

DIMETHYL SULFOXIDE ACCELERATES MUSTARD GAS-INDUCED SKIN PATHOLOGY

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SUMMARY

Dimethyl sulfoxide (DMSO), when used as a percutaneous carrier or as a required solvent for drugs, may have modulating effects on drug mechanisms and untoward effects on subject tissues. In this collaborative study, anhydrous DMSO was used as a vehicle for peptide caspase inhibitors of sulfur mustard gas (HD)-induced apoptosis in hairless guinea pig skin. Results of the inhibition study are the subject of a companion report. In this present manuscript we describe the observed morphological effects of DMSO on hairless guinea pig skin when used as a drug vehicle and as pretreatment to HD exposure. DMSO was applied topically to skin sites as a vehicle control 30 minutes prior to neat HD-vapor exposure (7-8 minutes). Unexposed skin sites also treated for 30 minutes with DMSO were used as controls. Selected exposed and unexposed skin sites, harvested at 6 hours and 24 hours postexposure, were either paraffin-processed for routine histopathology or epoxy embedded for ultrastructural pathology. HD-exposed skin sites pretreated with DMSO presented a marked acceleration and exacerbation of characteristic HD-induced pathologies. At six hours postexposure, epidermal basal cells presented pyknotic nuclear and degenerative cytopathologies usually not seen until later times. Also at this same time, microvesicles in the basement membrane zone, usually not in evidence until 10-12 hours postexposure, were a persistent feature of the pathology. At 24 hours postexposure, cleavages of the dermal-epidermal junction were more expansive than with typical HD exposure. In addition, unexposed control sites treated with DMSO (but not with HD) presented an exaggerated inflammatory response of the papillary dermis. The "inflammation-primed" dermis induced by DMSO may be largely responsible for the acceleration and exacerbation of the characteristic HD-induced skin pathology observed in this study.

INTRODUCTION

Although dimethyl sulfoxide (DMSO), a by-product of the wood industry, has been in use as a pharmaceutical for several decades, its mechanism-of-action is one of the least understood. Dozens of well-documented, but seemingly contradictory, physiological and morphological actions have been attributed to DMSO, including: enhances cellular penetration, promotes membrane transport, stabilizes membrane integrity, improves percutaneous penetration, accelerates drug absorption, enhances synergistic drug effects, reduces concomitant drug action, is an obligatory solvent for selected drugs, is radio-protective, is cryoprotective, is a vasodilator, is anti-inflammatory, stimulates histamine release, is an antioxidant, and, encourages cellular differentiation and maturation. (Jacob, and Herschler, 1986; Kolb et al 1967, Maibach and Feldman, 1967; Rosen et al, 1965; Sandborn et al, 1975; Spruance et al 1983) All these actions have found extensive applications in the armamentaria of veterinary

and medical clinical practices and in primary medical research. Clinical beneficial effects of DMSO have been demonstrated in the treatment of interstitial cystitis, scleroderma, lupus, ulcerative colitis, arthritis, burns and scar tissue reduction. (Jacob, March 2002) Perhaps the most applied use in primary investigative research is as a percutaneous penetrant and drug solvent. In a collaborative temporal study of HD-induced apoptosis, DMSO was used as an obligatory solvent for the anti-apoptotic, anti-caspase drugs z-“VAD”-fmk (N-benzyloxycarbonyl-Val-Ala-asp-fluoromethylketone trimethylketone tripeptide) and Ac-DEVD-“CHO” (N-acetyl-Asp-Glu-Val-Asp aldehyde tetrapeptide). Within that experimental paradigm, DMSO, as a vehicle control, was applied topically 30 minutes prior to HD exposure of skin sites. This manuscript describes morphological effects of DMSO as a topical pretreatment to HD exposure and as a topical treatment to unexposed skin. Results of caspase inhibition on HD-induced apoptosis are described in a subsequent publication.

