*International Journal of Pharmaceutics*, 48 (1988) 141–147
Elsevier



IJP 01622

# Preclinical studies of iododeoxuridine in dimethyl sulfoxide for the topical treatment of cutaneous herpes simplex virus infections

Donna J. Freeman [1], Nitin V. Sheth [1,2] and Spotswood L. Spruance [1]

[1] *Division of Infectious Diseases, Department of Medicine, and Center for Infectious Diseases, Diagnostic Microbiology and Immunology, School of Medicine, and the* [2] *Department of Pharmaceutics, College of Pharmacy, University of Utah, Salt Lake City, UT (U.S.A.)*

(Received 20 October 1987)
(Modified version received 22 March 1988)
(Accepted 18 May 1988)

*Key words*: Skin penetration; Dimethyl sulfoxide; Iododeoxyuridine; Herpes simplex virus; Topical therapy

## Summary

The topical use of iododeoxyuridine (IDU) in dimethyl sulfoxide (DMSO) for the treatment of cutaneous herpes simplex virus (HSV) infections has been advocated for over 20 years, but the clinical efficacy of this combination remains controversial. The following studies were undertaken to understand better how variable formulations of IDU in DMSO and different rates of percutaneous drug delivery affect clinical efficacy. The flux of IDU from DMSO through excised guinea pig skin was measured in Franz diffusion cells, with varying experimental conditions and formulations of IDU, DMSO and water. Contrary to predictions derived from Fick's law, the flux of IDU did not continue to increase with increasing drug concentration beyond 20% IDU. Higher rates of drug flux were measured when the in vitro experimental conditions more closely replicated those of a skin surface in vivo, that is with the receiver solution at 37 °C and skin exposed to ambient conditions. Three IDU-DMSO formulations tested in vitro were used to treat an experimental dorsal cutaneous HSV infection of guinea pigs. The flux values determined at 37 °C in the open diffusion cells showed better correlation with treatment efficacy. The results of these experiments may explain in part why very high concentrations of IDU in DMSO may not necessarily be more clinically effective beyond an optimal drug concentration and why results with different IDU-DMSO formulations have been variable.

## Introduction

Iododeoxyuridine (5-iodo-2′-deoxyuridine, idoxuridine, IDU) is a structural analogue of the natural deoxyribonucleoside thymidine. IDU was first synthesized by Prusoff in 1959 (Prusoff, 1959), and the inhibitory effect of IDU against herpes simplex virus (HSV) replication was reported shortly thereafter (Herrmann, 1961). Kaufman et al. (1962) observed that a topical solution of IDU was effective in shortening the course of experimental herpetic keratitis in rabbits. A 0.1% ophthalmic formulation of IDU was subsequently made available for clinical use in the treatment of herpes keratitis.

IDU in various topical preparations, including solutions, creams and ointment bases with concentrations of IDU ranging from 0.1% to 10%, has not demonstrated clinical efficacy in the treatment

*Correspondence*: D.J. Freeman. *Present address*: Department of Infectious Diseases and Microbiology, Graduate School of Public Health, 418 Parran Hall, University of Pittsburgh, Pittsburgh, PA 15261, U.S.A.

of mucocutaneous HSV infections (Hall-Smith et al., 1962; Burnett and Katz, 1963; Ive, 1964; Juel-Jensen and MacCallum, 1964; Kibrick and Katz, 1970). One possible explanation for this lack of efficacy, despite the in vitro activity of IDU against HSV and its utility in ocular herpes, is poor delivery of the relatively insoluble drug through the stratum corneum barrier layer of the skin to the infected cells in the basal epithelial cell layers. Juel-Jensen and MacCallum showed that if IDU delivery through the skin is guaranteed by use of a spray gun, lesion duration may be significantly reduced (Juel-Jensen and MacCallum, 1965).

