

Shorter Methods for Evaluating Antidandruff Agents

JAMES J. LEYDEN, M.D., KENNETH J. MCGINLEY, and
ALBERT M. KLIGMAN, M.D., Ph.D.*

Presented December 2, 1974, SCC Annual Meeting, New York City

Synopsis—Two schedules are described in which the efficacy of ANTIDANDRUFF AGENTS can be assessed in panels of 10 subjects.

The first employs shampooing twice weekly for 3 weeks with clinical grading and CORNEOCYTE COUNTS (quantification of desquamating cells) once weekly and after a one-week follow-up. In the second, the test agent is applied once daily for 5 weekdays for 2 weeks. Corneocyte counts and CLINICAL GRADING are done 4, 8, and 12 days after the last exposure.

With the corneocyte technique, both schedules can be employed to evaluate CYTOSTATIC AGENTS on nondandruff subjects.

INTRODUCTION

Evaluation of antidandruff preparations has been hampered by the lack of standardized schedules and, especially, by the lack of reliable modes of assessment. Gross visualization is the usual means of appraisal. It is hardly surprising that different observers often disagree when evaluating the same agent.

Recently, we described an objective procedure for quantifying desquamating horny cells and demonstrated its usefulness in the appraisal of antidandruff agents (1). This procedure, the corneocyte count, employs a nonionic detergent to sample a defined area of the scalp. The surfactant disperses the collected horny cells and allows them to be counted in a hemocytometer. Corneocyte counting adds objectivity to the evaluation of antidandruff agents, but does not supplant careful clinical evaluation. In dandruff there is both an increased shedding of horny cells and a greater quantity of visible

*Department of Dermatology, School of Medicine, University of Pennsylvania, Philadelphia, Pa.

scales or squames (2). Corneocyte counting measures the quantity of horny cells, while clinical grading assesses the amount of scales.

We have previously demonstrated that with effective antidandruff agents, twice weekly shampooing for 1 month results in significant decreases in grades and counts. In this report, we will describe two shorter modifications, namely, daily shampooing for 10 days and twice weekly shampooing for 3 weeks. In addition, we shall show that agents exerting cytostatic effects may be studied in subjects free of dandruff by measuring the effect on the corneocyte count using either of the 2 schedules.

Clinical Grading

The grading scheme, based on a 10-point scale, has been previously described (1). The amount of scaling is estimated by scraping the scalp at multiple sites with a wooden tongue blade. Subjects must not have used an active agent for 1 month. Pretreatment grading is done 4 days after a bland shampoo. Test panels consist of 10 subjects with grade 5 dandruff or higher (grade 4 represents mild dandruff and grade 5 moderate dandruff).

Corneocyte Count

This procedure has been described in detail elsewhere (3). One ml of buffered 0.1%-Triton-X-100 is placed in a 3.8 square centimeter glass cup placed on a small area of the scalp from which the hair has been clipped. The surface is rubbed 1 min with a teflon rod, the wash fluid is aspirated, and after repeating this procedure, the two samples are pooled. The cell suspension is agitated mechanically, and the cells counted in a Fuchs-Rosenthal hemocytometer. Only one site is sampled since we have demonstrated that the counts do not vary in different areas (1).

Statistics

Because corneocyte counts are log-normally distributed, the values are transformed into logarithms and expressed as the geometric mean count per square centimeter. Mean grades were calculated arithmetically. Statistical significance for reduction of corneocyte counts is measured by the students' T-test.

Twice Weekly Shampooing—Three Week Schedule

The protocol is outlined in Table I. Two pretreatment bland shampoos are employed to achieve base line levels. The subjects are then shampooed twice weekly 6 times. Grades and counts are obtained on days 0, 7, 14, and 21—always 4 days after the last shampoo. Bland shampoos are then resumed on days 22 and 24 with a final assessment on day 28.

