

Induction of delayed hypersensitivity to nitrogen mustard in the guinea-pig

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SUMMARY

The acquisition of allergic contact dermatitis limits the therapeutic usefulness of topical nitrogen mustard in man. A reproducible experimental model for this syndrome has been provided in the guinea-pig. Guinea-pigs were sensitized to nitrogen mustard (HN_2) by means of the repeated epicutaneous administration of sensitizer to a skin site, and the injection into that site of complete Freund's adjuvant. Application of dimethyl sulphoxide before HN_2 to the sensitization or challenge site facilitated the sensitization and challenge procedures. Positive challenge could be elicited by the intradermal injection of a non-toxic amount of HN_2 . The methodology extends to the study of a variety of other sensitizers and potential sensitizers.

Nitrogen mustard (mechlorethamine, $\text{CH}_3\text{-N}(\text{CH}_2\text{CH}_2\text{Cl})_2$) is an alkylating agent, widely used in cancer chemotherapy. It is unstable at alkaline or neutral pH, but relatively stable in acid solutions. It forms covalent reaction products with a wide variety of biological materials, including nucleic acids and proteins. It is likely that the major biological effects produced by this drug result from its forming irreversible cross linkages, since, generally, monovalent alkylating agents are relatively inactive (Wheeler & Alexander, 1969).

Topical nitrogen mustard has been used with considerable success in the treatment of mycosis fungoides (Haserick, Richardson & Grant, 1959; Madison & Haserick, 1962; Van Scott & Writters, 1970). Repeated application to lesions of mycosis fungoides, particularly to those in the erythematous or plaque stage, may cause them to resolve, sometimes for a long period of time. Topical nitrogen mustard has also been reported to induce healing in stable plaques of psoriasis (Epstein & Ugel, 1970).

The principal toxic side effects of parenterally administered nitrogen mustard, that limit the amount of drug that can be given, are bone marrow depression and gastro-intestinal ulceration. Significant absorption of nitrogen mustard does not occur when it is applied to the skin. However, the induction of allergic contact dermatitis is a frequent complication of therapy with topical nitrogen mustard. For instance, of the patients of Epstein & Ugel (1970) who were treated with topical nitrogen mustard, the drug had to be discontinued in half because they became sensitized to the medication. Further, beyond the usual hazards and discomforts of allergic contact dermatitis, in psoriasis there is the real possibility

of a Koebner phenomenon developing at sites of contact dermatitis. A recent attempt to induce specific immune tolerance to nitrogen mustard in patients by giving the drug intravenously prior to topical therapy was reported as unsuccessful (Baer, Michaelides & Prestia, 1972).

An animal system in which the acquisition and expression of allergic contact sensitivity to nitrogen mustard could be studied, would seem desirable. We now report experiments in which allergic contact dermatitis to nitrogen mustard has been induced in guinea-pigs with some regularity.

MATERIALS AND METHODS

The animals were closed-colony, randomly bred, Hartley strain, or Family 13 derived, guinea-pigs. In different experiments, either males or non-pregnant females weighing between 300 and 500 g were used. The animals were fed fresh Purina Guinea-pig Chow supplemented with lettuce thrice weekly; water *ad libitum* was provided. The guinea-pig colony was clinically free of streptococcal C cervical adenitis and the animals were considered generally healthy as judged by consistent weight gain and negligible unintentional losses.

The guinea-pigs were sensitized by a variant of the 'split adjuvant' technique, that is by the separate successive administration of allergen (Mustargen®, Merck) and complete Freund's adjuvant to the skin (5 mg heat-killed tubercle bacilli of the H37Ra strain in 10 ml of a solution consisting of 8.5 ml mineral oil and 1.5 ml Arlacel A, Difco) (Maguire & Chase, 1967). Challenges were made to new, freshly clipped sites on the back, the appearance of the induced reactions being observed and recorded daily for several days. Later, the intensity of the inflammation (erythema, induration) was scored: 0, ±, +, ++, ... +++++ (Chase, 1954). Readings less than '±' were described as 'strong trace', 'trace', 'faint trace', or 'very faint trace' to differentiate these reactions from those in which the challenge site appeared normal, i.e. '0'. Intradermal reactions were evaluated for size, with calipers, and for thickness, by palpation. Unsensitized toxicity control guinea-pigs were always tested in parallel. Skin biopsies were taken under light ether anaesthesia and fixed in neutral buffered formalin. The biopsy specimens were processed to sections by routine histopathological techniques.

