ELSEVIER

International Journal of Pharmaceutics 105 (1994) 227–233

international journal of pharmaceutics

Topical formulation development of a novel thymidylate synthase inhibitor for the treatment of psoriasis

Leo Pavliv *, Ken Freebern, Terry Wilke, Chin-Chih Chiang, Bhasker Shetty, Praveen Tyle

Agouron Pharmaceuticals, Inc., 3565 General Atomics Court, San Diego, CA 92121, USA

(Received 10 May 1993; Modified version received 8 October 1993; Accepted 4 November 1993)

Abstract

A topical anhydrous semisolid was developed for a novel chemical entity to maximize delivery of drug in the target organ, the skin. Excipients were selected based on increasing concentration of drug into the skin and the ability to form semisolids. Using in vitro skin studies, the semisolid product delivered approx. 3-times more drug into the skin than a previous clinical solution formulation without significantly increasing receptor values. In vivo rat studies indicate the semisolid product delivered approx. 8-times more drug than the previously tested clinical solution formulation.

Key words: Psoriasis; Topical formulation; Thymidylate synthase inhibitor

1. Introduction

AG-85 {N,N^4-(3-dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl)-N^4-(prop-2-ynyl)sulfanilyl)indole} is a rationally designed novel chemical based on the three-dimensional structure of the target enzyme, thymidylate synthase (see Fig. 1). AG-85 showed potent in vitro inhibition of the enzyme thymidylate synthase (K_i 8.1 nM) and growth inhibition of continuous tumor cell lines and human keratinocytes (Appelt et al., 1991). AG-85's potent inhibitory activity indicated it as a potential treatment of psoriasis.

A topical solution consisting of 0.1% (w/v) AG-85 in propylene glycol and ethanol (20:80 v/v) was selected for use in the first clinical trial. The clinical trial consisted of 10 evaluable patients with mild to moderate psoriasis. Evaluation criteria consisted of erythema, scaling and induration, as well as, a global evaluation and sub-

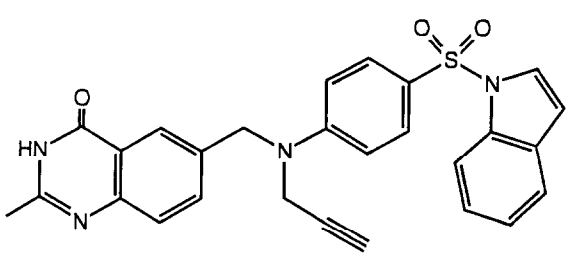

Fig. 1. Chemical structure of AG-85.

* Corresponding author. Tel.: (619) 622-3177; Fax: (619) 622-3299.

SSDI 0378-5173(93)E0332-K

jective scoring of pruritis. Each patient received an application of the 0.1% AG-85 solution, vehicle (propylene glycol and ethanol 20:80 v/v) and fluocinonide 0.1% (Lidex®, Syntex Corp.) as an active control, each applied to a separate psoriatic plaque. Solutions were applied twice weekly for 6 weeks and occluded with an aluminum disc.

Significant improvements in psoriasis were observed for all three study medication sites. The fluocinonide treated site exhibited a statistically significant improvement over both the AG-85 and vehicle treated sites at times of best and last response. Due to the clinical results, it was decided to reformulate the product in an attempt to maximize delivery of AG-85 into the skin. To avoid any possible effects of and need for occlusion in future studies, only semisolid formulations were examined.

Often, formulation development of topical products is not conducted by optimizing drug delivery to the target site, the skin. Instead, formulations are evaluated based on the drug's flux across the skin and formulations are selected based on maximizing flux (Kumar et al., 1992; Shah et al., 1992). Although the etiology of psoriasis remains unclear, the basic pathology of psoriasis is a hyperproliferation of the psoriatic epidermis (Tung and Maibach, 1990). Psoriatic plaques have been reported to be more permeable than normal skin for some drugs, e.g., anthralin (Wang et al., 1987) and lonapalene (Lehman et al., 1992), and equivalent to normal skin for other drugs, such as hydrocortisone (Wester et al., 1983). No definite correlation can be made between the permeability of normal vs psoriatic skin because it will differ depending on the drug, formulation and severity of disease (Maibach and Surber, 1992). Formulation development with AG-85 was based on maximizing delivery of drug to the epidermis and dermis of normal human cadaver skin, realizing the values may be different in psoriatic skin.