Table I
Twice Weekly Three-Week Treatment Schedule

Day	Pretreatment			Treatment					Posttreatment		
	-7	-4	0	3	7	10	14	17	21	24	28
Bland shampoo	x	x							x	x	
Test shampoo			x	x	x	x	x	x			
Grade			x		x		x		x		x
Corneocyte count			x		x		x		x		x

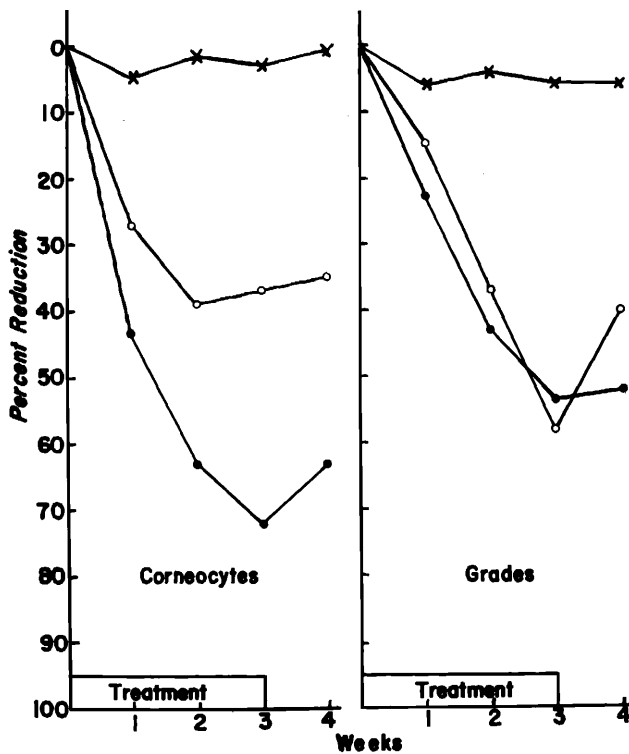


Figure 1. Graphic representation of data in Table I, expressed as percentage reductions from original level. Effectiveness of both shampoos is immediately apparent in contrast to control. Grades and counts are still significantly decreased 1 week after the last shampoo: (x) control; (o) 2 per cent ZPT; (*) 2.5 per cent SeS

The results obtained by the use of three agents are shown in Fig. 1 and Table II. The bland shampoo produced no change in the clinical grade or corneocyte count. A significant reduction in the count and grade was evident in 1 week with both 2.5 per cent selenium sulfide (SeS) and 2 per cent zinc

Table II
Twice Weekly—Three-Week Schedule

Shampoo	Day	Dandruff				
		0	7	14	21	28
A ^a Control shampoo #10						
Grade		5.2	4.9	5.0	4.9	4.9
Corneocytes	GM ^d	1,013,000	860,000	894,800	883,900	898,600
	SD ^e	0.14	0.13	0.08	0.12	0.16
B ^b +2 per cent ZPT #12						
Grade		5.2	4.6	3.4	2.3	3.3
Corneocytes	GM	1,074,000	785,900	658,900	674,100	695,000
	SD	0.10	0.09	0.12	0.12	0.11
C ^c ++2.5 per cent SeS #12						
Grade		5.4	4.2	3.1	2.5	2.6
Corneocytes	GM	1,205,000	683,100	444,600	340,200	444,000
	SD		0.22	0.21	0.13	0.24
Non-Dandruff						
A Control shampoo #12						
Corneocytes	GM	788,300	707,000	733,000	767,000	794,000
	SD	0.25	0.23	0.21	0.25	0.22
B 2 per cent ZPT #13						
Corneocytes	GM	833,900	463,500	549,600	489,300	502,900
	SD	0.18	0.16	0.18	0.31	0.19
C 2.5 per cent SeS #12						
Corneocytes	GM	799,300	589,300	489,100	319,400	384,300
	SD	0.11	0.24	0.16	0.24	0.15

^aJohnson & Johnson Baby Shampoo.

^bHead & Shoulders.

^cSelsun.

