CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

20-941

MICROBIOLOGY REVIEW

JUL 6 1998

REVIEW FOR HFD-540

OFFICE OF NEW DRUG CHEMISTRY
MICROBIOLOGY STAFF HFD-805
Microbiologist's Review # 1 of NDA 20-941
June 29, 1998

A. 1. **APPLICATION NUMBER:** NDA 20-941

APPLICANT: LIDAK Pharmaceuticals
11077 North Torrey Pines Road
La Jolla, CA 92037

2. **PRODUCT NAME:** LIDAKOL®

3. **DOSAGE FORM:** LIDAKOL® (*n*-docosanol 10% cream) topical cream in 2, 5, and 15 gram epoxy-lined aluminum tubes, and in 1 gram laminate pouch.

4. **METHOD OF STERILIZATION:** None (non-sterile product). The product is preserved with benzyl alcohol (2.7%).

5. **PHARMACOLOGICAL CATAGORY and/or PRINCIPLE INDICATION:** The drug product is indicated for the treatment of oral-facial herpes simplex.

6. **DRUG PRIORITY CLASSIFICATION:** S

B. 1. **DATE OF INITIAL SUBMISSION:** November 26, 1997

2. **DATE OF CONSULT:** April 23, 1998

3. **RELATED DOCUMENTS:** (none)

4. **ASSIGNED FOR REVIEW:** April 28, 1998

C. **REMARKS:** The n-docosanol 10% cream intended for commercial distribution is the third generation formulation, and is the subject of the NDA.

APPEARS THIS WAY
ON ORIGINAL

D. CONCLUSIONS:

The application is recommended as "approvable" for issues concerning microbial limits, preservative effectiveness, and microbiology issues relating to drug product stability. Specific comments are provided in "E. REVIEW NOTES" and deficiencies are provided in "Microbiologist's Draft of Letter to the Applicant."

/S/

6/29/98

Neal Sweeney, Ph.D.

/S/ 7/6/98

cc:

Original NDA 20-941
HFD-540/ Division File
HFD-540/CSO/K.D. White
HFD-805/Consult File/N. Sweeney

Drafted by: Neal Sweeney, June 29, 1998
R/D initialed by P. Cooney June 29, 1998

APPEARS THIS WAY ON ORIGINAL

JUL 13 1998

MICROBIOLOGIST'S REVIEW
DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)

NDA 20-941

REVIEWER: N. Biswal
CORRESPONDENCE DATE: 12/19/97
CDER RECEIPT DATE: 12/22/97
REVIEW ASSIGN DATE: 1/21/98
REVIEW COMPLETE DATE: 7/10/98

SPONSORS: Lidak Pharmaceutical
11077 North Torey Pines Rd
La Jolla, CA 92037

SUBMISSION REVIEWED: New (N-000, for Consultation from HFD-540)

DRUG CATEGORY: Antiviral

INDICATION: Recurrent Oral-Facial Herpes Simplex

DOSAGE FORM: Topical, 10% Cream

PRODUCT NAMES:

a. PROPRIETARY: LIDAKOL®

b. NONPROPRIETARY: Behenyl alcohol

c. CHEMICAL: n-Docosanol

STRUCTURAL FORMULA: $CH_3(CH_2)_{20}CH_2OH$

Mol. wt.: 326.61

Mol. Formula: $C_{22}H_{46}O$

SUPPORTING DOCUMENTS: [redacted]

INTRODUCTION: In this New Drug Application (NDA) the sponsor has requested approval of Lidakol® cream for the treatment of recurrent oral-facial herpes simplex virus (HSV) infection. Each gram of Lidakol cream contains 100 mg of n-docosanol (the active ingredient), [redacted] of sucrose stearate, [redacted] mineral oil [redacted] propylene glycol, [redacted] water. The sponsor has suggested that treatment should be initiated as soon as the oral-facial herpes lesions appear by topically applying the cream 5 times/day for a maximum of 10 days.

Herpes simplex viruses are members of Herpesviridae family capable of causing a variety of acute, latent and recurrent infections in man and animals. These viruses, belonging to the alpha herpesvirinae subfamily, have a variable host range, rapid growth in cell culture with relatively short reproductive cycle and efficient destruction of infected cells. There are two serotypes of HSV, HSV-1 and HSV-2, formally designated under ICTV rules as human herpesviruses 1 and 2. HSV-1 is more frequently (but not exclusively) associated with the primary infection of the mouth and lip, while HSV-2 is invariably associated with genital diseases.

The Virion: HSV is composed of four elements: (I) a core containing viral DNA is electron opaque, (ii) an icosahedral capsid surrounding the core, (iii) an amorphous tegument surrounding the capsid, and (iv) an outer envelope with spikes on its surface. Purified HSV-1 contains at least 33 structural proteins which are made after virus infection. At least 11 of these proteins (gB, gC, gD, gE, gG, gH, gI, gJ, gK, gL and gM) are glycosylated, and are on the surface of the virion. Four of these glycoproteins (gB, gD, gH, and gI) may be essential for virus attachment and entry in cells cultured *in vitro*.

The genome of HSV is a linear double stranded DNA of about 150 kb pairs with a G+C content of 68% for HSV-1 (ρ_{CsCl} = 1.725 g/ml) and 69% for HSV-2 (ρ_{CsCl} = 1.727 g/ml). It consists of two unique long (L) and short (S) covalently linked sequences, each bounded by inverted repeats. Inversion of the L and S components leads to the production of four linear isomers of the viral DNA molecule. While the entire viral genome has been sequenced, the significance of the repeated sequences and genome inversion to the biology of HSV remains to be elucidated. The genetic maps of HSV-1 and HSV-2 are largely collinear, but they differ in restriction endonuclease cleavage sites and in apparent sizes of viral proteins. At the DNA levels, various strains of either HSV-1 or HSV-2 also differ from each other due to base substitution and variability in the number of repeated sequences present in a number of regions of the viral genomes. Restriction endonuclease polymorphism has been extensively used in several epidemiologic studies of HSV infection of and transmission in the human population.

Viral Replication: Infection of HSV ordinarily involves a lytic cycle of replication in the epithelial cells followed by a latent cycle in the neuronal cells. In the permissive cells, the replicative cycle is initiated by attachment of the virus surface proteins to cell surface heparan sulfate proteoglycans followed by sequential interactions between viral glycoproteins and corresponding cellular receptors. Depending upon the viral strains, this cooperative interactions between viral and cell components may lead to fusion of the virus envelope proteins with the cellular plasma membrane which internalizes the nucleocapsids into the cytoplasm of the cells. Thus HSV attachment to the cell surface may activate a multi-step penetration process mediated by a number of viral surface glycoproteins which leads to fusion of the viral envelope and the cell plasma membrane. While it is recognized that there are multiple attachment pathways available to HSV, ability to assign a particular viral surface protein (glycoprotein) the

responsibility for attachment to specific cell surface receptor(s) is yet to be clearly defined. Extensive use of monoclonal antibodies, liposomes containing viral proteins, and temperature sensitive mutants have implicated glycoproteins gB, gD, gH, gK and gL in HSV-induced cell fusion and in virus entry. Upon entry into the host cell, viral capsids are transported to the nuclear pores and the viral DNA is released into the nucleoplasm. Viral DNA is transcribed in the nucleus and all viral proteins are synthesized in the cell cytoplasm.

HSV gene expression and regulation: In general, HSV gene expression may be divided in to three sequentially expressed kinetic classes: **α (immediate early), β_1 and β_2 (delayed early), and γ_1 and γ_2 (late)** based on the time of expression after virus infection. α genes are the first to be expressed in the absence of viral protein synthesis. There are 5 α proteins: infected cell polypeptides (ICPs) 0, 4, 22, 27, and 47. These proteins, with the exception of α47, have been shown to have regulatory functions, and functional α proteins are required for the expression of subset β and γ gene products.

Delayed early β_1 and β_2 genes are expressed between 5 and 7 hours post infection. Large component of the viral ribonucleotide reductase (ICP6) and the major DNA binding protein (ICP8) are the examples of β_1 gene products. β_2 proteins include, among others, the viral thymidine kinase (TK) and DNA polymerase whose appearance signals the onset of viral DNA synthesis and viral nucleic acid metabolism. These two virus-coded enzymes, TK and DNA pol, have been quite essential for the development of a number of nucleoside analogues such as acyclovir and penciclovir for selective antiviral action against HSV infection.

Late γ_1 transcripts are detected between 7 and 8 hour postinfection and are only minimally affected by inhibitors of DNA synthesis. **γ_2 transcripts** are detected late in infection and are not expressed in the presence of inhibitors of viral DNA synthesis. Late in infection, structural components of the capsid begin to accumulate, different viral components are assembled and newly synthesized virions are released.

After the initial lytic infection of the epithelial cells, HSV is capable of establishing latent infections in neuronal cells with subsequent recurrences. It is still not clear how the virus escapes from its latent state to initiate a variety of complex pathogenic processes in different organs ranging from simple fever blister to fatal encephalitis. Animal models have become useful for investigation of the intricate molecular mechanism(s) of viral latency, including establishment, maintenance, and disruption of the latent state to reactivate infectious virus under various experimental conditions such as trauma, surgery, irradiation, and exposure to immunosuppressive drugs.

Diagnosis of HSV infections is based on clinical observations (lesions and symptoms) along with confirmation by virologic tests, which are designed to detect the presence of infectious virus, viral specific proteins (antigens), nucleic acids or antibodies. While none of the currently available methods of viral detection methods has been formally standardized, quite a number of highly specific, sensitive and rapid detection methods and materials are now available to the clinical laboratories.

The unequivocal diagnosis of HSV infection is the **detection of infectious virus** through cell culture. However, it takes relatively long time to detect the cytopathic effect in infected cells. In addition, a number of adverse conditions of virus collection, storage, transportation are known to seriously influence the titer of infectious virus in clinical specimens.

Immunodetection of viral proteins through immunofluorescence, radioimmunoassay or enzyme immunoassay methods has become quite sensitive and specific in clinical laboratories. However, the optimal sensitivity of these methods require collection and processing of the clinical specimens from fresh lesions. [redacted] HSV ELISA test [redacted] is one of such methods which utilizes a polyclonal anti-HSV rabbit serum to capture HSV antigens directly in the clinical specimens which is subsequently detected by a biotinylated monoclonal anti-HSV antibody in an enzyme-linked immunosorbent assay (ELISA). The colorimetric assay results are read through a spectrophotometric [redacted] While this entire test takes about 4 hours, it is not as sensitive as the culture method when test material contains < 200 plaque forming units (PFU) of virus and it does not distinguish HSV-1 from HSV-2.