Dimethyl sulfoxide (DMSO) is an organic solvent well known to enhance the penetration of many compounds through skin (Stoughton and Fritsch, 1964). Topical formulations of 1–20% IDU in vehicles of 90% to 100% DMSO have been reported to decrease lesion severity in both oral and genital recurrent HSV infections (Goldman and Kitzmiller, 1965; MacCallum and Juel-Jensen, 1966; Parker, 1977). In contrast, Silvestri et al. (1982) have found more recently that topical 30% IDU in DMSO (no water dilution) was both ineffective and painful when used to treat genital herpes.

Clinical studies using topical IDU in DMSO to treat mucocutaneous HSV infections have yielded confusing and variable results, and no study of these antiviral formulations to date has satisfactorily explained this variability. The data presented here are the results of a series of experiments with different formulations of IDU in DMSO designed to quantitate the extent to which DMSO enhances the penetration of IDU through excised guinea pig skin. The efficacy of topical IDU in DMSO against an experimental dorsal cutaneous HSV-1 infection of guinea pigs is then correlated with the in vitro findings.

## Materials and Methods

*Experimental animals and virus*

Hartley strain, outbred, female albino guinea pigs, 200–250 g each, were obtained from Charles River Breeding Laboratories, Inc., Wilmington, MA; the mean age of these animals was 17 weeks. Following virus inoculation and during treatment, animals were housed in individual cages. The virus used throughout these studies is the laboratory strain HSV type 1 (HSV-1) E115 (originally obtained from A. Nahmais, Atlanta, GA). Virus stock for inoculation of guinea pigs was grown in Vero cells and contained $5–10 \times 10^7$ pfu/ml. The concentration of IDU that inhibits HSV-1 E115 plaque formation by 50% in Vero cells is 0.18 mg/liter (Freeman et al., 1986).

*Antiviral drugs and drug formulations*

IDU and DMSO were obtained from Research Industries Inc., Salt Lake City, UT. [$^3$H]IDU was obtained from Moravek Biochemicals Inc., Brea, CA, at a specific activity of 10 Ci/mmol and concentration of 1 mCi/ml. The purity of the [$^3$H]IDU was confirmed by thin-layer chromatography on a fluorescent silica gel plate with an upper phase of ethyl acetate–formic acid–water (60 : 5 : 35) liquid phase. A single peak contained 96–97% of the activity. Radiolabeled formulations of IDU were prepared by stirring 5 $\mu$l of [$^3$H]IDU with 2 ml each of the liquid test formulations. The resultant specific activity of the formulations was 50–100 CPM/$\mu$g IDU. The radiolabeled formulations of IDU were used for the in vitro drug diffusion experiments. IDU test formulations were prepared by dissolving appropriate concentrations of IDU in stock solutions with varying concentrations of DMSO and water.

*Penetration of IDU through guinea pig skin in vitro*

Guinea pigs were sacrificed with ether and shaved closely using electric clippers, with care taken to ensure that the stratum corneum layer of the epidermis was not damaged during clipping. Full-thickness skin was removed from the dorsum by dissection, and specimens were used within an hour. Franz diffusion cells (Crown Glass, Somerville, NJ) were used in these studies. The receiver chamber was filled with 0.15 M NaCl, 0.01% thimerosal (Sigma Chemicals, St. Louis, MO). Guinea pig skin was clamped across the 1.6 cm diameter opening at the top of the cell with the stratum corneum facing upwards. The temperature of the receiver solution was either kept at

37 °C by circulating water through the outer jacket or left at room temperature (25 °C). Samples of receiver solution were withdrawn through a side port, and mixing of the receiver solution was achieved with an elongated magnetic stir bar.

At time zero, 100 μl of the IDU-DMSO solution being tested was applied to the exposed stratum corneum surface. The upper glass chamber was either sealed with paraffin film or left open to ambient conditions. Samples (200 μl) were withdrawn from the receiver chamber at intervals and assayed for radioactivity in a Beckman scintillation counter. The concentration of IDU was calculated from the CPM measured.

The penetration of IDU through the excised skin was described by a plot of the IDU concentration (mg/liter) in the receiver chamber against time (h). Drug flux ($J$, μg IDU/(cm$^2$ · h)) was calculated from the slope (mg IDU/(liter · h)), the volume of the receiver chamber (ml), and the area of the skin surface through which diffusion was taking place (cm$^2$).