^dGeometric mean.

^eStandard deviation: the values enclosed are statistically different from the pretreatment figures.

Table III
Intensive Shampooing Schedule

Day	Pretreatment		Treatment												Posttreatment			
	-7	-4	0	1	2	3	4	5	6	7	8	9	10	11	12	16	20	24
Bland shampoo	x	x																
Test shampoo			x	x	x	x	x	x			x	x	x	x	x			
Grade			x													x	x	x
Corneocyte count			x													x	x	x

pyrithione (ZPT). With ZPT, the corneocyte count fell from a geometric mean of 1,074,000 to 677,000 cells per square centimeter at the end of 3 weeks. A significant reduction was still present at the 1 week follow-up (695,000 cells per square centimeter). SeS produced an even greater fall by the third week (the count fell from 1,205,000 to 340,200 cells per square centimeter). The 1-week follow-up corneocyte count was only 444,000 cells per square centimeter.

Both agents produced a sharp decrease in clinical grades, SeS having the greater effect. With ZPT, the clinical grades started to increase 1 week post-treatment, while with SeS there was no evidence of relapse.

Comment

This assay approximates the manner in which a patient would normally use a product. We view it as a suitable procedure for determining the efficacy of finished formulations.

Intensive Shampooing Schedule

The protocol is detailed in Table III. The scalp is shampooed 10 times in a 12-day period (5 days a week for 2 weeks). Corneocyte counts and clinical grades are done on day 0 and then 4, 8, and 12 days after the last shampoo. The scalp is *not* shampooed during this 12 day follow-up period.

Four days after the last shampoo (Table IV), both 1 per cent ZPT and 2.5 per cent SeS produced highly significant reductions in the counts and grades. However, by 8 days after the last shampoo, the corneocyte count

Table IV
Intensive Shampooing

Shampoo	Pretreatment		-4	p ^a	Posttreatment			
	Number	Day 0			-8	p	-12	p
A ^b Control shampoo								
Nondandruff	12	629,800	494,100	NS ^c	546,300	NS	437,200	NS
Dandruff	12	989,100	893,100	NS	1,042,000	NS	942,200	NS
D ^c 1% ZPT								
Nondandruff	10	650,300	249,800	<0.01	477,400	NS	688,700	NS
Dandruff	12	1,054,000	493,900	<0.01	721,000	NS	852,100	NS
E ^d 5% SES								
Nondandruff	10	495,700	160,300	<0.001	130,700	<0.001	115,400	<0.001
Dandruff	11	1,134,000	406,200	<0.001	307,200	<0.001	348,100	<0.001

^aP value calculated on basis of students' T-test.

^bJohnson & Johnson Baby Shampoo.

^cDanex.

^dExsel.

^eNonsignificant.

and clinical grade in those subjects treated with ZPT had returned to pre-treatment levels, while SeS was still highly effective. In the case of 2.5 per cent selenium shampoo, the counts and grades were still significantly depressed 12 days after the last shampoo.

Comment

With this intensive procedure, effectiveness can be ascertained in 18 days (at the 4 day follow-up). A complete test (12 day follow-up) takes 1 month. This schedule is well adapted for comparisons of effectiveness, especially with regard to persistence of action. It serves as a rapid test for screening new agents and for evaluating formulation changes, whether of the vehicle or the concentration of the active ingredients.

When the procedure was shortened to only 5 daily shampoos, neither ZPT nor SeS were effective. Intensive shampooing also may reveal irritancy potentialities. In that case, the corneocyte counts will go up instead of down.

Assessment of Cytostatic Effects in Nondandruff Subjects

Most, but possibly not all, agents which are beneficial in dandruff exert a cytostatic effect; they decrease the rate at which horny cells are manufactured by the epidermis. It should be possible, therefore, to demonstrate decreased epidermal proliferation in subjects without dandruff, using the corneocyte count as the sole measurement.