Identification of viral nucleic acids (DNA or mRNA) through hybridization (and PCR amplification of conserved DNA sequences, when necessary) are quite sensitive and specific. Usually radiolabeled or biotinylated nucleic acid probes are used to specifically hybridize with the corresponding viral DNA or RNA (present in clinical specimens) in southern blot or *in situ* hybridization procedures. While these methods are more sensitive than the virus culture method, precise standardized procedures of PCR amplification and hybridization are required to avoid false positive results.

Serologic methods for the diagnosis of either HSV include testing of serum specimens for antibodies (IgG or IgM) to the corresponding viruses or viral proteins. The most important aspect of assays for viral antibodies is to determine the immune status of the patients whose history of virus infection is not known or uncertain. While a number of classic methods (such as virus neutralization) are still being used, commercially available methods involving enzyme immunoassay or radioimmunoassay are highly sensitive and specific enough to be routinely used to detect the presence of viral antibodies in patient's serum.

Treatment of HSV infections have been facilitated with the approval of nucleoside analogues such as acyclovir and its prodrug valacyclovir, penciclovir and its prodrug famciclovir. In this NDA the sponsor has claimed that the new drug Lidakol may prove to be an alternative to the nucleoside analogues because of its different mechanism of antiviral action involving the initial stage(s) of virus replication. To substantiate this claim the sponsor has provided the following information on the preclinical antiviral activity of Lidakol.

A. MECHANISM OF ANTIVIRAL ACTION

The sponsor has introduced Lidakol as an antiviral without any direct virucidal activity. To exert antiviral activity, the host cells must be pre-exposed before viral infection to massive concentrations [redacted] of its active ingredient, n-docosanol, along with a surfactant such as [redacted] for as long as 24 hours. The mode of antiviral action of n-docosanol may be related to its ability to "interfere with one or more steps of viral entry" in to the host cell. Preliminary results have provided suggestive evidence that the drug apparently does not affect the initial step of virus binding to the cell surface but entry of virus particles into the target cell cytoplasm may be inhibited through interference with the normal process of virus and cell fusion. However, the following review of the reports submitted by the sponsor (designated as Lidak Reports) shows that the precise mechanism by which n-docosanol exerts its antiviral activity remains to be elucidated.

A.1. To determine that n-docosanol was not directly virucidal, HSV-2 (strain MS) was incubated with a wide range of concentrations n-docosanol suspended [redacted] for 1

to 4 hours at 37 C. As determined by plaque reduction assays in monolayers of Vero cells, the infectivity titers of such n-docosanol treated HSV-2 preparations were not significantly different than those of the untreated control HSV-2 preparations (Lidak Report 102). On the other hand, it was discovered that the host cells must be preincubated with massive amounts of n-docosanol for extended period of time to demonstrate any antiviral activity (Lidak Report 105). As a result, the sponsor has included a number of Lidak Reports which document the optimal conditions for n-docosanol to become effective as an antiviral agent.

A.2. Requirements for Optimal Antiviral Activity by n-Docosanol

A.2.1. Preincubation of Host Cells Prior to Virus Infection

To determine the optimal time of addition of n-docosanol to host cells, a series of experiments were conducted and submitted (in Lidak Reports 102 to 106) earlier in IND supplements for review. In Lidak Report 105 the temporal relationship of target cell treatment and antiviral activity of Lidakol was demonstrated. Vero cells ($6X10^5$) cells in 1.8 ml of growth medium per well) were plated in 35 mm wells of 6 well plates to form a monolayer at 37 C. n-Docosanol (18 mM, 6 mg/ml) suspended in [redacted] was added to the monolayers of Vero cells at 24, 6, 4, 2, 1, 0.5 or 0.0 hours prior to infection with about 175 plaque forming units (PFU) of HSV-2 (MS strain). As controls, acyclovir (ACV, 2 µg/ml) [redacted] was added at the time of HSV-2 infection. Cultures were incubated further at 37 C for 42-44 hours, washed, fixed, stained and the number of plaques formed in treated wells was compared with the number of plaques formed in untreated wells, and the percent inhibition was determined. Results presented in Fig 5 of Lidakol Report 105 (Vol 2.10) demonstrated that addition of 18 mM n-docosanol to Vero cells at the time of HSV-2 infection (0 hour) resulted in only partial inhibition (~26%) of HSV-2 infection which was not significantly altered even if the cells were pretreated with n-docosanol for 6 hours. Preincubation of the host cells with Lidakol for 24 hours, however, resulted in 68% inhibition of plaque formation by HSV-2. Pretreatment of cells with [redacted] alone for 24 hours did not alter the plaque forming ability of HSV-2 while addition of ACV at the time of virus infection inhibited HSV-2 infection by 78%. From these results the sponsor has concluded that the optimal antiviral effect of the test drug was achieved when the target Vero cells are pre-treated with the test drug for more than 6 hours.

A.2.2. Effect of Vehicles for n-Docosanol for Optimal Antiviral Activity

The sponsor has investigated the possibility of a better vehicle for the test drug that would increase its antiviral activity. [redacted]

Table 1. Antiviral Activity of Different Formulations of n-Docosanol against HSV-1 Infections*.

Experiment	Formulation	Concentration (mM)	Preincubation (Hours)	% Inhibition of Plaques
Report 107			0	36
			24	68
LK 229			6	27
			24	84
			6	77
			24	>99
LK 303			0	22
			3	43
			6	40
			0	55
			3	66
			6	74

* Adapted from Table 1, page 4 of Lidak Report 120, Vol 2.10)

These results led the sponsor to conclude that Formulation E containing n-docosanol suspended in the [redacted] is superior to the formulations containing [redacted] In addition, as discussed later in Section A.5.4 below, the sponsor has also demonstrated that the median inhibitory concentrations (ED_{50}) of n-docosanol against HSV-2 (MS strain) replication by n-docosanol (as determined by plaque reduction assays in Vero cells) was [redacted]

Comments: [redacted]

2. The sponsor has not explained the contradictory observation by independent investigator [redacted] who has reported that "the solubilizing medium, [redacted] was not a factor in contributing to the antiviral effect" of n-docsanol (page 067, paragraph 5, Vol 2.10)

3. It should be pointed out that in many of the manuscrpts, investigators have used several terminologies such as ED_{50}, ID_{50} and EC_{50} interchangeably throughout this submission to define median inhibitory concentrations of n-docosanol against virus infections *in vitro*.

A.2.3. Effect of Host Cells

While investigating the relatedness of the metabolic conversion and anti-viral activity of n-docosanol, the sponsor has reported that monkey kidney (Vero) cells were superior to

bovine kidney (MDBK) cells in that n-docosanol was 3- to 4-fold more effective in inhibiting HSV-2 induced plaques in Vero cells (ED_{50} = 5.6 mM) than in MDBK cells (ED_{50} >20.0 mM) (see Section A.5). In addition, evidence has been presented to claim that n-docosanol could exert anti-HSV activity much more efficiently in human fibroblasts lines (cells derived from normal skin (ATCC #CRL-1900), fetal foreskin (Hs68, ATCC #CRL-1635), and fetal lung (HFL-1, ATCC #CCL-159) than in Vero cells possibly because all human fibroblast cell lines were superior to Vero cells in incorporating more n-docosanol (see Section A.5).

Comment: 1. Cells of human origin are the natural hosts for HSV infection. With the discovery that n-docosanol is more active in human cells to inhibit HSV infection, it is not clear why monkey kidney Vero cells continued to be used in most of the subsequent experiments.

A.2.4. Timing of Removal of n-Docosanol from Host Cells

In a previously submitted LIDAK Report 107, the sponsor had confirmed that for optimal anti-HSV activity, the host Vero cells must be preincubated with n-docosanol for about 24 hours. More importantly, it was reported that the test drug must be present at the time of virus infection and may be removed from culture 16 hours post-viral addition without loss of antiviral activity. These experiments were conducted by using Vero cells incubated in the presence of test drug at 37 C pre- or post-infection with HSV-2 (MS strain). However, with a new hypothesis (by the sponsor) that n-docosanol must be modified to a metabolically active form which would "adequately inhibit replication of the initial round of attached virus," these results "seemed incongruous" (pages 123-124, Vol 2.10). A series of new experiments were designed to determine the half-life of n-docosanol in the host cells.

In the new Lidak Report 120 (which was not submitted for review before), the sponsor has described a number of experiments (in pages 124-140) which determined the time when n-docosanol could be removed from host cells (relative to the time of HSV infection) without loss of antiviral activity. Experiment LK253 (Lidak Report 120, Vol 2.10, page 130) was conducted to evaluate the effect on HSV-1 plaque production when n-docosanol was removed either before virus infection or following 2 hrs of chilling at 4 C with the virus. Vero cells were preincubated for 24 hours with 18 mM n-docosanol at 37 C, virus (unspecified amount) was added, and cultures were chilled for 2 hours at 4 C. Growth medium containing unattached virus and unincorporated drug suspension was then removed and cultured cells were transferred to 37 C for 42-44 hrs, washed, stained and HSV-1 induced plaques were enumerated by using a dissecting microscope. Results of this experiment showed that in cells treated with 18 mM n-docosanol, plaque reduction was inhibited by 99%; if the drug was removed prior to virus addition and virus was not removed, plaque production was inhibited by 33%. However, if the virus was removed following the 2 hr incubation at 4 C, plaque formation was reduced by 82%. Thus the presence of n-docosanol in the culture media was not required at the time of HSV addition.

In another series of experiments (LK301) the lifetime of the antivirally active species of n-docosanol in Vero cells was determined. n-Docosanol (9 mM) was incubated with Vero cells for 21 to 27 hours prior to HSV addition. Drug was removed at various time intervals (6, 3, 2, 1 and 0 hr) before addition of the Macintyre strain of HSV-1. During the interim between drug removal and virus addition, cells were incubated at 37 C. Unspecified amount of virus was added to cells, and after two hours of incubation at 4 C unattached virus was removed. The results of these experiments indicated that the active drug species turned over with a half-life of approximately 3 hours, and no viral inhibition was observed if virus was added 6 hours after

drug removal. The sponsor has concluded that these studies are consistent with other experimental results that show that antiviral activity may be dependent on drug metabolism, and suggest a limited lifetime for the antivirally active species.