The objectives of this project were to (1) select solubilizer(s) that maximize AG-85 solubility and delivery into the skin, (2) evaluate AG-85 delivery enhancers, (3) examine excipients that form pharmaceutically elegant semisolid topicals, (4) examine the effect of varying concentrations of AG-85 on percutaneous absorption and (5) compare different formulations for their effect on delivering AG-85 into the skin using in vitro and in vivo models.

2. Experimental

2.1. Materials

2.1.1. Percutaneous absorption studies

Cryopreserved human cadaver skin was obtained from The New York Firefighters Skin Bank, NY. Polyethylene glycol 400 NF, methanol (Optima grade), acetonitrile (Optima grade), HPLC grade water and ammonium acetate were all purchased from Fisher Chemical Co. AG-85 used in all the studies was synthesized at Agouron Pharmaceuticals, Inc. [^{14}C]AG-85 was purchased from Amersham plc.

2.1.2. Excipients studied

Caprylic/capric triglycerides (CCT) and cetyl alcohol were obtained from Henkel Corp., Ambler, PA. Lauryl lactate was purchased from Van Dyk & Co., Inc., Belleville, NJ. Hydrogenated vegetable oil (Wecobee® M) was purchased from Stepan Co., Maywood, NJ. PEG-8 caprylic/capric triglycerides (Labrasol®), diethylene glycol monoethyl ether (Transcutol®), glyceryl behenate (Compritol® 888 ATO), saturated polyglycolyzed glycerides (Labrafil® M-2130CS) and glycols palmitostearate and phosphated polyoxyethylenated fatty alcohols (Sedafos® 75) were all obtained from Gattefosse Corp., Westwood, NJ (Ritschel and Hussain, 1988). PEG-6 caprylic/capric triglycerides (Softigen® 767) were supplied by Huls America, Inc., Piscataway, NJ. Stearic acid was purchased from Fisher Scientific, Fair Lawn, NJ. Glyceryl monostearate and polyoxyethylene stearate (Arlacel®165) were obtained from ICI Americas, Inc., Wilmington, DE.

2.2. Methods

2.2.1. AG-85 HPLC assay

AG-85 was quantified by reverse-phase high-pressure liquid chromatography (HPLC). The

HPLC system consisted of a Gilson Model 715 system with a Zorbax reverse-phase CN column (4.6 × 250 mm, 5 μ) using 45%/55% (v/v), acetonitrile/0.1 M ammonium acetate as the mobile phase at a flow rate of 2.0 ml/min. 20 μl of sample solutions were injected. Peak areas of AG-85 were detected at 300 nm. Standard curves of AG-85 in methanol and 60% polyethylene glycol 400/water (v/v) were linear in the range of 0.8–4.1 μg AG-85/ml (Wilke et al., 1994).

2.2.2. Percutaneous absorption experiments

Experiments were conducted with cryopreserved human cadaver skin from the trunk of white male adults. Skin samples were thawed at room temperature, cut into approx. 1 cm^2 pieces and soaked in distilled water for approx. 5 min prior to mounting on Franz diffusion chambers (Crown Glass Co., Inc., Somerville, NJ). Total exposed skin surface was 0.9 cm^2. Skin thickness was measured using a micrometer and finding the difference in thickness between two glass slides with and without skin placed between them. To maintain sink conditions, the receptor fluid consisted of 60% polyethylene glycol (PEG) 400 in distilled water. Receptor solutions were continually stirred and maintained at 37°C with a recirculating water bath. Accurate weights of AG-85 containing products, solutions or semisolids, were applied to mounted skin samples in the Franz diffusion cells. AG-85 skin penetration was measured by collecting receptor solutions at specified times after application of product, filtering and analyzing by HPLC. To remove AG-85 not associated with the skin, skin samples were prepared by washing the skin surface three times with 60% PEG 400 solution, drying with absorbent wipes and tape stripping three times with cellophane tape. The samples were then cut into small pieces and AG-85 extracted by sonicating for 3 h with 2.0 ml methanol. The AG-85 solution was filtered through a 0.45 μ nylon membrane (Gelman acrodisc) and analyzed by HPLC. A study was conducted where the epidermis was separated into two different layers using a pair of tweezers. The skin was not tape stripped in this study. All other methods remained the same.