We evaluated ZPT and SeS shampoos on nondandruff subjects, whose clinical grades were 3 and below with the twice weekly treatment schedule. Twelve subjects used a bland shampoo; 13 used 2 per cent ZPT; and 12 used 2.5 per cent SeS. In the intensive shampooing schedule, 32 subjects were divided into 3 groups and evaluated with either a bland shampoo; 1 per cent ZPT, or 2.5 per cent SeS.

With the twice weekly schedule, both ZPT and SeS significantly suppressed the production of horny cells (Tables II and IV). Two per cent ZPT produced a significant reduction in the corneocyte count in 1 week's time, which was still apparent 1 week after the last shampoo (Table II). SeS produced an even greater reduction, reducing the corneocyte count from 779,300 to 319,400 cells per square centimeter in 3 weeks and to 384,300 cells per square centimeter at the 1 week follow-up measurement (Table II).

In the intensive shampooing schedule, 4 days after 10 shampoos with 1 per cent ZPT, there was significant reduction in the corneocyte count (from 650,300 to 249,800). By 8 days, the mean count had returned to the original level. On the other hand, 2.5 per cent SeS, produced a decrease in the corneocyte count, which remained significantly low 8 and 12 days after shampooing (Table IV).

Comment

With ZPT and SeS, the same pattern of corneocyte reduction was obtained in subjects with and without dandruff. Because subjects with grades of less than 5 are about 5 times more numerous than subjects with moderate to severe dandruff, it is advantageous to employ these methods in the evaluating of agents which exert their effect through cytostatic activity.

DISCUSSION

Our aim has been to devise simple, workable assays for evaluating anti-dandruff preparations. Agents which truly affect dandruff must do more than merely remove scales. With any surfactant, shampooing daily or every other day, will remove scales as fast as they are formed; the condition simply becomes invisible. Agents, which affect the basic process, must either suppress the production of horny cells or prevent them from aggregating into large flakes. In dandruff subjects, we employ both the subjective method of clinical grading and the objective corneocyte count. The twice weekly 3-week schedule is utilized for evaluation of final formulations, while the intensive shampooing schedule is perhaps best suited for screening and evaluating formulation changes. Cytostatic agents can be evaluated by either schedule in subjects free of dandruff.

To date, our studies have concentrated on two time tested and proven agents, SeS and ZPT, which now serve as our bench-marks for comparison of the efficacy of other agents. However, even within these standards, there can be significant variations with different formulations. For example, one formulation of ZPT (product D, 1 per cent ZPT) proved equally effective as 2 per cent ZPT (product B). Obviously, the vehicle plays an important role in the effectiveness of the final product. The two formulations of 2.5 per cent SeS were equivalent. We have also had other experiences in which various other formulations of these agents resulted in a difference both in clinical efficacy and ability to suppress the production of horny cells.

It is possible that there are agents, which do not work by suppressing the production of horny cells, but rather prevent aggregation of cells into visible flakes. Coal tar might be one of these. With such agents, the corneocyte count might remain unchanged, while the clinical grade could decrease. At the moment, we lack adequate experience with agents that are commonly used in antidandruff shampoos such as tars, sulfur, salicylic acid, and quaternary ammonium salts. These formulations are now being investigated.

ACKNOWLEDGMENT

We would like to thank Mr. Ferdinand Huber for his expert assistance.

(Received March 27, 1975)

REFERENCES

- (1) A. M. Kligman, R. R. Marples, L. R. Lantis, and K. J. McGinley, Appraisal of efficacy of antidandruff formulations, *J. Soc. Cosmet. Chem.*, **25**, 73 (1974).
- (2) A. M. Kligman, K. J. McGinley, and J. J. Leyden, The nature of dandruff, *J. Soc. Cosmet. Chem.*, in press.
- (3) K. J. McGinley, R. R. Marples, and G. Plewig, A method for visualizing and quantitating the desquamating portion of the human stratum corneum, *J. Invest. Dermatol.*, **53**, 107 (1969).