Comments: 1. At 37 C physiologic temperature, host Vero cells must be pre-exposed to n-docosanol for more than 6 hours and must be present continuously for 16 hours before the drug could be removed without loss of antiviral activity (Lidak Report 107). In the current series of experiments it was determined that by simply lowering the temperature to 4 C during virus attachment, the presence of the drug during virus infection was not needed and that the antivirally active form of n-docosanol had a half-life of approximately 3 hours (Lidak Reports 118 and 120). If indeed the putative antivirally active form of the test drug was formed during the prolonged preincubation, the sponsor has not explained how Formulation E[redacted] successfully inhibited 55% of HSV-plaque formation without any preincubation of the host cells (see Table 1 above).

2. The sponsor has not provided any information on concentration of the virus (in terms of the multiplication of infection) used in these experiments.

A.3. Effect of n-Docosanol on HSV attachment to host Vero cells

Initial experiments demonstrated that radioactive n-docosanol [redacted] was irreversibly incorporated into cultured Vero cells in a concentration- and time-dependent manner although the efficiency of n-docosanol uptake was less than 0.1% of the radiolabel added to culture. (Lidak Reports 106, 107 and 109). In order to determine whether the test drug interferes with the attachment of HSV to host cells, the following experiment was conducted.

Vero cells (2.5×10^5 per 16-mm culture dish) were incubated with n-docosanol [redacted] suspended in [redacted] for 24 hours before addition of [redacted] labeled HSV-1 (Macintyre strain, 5×10^5 PFU) for 1.0 or 2.5 hours. As controls, the cells were incubated with either the vehicle [redacted] or only the growth media. The amount of cell-associated radioactivity was determined by scintillation counting after extensive washing to remove unattached virus particles. Vero cells without n-docosanol preincubation bound only [redacted] labeled virus after 1 hour and [redacted] after 2.5 hours after virus infection. This binding was not changed, however, either by the concomitant presence of n-docosanol or the vehicle control.

This study was extended to determine whether the test drug inhibited the early or late stages of the viral replicative cycle. Experiments described Lidak Report 109 were designed to determine the effect of n-docosanol [redacted] on (I) plaque production in the primary culture; (ii) virus production as based on a secondary plaque reduction assay; (iii) viral core and envelope protein production as determined by ELISA on culture fluid using rabbit anti-HSV antibodies; and (iv) production of the immediate-early protein ICP4 in cells as assayed by immunofluorescence using a murine monoclonal antibody specific for ICP4.

1. Vero cells were incubated separately with n-docosanol at 0.75, 1.5, 3.0, 7.5, and 15.0 mM concentrations with [redacted] vehicle, with the vehicle as control, 24 hours before the addition of HSV-2. When cultures were assayed for plaque production, n-docosanol exhibited a dose-dependent inhibition of HSV plaque formation, plaque size, and production of infectious particles with an average ED_{50} value of 6 mM.

2. Vero cells (3 x 10^5/ml) were plated in 16-mm wells in medium alone or in medium containing n-docosanol in [redacted] vehicle. After 24 hours incubation, [redacted] acyclovir-resistant HSV-2 (MS strain) were added to the cultures. The cultures were incubated for an additional 44 hours after which the culture fluids were harvested to assay for infectious virus production in secondary plaque assays. Monolayers were fixed and stained and scored for the presence of direct plaques. The drug was demonstrated to be as effective against an acyclovir-resistant strain as against wild-type HSV-2 (MS strain). The control suspension had no inhibitory activity against either virus type, and acyclovir (5 µg/ml, added concurrently with HSV) exhibited pronounced inhibition against wild-type HSV-2.

3. The amount of HSV-1 core and envelope protein antigens were determined by ELISA of primary culture fluids obtained 44 hours following infection with HSV-1 from untreated Vero cell cultures or from Vero cells treated with 15 mM n-docosanol or the [redacted] vehicle control only. The values are expressed as means of duplicate determinations for each culture condition. The result show that there was about 80% reduction in the synthesis of viral core and evelope (late) proteins by n-docosanol-treated cell cultures.

4. Vero cells (6 x 10^5/ml) were plated in 35-mm dishes in the presence of medium alone, 15 mM n-docosanol suspended in [redacted] vehicle control. After 24 hours incubation, HSV-1 was added at a multiplicity of infection (MOI) of 0.33 infectious virus particles/cell. The cultures were incubated 6 hours at 37 C, then washed, fixed, and stained for the presence of the immediate early (α) protein ICP4. The data are presented as the average percent of cells positive for nuclear fluorescence. The number of cells expressing ICP4 was reduced by 68% in cultures treated with n-docosanol compared to untreated or control-treated cultures. The sponsor has concluded that these results confirmed that early stages of viral replication were significantly inhibited in n-docosanol-treated cells. Hence, the mechanism for the antiviral activity of n-docosanol may be substantially different than the mechanism of action of acyclovir and other agents which inhibit DNA replication.

A.4. Effect of n-Docosanol on viral entry process involving fusion of HSV envelope and cellular plasma membrane (Lidak Report 118, Vol 2.10).

A number of experiments were conducted to confirm that n-docosanol did not inhibit HSV attachment and to provide evidence that the fusion of viral surface protein(s) with cellular plasma membrane may be inhibited. A stock suspension of [redacted] for the experiments described below (pages 077-104, Vol. 2.10)

A.4.1. Effect of n-Docosanol on HSV attachment to host Vero cells.

Duplicate cultures of Vero cells (6 x 10^5 cells/well in 35-mm wells) were incubated for 24 hours at 37 C in the presence of growth medium alone, medium containing 7.5 mM n-docosanol, the corresponding amount of control suspension, or acyclovir (40 µM). Before being infected, one set of two cultured cells was treated with heparin [redacted] as positive controls to specifically block HSV receptors on cell surface. All the treated and untreated cells were infected with [redacted] cell of HSV-1 (Macintyre strain) labeled with [redacted]. The cells were incubated for 3 hours at 4 C to allow for virus binding and were then washed. Cell-associated radioactivity was determined by scintillation counting. Untreated Vero cells bound [redacted] Results presented in Fig 2, page 100 of Vol. 2.10 shows that n-Docosanol and its vehicle had

no effect on virus attachment. [redacted] which specifically blocks HSV-receptor such as [redacted] inhibited the attachment of radioactive HSV by 96%. Acyclovir, which inhibits viral DNA replication but not viral attachment, also had no effect on the amount of radioactivity bound to Vero cells. Thus, the sponsor has concluded that this experiment confirmed that n-docosanol does not affect the attachment of HSV-1 to Vero cell surface.

A.4.2. Reduced expression of β-gal in n-docosanol treated Hep-2 cells infected with HSV-1-(KOS)gL86

To determine the effect of n-docosanol on viral entry into the target cells, a viral construct, HSV-1 (KOS)gL86, in which gL ORF is replaced with lacZ under control of the CMV promoter, was utilized. This replication defective mutant of HSV-1 is propagated in gL-expressing cells and is fully infectious but can undergo only one round of replication in non-complementing cells. Infection of human HEp-2 cells by this mutant results in the production of β-galactosidase which is easily detected by blue color development after the addition of the appropriate substrate [redacted] The intensity of the color signal is dependent upon viral input. [redacted] (which blocks viral binding to specific cell receptors) and anti-HSV acyclovir (inhibitor of viral DNA replication in virus infected cells) were included in this experiment as controls.

Approximately 2.6 x 10^6 HEp-2 cells/0.5 ml growth media were seeded in each well of 24-well plates. After cell attachment, n-docosanol (1, 2, 4, 6, 8, and 10 mM) in [redacted] equivalent volumes of [redacted] was added to the cells in 0.5 ml media. Following 24 hours incubation at 37 C, cells were infected with at least [redacted] of HSV-1(KOS)gL86 per cell. Infection at 37 C was continued for 5 to 6 hours, the cells were then washed, fixed with formaldehyde (2%)and gluteraldehyde(0.2%) and permeabilized with detergents (0.02% NP-40 and 0.01% deoxycholate) and [redacted] was added for development of blue color. After removing the unutilized substrate, the plates with blue color were photographed and enumerated. To quantitate the blue color intensity, DMSO was used to solubilize the incorporated dye, and the absorbency at OD_{600} was recorded.

Results of this study presented in Figs. 3 and 4 (Lidak Report 118) demonstrated that n-docosanol pretreatment of cells caused an 80% reduction in blue color development compared to untreated cells. The concentration of n-docosanol that inhibited color development by 50% (EC_{50}) was about 7 mM. The vehicle, [redacted] itself was not inhibitory, rather it increased the color intensity by as much as 40% [redacted] which blocks viral binding, completely inhibited the color development. Combined with the observation that n-docosanol was not able to inhibit viral attachment, this experiment indicated that n-docosanol blocked a step of viral entry into host cell without affecting viral attachment.

Comments: 1. The sponsor has calculated that the EC_{50} value of n-docosanol to be "roughly equivalent to the observed EC_{50} values, 4 and 9 mM, for inhibition of HSV production and plaque formation, respectively, in Vero cells." The later EC_{50} values (4 mM and 9 mM) were supposed to have been published by Katz *et al.*, cited in Reference 4. This comparison seems to be erroneous since the investigators of the reference did not use [redacted] and human cell lines to establish the EC_{50} values of n-docosanol. They have used [redacted] as the surfactant to determine the EC_{50} value of v-docosanol in Vero cells.
2. While 9 mM n-Docosanol in [redacted] inhibited 55% HSV-1 plaque formation with six hours of preincubation of host cells (see Table 1 above), it is not clear why a range of [redacted] n-docosanol in [redacted] needed 24 hours of pre-exposure to inhibit color development by 50%.

A.4.3. n-Docosanol Inhibited HSV-2 (333) Infectivity of CHO-IEβ8 cells.

This experiment was very much similar to the one described above except that the lacZ gene was under the control of HSV-1 ICP4 promoter in a special CHO-IEβ8 cell line. In this cell line β-gal gene is expressed immediately after penetration of HSV proteins into the infected cell cytoplasm. It should be recalled that infected cell polypeptide 4 (ICP4) is an immediate early (IE, α) gene product of HSV whose synthesis can be detected immediately after virus infection (peak period of synthesis may be about 2 to 4 hours post infection).

CHO-IEβ8 is a cell line selected by transfection with a [redacted] selectable marker and lacZ under control of the HSV-1 ICP4 promoter. Only upon infection by HSV and immediately after viral entry into the cytoplasm of this cell line, viral protein 16 (VP16) transactivates ICP4 promoter and β-gal expression is induced. Color (blue) development results upon addition of [redacted] As noted earlier, the intensity of the color signal is dependent upon the viral input which can be influenced by agents such as [redacted] antibodies to viral gD.