*Animal inoculation and treatment regimens*

Guinea pigs were inoculated with 20 μl undiluted stock HSV-1 E115 in 4 different areas on the depilated dorsum by multiple shallow punctures following the model described by Hubler et al. (Hubler et al., 1974) and as described in detail in previous studies from this laboratory (Spruance et al., 1984). The day of inoculation was designated day 0. Treatment was begun 24 h after virus inoculation and continued for a total of 3 days (days 1–3). Treatments were given 4 times a day; 100 μl of each test formulation was applied at these times, a quantity sufficient to cover each infected area. Each drug formulation and its corresponding placebo vehicle were always tested opposite each other at the same rostral–caudal level on the guinea pig dorsum. While each animal provided two pairs of treatment sites, a given formulation or vehicle was tested only once on each animal. IDU-DMSO solutions used for treatment contained a minimum of 5% water in the vehicle to minimize the erythematous reaction which undiluted DMSO solutions may cause on contact with skin.

On day 4 after completion of the treatment regimen, the dorsum of each animal was again depilated, and each infection site was evaluated with respect to number of lesions, total lesion area, and titer of virus in skin excised from the treatment site.

*Statistical procedures*

Paired data (drug and drug vehicle) were evaluated by the Wilcoxon signed rank test. The percent differences in various measures of lesion severity effected by each formulation in comparison to its vehicle control were compared with the effects of other formulations by the Mann–Whitney rank-sum procedure. Other data were analyzed by Student's $t$-test. All probability determinations were two-tailed, and a $P$-value of $\leqslant 0.05$ was considered to be significant.

## Results

*Penetration of IDU through guinea pig skin in vitro*

The rates of percutaneous IDU delivery to the receiver solution (flux values, $J$) for each set of experimental conditions are presented in Tables 1–3. Table 1 shows the effect of varying IDU concentrations in DMSO undiluted by water on the rate of drug penetration. These experiments were performed with the top of the diffusion cell

TABLE 1

*Influence of different concentrations of IDU in DMSO on the penetration of IDU through guinea pig skin in vitro*

| IDU (%) | DMSO (%) | Flux ($J$) μg/(cm$^2$·h) | Permeability constant ($K_p \cdot 10^{-5}$) |
|---|---|---|---|
| 2 | 98 | 2.81 ± 0.61 | 14.05 ± 3.05 |
| 5 | 95 | 3.65 ± 0.62 | 7.29 ± 1.24 |
| 10 | 90 | 5.93 ± 0.83 | 5.93 ± 0.83 |
| 20 | 80 | 15.51 ± 1.81 | 7.75 ± 0.91 |
| 30 | 70 | 7.91 ± 1.14 | 2.64 ± 0.38 |
| 40 | 60 | 4.67 ± 0.33 | 1.16 ± 0.08 |

Experiments were performed with top of diffusion cell sealed, receiver solution at 25 °C. $K_p = \{J[\mu g/(cm^2 \cdot h)]\}/\{\text{concentration } [\mu g/cm^3]\} = cm/h$. For all experiments, $n = 3$, mean ± S.E.M.

TABLE 2

*Influence of experimental conditions on the flux of 2% IDU in 58.8% DMSO, 39.2% water through guinea pig skin in vitro*

| Receiver chamber temperature (°C) | Top sealed | Top open |
|---|---|---|
| 25 | 0.05 ± 0.03 | 0.53 ± 0.38 |
| 37 | 0.25 ± 0.16 | 1.26 ± 0.57 |

Flux in $\mu g/(cm^2 \cdot h)$; $n = 3$, mean ± S.D.

sealed and the receiver solution maintained at room temperature (25 °C). The flux of IDU tended to increase with increasing IDU concentration in the formulation up to 20% IDU. However, flux decreased thereafter as the IDU concentration was increased to 40%. The concentration of IDU in all these formulations is well below the saturation solubility (⩾ 80%, N.V.S., unpublished data). The permeability constants ($K_p$) tended to drop with increasing IDU concentration. A 12-fold greater permeability constant was observed with 2% IDU compared to 40% IDU.