MATERIALS AND METHODS

Six randomized skin sites of six hairless guinea pigs (HGP) were exposed to anhydrous DMSO, as a pretreatment to HD exposure, according to the following protocol. Anhydrous DMSO (18 microliters, μL) was applied for 30 min to a 12 mm skin-exposure area sealed by double-sided Blanderm® tape and covered with an “Edgewood Vapor Cup”. Pretreated sites were then exposed to neat HD-vapor for 7-8 minutes by standardized “Edgewood Vapor Cup” methodology.(Mershon et al, 1990) Exposed sites were harvested at 6 hours and 24 hours postexposure, fixed in combined aldehydes (1.6% formaldehyde, 2.5% glutaraldehyde in 0.1 Molar cacodylate buffer), dehydrated in graded ethanols and either paraffin-processed for light microscopic histopathological study (or post osmicated and epoxy-embedded for transmission electron microscopy, TEM, ultrastructural analysis). Only the light microscopy results are presented here.

The use of DMSO as a vehicle control was addressed: unexposed skin sites were treated with DMSO for 30 min, were harvested and processed similarly, and were used as controls.

Morphological data are presented in photomicrographs and data comparisons were made with earlier published studies of HD-induced skin pathology and with unpublished data gathered from related DMSO-HD investigations.

RESULTS

All HD-exposed skin sites pretreated with DMSO appeared to have qualitatively significant accelerations and exacerbations of dermal inflammatory responses and epidermal cytopathologies when compared with similar postexposure times for skin not treated with HD. At six hours postexposure, degenerative cytopathologies in the epidermal basal cells appeared that are not seen until later times when no DMSO pretreatment was used. At this time, and of special interest, microvesicles appeared at the basement membrane zone. Microvesication, a characteristic of HD skin pathology, is typically considered a latent feature of the pathology and is not usually seen until 10 – 12 hours postexposure. At 24 hours postexposure, cleavage and separation of the epidermis and dermis were more expansive than with non-pretreated HD exposures. In addition, control sites treated with DMSO for 30 min but not exposed to HD (see above), although not presenting detectable skin surface irritancy, presented exaggerated inflammatory responses of the papillary dermis usually not associated with control skin. The

leading cellular elements of the “inflammation – primed” dermis induced by DMSO were neutrophils and eosinophils. (See figures 1, 2, 3, 4, 5, 6).

DISCUSSION / CONCLUSIONS

The pathology associated with HD-vapor skin exposures of the HGP animal model is now well documented. (Petrali and Oglesby-Megee, 1997; Petrali et al, 2003) Typically there is a sequential prevesication-vesication process. The prevesication phase involves the early targeting of selected epidermal basal cells resulting in apoptotic-necrotic epidermal cell death and primary or secondary compromises of attachment proteins of the basal cell microenvironment and its basement membrane. The vesication phase begins with an apparent pathological cascade of microvesications at the dermal-epidermal junction that prognerates cleavage of the epidermal-dermal junction and leads to eventual formations of microblisters, and perhaps extrapolative bullae formations in man. DMSO, with its reported properties of enhancing membrane transport and accelerating drug absorption (see above), would be expected to produce a change in the magnitude of HD toxicity. (Wormser et al, 1996) As we found in the present study, however, DMSO pretreatment resulted in a telescopic sequencing and acceleration of the known pathology rather than simply a change in magnitude. For example, microvesicle formation at the microenvironment of epidermal basal cells and the basement membrane occurred at six hours postexposure, as opposed to the usual 10-12 hours postexposure period. Although this study did not provide for the harvesting of postexposure times between six hours and 24 hours, we suspect that early microvesications probably led to early cleavages of the dermal-epidermal junction. This early microvesication may account for the observation that epidermal-dermal separations appeared more expansive at 24 hours postexposure when compared to HD exposures without DMSO pretreatment.

Although this morphological *in-vivo* study did not address the effects of DMSO on caspase activation, one of the authors studied the effects of DMSO on caspase-3 activation *in-vitro* as a result of HD exposure in cultured normal human epidermal keratinocytes (NHEK). (Ray, 2005) In that study, caspase-3 activity was determined by a fluorometric assay based on enzymatic hydrolysis of a fluorogenic caspase-3 substrate AC-DEVD-AMC (acetyl-Asp-Glu-Val Asp-7-amido-4 methylcoumarin). Inclusion of 1 percent DMSO in the assay medium had no effect on capase-3 activation in NHEK exposed to 0.3mM HD, which is considered the *in vitro* equivalent of an *in vivo* vesicating dose of HD.