In this experiment, the effect of n-docosanol on the entry of a [redacted] HSV-2(333) into CHO-IEβ8 cells was investigated. Approximately 2.6 x 10^5 CHO-IEβ8 cells/well in 0.5 ml growth medium were seeded into 24-well plates. After cell attachment, n-docosanol with final concentrations ranging from 3, 6, 12, 18, 24, and 30 mM in [redacted] equivalent volumes [redacted] or no agent was added in a total of 0.5 ml of growth media. After 24 hours at 37 C, cells were infected with at least HSV-2(333)/cell. After 5 to 6 hours of incubation at 37 C, cells were fixed with formaldehyde (2%) and gluteraldehyde (0.2%) and permeabilized with detergents (0.02% NP-40 and 0.01% deoxycholate), [redacted] was added for development of blue color. After removing the substrate, photographs were taken and colored plates were enumerated. Quantitation of the color was done after DMSO solubilization as described above.

Results presented in Fig. 5 of Lidak Report 118 (Vol. 2.10) demonstrated that 30 mM n-docosanol inhibited color production 40% compared to untreated cells, and 55% compared to [redacted] treated cells. This observation again narrowed a point of inhibition by n-docosanol to an event occurring after viral attachment but prior to release of virion proteins (an immediate post-entry event) and manifestation of VP16 transactivator activity. Treatment of cells with vehicle alone resulted in a slight increase in absorbance at 600 nm, and [redacted] effectively inhibited this signal of entry, as expected, since it effectively inhibits viral binding to target cells.

Comment: It is not clear why 30 hours of pre-exposure of 30 mM n-docosanol in [redacted] inhibited only 40 - 55% of color development after HSV-2 infection while [redacted] "effectively inhibited" the color development (signal of virus entry). In addition, it should be recalled that when suspended in [redacted] n-docosanol (formulation E, Table 1 above) was able to inhibit a higher percentage (55%) of plaque formation by HSV-1 even at 0 hour exposure which increased to 74 % with 6 hours of pre-exposure of host cells.

A.4.4. Inhibition of fusion of HSV-2 with host NC-37B cell surface by fluorescence assay

In this experiment the possibility that n-docosanol may inhibit viral entry by altering target cell membranes to prevent effective fusion of viral particles was investigated. The outer envelope of the infectious virus was labeled with a fluorescent probe [redacted] and added to a human B cell line treated with or without the test drug n-

docosanol. The assumption is that if there is viral fusion with cell membrane, then the fluorescent probe diffuses from the viral envelope to the larger cell membrane. This relieves the self-quenching of the probe and causes an increase in fluorescence intensity in the cell membrane which can be measured by a fluorescence-activated cell sorter.

HSV-2 (MS strain) was labeled with the fluorescent probe [redacted] according to a method described by Hoekstra *et al.*, (Biochem. 23:5675-5681, 1984). NC-37 human B cells (ATCC #CCL-214) (2.5 X 10^5 per ml in a total of 25 ml growth medium per flask) were incubated at 37 C for 24 hours with 15 mM n-docosanol in [redacted] or an equivalent volume of the vehicle [redacted] (page 097, Vol. 2.10), or with no added agent. After incubation, the cells were centrifuged and resuspended at a concentration of 1X 10^6/ml. After chilling aliquotes of 0.2 ml of these cells for 20 minutes at 4 C, 0.1 ml of R-18 labeled HSV-2 was added and chilling of the cell/virus mixture was continued for another 3 hours. n-Docosanol (15 mM) in [redacted] at original concentration in 3 ml growth medium was added and incubated at 37 C for various periods of time. Cells were then washed by centrifugation at 4 C, fixed in 10% formalin and resuspended in phosphate buffered saline (PBS) containing 10% fetal calf serum. Fluorescence intensity was measured by using a fluorescence-activated cell sorter [redacted]

Results presented in Fig. 6 (Lidak Report 118, Vol 2.10, not submitted before) demonstrate that while addition of [redacted] labeled HSV-2 to untreated control NC 37 human B cells resulted in an increase in fluorescence intensity, pretreatment of such cells with 15 mM n-docosanol inhibited the fluorescence response by 50% compared to cells receiving no treatment. Treatment with the vehicle [redacted] alone was not inhibitory; rather there was an increase in the relative fluorescence intensity. Compared to the vehicle control alone, the sponsor has calculated that n-docosanol inhibited the fluorescence by as much as 76 % . Without providing any data, it is stated that anti-gD monoclonal antibody (a specific inhibitor of virus penetration) at a 1:40 dilution completely blocked the increase in fluorescence. In addition, the compound was not inhibitory if added only during the fusion process thus requiring a preincubation period for its effectiveness. The sponsor has, therefore, concluded that fusion of HSV virions to host cell membranes is significantly inhibited in n-docosanol treated cells.

Comment: 1. This important experiment lacks some basic information such as infectivity titer of the [redacted] labeled HSV-2, the choice of NC 37 human B cells for infection and the multiplicity of virus infection. In addition, there is no visual fluorescent microscopic evidence that upon fusion, the fluorescent probe from the viral envelope indeed diffused to the host cell membrane and that n-docosanol reduced the fluorescence while the vehicle [redacted] intensified it.

2. Even if one assumes that tne cell membranes became fluorescent and the test drug was effective in blocking the fluorescence, the sponsor has not provided any real data on the florescence intensity, as measured by [redacted] In addition, real data on the blockage of fluorescence by anti-gD monoclonal antibody before, during and after the fusion of the viral glycoprotein with host cell membrane are missing.

3. As already noted (several times), it is not clear why a 24 hour pre-exposure of host cells to 15 mM n-docosanol in [redacted] was necessary to inhibit the putative fusion of the virus surface protein(s) with host cells by only 50% when Formulation E of n-docosanol was perhaps a little more efficient in inhibiting plaque formation by HSV by about 55% without any pre-exposure of host cells.

A.5. Metabolism of n-docosanol in uninfected host Vero cells

Pope *et al.*, (J. Lipid Res., 1996, 37:2167-2178, Lidak Report 119, Vol. 2.10) have extended the studies (reported earlier) on the fate (uptake, distribution, and metabolism) of n-docosanol in the host Vero cells in greater detail.

A.5.1. To determine the intracellular fate of n-docosanol, Vero cells were incubated with [redacted] for 24 hours, extensively washed, sonicated and the cellular components (membrane, cytoplasmic components and nuclei) were separated by differential centrifugation. Measurement of radioactivity incorporated into various compnents revealed that **73% of radioactivity was localized in membranous fractions, 27% was associated with the cytoplasmic fraction and less than 1% of radioactivity was associated with nuclear fraction.**

A.5.2. To assay for possible metabolic conversions of cell-associated n-docosanol, Vero cells (6 x 10^5 cells/well in 35-mm wells) were incubated with [redacted] for 24 hours, washed, the lipids were extracted and analyzed in a silica thin layer chromatography (TLC) system. Radioactivity of the lipid extracts was located either at the position of the n-docosanol standard (53%) or at the point of origin (47%). No radioactivity above background was detected at the position of n-docosanoic acid. The investigators claim that the results were reproducible and indicated that Vero cells metabolize a large portion of incorporated n-docosanol.

Comment: If the ultimate goal of these experiments are to determine whether metablite(s) of n-docosanol are involved in the inhibition of viral entry into the host cell (through fusion with host cell membranes), it is not clear why the sponsor did not continue to isolate and analyze the cellular membrane fraction, rather than total cells, for characterization of n-docosanol metabolites.

[redacted]

A.5.4. Role for the polar metabolites in the antiviral activity of n-docosanol.

To investigate "the possibility that the enzymatic conversion of n-docosanol is a necessary prerequisite for its antiviral activity" and to "demonstrate that efficiency of metabolic conversion directly correlates with the magnitude of antiviral activity of n-docosanol" the following experiments were conducted.

[redacted]

The total amount of radioactivity incorporated per cell was the same regardless of the surfactant used. However, the rate and extent of metabolism to the more polar material was three times as great when n-docosanol was [redacted] During a prolonged (48-hour) chase in the absence of added drug, all of the radiolabeled [redacted] was incorporated into the polar material. Exposure to a combination of metabolic inhibitors [redacted] was shown to inhibit both uptake and metabolism of n-docosanol (data not shown).

ii. To investigate the possibility that differences in the mechanism of action of n-docosanol suspended with [redacted] may be correlated, the antiviral activities of the two types of suspension was compared by assayng for the inhibition of plaque formation by HSV-2 in Vero cells. Results of this experiment demonstrated that the ED_{50} values of n-docosanol [redacted] Since the ED_{50} values of n-docosanol against HSV-2 in Vero cells were approximately 3 times higher when in [redacted] the sponsor has concluded that the 3 times higher ED_{50} vales with [redacted] may be correlated with the 3-fold higher metabolic conversion of n-docosanol observed in the previous experiment.

Table 2: Dependence of the Antiviral Activities of n-Docosanol in various Cell Types and Surfactant.*

Cell type	Surfactant	ED_{50} (mM)	SD (mM)	Number of Determinations
Vero	[redacted]	15.3	3.3	2
Vero		5.6	1.7	3
MDBK		>20	NA	3
Normal skin fibroblast		10.9	2.9	3

* Adapted from Table 1 by Pope *et al.*, (J. Lipid Res., 1996, 37:2167-2178).
MDBK, Madin-Darby bovine kidney cells
NA, Not Applicable
SD, Standard Deviation

The sponsor has also observed a correlation between metabolic conversion of n-docosanol and anti-viral activity in a comparative analysis of Vero cells and the epithelial-like Madin-Darby bovine kidney cell line, (MDBK, ATCC #CCL-22) which exhibits a relative resistance to the

anti-HSV activity of n-docosanol. As demonstrated in Table 2 above, n-docosanol was 3 to 4-fold more effective in inhibiting HSV-2 induced plaques in Vero cells (ED_{50} = 5.6 mM) than in MDBK cells (ED_{50} >20.0 mM). To determine the total cellular uptake and relative metabolism of n-docosanol, separate monolayers of MDBK and Vero cell (3×10^5 cells/well in 35-mm wells) were incubated in the presence of [redacted] cells were washed, harvested, lipids were extracted, and analyzed by TLC as described earlier. Both cellular uptake and relative conversion to polar metabolites were higher in Vero cells, and the differences increased with longer incubation times. MDBK cells metabolized 10%, 17%, and 19% less n-docosanol at 24, 48, and 72 hours, respectively, than Vero cells. The ultimate effect of decreased uptake combined with decreased metabolism was that after 72 hours, Vero cells contained almost 4-fold higher amounts of the phosphatide metabolites of n-docosanol.