2.2.3. Solubility studies

Initial solubility studies were performed by adding AG-85 to the excipient, heating to approx. 70°C and sonicating for 6 h. After cooling to room temperature, the suspensions were then filtered through 0.45 μ nylon membranes and assayed by HPLC.

2.2.4. Product preparation

AG-85 solutions were prepared by slow addition of drug into the appropriate solubilizers with vigorous mixing using a Lightnin® propeller mixer or a magnetic stirrer. AG-85 solutions in Transcutol®, Labrasol® or Softigen® 767 required heating to approx. 90–110°C. Semisolids were manufactured by combining a heated AG-85 solution (70°C) with melted semisolid ingredients (70°C). These products were mixed and cooled until a uniform semisolid formed.

2.2.5. In vivo skin penetration (rats)

A known amount of AG-85 1.25% cream or 0.1% solution, both containing [^{14}C]AG-85 (31.06 μCi/mg), was applied onto a shaved 10 cm^2 area on Sprague Dawley rats and the application site covered with a tent made from filter paper and aluminum foil. Elizabethan collars prevented the rats from disturbing the treatment areas. Animals were euthanized with CO_2 at 2 and 24 h. The application site was then washed with soap and water to remove residual product. Skin from the application and surrounding area was dissected and digested in Soluene 350 (Packard Instruments). [^{14}C]AG-85 was analyzed by liquid scintillation techniques using a Beckman Model LS 1801 Liquid Scintillation Counter.

3. Results and discussion

3.1. Solubilizer selection

AG-85 has very limited solubility in most hydrophilic and lipophilic excipients. Only four of the excipients tested (Transcutol®, Labrasol®, Softigen® 767 and polyethylene glycol 300), demonstrated an AG-85 solubility greater than 1%. Filtered saturated solutions of AG-85 in

Table 1
In vitro comparison of solubilizers (human cadaver skin)

Product	μg AG-85/cm² (mean ± SD) (24 h; n = 3)
AG-85 Sat. in Transcutol®	1.88 ± 0.65
AG-85 Sat. in Labrasol®	1.57 ± 0.18
AG-85 Sat. in Softigen® 767	1.21 ± 0.05
AG-85 Sat. in Transcutol®/ Labrasol® (1:1)	2.54 ± 1.51
AG-85 Sat. in Transcutol®/ Softigen® 767 (1:1)	1.26 ± 0.05
AG-85 Sat. in Labrasol®/ Softigen® 767 (1:1)	1.20 ± 0.29

these excipients contained 2.9, 2.0, 1.5 and 2.9% AG-85, respectively. Although the solubility of AG-85 in polyethylene glycol was acceptable, it was not used in these studies because earlier studies carried out in our laboratories showed poor skin permeation from polyethylene glycol containing solutions.

Saturated solutions of AG-85 were made in each of the three solubilizers (Transcutol®, Labrasol® and Softigen® 767) based on solubility, as well as 1:1 combinations of the solubilizers with AG-85. Table 1 shows the amount of AG-85 in the intact skin from an in vitro percutaneous absorption experiment. No effort was made to separate the skin into the epidermal and dermal layers. AG-85 receptor solution values were insignificant, averaging less than 0.5 μg/ml. A combination of Labrasol and Transcutol (1:1) was selected as the AG-85 solubilizer due to AG-85 skin levels at least 35% higher than the other individual or combination of solubilizers tested.

Table 2
In vitro comparison of two candidate delivery enhancers (human cadaver skin)

Product	μg AG-85/cm² (mean ± SD) (24 h; n = 3)
AG-85 Sat. in 90% Labrasol® 10% lauryl lactate	3.26 ± 1.21
AG-85 Sat. in 90% Labrasol®/ Transcutol® (1:1) and 10% lauryl lactate	4.74 ± 1.41
AG-85 Sat. in 90% Labrasol® 10% caprylic/capric triglycerides (CCT)	1.72 ± 0.70
AG-85 Sat. in 90% Labrasol®/ Transcutol® (1:1) and 10% CCT	2.86 ± 2.00