Superimposed upon the accepted DMSO properties of enhanced membrane transport and accelerated drug delivery, the exaggerated, but problematic, inflammatory response of the papillary dermis following pretreatment with DMSO was noteworthy. The resultant “inflammation-primed” dermal microenvironment, complete with histamine releasing elements, neutrophils, eosinophils and macrophages, may largely account for the accelerated HD-induced pathology observed in this study, especially the microvesication. Perhaps a beneficial laboratory-based research use of DMSO pretreatment to HD exposure might be DMSO's use as a cytological tool to magnify and more fully reveal the early and more fleeting events of HD toxicity. Pathological presentations that might be more vigorously investigated using this pretreatment are HD-induced apoptosis, HD-targeting of selected basal cells, pathogenesis of microvesication, and influences of the inflammatory response.

NOTE: In conducting the research described in this report, the investigators complied with the regulations and standards of the Animal Welfare Act and adhered to the principles of the Guide for the Care and Use of Laboratory Animals, National Research Council, 1996. The facility where this research was conducted is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. The findings contained herein are the private views of the authors and are not to be construed as official or reflecting the views of the U.S. Army or the Department of Defense.

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REFERENCES

Jacob, S.W., and Herschler, R (June, 1985). "Pharmacology of DMSO." <http://www.dmsol.org/articles/information/herschler.htm> (2004)

Jacob, S.W. (March 2002) "Current status of Dimethyl sulfoxide (DMSO)." <http://www.dmsol.org/articles/information/jacob.htm> (2004)

Kolb, K.H., Jaenicke, G., Kramer, M., and Schulze, P.E. (1967) Absorption, distribution and elimination of labeled dimethylsulfoxide in man and animals. *Ann NY Acad Sci* 141:85-95.

Maibach, H.I. and Feldman, R.J. (1967) The effect of DMSO on percutaneous penetration of hydrocortisone and testosterone in man. *Ann NY Acad Sci* 141:423-427.

Mershon, M.M., Mitcheltree, L.W., Petrali, J.P., Braue, E.H., and Wade, J.V. (1990) Hairless guinea pig bioassay models for vesicant vapor exposure. *Fundam Appl Toxicol* 15:622-630.

Petrali, J.P. and Oglesby-Megee, S. (1997) Toxicity of Mustard Gas Skin Lesions. *Microsc Res Tech.* May 1; 37(3):221-228.

Petrali, J.P., Kan, R.K., Hamilton, T.A. and Pleva, C. Morphological Expressions of Mustard Gas-Induced Skin Lesion. *Proceedings North American Congress on Clinical Toxicology*, Chicago, IL, June 2003.

Ray R, Hauck S, Kramer R, Benton B. A Convenient Fluorometric Method to Study Sulfur Mustard-Induced Apoptosis in Human Epidermal Keratinocytes Monolayer Microplate Culture; *Drug Chem Toxicol.*, 1:105-116, 2005

Rosen, H., Blumenthal, A., Panasevich, R., and McCallum, J. (1965) Dimethyl sulfoxide (DMSO) as a solvent in acute toxicity determinations. *Proc Soc Exp Bio Med* 120:511-514.

Sandborn, E.B., Stephens, H., and Bendayan, M. (1975) The influence of dimethylsulfoxide on cellular ultrastructure and cytochemistry. *Ann NY Acad Sci* 243:122-138.

Spruance, S.L., McKeough, M.B., and Cardinal, J.R. (1983) Dimethyl sulfoxide as a vehicle for topical antiviral chemotherapy. *Ann NY Acad Sci* 411:28-33.

Wormser, U., Green, B.S., Arad-Yellin, R., Brodsky, B., Shatz, I., and Nyska, A. (1996) In-vivo and in-vitro toxicity of newly synthesized monofunctional sulfur mustard derivatives. *Toxicology*. Apr 15; 108(1-2):125-128.