Antiviral activity of n-docosanol was shown to correlate better with the level of polar metabolites formed rather than the level of n-docosanol incorporated into host cells. This was demonstrated in several human fibroblast cell lines including those cells derived from normal human skin (ATCC #CRL-1900), fetal foreskin (Hs68, ATCC #CRL-1635), and fetal lung (HFL-1, ATCC #CCL-159). In these studies it was shown that the human fibroblasts incorporated more radiolabeled n-docosanol [redacted] in 72 hours) than the same number of Vero cells [redacted] in 72 hours). However, the amount of metabolites formed was roughly equivalent [redacted] in 72 hours). From these combined experimental results the sponsor has concluded that anti-HSV activity of n-docosanol is quantitatively proportional to the amount of metabolism of n-docosanol to polar material which exhibits the chromatographic properties of phosphatidylcholine and phosphatidylethanolamine.

Comments: 1. Maximal anti-HSV activity of n-docosanol was demonstrated when host cells of human origin were pre-exposed to the drug [redacted] The sponsor has attributed the increased antiviral activity of n-docosanol to the efficiency with which n-docosanol is maximally metabolized to polar compounds (such as phosphatidylcholine and phosphtidylethanolamine) under such experimental conditions. These are circumstantial evidence which suggest (but do not experimentally prove) that the "antiviral activity of n-docosanol involves cellular uptake and metabolism of the drug." Further exprimental evidence is needed that would unambiguously establish that the polar metabolites of n-docosanol located at or isolated from the host cell membranes interact with specific viral targets to inhibit the initial step(s) of virus replication.

2. With greater efficiency with which n-docosanol [redacted] suspension is putatively metabolized to exert its anti-HSV activity in host cells of human origin, it is not clear why human cells and the [redacted] were not routinely used to determine the mechanism by which n-docosanol exerts its anti-HSV activity.

3. To exert optimal antiviral activity, host cells were usually pre-exposed to n-docosanol for 24 hours or less before virus infection. However, the sponsor has not explained why the polar metabolites were analyzed from cells labeled with radioactive n-docosanol for 72 hours, instead of 24 hours or less.

4. If the polar metabolites of n-docosanol are formed by "enzymes of lipid metabolism" and are responsible for the anti-HSV activity of n-docosanol, there is no explanation why Formulations A [redacted] of n-docosanol inhibited 36 % and 55%, respectively, plaque formation by HSV-1 added only at the time of virus infection and without any pre-exposure of the host cells.

5. Even if the metabolites formed after 72 hours may be the same as during the 24 hours of labeling, there is no experimental evidence that the metabolites (phosphatidylcholine and phosphtidylethanolamine) are indeed reponsible for the antiviral activity of n-docosanol. In addition, it is not clear why the polar metabolites were isolated from total cell extracts rather than the membrane fractions of the host cells where viral entry through fusion to host cell membrane takes place.

6. In this section, the sponosor enthusiastically started to investigate "the possibility that the enzymatic conversion of n-docosanol is a necessary prerequisite for its antiviral activity." However, the sponsor has yet to demonstrate that indeed n-dcosanol is enzymatically converted in the host cells to antivirally active form(s). On the contrary, the sponsor has stated that "due to the chemical structure of the molecule, which possesses no reactive groups other than a primary alcohol, enzymatic hydrolysis is unlikely" (see subsection 7.4, page 089, Vol 2.9).

B. ANTIVIRAL ACTIVITY *IN VITRO*

B.1. Spectrum of Antiviral Activity: The sponsor has presented evidence that n-docosanol is an inhibitor of a broad spectrum of lipid-enveloped, DNA- and RNA- containing viruses including a number of human herpesviruses, human immunodeficiency virs type 1 (HIV-1), respiratory syncytial virus, and influenza A virus(see Table 3 below). As noted earlier in Section A, the test drug must be homogeneously suspended in a surfactant like [redacted] and the host cells must be pre-exposed to massive amounts of the suspended drug for as long as 24 hours to exert its antiviral activity. Thus the sensitivity test results, expressed as the concentration of n-docosanol required to inhibit by 50 % the replication of virus (ED_{50}) in host cells cultured *in vitro*, vary greatly depending upon the surfactant used to suspend the drug, particular assay method used, the cell type employed, and the virus strain. The ED_{50} values of of n-docosanol against various repesentative viruses are presented in Table 3 (see below), Against various laboratory strains and clinical isolates of HSV-1 and HSV-2, the ED_{50} values of n-docosanol ranged from [redacted]

B.2. Results presented in Table 3 also show that acyclovir resistant (ACV) mutants of both HSV-1 and HSV-2 are equally susceptible to n-docosanol as the [redacted] virus strains with ED_{50} values ranging from [redacted] From all these results along with those from Lidak Reports 110 and 117, the sponsor has concluded that n-docosanol exhibits preferential inhibtory activity for lipid-enveloped viruses which enter into the host cell by fusion with host cell membrane rather than by endocytosis (exception being influenza A virus). Non-enveloped viruses (e.g. poliovirus and adenovirus) were resistant to n-docosanol.

To extend these results and to confirm the spectrum of viruses susceptible to the inhibitory activity of n-docosanol, several enveloped viruses were also tested by Dr. Dale L. Barnard at the [redacted] Vol 2.10, pages 065-076). Initially the following viruses were investigated: Semliki forest virus (SFV) in Vero cells, vaccinia virus (LED strain) in Vero cells, ovine ecthyma virus (OEV; vaccine strain) in primary sheep testes cells, and vesicular stomatitis virus (VSV; [redacted] in LLC-MK2 cells. As control, efficacy of n-docosanol against HSV1 (KOS) was also assessed in Vero cells. For these studies, a range of concentrations of n-docosanol [redacted] was suspended in the surfactant, [redacted] Cells were cultured in 96-well plates (5×10^4 cells/well) for 24 hours before the addition of virus (multiplicity of infection [MOI] = 0.01 to 0.001). After 24

hours, the tet drug was removed, fresh medium was added and the cells were incubated until virus controls showed 100% cytopathic effect (CPE) as observed by light microscopy (4 to 6 days). Control drugs known to be effective against corresponding viruses (e.g, acyclovir for HSV-1; (S)-9-(3-hydroxy-2-phosphonylmethoxypropyl)-adenine (HPMPA) for ovine ecthyma virus and vaccinia virus; and ribavirin for SFV and VSV) were also included in this study. The activity of the [redacted] alone was also assessed separately for all viruses. Cells receiving no virus served as drug cytotoxicity controls. After scoring by microscopic examination, a neutral red assay was also used to estimate inhibition of CPE and cytotoxicity. For all CPE assays, the ED_{50} values (noted as EC_{50} values in the report) were calculated by regression analysis using the means of duplicate CPE ratings at each concentration of compound. The 50% cytotoxic doses (IC_{50}) were also determined and the therapeutic index or selective index (SI) was estimated.

Table 3. ED_{50} Values of n-Docosanol Against Various Viruses*

Virus (strain)	Host Cell	ED_{50} (mM)	Assay Method
HSV-1 (Macintyre)	Vero	6.0 - 9.0	Plaque Reduction
HSV-1 (Macintyre)	Human fetal foreskin	6.0 - 9.0	Plaque Reduction
ACV^r HSV-1 (Macintyre)	Vero	6.0 -9.0	Plaque Reduction
HSV-1 (KOS)	Vero	9.0	CPE (microscopic)
HSV-1 (KOS)	Vero	12.0	Neutral Red
HSV (Clinical, oral)	Human fetal foreskin	15.0 (ED_{97})	Plaque Reduction
HSV-2 (MS)	Vero	6.0 - 9.0	Plaque Reduction
ACV^r HSV-2 (MS)	Vero	6.0 - 9.0	Plaque Reduction
HSV-2 (MS)	Human fetal forskin	6.0 - 9.0	Plaque Reduction
HSV-2 (MS)	MDBK	15.0 -24.00	Plaque Reduction
HSV-2 (Clinical, genital)	Human fetal foreskin	9.0	Plaque Reduction
HHV-6	Human PMBC (PHA)	6.0	p41- ELISA
CMV (Human)	MRC-5	6.0 - 9.0	Plaque Reduction
CMV (Murine)	Murine SC-1	15.0 (ED_{90})	Plaque Reduction
VZV	MRC-5	3.0	Plaque Reduction
HIV (III_B)	Human PMBC (PHA)	9.0	p24 - ELISA
Respiratory Syncytial Virus	Vero	10.0	Syncytia Reduction
Influenza A	MDBK	4.0	Immunofluorescence
Influenza A	Vero	7.5	Immunofluorescence
Poliovirus	Vero	>15.0	Plaque Reduction
Vaccinia Virus	Vero	>22.0	Plaque Reduction
Vesicular Stomatitis Virus	LLC-MK2	>30.0	CPE
Semliki Forest Virus	Vero	>30	CPE
Adenovirus	Vero	>15.0	Immunofluorescence

* Adapted from Table 1, Lidak Report 118, Vol. 2.10)

Results from both the microscopic CPE and neutral red assays were consistent with one another and confirmed the antiviral activity of n-docosanol against HSV-1 (KOS strain) with an ED_{50} value of 4.5 to 9.0 mM. While there was slight inhibition of CPE by ovine ecthyma virus

there was no detectable inhibition of CPE induced in SFV, VSV, or vaccinia virus cultures treated with n-docosanol (ED_{50} >30 mM). All of these viruses were inhibited by the corresponding positive control drugs. There was little drug cytotoxicity with n-docosanol and the IC_{50} value could only be estimated as >30 mM, the highest concentration examined and only minimal values for selective indices of >3 to >7 could be determined from this study.

These data support the theory that n-docosanol treatment of target cells may inhibit the replication of lipid-enveloped viruses that fuse with the plasma membrane as a primary means of entering target cells since several lipid-enveloped viruses which enter cells via endocytic pathways were resistant to inhibitory effects. However, the investigator also concluded that the multiplicity of infection (0.001) used in the assay may have overwhelmed the inherent antiviral properties of n-docosanol. To test this possibility a second series of experiments were conducted by using a lower moi of viruses.

[redacted] Report II was entitled, "Effects of multipticity of infection (MOI) on the antiviral activity of 1-docosanol against selected enveloped viruses that enter cells by receptor-mediated endocytosis." Vaccinia virus, VSV and Semliki forest virus found resistant to the inhibitory effects of n-docosanol in the previous study were re-examined for susceptibility at a lower multiplicity of infection (MOI) to confirm that the viral resistance to inhibition would persist with a lower viral load. The investigator hypothesized that the lack of inhibitory effect would be a characteristic of the specific virus and not that the amount of virus added overwhelmed the protective effect of n-docosanol on the host cells. Thus the assay for inhibition of the viruses were conducted as described in the previous study except that the viruses were added at a MOI's of 0.001 to the first set of cultured cells and at an MOI of 0.0005 to the second set. To validate the assay, HSV-1 was included as a control virus known to be inhibited by n-docosanol.