Table 3
In vitro comparison of three candidate semisolid excipients (human cadaver skin)

Product	μg AG-85/cm² (mean + SD) (24 h; n = 3)
1% AG-85 semisolid with Arlacel® 165	3.12 + 0.67
1% AG-85 semisolid with Sedafos® 75	2.61 ± 0.79
1% AG-85 semisolid with Wecobee® M	1.69 + 0.33
1% AG-85 semisolid with stearic acid and cetyl alcohol	1.85 + 0.24

3.2. Drug delivery enhancer selection

Lauryl lactate and caprylic/capric triglycerides (CCT), two pharmaceutically acceptable excipients, were examined to measure their effect on AG-85 skin and receptor values. Saturated solutions of AG-85 in Labrasol or Labrasol and Transcutol containing either 10% lauryl lactate or 10% (CCT) were studied. AG-85 skin values from in vitro percutaneous absorption studies comparing the potential delivery enhancers are shown in Table 2. Lauryl lactate containing solutions delivered at least 66% more AG-85 into the skin than solutions with CCT and was selected for use in the cream.

Table 4
AG-85 concentration optimization in vitro comparison (human cadaver skin)

Product	μg AG-85/cm² (mean ± SD) (24 h; n = 3)
1.25% AG-85 cream	5.54 ± 1.00
1.0% AG-85 cream	5.32 ± 0.79
0.625% AG-85 cream [a]	2.80 ± 0.85
0.50% AG-85 cream [b]	2.19 ± 0.15

[a] Same saturation concentration as 1.25% cream.
[b] Same saturation concentration as 1.0% cream.

Table 5
AG-85 topical cream, 1.25%

Ingredient	Chemical name	Supplier	Percent
Labrafil® M-2130CS	saturated polyglycolyzed glycerides	Gattefosse	25.00
Labrasol®	PEG-8 caprylic/capric triglycerides	Gattefosse	24.37
Transcutol®	diethylene glycol monoethyl ether	Gattefosse	24.37
Arlacel® 165	glyceryl monostearate and polyoxyethylene stearate	ICI	10.00
Ceraphyl® 31	lauryl lactate	Van Dyk	10.00
Compritol® 888 ATO	glyceryl behenate	Gattefosse	3.00
Benzyl alcohol, NF	benzyl alcohol, NF	Spectrum	2.00
AG-85	AG-85	Agouron	1.25
BHT	butylated hydroxytoluene, FCC	Spectrum	0.01

3.3. Semisolid excipient selection

A number of semisolid forming excipients were tested to evaluate their effect on AG-85 delivery. Formulations tested contained the three previously selected excipients, Transcutol®, Labrasol® and lauryl lactate along with various semisolid excipients. Creams were prepared with different concentrations of these excipients, with the goal of forming pharmaceutically elegant products with similar consistencies. The concentration of AG-85 remained the same.

Table 3 summarizes the results of a study comparing four alternative semisolid formulations. Semisolids containing either stearic acid with cetyl alcohol or semisolids containing Wecobee® M delivered less AG-85 than the other excipients tested. Substitution of the anionic excipient, Sedafos® 75 with the nonionic Arlacel® 165 resulted in approx. 20% higher AG-85 skin values. Therefore, Arlacel® 165 was selected as a semisolid excipient for the cream.

3.4. AG-85 concentration level

Various concentrations of AG-85 were tested in semisolids. Increasing concentrations of AG-85 in the products resulted in higher levels of AG-85 in the skin. Studies were also conducted maintaining the same level of AG-85 saturation in the cream while decreasing the amount of AG-85 in the cream. This was accomplished by substituting half the AG-85/Labrasol/Transcutol solution used in the cream with Labrafil® 2130 CS, a semisolid forming excipient in the cream. AG-85 has negligible solubility in Labrafil® 2130 CS. Products containing more AG-85 but at the same saturation concentration, also delivered significantly more AG-85. Table 4 lists the results of two experiments comparing AG-85 concentration

Table 6
In vitro comparison between 1.25% cream and 0.1% solution (human cadaver skin))