FIGURES

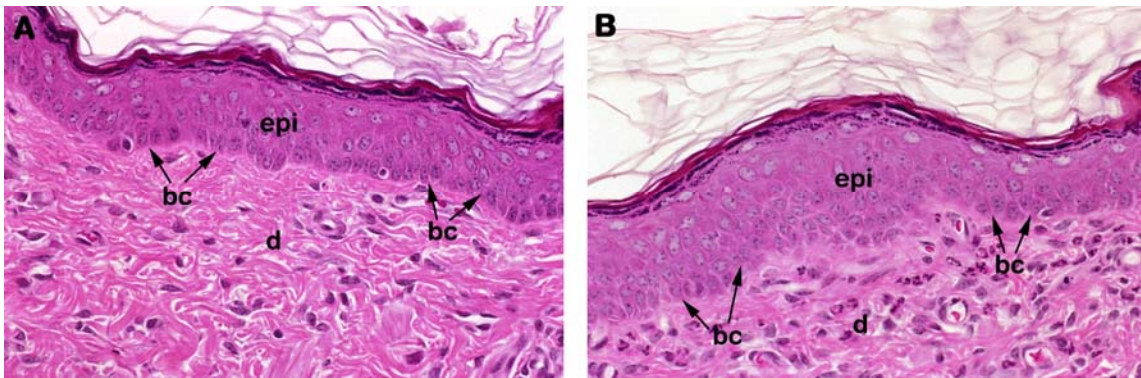


Figure 1. A, B. Light microscopy of untreated control HGP skin; epidermis (epi), dermis (d), basal cells (bc)

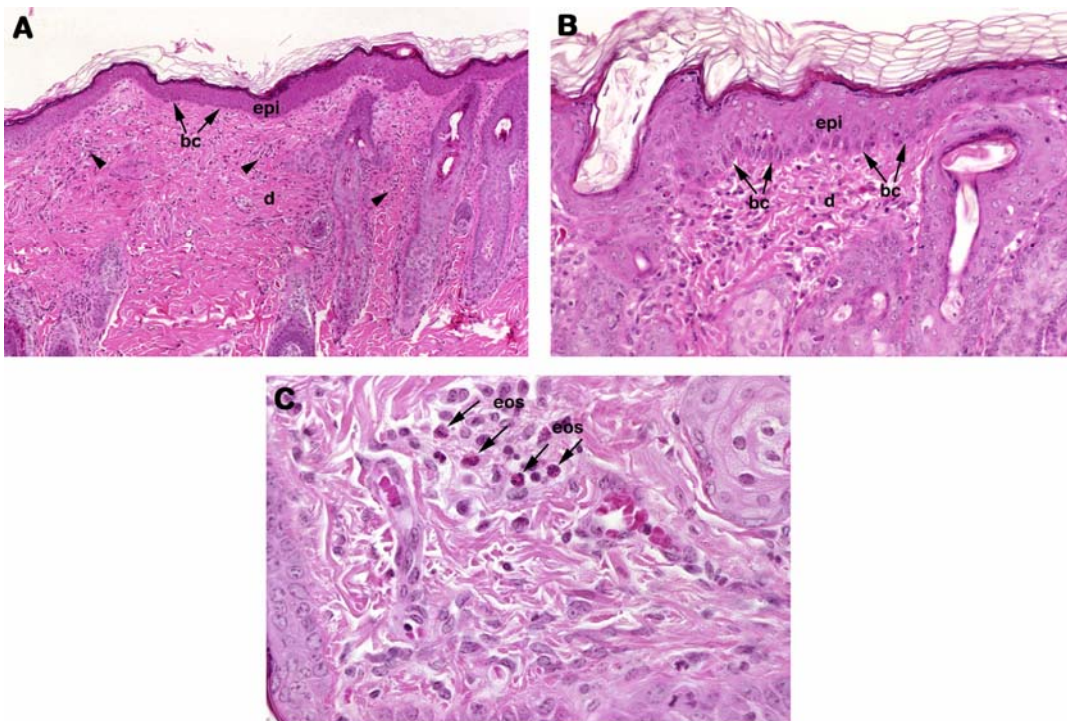


Figure 2. A, B, C. Light microscopy of DMSO-treated HGP control skin; epidermis (epi), dermis (d), basal cells (bc), dermal inflammatory cellular elements (arrow heads) recognized as eosinophils (arrows)