The inhibition of HSV-1 by n-docosanol was largely independent of the viral MOI and the ED_{50} values ranged from [redacted] On the other hand, lowering the moi resulted in at least 5 fold lower EC_{50} value of acyclovir under similar conditions of experimentation. As in the previous study, no antiviral effects were detected against vaccinia virus, vesicular stomatitis virus, or Semliki forest virus regardless of MOI. Known inhibitors of the viruses were either active as expected or appeared to be more inhibitory than usual.

These results confirmed that n-docosanol does not inhibit the replication of these lipid-enveloped viruses which enter cells by endocytic pathways. It was also concluded that perhaps those lipid-enveloped viruses which enter cells via endocytic pathways may resist the antiviral effects of the compound. However, since Influenza A virus (which enters the cells by receptor-mediated endocytosis) was susceptible to n-docosanol, the investigator has suggested that "it might be wise to evaluate 1-docosanol" against a number of other viruses including influenza groups B and C, paramyxoviruses, flavivirus of the Togaviridae and against one virus from Bunyaviridae, Coronavridae and Retroviridae.

Comments: 1. Dr. Barnard has pointed out that HSV-1 "may use both the fusion pathway or the receptor-mediated endocytosis pathway depending on the virus strain" (paragraph 1, page 066, Vol. 2.10). Thus the assumption that all strains of lipid-enveloped HSV-1 are susceptible to n-docosanol may not be entirely correct.

2. Virus strains capable of entering host cell by fusion require high multiplicity of infection because it is mediated by proteins present in the infecting virion and occurs within 1 to 2 hours

of infection. This process is also called "fusion from without." On the other hand, viruses which induce fusion of host cells from within (e,g., during syncytia formation) reqire new viral protein synthesis over a long period of time. To demonstrate that n-docosanol was inhibitory to viral infectivity because of its abilty to inhibit the fusion process, it would have been more appropriate to use much higher mulitplicity of infection (> 20- 800) as were correctly used in experiments reviewed in Secton A.4 above where viral entry through fusion was investigated.

B.3. Therapeutic Index of n-Docosanol

Therapeutic index is defined as the drug's potential for cytotoxicity versus its antiviral activity as a function of drug concentration. As has been discussed earlier, the ED_{50} values of the drug varied from [redacted] depending upon the virus strains, host cell lines, the vehicle of the drug used and the laboratory performing the virus assay. A similar situation also exists for the determination of the concentration of the drug that effectively inhibits host cell replication by 50% (IC_{50}). Katz *et al.*, (Proc. Natl. Acad. Sci. USA, 1991, 88:10825-10829) have reported that n-docosanol [redacted] had no appreciabe inhibitory effect on the growth of Vero cells cultured *in vitro* (at up to 100 mg/ml) "or several other nucleated human and murine cell lines (up to 25 mg/ml). Therefore the therapeutic index (or selectivity index) of the drug may vary from 1 to 17 for laboratory strains as well as clinical isolates of HSV-1 and HSV-2. On the other hand, Dr. Barnard of [redacted] has presented evidence (Table 1 in page 069 of Vol. 2.10) that the selectivity index of n-docosanol may range from [redacted] depending on the assay procedures used.

Comment: The low therpeutic indices may indicate thet the test drug would be toxic to the host cells. However, large amounts [redacted] of the drug was used to demonsrate its antiviral activity and there is no clear experimental evidence on the intracelluar concentrations of n-docosanol which were active against either the infectious virus or the host cells.

C. Assessment of Resistance

The sponsor has stated that preliminary attempts to induce laboratory strains of HSV, resistant to the antiviral effects of *n*-docosanol have been unsuccessful and that the emergence of HSV strains resistant to the test drug appears unlikely because of the drug's mechanism of action which is not dependent upon any viral specific genes or gene products. It may modulate the host cell to prevent entry of the virion and is unlike that of other antiviral agents that work after the virus has entered the cell and begins replicating.

C.1. Clinical Laboratory Susceptibility Test Methods

The sponsor has stated that no susceptibility test methods have been developed for 10% LIDAKOL cream.

D. Enzyme Hydrolysis Rates

In subsection 7.4 (page 089, Vol 2.9) the sponsor has made a simple statement that there is no evidence for virus-induced enzymatic hydrolysis of n-docosanol and that "due to the chemical structure of the molecule, which possesses no reactive groups other than a primary alcohol, enzymatic hydrolysis is unlikely."

Comments: 1. The sponor has been very vigorus to conclude that "anti-HSV activity of n-docosanol is quantitatively proportional to the amount of metabolism of n-docosanol to polar material which exhibits the chromatographic properties of phosphatidylcholine and phosphatidylethanolamine" (page 042-043, Vol 2.9) and that "the anti-HSV activity of n-docosanol involves cellular uptake and metabolism of the drug" (Lidak Report 119, Vol 2.10). While the sponsor has yet to demonstrate that the metabolites of n-docosanol are indeed responsible for the antiviral activity of n-docosanol, it is likely that celluar enzyme(s), in contrast to virus-induced enzyme(s), may be involved in the metabolism of n-docosanol.

2. If "enzymatic hydrolysis" of n-docosanol structure is "unlikely," the sponsor has not explained how the "polar metabolites" were formed in cells exposed to n-docosanol. On the other hand, if cellular enzmes are indeed involved in the metabolism of n-docosanol, the prolonged pre-exosure to the drug may induce mutations in cellular enzymes which may not metabolize n-docosanol to its antivirally active form.

D. ANTIVIRAL ACTIVITY *IN VIVO*

D.1. Against HSV Infctions in Guinea Pig Models:

Effectiveness of topical *n*-docosanol cream for the treatment of HSV-induced cutaneous disease was evaluated in two guinea pig models; hairless guinea pigs and conventional (Hartley, or haired) guinea pigs. In general, guinea pigs were inoculated with either HSV-1 or HSV-2 on their backs, and then treated with with various formulations of n-docosanol cream. The number of vesicles appearing during the course of the treatment with the test drug were recorded and compared with the number of vesicles at untreated sites to estimate the effectiveness of n-docosanol. Some of the experiments described were also used to evaluate effectiveness as part of the formulation development process of *n*-docosanol. Antiviral activities of the following formulations of Lidakol cream were compared with 5% acyclovir ointment (Lidak Report 115):

1. The topical cream emulsion contained on a percent w/w basis 10% n-docosanol, 11% sucrose stearate, 5% sucrose cocoate, 8% mineral oil, 5% propylene glycol USP, 2.7% 2-ethyl-1, 3-hexanediol, and the remainder water.

2. Another topical cream emulsion which was used earlier in clinical trials contained on a percent w/w basis 10% n-docosanol, 5% sucrose stearate, 8% mineral oil, 5% propylene glycol USP, 2.7% benzyl alcohol, and the remainder water.

The vehicle control used in some of the experiments contained these same ingredients except that n-docosanol was omitted. In some other experiments n-docosanol was also suspended [redacted] The mixture was sonicated for 21 min which resulted in a suspension consisting of very fine globular particles. The control vehicle for this suspension contained [redacted]

Effectiveness of Lidakol cream preparations were investigated against HSV-1 (Macintyre strain) as well as HSV-2 (MS strain) in hairless and Hartley female guinea pigs (250-400 gm). The backs of the Hartley guinea pigs were shaved and cleaned with ethanol and sterile saline. Seventy-five microliters of saline containing [redacted] of HSV was inoculated into the skin

with a tattoo instrument to eight (4 cm x 4 cm) sites on the backs of guinea pigs. Two hours after virus inoculation, animals were grouped to receive treatment with 200 µl of n-docosanol cream, or vehicle control, or acyclovir 5% ointment with gentle circular rubbing and continued three times daily for several days. The inoculated sites were evatuated for vesicle formation at various time points. No virologic end points were evaluated.

From the results (presented in several figures) of this study, the sponsor has concluded that 10% n-docosanol cream and 5% acyclovir ointment exhibited comparable inhibitory activity on HSV-1-induced cutaneous vesicles in the guinea-pigs. Small numbers of vesicles were observed in all the sites 1 day after inoculation with HSV-1. While the untreated sites exhibited a dramatic increase in vesicle number the second day post-inocuiation, comparable inhibition of vesicle formation was observed on the second day in the sites treated with n-docosanol cream or acyclovir ointment. By the third day after inoculation substantial drying of the vesicles was observed in the untreated sites, but a significant number of active vesicles could still be observed. The sponsor has further stated that the total disease course, defined as the area under the curve (AUC) derived from the plot of vesicle number vs. time post-infection, was significantly decreased in the acyclovir ointment- and n-docosanol cream-treated sites as contrasted the untreated sites.

Results obtained with the HSV-1 infection were also confirmed with the demonstration that 10% n-docosanol cream, but not the vehicle control, could inhibit HSV-2-induced vesicle formation in hairless guinea pigs when applied three times daily. It is claimed that significantly fewer vesicles were observed in the sites treated with n-docosanol cream as contrasted to either the untreated or the vehicle control-treated sites. Mean vesicle numbers started to decrease in all of the groups by 3 days after virus inoculation, but mean vesicle number in the group treated with n-docosanol cream was still significantly lower than that of the untreated or vehicle-treated groups of sites. Total disease course (AUC) was significantly decreased in the n-docosanol-treated sites as contrasted to the untreated sites or the sites treated with the vehicle control. The results also show that a) n-docosanol formulated in [redacted] exerts anti-HSV-1 activity in the hairless guinea pig model system, b) [redacted] itself has no apparent effect on HSV-1-induced vesicle formation and resolution, and c) treatment with n-docosanol can be delayed for up to 2 days after virus inoculation and still can inhibit HSV-induced vesicles.

The sponsor has extended these studies to evaluate two additional cream preparations: (1) the stearic acid cream preparation that was used as a placebo in a phase III clinical trial, and (2) polyethyne glycol (PEG) placebo in the HSV-2/guinea pig model system. Lidakol cream (10%) served as positive control.

1. The 10% stearic acid (97% pure) cream contained the same ingredients except n-docosanol.

2. The polyethylene glycol (PEG) preparation consisted of 70% PEG 400 NF (a liquid at room temperature) and 30% PEG 3350 NF (a solid at room temperature).