Product		μg AG-85/cm^2 after 24 h (mean ± SD)			Mean value from all points
		Study 3392	Study 3692	Study 3992	
0.1% AG-85	receptor	0.14 ± 0.20	0.54 ± 0.32	0.66 ± 0.52	0.46 ± 0.41
solution	skin	2.88 ± 0.64	3.37 ± 1.22	3.27 ± 1.26	3.32 ± 1.21
(80% ethanol 20% PG)		(n = 3)	(n = 5)	(n = 3)	(n = 11)
1.25% AG-85	receptor	0.16 ± 0.23	0.58 ± 0.36	1.02 ± 0.36	0.67 ± 0.47
anhydrous	skin	8.26 ± 0.94	7.87 ± 1.54	11.51 ± 1.80	9.40 ± 2.33
cream (Table 5)		(n = 3)	(n = 6)	(n = 6)	(n = 15)

Table 7
In vitro comparison between 1.25% cream and 0.1% solution (human cadaver skin)

Product	μg AG-85/cm² after 24 h (mean ± SD)		
	Receptor	Epidermis	Dermis
0.1% AG-85 solution (80% ethanol : 20% PG) ($n = 4$)	0.30 ± 0.36	8.59 ± 2.37	1.12 + 0.45
1.25% AG-85 anhydrous cream (Table 5) ($n = 7$)	0.22 ± 0.24	17.14 ± 8.87	3.86 ± 1.62

on delivery of drug into the skin. Based on these experiments, an AG-85 concentration of 1.25% in an anhydrous semisolid was selected to be used in the cream.

3.5. *In vitro product evaluation*

Excipients were selected to produce a semisolid that delivered more AG-85 into the skin than the previous clinical solution formulation. An anhydrous cream, composed of the excipients listed in Table 5, was manufactured and compared against the solution formulation used in the first clinical study, using in vitro percutaneous absorption studies. The cream delivered approx. 3-times more AG-85 into human cadaver skin compared to the previous clinical solution formulation. Receptor solution values for the two products were similar, less than 1 μg (Table 6). These low receptor solution values indicate a low likelihood for systemic toxicity with this compound (see Table 6). An additional in vitro study was conducted in which the epidermis was separated from the dermis to determine how much AG-85 was present in each. Epidermal AG-85 values were 17.14 μg/cm² for the cream vs 8.59 μg/cm² for the solution, approximately twice as much for the cream (Table 7). These AG-85 epidermal values (Table 7) are considerably higher than those observed in the non separated skin (Table 6) and may be due to lack of tape stripping the skin prior to separation. AG-85 concentration in the dermal layer was 3.86 μg/cm² for the cream and 1.12 μg/cm² for the solution, approx. 3.5-times more AG-85 with the cream.

Table 8
In vivo skin penetration (10 cm²) (rats)

Time (h)	AG-85 cream, 1.25%	AG-85 solution, 0.1%
2	111 ± 41.3 [a]	34 ± 11.3 [b]
24	370 ± 97.4 [a]	45 ± 6.1 [b]

[a] Mean ± SD of data from four rats.
[b] Mean ± SD of data from two rats.

3.6. *In vivo product evaluation (rats)*

AG-85 topical cream, 1.25% was compared to the previous clinical solution formulation in rats after dermal application of both products. The values listed in Table 8 are mean values from four rats receiving 1.25% [^{14}C]AG-85 cream and two rats receiving the 0.1% [^{14}C]AG-85 solution. After 2 and 24 h the cream delivered approx. 3- and 8-times more AG-85, respectively, into the skin than the topical solution. Equalizing for surface area between the in vitro and in vivo studies the in vivo rat study showed 24 h AG-85 skin values with the cream approx. 4-times higher (37 μg/cm²) than the in vitro studies using human cadaver skin (8.3 μg/cm²). However, for the solution in vivo studies showed approximately only a 40% increase (4.5 μg/cm²) compared to the in vitro studies (3.2 μg/cm²). The observed increase in permeability in rat skin compared to human skin is consistent with previously published studies. Although rats were used in this study, they are not typically good in vivo models for man due in part to the increased permeability of their skin (Barry, 1983; Bronaugh and Maibach, 1985).