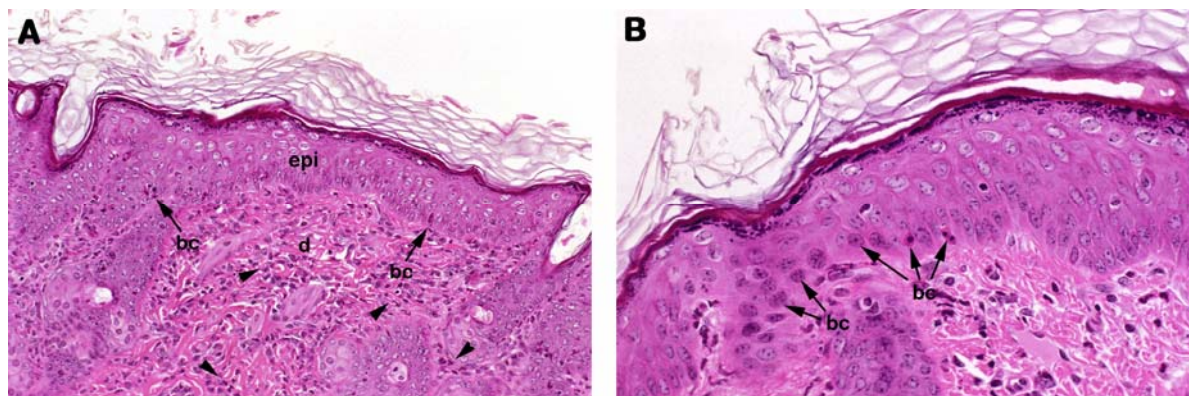


Figure 3. A, B. Light microscopy of HD exposed HGP skin 6h postexposure: epidermis (epi), dermis (d), basal cells (bc), inflammatory elements (arrows)