Table 2 shows the effects of varying temperature and atmospheric conditions on the rate of penetration of IDU through guinea pig skin from a solution of 2.0% IDU, 58.8% DMSO, 39.2% water. By changing the conditions in the top chamber of the diffusion cell from sealed to open atmospheric environment, the flux of IDU was increased 10-fold at 25 °C and 5-fold at 37 °C. With the top of the cell covered, elevation of the temperature from 25 °C to 37 °C increased the flux of IDU 5-fold; with the top of the cell open to ambient conditions, a greater than 2-fold increase in the flux was observed when the temperature was elevated from 25 to 37 °C.

TABLE 3

*Influence of DMSO concentration on the flux of 5% IDU through guinea pig skin in vitro*

| IDU-DMSO formulation | | | Flux, $\mu g/(cm^2 \cdot h)$ | |
|---|---|---|---|---|
| IDU (%) | DMSO (%) | Water (%) | 25 °C, sealed | 37 °C, open |
| 5 | 66.50 | 28.50 | 0.18 ± 0.08 | 2.36 ± 0.74 |
| 5 | 76.00 | 19.00 | 0.71 ± 0.57 | 2.40 ± 0.18 |
| 5 | 90.25 | 4.75 | 2.81 ± 0.64 | 3.69 ± 0.57 |

Flux values are mean ± S.D., $n = 3$–4.

Table 3 shows the effect of changing in vitro experimental conditions on the rate of IDU penetration through guinea pig skin from three 5% IDU formulations. At 25 °C with the top of the cell sealed, the flux of IDU increased 16-fold as the DMSO in the formulation increased to 90.25% DMSO. When experiments were conducted at 37 °C with the top of the cell open to ambient conditions, the changing DMSO concentration had a much smaller effect on flux, and all 3 formulations showed similar, relatively high, rates of drug delivery. The differences in IDU flux between the 5% IDU-DMSO formulations at 37 °C were not significant.

TABLE 4

*Effect of topical therapy with IDU in DMSO on the severity of an experimental cutaneous HSV-1 infection of guinea pigs*

| Treatment group [a] | Measure of lesion severity [c] | | | | | |
|---|---|---|---|---|---|---|
| | No. of lesions | % | Total lesion area ($mm^2$) | % [b] | Lesion virus titer, $\log_{10}$ pfu/ml | % |
| 5% IDU/70% DMSO | 16 ± 4 | 60 | 19 ± 10 | 78 | 2.8 ± 0.6 | 94 |
| 70% DMSO vehicle | 40 ± 5 | | 85 ± 30 | | 4.0 ± 0.4 | |
| 5% IDU/80% DMSO | 13 ± 4 | 68 | 25 ± 11 | 68 | 2.4 ± 1.3 | 97 |
| 80% DMSO vehicle | 41 ± 9 | | 79 ± 23 | | 3.9 ± 0.4 | |
| 5% IDU/95% DMSO | 8 ± 3 | 78 | 8 ± 5 | 90 | 2.3 ± 1.0 | 98 |
| 95% DMSO vehicle | 37 ± 8 | | 79 ± 39 | | 4.0 ± 0.5 | |

[a] Composition (%, w/w) of IDU-DMSO formulations is the same as in Table 3; 70, 80, and 95% DMSO vehicle refers to stock solutions of DMSO, water used for placebo treatment and, with addition of 5% IDU, for active treatment.
[b] Percent reduction with treatment; $P = 0.01$–$0.02$.
[c] Mean ± S.D., $n = 8$.

*Efficacy of topical IDU-DMSO in the dorsal cutaneous guinea pig model*

Twelve animals were inoculated with HSV-1 E115 as described above. The same 3 formulations of 5% IDU in DMSO described in Table 3 were assessed, and these results are presented in Table 4. Each of the IDU formulations was significantly more effective than placebo treatment ($P = 0.01–0.02$). When the relative efficacy of each formulation was compared to the others, the differences between 5% IDU in 70% DMSO and in 95% DMSO were significant for reduction in lesion number and lesion area ($P = 0.01$ and 0.02, respectively), while the IDU formulations in 80% DMSO and 95% DMSO were significantly different from each other only for reduction in lesion area ($P = 0.002$). There were no significant differences in efficacy between 5% IDU in the 70% and 80% DMSO vehicles.

