3	4.1	0.3
DMSO 12% (<i>n</i> =10)	3×1	0.6	8.64	81.8	3.6	8.6	2.3	3.7

^{a)} 10 µg injected intradermally in sensitized animals, caused an immediate allergic reaction (14).

^{b)} 0.2% in sensitized animals elicits a delayed type allergic reaction (12).

^{c)} Produces a toxic reaction (15).

^{d)} Irritant reaction is caused by repetitive application of 2% SLS (13).

^{e)} A lesser amount of cells found in the reaction than that found in normal skin.

thelium but also recruit basophils and other granulocytes after DMSO application (27, 28).

DMSO 12% in a single application gave neither a macroscopic nor a microscopic reaction. Only multiple applications of 12% DMSO resulted in erythema and a dermal cellular infiltrate. The cellular response was unlike a NIICR reaction since the percentage of mononuclear cells was high and basophil and eosinophil numbers were low. This dermal reaction pattern is similar to that previously seen in model irritant reactions to 1% and 2% sodium lauryl sulfate (SLS) (Table 3). Despite the lower macroscopic score in the 12% DMSO reaction than in the SLS reaction, the cellular differential argues for a similar mechanism – an irritant reaction. We suggest that the 100% DMSO single application and the 12% DMSO multiple applications represent extremes of the pathogenetic capabilities of DMSO. The dermal inflammatory cell infiltrate for the 50% and 25% DMSO application were less easily interpreted and may represent mixed reaction types pathogenetically. Whilst DMSO is a relatively coarse example of how a sub-

stance can induce pathogenetically different reactions at different concentrations (10), other examples can be expected to be more subtle, or of more individual relevance in a clinical setting.

Our model (11–15) has been useful in studying the pathogenetic mechanisms of known agents in defined concentrations and application conditions and allows a comparison of the cellular dermal infiltrate reaction types at given time points. A convenient way of comparison between reactions in which the total cell response may differ is to express the differentiated cell response in percent (Tables 4, 5). 2 rapid reaction types, the Type I reaction to ovalbumin and the present NIICR (100% DMSO) are compared to a Type IV delayed allergic reaction caused by oxazolone 3 h after provocation (Table 4). The different immunological and inflammatory mechanisms produce a cell differential which varies markedly between these reaction types. The neutrophil granulocyte, although seen in both rapid reactions, is more predominant in the Type I reaction. The basophil is the predominant granulocyte in the NIICR reac-

tion. A feature in common for the 2 rapid reaction types was the absence of mast cells. Thus, the abundance of basophils in the NIICR reaction represents a clear point of differentiation from the other 2 reaction types at this early time point.

At 24 h (Table 5), these same reaction types show a different cell profile than that seen at 3 hours. The NIICR (100% DMSO) cell profile is now similar to the Type IV reaction. One implication of this finding is that, ideally, an early reaction (1–3 h) should be excluded before a reaction at 24 h is accepted on histological (or possibly even naked-eye) findings as a delayed Type IV allergic reaction. A Type I allergic mechanism may be indicated by a large population of eosinophils at 24 h.

At the very least, these findings demonstrate how different and quite distinct dermal inflammatory histological patterns can lie behind a similar macroscopic appearance. The range of inflammatory cell participation is the net result of the diversity of potential inflammatory mechanisms which may be operating when the skin reacts to contact with an exogenous substance. Individual variability in reacting to these substances, already visible in this experimental model can be expected to be even more obvious in the (outbred) human situation. Extrapolation to the human situation must of course be done with caution, but the information from the present study can offer valuable background information for continued studies in a clinical situation.

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