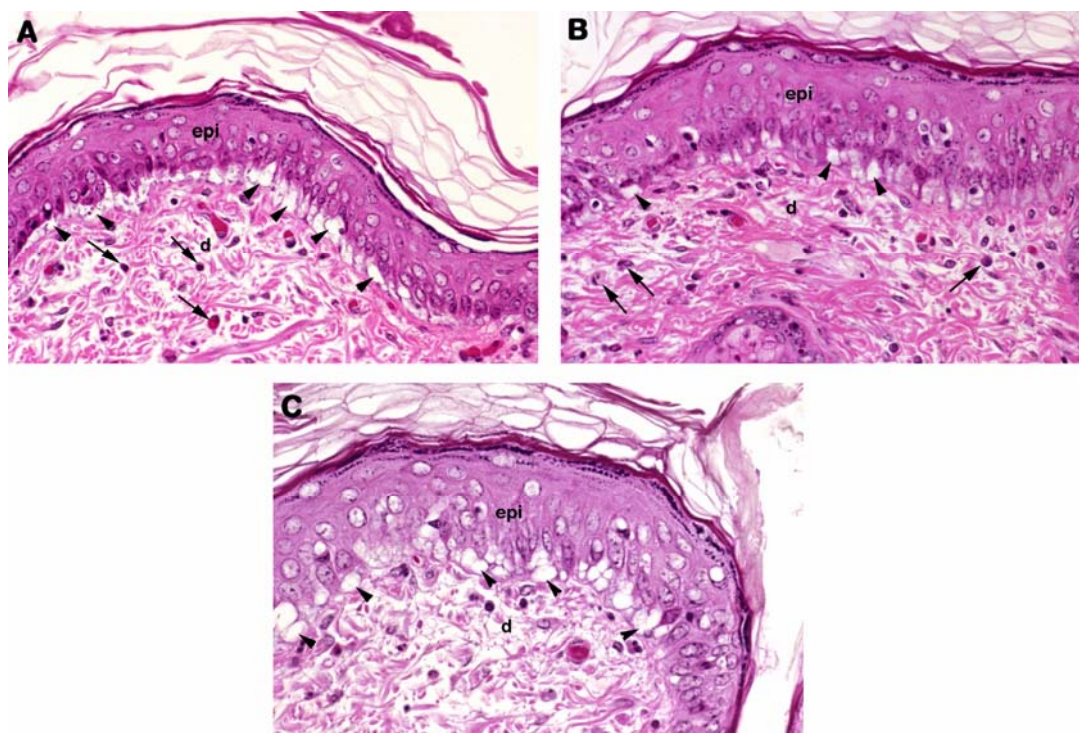


Figure 4. A,B,C. Light microscopy of DMSO-HD exposed HGP skin 6h postexposure; epidermis (epi), dermal inflammatory elements (arrows), basal cells with microvesicles (arrow heads)

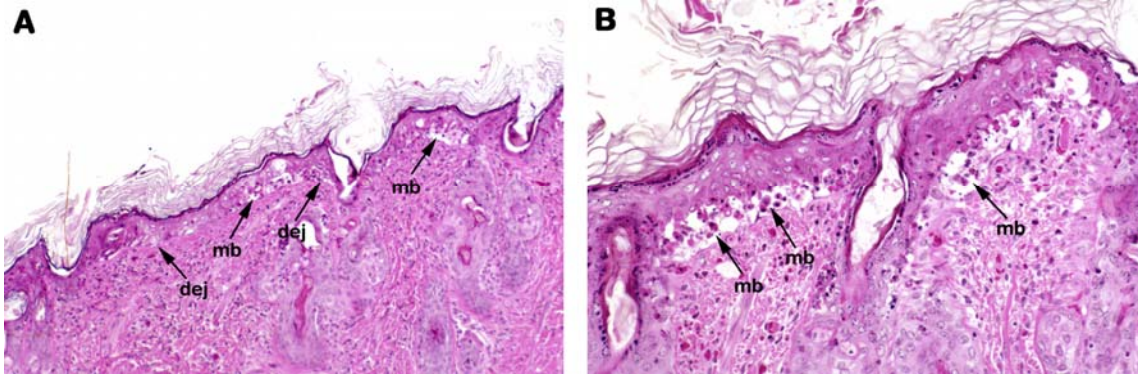


Figure 5. A, B. Light microscopy of HD exposed HGP skin 24h postexposure; microblisters (mb), dermal epidermal junction (dej)

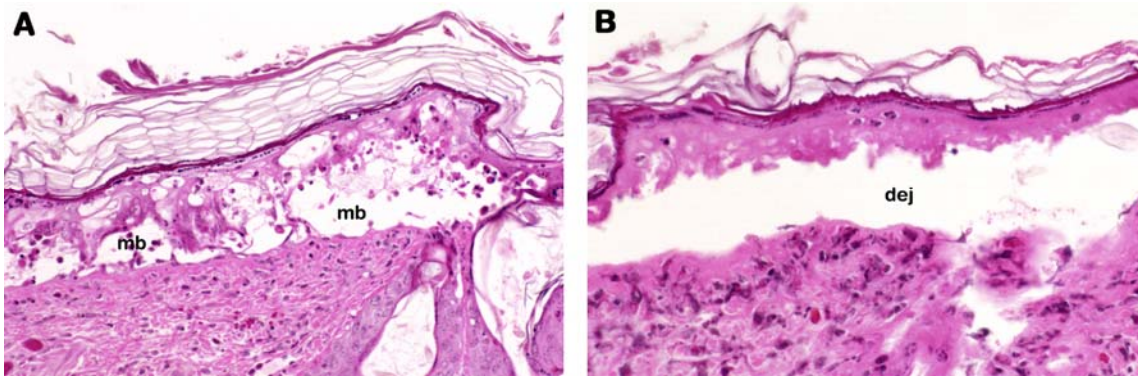


Figure 6. A, B. Light microscopy of DMSO-HD exposed HGP skin 24h postexposure with exacerbated microblisters (mb) and expansive cleavage at the dermal-epidermal junction (dej)