## Discussion

These studies demonstrate that several variables affect percutaneous drug delivery enhanced by DMSO, both in the Franz cell diffusion studies and in the living animal model. We found that the flux of IDU tended to increase with drug concentration from DMSO vehicle up to 20% IDU, but then decreased as the drug concentration was further increased to 40%. The saturation solubility of IDU in DMSO exceeds the concentrations of IDU studied by two-fold.

Fick's law of diffusion states that the rate of penetration per unit area ($J$) is proportional to a permeability constant ($K_p$) times the penetrant concentration ($C$) (Scheuplein and Blank, 1971). According to this principle, we would expect the flux of IDU to increase until the saturation concentration of IDU in DMSO is reached. We found that $K_p$ tended to decrease as the concentration of IDU increased (Table 1), indicating that $K_p$ is not invariant with drug concentration. This observation shows that Fick's law alone is not sufficient to interpret the experimental data. Similar results with DMSO as the solvent and *O*-ethyl S-2-diisopropyl-aminoethyl methylphosphonothiolate (VX) as the penetrant have been observed by Creasy et al. (1978). The penetration-enhancing properties of DMSO are well recognized (Stoughton and Fritsch, 1964). It has been postulated that DMSO interacts with the stratum corneum either by causing it to swell (due to DMSO's hygroscopic properties) or by solubilizing and eluting components from the horny structure (Bergstrom-Kurihara et al., 1986). In either case, the diffusional resistance of the stratum corneum would be reduced (Creasy et al., 1978).

One assumption we have made in calculating $K_p$ for our data is that the IDU concentration applied to the stratum corneum remained constant. In the Franz cell, conditions on the donor side, particularly with a single bolus dose of drug, are not constant; this dynamic aspect is even more of a factor in topical therapy on living skin. The changing $K_p$ which we found is likely to be due to a combination of effects, including the possibility that the effect of DMSO on permeability at high concentrations of IDU wanes because the drug is so soluble that it acts as a diluent of DMSO.

The data in Table 2 show the effect of different experimental conditions on the measurement of flux in vitro. Raising the temperature of the receiver chamber increased the rate of penetration of IDU by 5 times in the sealed cells and greater than two-fold in the open cells. One reason for the increase in flux at higher temperature is the increasing molecular diffusivity of the drug, which is determined in part by temperature (Stoughton, 1965; Poulsen, 1973). In other studies with different antivirals, we have noted a similar temperature effect on drug flux (Spruance et al., 1985; Sheth et al., 1986).

When the flux was measured from the same 2% IDU-DMSO formulation at a set temperature, the exposure of the skin surface to ambient conditions increased the rate of drug delivery by five-fold and ten-fold for 37 °C and 25 °C, respectively. The reasons for this increase are not clear, but lower ambient humidity in the top chamber of open cells may allow water to evaporate from the IDU-DMSO formulation, increasing the effective concentrations of both IDU and DMSO on the surface of the skin and hence, creating a steeper diffusion gradient across the skin.

Examination of the results in Table 3 shows that in experiments done at 25 °C in sealed cells, the flux of 5% IDU increases as the DMSO concentration increases. This suggests that higher concentrations of DMSO in the formulation enhance IDU penetration and is consistent with previous reports (Stoughton and Fritsch, 1964). However, when the same formulations were studied at 37 °C with the cells open to ambient conditions, the rates of penetration were not significantly different from each other. Again, this may be due in part to water evaporation from the skin surface.

These data emphasize an important point about the use of in vitro skin diffusion results to predict in vivo efficacy. In the guinea pig model of dorsal cutaneous HSV infection, all three 5% IDU-DMSO formulations were effective topical therapies, with the highest DMSO concentration formulation showing a slight advantage. We have previously shown that efficacy in this model is in part correlated with drug flux measured in Franz cells (Freeman et al., 1986; Freeman and Spruance, 1986). When the results in Tables III and IV are examined, it appears that the measurement of drug flux at 37 °C with the skin surface exposed to ambient conditions is the better in vitro system for predicting in vivo efficacy, perhaps because conditions more closely resemble those on the living animal's infected back.