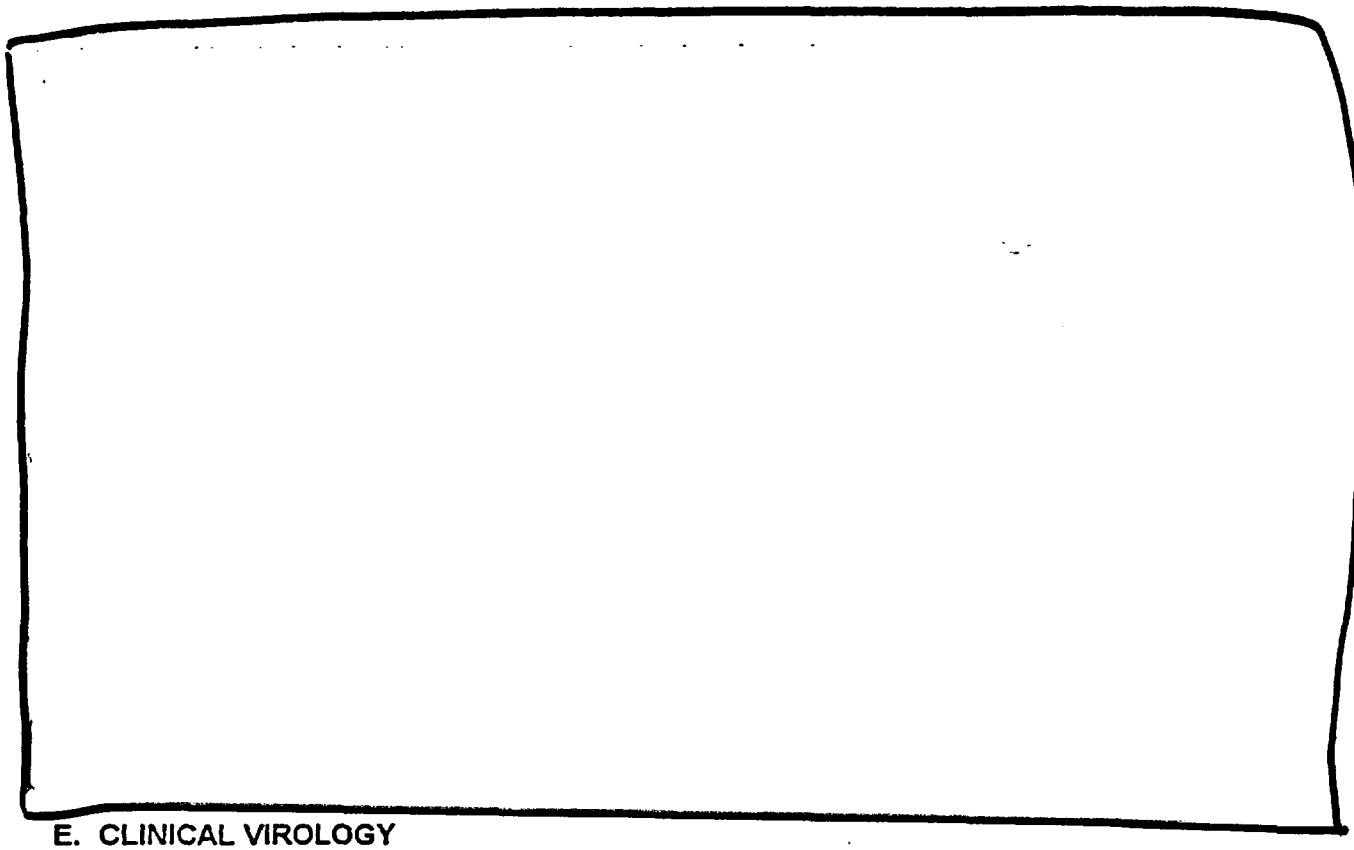
Seventy-five microliters of saline containing [redacted] of HSV-2 (MS strain) were used to infect the backs (4 cm x 4 cm sites) of four male hairless guinea pigs (200-300 gm) with a tattoo instrument. Each animal had 6 inoculation sites. Treatment with the indicated preparation was initiated 24 hours after virus inoculation. The sites were evaluated for vesicle numbers and irritation at the indicated time points. Vesicles were defined as white, fluid-filled

pustules. Irritation was scored on a scale of 0-4; normal skin (no erythema) was rated as zero, mild erythema was rated as 1, moderate erythema was rated as 2, severe erythema was rated as 3, and severe erythema with bleeding was rated as 4.

Results presented demonstrate that application of the stearic acid cream 3 times daily did not significantly inhibit HSV-2 cutaneous lesions in hairless guinea pigs; although, the positive control, the 10% n-docosanol cream, exhibited activity with 3 times daily application. However, when the stearic acid cream application was increased to 5 times daily, antiviral activity was observed. Greater anti-HSV activity was also observed with 5 times daily application of the n-docosanol cream as well; however, statistical significance for this observation has not been established. Under similar conditions, the PEG preparation did not exhibit activity even when applied 5 times daily. These results indicated to the sponsor that the stearic acid cream can exhibit anti-HSV activity *in vivo* when applied 5 times daily and the PEG preparation appears to be without activity and may be suited for use as a placebo in a human clinical trial.

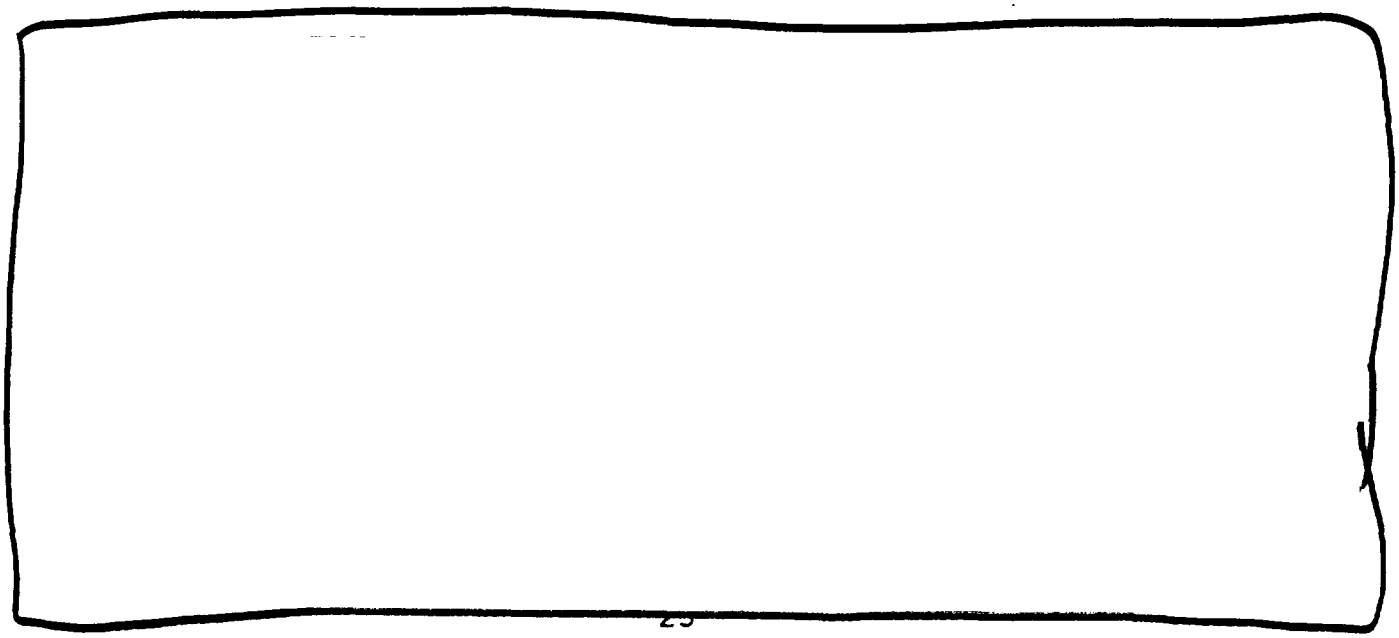
Comment: According to the sponsor, n-docosanol suspended [redacted] had the demonstrated maximal antiviral activity *in vitro*. It is not clear why [redacted] was not included in any of the Lidakol cream formulations for efficacy tests *in vivo*.

[redacted]



E. CLINICAL VIROLOGY

The sponsor has conducted a number of clinic- or patient- initiated clinical studies to determine the efficacy and safety of Lidakol in the treatment of oral-facial recurrent herpes simplex virus infection. Only abbreviated summarized reports of these studies are included for microbioogy review. None of the studies have included any virologic endpoint to evaluate the effectiveness of Lidakol in a clinical setting. All the efficacy parameters were clinical in nature; therefore please see the review of the medical officer for the evaluation of the effectiveness of Lidakol cream in the treatment of oral-facial recurrent HSV infection.



2 pages of revised draft labeling have been redacted from this portion of the document.

Antiviral Activity In Vitro and In Vivo: n-Docosanol exhibits antiviral activity against HSV-1 and HSV-2. Sensitivity test results, expressed as the dose of drug required to inhibit growth of the virus by 50% (ID_{50}) in cell culture *in vitro* are shown in Table I. These test results are known to vary depending upon a number of factors such as virus strain, host cell, virus assay protocol and the vehicle used to suspend the drug. *In vivo* antiviral activity has been demonstrated against cutaneous infections of guinea pigs with either HSV-1 or HSV-2.

Table 1

In Vitro Antiviral Activity of n-Docosanol

Virus Type (Strain)	Cell Type	ID_{50} (mM)	Method of Assay
HSV-1 (Macintyre)	Vero	6-9	Plaque reduction
HSV-1 (Macintyre)	Human fetal foreskin	6-9	Plaque reduction
HSV-1 (Macintyre) acyclovir-resistant	Vero	6-9	Plaque reduction
HSV-1 (KOS)	Vero	9	Cytopathic effect (microscopic examination)
HSV-1 (KOS)	Vero	12	Neutral red
HSV-2 (MS)	Vero	6-9	Plaque reduction
HSV-2 (MS)	Human fetal foreskin	6-9	Plaque reduction
HSV-2 (MS)	MDBK	15-24	Plaque reduction
HSV-2 (MS) acyclovir-resistant	Vero	6-9	Plaque reduction
HSV (oral clinical isolate)	Vero	15	Plaque reductionl
HSV (genital clinical)	Human fetal foreskin	9	Plaque reduction

Drug Resistance: The emergence of HSV strains resistant to the antiviral effects of n-docosanol has not been determined.

G: CONCLUSIONS

G.1. The sponsor is seeking approval of Lidakol (a 10% cream formulation of n-docosanol) for the treatment of recurrent oral-facial herpes simplex virus infection. Initial experiments have provided evidence that n-docosanol is not directly virucidal since it did not adversely influence the infectivity titer of HSV when incubated together for 1 to 4 hours.