RESULTS

In a typical experiment, we prepared a sensitization site on the right dorsal flank of eight male Family 13 derived guinea-pigs. The skin was clipped with animal clippers and then close-shaved with a safety razor. Then 0.1 ml of a freshly prepared 0.1% solution of nitrogen mustard in distilled water was applied to a 1 cm square filter paper (Whatman No. 3) and this in turn put on the shaved site. The filter paper was covered with occlusive tape (Blenderm®, Minnesota Mining Company) and this fixed in place with a stretch adhesive (Elastoplast®, Duke Laboratories) and regular adhesive tape. The allergen was reapplied to the filter paper on days 2, 4, and 7, and the dressings removed on day 9. On day 4, complete Freund's adjuvant, 0.1 ml was injected intradermally on either side of the sensitization site (Maguire, 1973). Boosting applications of sensitizer were made on days 21 and 28 under closed dressing. On day 35, animals were tested intradermally with nitrogen mustard 0.001%, (0.1 ml in volume) intradermally. They were further tested on day 45 topically on two sites with 0.01 ml of 1% nitrogen mustard (HN₂); this solution was pipetted and spread over an area measuring about 1 cm diameter; on one site, 0.01 ml of a solution containing nine parts dimethyl sulphoxide (DMSO) and one part water was applied about 10 min prior to challenge with HN₂. The results of these testings are outlined in Table 1.

In another experiment, we sensitized two groups of male Hartley guinea-pigs to HN₂ using the open application to the skin of a 1% (w/v) solution of HN₂ in normal saline. In one of the experi-

mental groups, we pretreated the sensitization site with DMSO prior to the percutaneous administration of the HN_2 solution (Table 2). Thus, DMSO (90%, 0.01 ml) was applied to a single clipped site on the right rear dorsal flank on days 0, 1, 2, 3, 4 and 7. On each of these six days, about 10 min later, HN_2 (1%, 0.01 ml) was pipetted onto the same site (guinea-pigs nos. 278–281) or onto a clipped site in a symmetrical location on the left side (guinea-pigs nos. 282–285). On day 4, complete Freund's adjuvant (0.1 ml) was injected into the HN_2 sensitization site in all eight animals. Testings were made on day 16 with 0.5 ml HN_2 (with and without DMSO pretreatment of the challenge site), and on day 22 by the intradermal route with 0.1 ml of 0.0001% HN_2 in normal saline. The results are summarized in Table 2. Clearly, guinea-pigs could be sensitized to HN_2 by this open technique, and the efficiency of the method was much improved by prior treatment of the sensitization site with DMSO.

TABLE 1. Challenge reactions of guinea-pigs sensitized to nitrogen mustard under occlusive dressing

Guinea-pig no.	Intradermal, T-35		Topical, T-43	
	HN_2	HN_2	DMSO, HN_2	DMSO
193	11	+++	++++±	[tr]
194	9	++	(++++)	f.tr
195	8	±	(++++)	f.tr
196	<3	[±]	(+++)	o
197	5	++	++++	o
198	[9]	+++	+++++	o
199	9	(±)	++++	f.tr
200	6	+±	[+++++]	[tr]
TC	o	---	---	---
TC	o	---	---	---
TC	---	o	±	o
TC	---	o	(±)	o

Eight guinea pigs were sensitized to nitrogen mustard by means of a modified split adjuvant technique using an occlusive dressing. The results of intradermal testing with 0.001% (w/v) HN_2 on day 35, and of epicutaneous testing with 1% HN_2 (to sites untreated and pretreated with 0.01 ml of a 90% DMSO aqueous solution 10 min previously) and with 90% DMSO alone on day 43 are shown. The diameters in millimeters of the intradermal reactions are recorded; the intensity of the inflammatory response evoked by the topical applications have been scored according to standard criteria. For f.tr read faint trace; tr, trace; tr, strong trace. Round brackets () indicates maximum reaction at 48 h, square brackets [], maximum at 72 h. For TC read unsensitized toxicity control.

Biopsy was made of the challenge reactions at 48 h, in guinea-pigs nos. 279 and 280 (Table 2). In sections of specimens derived from sites tested with nitrogen mustard alone, the epidermis showed spongiosis, extracellular oedema and exocytosis; in the upper dermis there was a round cell infiltrate. The histopathological changes were consistent with allergic contact dermatitis in the guinea-pig (Turk, 1967). The site challenged in succession with DMSO and HN_2 showed moderate to marked oedema of both dermis and epidermis; there were clefts, exocytosis and foci of necrosis in the epidermis, and the infiltrate contained polymorphonuclear leukocytes as well as lymphocytes.