While the measurement of drug flux is one useful correlate of therapeutic efficacy, the passage of antiviral drug through the epidermis, per se, is clearly not the desired pharmacokinetic endpoint. That endpoint is a concentration of antiviral drug inside infected cells that is greater than the virus inhibitory concentration of the agent. Based on our observed correlations of in vivo efficacy in a guinea pig model of HSV infection (Freeman and Spruance, 1986), we hypothesize that antiviral drug delivered through the skin periodically in high concentration to infected basal epidermal cells leads to the accumulation of nucleoside antivirals intracellularly. Phosphorylation of nucleosides creates a molecular "sink" inside infected cells so that drug is retained in the cell after the concentration in the intercellular space has fallen to zero.

We have also measured drug retention in the stratum corneum by assay of antiviral in adhesive tape strippings, and this measure also correlates with in vivo efficacy (Sheth et al., 1987). There is some evidence from our data that peak stratum corneum IDU levels correlate with clinical efficacy, but measurements at later time points (6–24 h) may show continuing high concentrations of retained drug but ineffective treatment of the clinical disease. This data suggests that some amount of drug becomes trapped in the stratum corneum, and that this reservoir does not release drug to reach locally infected cells and therefore is of no therapeutic significance.

The present studies have shown that IDU in a DMSO vehicle penetrates skin well in vitro, and this formulation likely allows the drug to reach the site of virus replication in the basal cell layers of the epithelium rapidly. Optimum concentrations of IDU and DMSO in the formulation should provide maximum penetration of the drug through the stratum corneum, which in turn may provide better therapeutic efficacy. Our studies suggest that in order to achieve favorable results in clinical trials with IDU and DMSO, it may be important to avoid concentrations of IDU higher than 20%.

IDU in DMSO formulations are effective in the treatment of cutaneous HSV-1 infections in the animal model. Further clinical studies with topical IDU in DMSO are under way to examine the therapeutic efficacy of a formulation based on these studies in the treatment of recurrent herpes labialis.

## Acknowledgements

The authors are grateful for the excellent technical assistance of M.B. McKeough. These studies were supported in part by a grant from Research Medical, Inc., Salt Lake City, UT.

## References

Bergstrom-Kurihara, T., Flynn, G.L. and Higuchi, W.I., Physicochemical study of percutaneous absorption enhancement by dimethyl sulfoxide: kinetic and thermodynamic determinants of dimethyl sulfoxide mediated mass transfer of alkanols. *J. Pharm. Sci.*, 75 (1986) 479–486.

Burnett, J.W. and Katz, S.I., A study of the use of 5-iodo-2′-deoxyuridine in cutaneous herpes simplex. *J. Invest. Dermatol.*, 40 (1963) 7–8.

Creasy, N.H., Battensby, J. and Fletcher, J.A., Factors affecting the permeability of skin: the relation between in vivo and in vitro observations. *Curr. Prob. Dermatol.*, 7 (1978) 95–106.

Freeman, D.J., Sheth, N.V. and Spruance, S.L., Failure of topical acyclovir in ointment to penetrate human skin. *Antimicrob. Agents Chemother.*, 29 (1986) 730–73.

Freeman, D.J. and Spruance, S.L., Efficacy of topical treatment for herpes simplex virus infections: predictions from an index of drug characteristics in vitro, *J. Infect. Dis.*, 153 (1986) 64–70.

Goldman, L. and Kitzmiller, K.W., Topical 5-iodo-2′-deoxyuridine in dimethylsulfoxide (DMSO), a new treatment for severe herpes simplex. *Ohio State Med. J.*, 61 (1965) 532–533.

Hall-Smith, S.P., Corrigan, M.J. and Gilkes, M.J., Treatment of herpes simplex with 5-iodo-2′-deoxyuridine. *Br. Med. J.*, 2 (1962) 1515–1516.