G.2. Optimal anti-HSV activity of n-docosanol is dependent upon a number of variables as follows:

G.2.A. Time of pre-exposure of host cells. The host cells must be pre-exposed to high concentrations [redacted] of n-docosanol in a suractant such as [redacted] for about 6 to 24 hours. However, pre-exposure of host cells is not absolute since 0 hour exposure of host Vero cells to Formulations A [redacted] of n-docosanol inhibited HSV-1 plaque formation by 30 or 55%, respectively (Table 1, Section A.2.2, page 6).

G.2.B. The surfactant used to suspend n-docosanol. When suspended in [redacted] n-docosanol was 3 times more effective as inhibitor of plaque formation by HSV-2 (MS strain) than when suspended in [redacted] (Sections A.2.2 and A.5.4). However, Dr. Barnard has indicated that [redacted] was not a factor in contributing to the anti-viral (HSV-1) effect of n-docosanol (page 067, paragraph 5, Vol 2.10).

G.2.C. Host cells used to determine antiviral activities of n-docosanol. Host cells of human origin (e.g., human fibroblasts) were found to be better than monkey kidney (Vero) cells which were better than bovine kidney (MDBK) cells (Table 2, Section 5.4. page 14).

These results should have prompted one to routinely use human fibroblasts (natural host for HSV infections) to analyze the mechanism by which n-docosanol suspended in [redacted] exerts its antiviral activity *in vitro*. It is not clear why the sponsor continued to use the experimentally proven inferior sytem involving the monkey kidney Vero cells to determine antiviral acivities of n-docosanol suspended [redacted] in most of the *in vitro* studies.

G.3. Initial experiments demonstrated that at 37 C physiologic temperature, host Vero cells must not only be pre-exposed to n-docosanol for more than 6 hours but must also be present continuously for 16 hours during- and post-infection, before the drug could be removed without loss of antiviral activity (Lidak Report 107). However, with a new hyptothesis (by the sponsor) that n-docosanol must be modified to a metabolically active form which would "adequately inhibit replication of the initial round of attached virus," these results "seemed incongruous" (pages 123-124, Vol 2.10). Therefore, a series of new experiments were designed to determine the half-life of n-docosanol in the host cells (Lidak Report 120, not submitted before). These studies have demonstrated that while prolonged preincubation was still a requirement, n-docosanol could be removed during the time of virus attachment if the temperature is lowered to 4C for 2 hours without significantly influencing its antiviral activity. Furthermore, the sponsor has also calculated a half life of "activated" n-docosanol to be about 3 hours. As noted earlier, these results with a putative antivirally active form of the test drug which is supposed to be formed during the prolonged preincubation has not explained how

Formulation E [redacted] of n-docosanol successfully inhibited 55% of HSV-plaque formation without any preincubation of the host cells.

G.4. n-Docosanol did not inhibit attachment of the virus to host cells but appears to have prevented virus entry into the host cells. In Lidak Report 118 (not submitted before), the sponsor has provided suggestive evidence that n-docosanol prevented viral entry by "inhibiting the fusion of virus surface protein with cellular plasma membranes." In one experiment where fusion of HSV-2 (labeled with a fluorescent probe, [redacted] with host NC-37B cell surface was measured by the intensity of fluorescence, details of the experimental protocol (e.g., choice of the host cell, titer of fluorescent labeled virus, multiplicity of infection and fluorescent microscopy) and some critical results on the flurescence intensity (with and without anti-gD monoclonal antibody treatment) as measured by [redacted] are missing. Even then, it is not clear why preincubation of host cells with 15 mM n-docosanol [redacted] for 24 hours inhibited fusion of the virus surface protein(s) with host cells by only 50%.

G.5. n-Docosanol undergoes cellular metabolism and polar metabolites isolated after 72 hours of radiolabeling, exhibited similar migration patterns (in silica gels) of phosphatidylcholine and phosphatidylethanolamine. As discussed earlier in Sctions A.2. and A.5.4, the anti-HSV activity of n-docosanol may be correlated with the polar metabolites formed in the host cells during the preincubation period. However, this conclusion is based upon circumstantial evidence which suggests, but does not prove, that "enzymatic conversion of n-docosanol is a necessary prerequisite for its antiviral activity." On the contrary, the sponsor has also stated that "due to the chemical structure of the molecule, which possesses no reactive groups other than a primary alcohol, enzymatic hydrolysis is unlikely" (see subsection 7.4, page 089, Vol 2.9). To prove that n-docosanol undergoes cellular metabolism to interfere with one or more steps of viral entry (by fusion), the metabolite(s) from the host cell membranes must be identified and their antiviral target(s) on the virus envelope must be characterized.

G.6. As noted earlier, the potency of n-docosanol to inhibit various laboratory strains and clinical isolates (including ACV-resistant strains) of HSV-1 and HSV-2 has been found to be dependent upon a number of factors including the host cells, preincubation of host cells. vehicle for the test drug, multiplicity of infection and assay method used. Thus, the ED_{50} values of n-docosanol against various strains of HSV have ranged from [redacted] However, the relationship between the *in vitro* sensitivity of HSV to n-docosanol and clinical response has not been established.

G.7. A variety of enveloped and non-enveloped viruses were also tested for suscetibility to n-docosanol. Non-enveloped (naked) viruses such as poliovirus, adenovirus were not susceptible to n-docosanol. Only those enveloped viruses (e.g., human herpesviruses, HIV-1, RSV) which, the sponsor believes, enter host cells by fusion were susceptible to n-docosanol with the ED_{50} values ranging from [redacted] Lipid-enveloped viruses which enter cells via endocytic pathways may resist the antiviral effects of the test drug wih the exception of influenza A virus. However, as discussed earlier, entry of viruses through virus-mediated fusion or receptor-mediated endocytosis is dependent upon a number of factors and there is no clear understanding yet as to the precise mechanism(s) by which enveloped viruses enter host cells. Even in this NDA application itself, Dr. Barnard has stated that HSV-1 may use both the fusion pathway or the receptor mediated endocytosis pathway depending on the virus strain" (5th sentence, first paragraph, page 066, Vol 2.10) and that enveloped influenza A virus which may enter its host cells by receptor-mediated endocytosis was also susceptible to n-docosanol. Clearly, more detailed and conclusive information on the mechanistics of inhibition

of the fusion process by any antiviral agent is needed to claim that n-docosanol is capable of inhibiting the viral entry mediated by fusion pathway of lipid-enveloped viruses (also see comments in Section B).

G.8. Katz *et al.*, (Proc. Natl. Acad. Sci. USA, 1991, 88:10825-10829) have reported that n-docosanol [redacted] had no appreciabe inhibitory effect on the growth of Vero cells cultured *in vitro* (at up to 100 mg/ml) or several other nucleated human and murine cell lines (up to 25 mg/ml). Therefore, the therapeutic index (or selectivity index) of the drug may vary from 1 to 17 for laboratory strains as well as clinical isolates of HSV-1 and HSV-2. On the other hand, Dr. Barnard of [redacted] has presented evidence (Table 1 in page 069 of Vol. 2.10) that the selectivity index of n-docosanol may range from [redacted] depending on the assay procedures used.

G.9. Topical application of n-docosanol has shown efficacy in both hairy (Hartley) and hairless guinea pig models of HSV infections. Formulation of n-docosanol with [redacted] as the vehicle, found to be superior in demonstrating anti-HSV activity *in vitro*, was not evaluated in any of the animal models. In addition, formulations of n-docosanol with polyethylene glycol, stearic acid and its salts used the animal models were not evlauted for their antiviral activities in any *in vitro* studies. Since the sponsor has demonstrated that the choice of the vehicle for n-docosanol [redacted] greatly influences its [redacted] metabolism and related antiviral activity, it is not clear how results obtained *in vitro* with entirely different formulatons will be related to the the results obtained in animal models *in vivo*. In addition, the relevance of animal models in predicting clinical efficacy in human subjects may not be consistently reproducible.

G.10. After presenting evidence that n-docosanol is not directly virucidal, the sponsor has presented evidence that 12% Lidakol cream may be useful as a "microbicide" in the prevention of vaginal transmission of HIV-1 infection since it prevented SIV infection in rhesus macaques model. The sponsor has not explained how the same n-docosanol which was not virucidal in all the studies in cultured cell *in vitro* and guinea pig models *in vivo* turned out to be a topical vaginal microbiocide that may prevent transmission of HIV. However, if the sponsor has any plan to develop n-docosanol as an anti-HIV agent, another NDA containing all the details of preclinical and clinical virology should be separately submitted for review.

G.11. The sponsor has stated that preliminary attempts to induce laboratory isolates of HSV resistant tc the antiviral effects of *n*-docosanol have been unsuccessful and no susceptibility test methods have been developed for LIDAKOL 10% cream in clinical studies. The sponsor has further stated that the emergence of HSV strains resistant to the antiviral effects of *n*-docosanol appears unlikely because of the drug's mechanism of action which is not dependent upon any viral specific genes or gene products. However, if the vigorous suggestion (of the sponsor) that "the anti-HSV activity of n-docosanol involves cellular uptake and metabolism of the drug" is experimentally proven to be correct, then one may expect that cellular, in contrast to viral, enzmes may be involved in the metabolism of n-docosanol and prolonged exosure to the drug may induce mutations in cellular enzymes leading to altered drug metabolism and its antiviral activity.

G.12. A number of clinical studies were conducted to evaluate the efficacy of Lidakol cream in the treatment of oral-facial HSV infections. None of the clinical studies have included any virologic end-points to determine the effectiveness of the test drug. It is also not clear whether clinical diagnosis of the lesions were based upon any virologic markers. Since efficacy of the

test drug was evaluated in a clinical setting for the first time, all patients should have been monitored for HSV infection and effctiveness of the test drug should have been evaluated by including any commonly practiced virologic methods such as virus culture, viral protein (antigen) or nucleic acid assay.

G. 13. The Package Insert for LIDAKOL cream has many microbiologic concerns which are detailed in Section F above. It is suggested that the microbiologic concerns be addressed and the package insert be rewritten as suggested in Section F.4.

H. RECOMMENDATIONS:

Pending completion of acceptable final draft labeling (see Section F above), this NDA is approved with respect to microbiology.

/S/

Nilambar Biswal, Ph.D.

CONCURRENCES:

HFD530/TLMicro /S/ Signature 7/13/98 Date

CC:

HFD530/Orig. NDA
HFD530/Zeccola, CSO

HFD540