TABLE 2. Challenge reactions in guinea-pigs sensitized to nitrogen mustard with and without pretreatment of the sensitization site with DMSO

Guinea-pig no.	T-16, topical		T-22, intradermal
	HN ₂	DMSO, HN ₂	HN ₂
278	+++	+++++	8
279	+++ ± (biopsy)	+++++ (biopsy)	10
280	++++ ± (biopsy)	++++ ± (biopsy)	9
281	(++)	(+++)	10
282	o	++	o
283	+	+++ ±	f.tr
284	+	++	tr
285	o	++	<3
TC	o	tr?	TC o
TC	o	tr	TC <3
TC	o	o	TC <3
TC	o	o	TC <3

Guinea-pigs were sensitized by the application of 0.01 ml of HN₂ to the skin utilizing complete Freund's adjuvant. In four of the guinea-pigs, the sensitization site was treated each time with 90% DMSO prior to the application of HN₂; the other four received DMSO to the opposite flank prior to HN₂. The results of topical testing on day 16 with 0.01 ml of 0.5% (w/v) HN₂ (to clipped sites with and without prior preparation with 0.01 ml 90% DMSO), and on day 22 with 0.01 ml of 0.0001% HN₂ in saline (intradermally) are indicated. The scoring system is outlined in Methods. TC: unsensitized toxicity control.

TABLE 3. Challenge tests to nitrogen mustard in recipients of cells from sensitized donors

Guinea-pig no.	Day				
	0	1	2	3	4
364	PE cells		+ ±	±, biopsy	---
365	PE cells		+ ±	+ ±, biopsy	---
366	Spleen cells		tr	tr	o
367	Spleen cells		f.tr.	o	o
368	---		prac. o	o	o
369	---		o	o, biopsy	---
370	---		o	o, biopsy	---
371	---	HN ₂	o	o	o

The results of contact tests with 1% HN₂ to recipients of viable cells from sensitized guinea-pigs, and to toxicity controls (no cells), are shown. The scoring system is given in Methods. Biopsies of the 48 h reactions in nos. 364, 365, 369 and 370 precluded 72 h readings in these animals. For 'PE' read 'peritoneal exudate'.

A limited attempt was made to transfer HN_2 hypersensitivity passively in the guinea-pig. Donor animals were the eight experimental guinea-pigs listed in Table 2. On day 32, they were injected intraperitoneally with 13 ml of medium weight mineral oil. Two days later, they were killed by acute cervical dislocation, and their spleen and peritoneal cells collected in separate pools by the method of Bloom & Chase (1967). 260×10^6 peritoneal exudate cells were injected intraperitoneally into each of two fresh guinea-pigs (nos. 364 and 365). Two further recipients (nos. 366 and 367) were each given 245×10^6 spleen cells, again intraperitoneally. The results of contact tests made with 0.01 ml of 1% HN_2 to clipped sites are shown in Table 3. The recipients of peritoneal cells, but not of spleen cells, showed definite positive reactions. Biopsies of the positive reactions revealed a mild dermatitis with a mononuclear infiltrate compatible with allergic contact dermatitis. Perhaps the use of a larger number of spleen derived WBCs would have resulted in a successful transfer. Peritoneal cells have been shown to be more active than spleen cells in transferring reactions of delayed hypersensitivity (Bloom & Chase, 1967). Waldorf, Haynes & Van Scott (1967) reported the successful transfer of contact sensitivity to nitrogen mustard in humans using about 10^9 blood leukocytes obtained from exquisitely sensitive donors.

DISCUSSION

Clinically, nitrogen mustard is a sensitizer of intermediate strength in man; it is a weaker sensitizer than dinitrochlorobenzene (DNCB) or picryl chloride, but stronger than materials such as formalin or procaine. This ordering appears to hold for the guinea-pig.

Guinea-pigs can be sensitized to an aqueous solution of nitrogen mustard by a closed technique or by an open method. In this context, a general difficulty with aqueous solutions is their poor penetration through the barrier layer of the epidermis. Occlusion facilitates such penetration and this is a major advantage of the closed technique (Magnusson & Kligman, 1970). Dimethylsulphoxide increases the penetration of materials through the skin by altering the physico-chemical constitution of the barrier layer (Stoughton & Fritsch, 1964; Kligman, 1966; Baker, 1968). Almost certainly, this accounts for the regular increase in the intensity of the challenge reactions in sites pretreated with DMSO; in fact, in our experiments, minor primary irritant reactions developed in several toxicity control animals that received in turn DMSO then HN_2 .