Herrmann, E.C., Plaque inhibition test for detection of specific inhibitors of DNA containing viruses. *Proc. Soc. Expt. Bio. Med.*, 107 (1961) 142–145.

Hubler, W.R. Jr., Felber, T.D., Troll, D. and Jarratt, M., Guinea pig model for cutaneous herpes simplex virus infection. *J. Invest. Dermatol.*, 62 (1974) 92–95.

Ive, F.A., A trial of 5 iodo-2′-deoxyuridine in herpes simplex. *Br. J. Dermatol.*, 76 (1964) 463–464.

Juel-Jensen, B.E. and MacCallum, F.O., Treatment of herpes simplex lesions of the face with idoxuridine: results of a double-blind controlled trial. *Br. Med. J.*, 2 (1964) 987–988.

Juel-Jensen, B.E. and MacCallum, F.O., Herpes simplex lesions of face treated with idoxuridine applied by spray gun: results of a double-blind trial. *Br. Med. J.*, 1 (1965) 901–903.

Kaufman, H.E., Martola, E-L and Dohlman, C., Use of 5-iodo-2′-deoxyuridine (IDU) in treatment of herpes simplex keratitis. *Arch. Ophthalmol.*, 68 (1962) 235–239.

Kibrick, S. and Katz, A.S., Topical idoxuridine in recurrent herpes simplex, with a note on its effect on early varicella. *Ann. N.Y. Acad. Sci.*, 173(1) (1970) 83–89.

MacCallum, F.O. and Juel-Jensen, B.E., Herpes simplex virus skin infection in man treated with idoxuridine in dimethyl sulphoxide. Results of double-blind controlled trial. *Br. Med. J.*, 2 (1966) 805–807.

Parker, J.D., A double-blind trial of idoxuridine in recurrent genital herpes. *J. Antimicrob. Chemother.*, 3, Suppl. A (1977) 131–137.

Poulsen, B.J., Design of topical drug products: biopharmaceutics. In *Drug Design, Vol. 4*, Academic, Inc., New York and London, 1973, pp. 149–192.

Prusoff, W.H., Synthesis and biological activities of iododeoxyuridine, an analog of thymidine. *Biochim. Biophys. Acta*, 32 (1959) 295–296.

Scheuplein, R.J. and Blank, I.H., Permeability of the skin. *Physiological Rev.*, 51 (1971) 702–747.

Sheth, N.V., Freeman, D.J., Higuchi, W.I. and Spruance, S.L., The influence of azone, propylene glycol and polyethylene glycol on in vitro skin penetration of trifluorothymidine. *Int. J. Pharm.*, 28 (1986) 201–209.

Sheth, N.V., McKeough, M.B. and Spruance, S.L., Measurement of the stratum corneum drug reservoir to predict the therapeutic efficacy of topical iododeoxyuridine for herpes simplex virus infection. *J. Invest. Dermatol.*, 89 (1987) 598–602.

Silvestri, D.L., Corey, L. and Holmes, K.K., Ineffectiveness of topical idoxuridine in dimethyl sulfoxide for therapy for genital herpes. *J. Am. Med. Assoc.*, 248 (1982) 953–959.

Spruance, S.L., McKeough, M.B. and Cardinal, J.R., Penetration of guinea pig skin by acyclovir in different vehicles and correlation with the efficacy of topical therapy of experimental cutaneous herpes simplex virus infection. *Antimicrob. Agents Chemother.*, 25 (1984) 10–15.

Spruance, S.L., Freeman, D.J. and Sheth, N.V., Comparison of topically applied 5-ethyl-2′-deoxyuridine and acyclovir in the treatment of cutaneous herpes simplex virus infection in guinea pigs. *Antimicrob. Agents Chemother.*, 28 (1985) 103–106.

Stoughton, R.B. and Fritsch, W., Influence of dimethyl-sulfoxide (DMSO) on human percutaneous absorption. *Arch. Dermatol.*, 90 (1964) 512–517.

Stoughton, R.B., Percutaneous absorption: influence of temperature and hydration. *Arch. Environ. Health*, 11 (1965) 551–554.