4. Conclusion

An anhydrous topical cream was developed that utilized in vitro percutaneous absorption

studies to maximize drug delivery into the target organ, the skin. Solubilizers, excipients and drug concentration were selected based on maximizing drug concentration in the skin. A 1:1 ratio of Transcutol® and Labrasol® demonstrated the highest AG-85 skin values and was selected as the solubilizer for AG-85. Products containing lauryl lactate resulted in the greatest AG-85 skin values which was therefore incorporated into the cream to increase drug levels in the skin. The semisolid forming excipients Arlacel® 165, Compitrol® 888 ATO and Labrafil® M-2130CS were each selected for this product based on their ability to form a pharmaceutically elegant cream and their effect on AG-85 skin values. Based on our drug concentration studies, a 1.25% concentration of AG-85 was selected as the optimal concentration for this product.

In vitro percutaneous absorption studies indicated the topical cream delivered approx. 3-times more AG-85 into human cadaver skin than the previous clinical solution formulation. In vivo rat studies indicated that the cream delivered approx. 8-times more AG-85 into the skin than the solution after 24 h. Based on this work, the 1.25% AG-85 anhydrous cream has been selected as the formulation for a second clinical study for the treatment of psoriasis.

5. Acknowledgments

The authors thank Stephanie Webber, Dominic Andrada and Maha Kosa in the design and execution of the in vivo rat study. We also thank Cindee Sandstoe for assistance in preparing the manuscript.

6. References

Appelt, K., Bacquet, R.J., Bartlett, C.A., Booth, C.L.J., Freer, S.T., Fuhry, M.A.M., Gehring, M.R., Herrmann, S.M., Howland, E.F., Janson, C.A., Jones, T.R., Kan, C.C., Kathardekar, V., Lewis, K.K., Marzoni, G.P., Matthews, D.A., Mohr, C., Moomaw, E.W., Morse, C.A., Oatley, S.J., Ogden, R.C., Reddy, M.R., Reich, S.H., Schoettlin, W.S., Smith, W.W., Varney, M.D., Villafranca, J.E., Ward, R.W., Webber, S., Webber, S.E., Welsh, K.M. and White, J., Design of enzyme inhibitors using iterative protein crystallographic analysis. *J. Med. Chem.*, 7 (1991) 1925–1934.

Barry, B.W., *Dermatological Formulations: Percutaneous Absorption*, Dekker, New York, 1983, pp. 138–145.

Bronaugh, R.L. and Maibach, H.I., *Percutaneous Absorption: Mechanisms, Methodology, Drug Delivery*, Dekker, New York, 1985, p. 150.

Kumar, S., Malick, A., Meltzer, N., Mouskountakis, J. and Behl, C., Studies of in vitro skin permeation and retention of a leukotriene antagonist from topical vehicles with a hairless guinea pig model. *J. Pharm. Sci.*, 7 (1992) 631–634.

Lehman, P., Tomlinson, R., Johnson, R., Johnson, J., Olerud, J., Akers, W. and Franz, T., Percutaneous absorption and metabolism of lonapalene in psoriatic skin. *Pharm. Res.*, 9 (1992) 1145–1151.

Maibach, H.I. and Suber, C., *Topical Corticosteroids*, Karger, Basel, 1992, pp. 139–140.

Ritschel, W.A. and Hussain, A.S., Influence of selected solvents on penetration of griseofulvin in rat skin, in vitro. *Pharm. Ind.*, 50 (1988) 483–386

Shah, V., Behl, C., Flynn, G., Higuchi, W. and Schaefer, H., Principles and criteria in the development and optimization of topical therapeutic products. *J. Pharm. Sci.*, 10 (1992) 1051–1054.

Tung, J. and Maibach, H., The practical use of methotrexate in psoriasis. *Drugs*, 5 (1990) 697–712.

Wang, J., Patel, B., Ehmann, C. and Lowe, N., The release and percutaneous penetration of anthralin products, using clinically involved and uninvolved psoriatic skin. *J. Am. Acad. Dermatol.*, 16 (1987) 812–821.

Wester, R. Bucks, D. and Maibach, H., In vivo percutaneous absorption of hydrocortisone in psoriatic patients and normal volunteers. *J. Am. Acad. Dermatol.*, 8 (1983) 645–647.

Wilke, T.L., Nguyen, T.H. and Tyle, P., HPLC method development for AG-85 and benzyl alcohol in anhydrous topical formulations. *Int. J. Pharm.*, 103 (1994) 187–197.