Pretreatment of the sensitization site with DMSO enhanced the acquisition of allergic contact sensitivity in guinea-pigs so treated. While this can be a useful experimental manipulation, the potential hazard for the worker handling DMSO and allergenic (or toxic) compounds at the same time must be kept in mind. We have previously described a technique for identifying potential contact allergens in the guinea-pig by repeated applications of test material under occlusion to a sensitization site and the injection of complete Freund's adjuvant next to the site. However, prolonged closed dressings are inconvenient and may irritate the skin of the guinea-pig. It is likely that as an alternative many materials (especially liquids) can more easily be investigated for their allergic potential by substituting for occlusive dressing the pretreatment of the sensitization site with DMSO.

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REFERENCES

- BAER, R.L., MICHAELIDES, P. & PRESTIA, A.E. (1972) Failure to induce immune tolerance to nitrogen mustard (intravenous administration preceding topical use in patients with psoriasis). *Journal of Investigative Dermatology*, **58**, 1.
- BAKER, H. (1968) The effects of dimethyl sulfoxide, dimethyl formamide and dimethyl acetamide on the cutaneous barrier to water in human skin. *Journal of Investigative Dermatology*, **50**, 282.
- BLOOM, B.R. & CHASE, M.W. (1967) Transfer of delayed-type hypersensitivity. A critical review and experimental study in the guinea-pig. *Progress in Allergy*, **10**, 151.
- CHASE, M.W. (1954) Experimental sensitization with particular reference to picryl chloride. *International Archives of Allergy and Applied Immunology*, **5**, 163.
- EPSTEIN, E. JR & UGEL, A.R. (1970) Effects of topical mechlorethamine on skin lesions in psoriasis. *Archives of Dermatology*, **102**, 504.
- HASERICK, J.R., RICHARDSON, J.H. & GRANT, D.J. (1959) Remission of lesions in mycosis fungoides following topical application of nitrogen mustard. *Cleveland Clinic Quarterly*, **26**, 144.
- KLIGMAN, A.M. (1966) The identification of contact allergens by human assay. II. Factors influencing the induction and measurement of allergic contact dermatitis. *Journal of Investigative Dermatology*, **47**, 375.
- MADISON, J.F. & HASERICK, J.R. (1962) Topically applied mechlorethamine on 12 dermatoses. *Archives of Dermatology*, **86**, 663.
- MAGNUSSON, B. & KLIGMAN, A.M. (1970) *Allergic Contact Dermatitis in the Guinea-Pig; Identifications of Contact Allergens*. Charles C. Thomas, Springfield, Illinois.
- MAGUIRE, H.C. JR & CHASE, M.W. (1967) Exaggerated delayed-type hypersensitivity to simple chemical allergens in the guinea-pig. *Journal of Investigative Dermatology*, **49**, 460.
- MAGUIRE, H.C. JR & CHASE, M.W. (1972) Studies on the sensitization of animals with simple chemical compounds. XIII. Sensitization of guinea-pigs with picric acid. *Journal of Experimental Medicine*, **135**, 357.
- MAGUIRE, H.C. JR (1973) The bioassay of contact allergens in the guinea-pig. *Journal of the Society of Cosmetic Chemists*, **24**, 151.
- STOUGHTON, R.B. & FRITSCH, W. (1964) Influence of dimethyl sulfoxide on human percutaneous absorption. *Archives of Dermatology*, **90**, 512.
- TURK, J.L. (1967) *Delayed Hypersensitivity*. North-Holland Publishing Co., Amsterdam.
- VAN SCOTT, E.J. & WRUTERS, P.L. (1970) Responses of mycosis fungoides to intensive external treatment with nitrogen mustard. *Archives of Dermatology*, **102**, 505.
- WALDORF, D.S., HAYNES H.A. & VAN SCOTT, E.J. (1967) Cutaneous hypersensitivity and desensitization to mechlorethamine in patients with mycosis fungoides lymphoma. *Annals of Internal Medicine*, **67**, 282.
- WHEELER, G. & ALEXANDER, J.A. (1969) Effects of nitrogen mustard and cyclophosphamide upon synthesis of DNA *in vivo* and in cell-free preparations. *Cancer Research*, **29**, 98.

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